MONOGRAPH
ON
DENTAL CARIES
AND
CARBOHYDRATES

M O N O G R A P H

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A N D

C A R B O H Y D R A T E S

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DENTAL CARIES AND CARBOHYDRATES

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DENTAL CARIES AND CARBOHYDRATES

Summary

A dictionary definition of caries reads: "tooth decay; the pathological process of localized destruction of tooth tissue by micro-organisms". A British panel on cariogenic foods, Committee on Medical Aspects of Food Policy (30) reported in 1969 a consideration of the carogenic action of foods; the following statement is taken from the report:

> "Although carious lesions, are primarily of environmental origin, their onset and rate of progression may be influenced in greater or lesser degree by the physical and chemical structure of the teeth. Dental caries is a disease which starts at the surface of the tooth, it penetrates the enamel and dentine, and if unchecked will eventually reach the dental pulp destroying the tooth. The onset of the initial lesion is due to demineralisation by the acid secretions of micro-organisms on the enamel surface of the tooth in a structure known as the dental plaque. Incontrovertible evidence exists that the micro-organisms of the plaque can convert fermentable carbohydrates into organic acids and it is these acids which lower the pH locally to a point where dissolution of the highly mineralised enamel cover occurs, causing the initial break on the surface of the enamel."

The report deals with the importance of cariogenic diets in young children, implicating sucrose as "the sugar most likely to promote caries". The panel was concerned only with rampant caries among young children and felt that this severe condition of the disease was increasingly prevalent. Rampant caries was defined as: "a lesion of acute onset involving many or all of the erupted teeth, rapidly destroying coronal tissue, often on surfaces normally resistant to decay and leading to the early involvement of the dental pulp and when involving the deciduous dentition occurs typically in very young children soon after their teeth have erupted.

Davies (41) discusses dental caries as an ecological problem, and observes that, as with all mass diseases, the health status of a community will be determined by a dynamic relationship among three factors: the agent of the disease, the host resistance and the environment (which may affect both agent and host resistance). The immediate environmental factor, the diet, is itself influenced by a large number of environmental factors, such as socio-economic status, climate, urbanization, war, taboos and fads, religious customs and methods of food processing and storage. "Thus dental caries is a multifactorial disease". The role of fermentable carbohydrates in the caries process has been established by four types of evidence: epidemiological, chemical, animal and human

studies. Additional discussion by Davies of diet and caries is summarized by the author: (1) Caries occurs when the state of balance between the agents which cause the disease overcome the resistance of the teeth to demineralisation. (2) The intensity of the attack by demineralising agents can be minimised by reducing the frequency which which sweet sticky foods are eaten. This can best be achieved by: (a) avoidance of sugar in a sticky form. If the sweet sticky food is to be eaten, it is better to take it at meals instead of between meals. (b) by eliminating sugar between meals or, if this is not practicable, taking it in liquid form instead of solid form. (c) by ending meals with firm fibrous foods and immediate toothbrushing followed by vigorous rinsing of the mouth with warm water. (3) The resistance of the teeth can be maximised by ensuring an adequate intake of fluoride during the period of tooth development preferably in the form of fluoridated water or, if this is not possible, in tablet form to the extent of 0.5 mg. fluoride ion for children under three years of age and 1 mg. fluoride ion for children aged three years and over.

Bibby (5) has surveyed the food relationships to recognized variations in caries prevalence that have occurred in relation to time, geography, social status, race, sex, or manner of life. Chronologically, there has been increased caries incidence related to the worldwide introduction and use of refined sugar and flour. Geographically, poorly nourished populations in Asian and South American countries seem to have less caries than populations in the more affluent Western world; rampant caries and the use of highly refined wheat flour seem to go together. Primitive peoples whose customs have been changed through contact with "civilized" cultures have frequently been noted to have concomitant increases in caries occurrence; again, this seems to have arisen through the introduction of refined sugar and wheat flour. Wartime changes in nutrition have been accompanied by changes in caries incidence, probably by decreased use of refined foods as well as by rationing. A higher caries attack rate among girls than among boys has been well established, but the reasons remain obscure. Institutionalized children have been shown to have less tooth decay than children of the same age group living with families. Studies such as the classical Vipeholm dental caries study reported by Gustafsson et al. (80) some twenty years ago have been useful. Many other curious relationships between caries and the manner of living have been noted. Though sugar use and extent of caries attack have been universally associated, other factors also must be considered, and meaningful conclusions are not yet possible.

Bibby and Shaw (6) had earlier, in 1965, observed that no simple explanation could adequately describe the "multifaceted etiology of dental caries". The dependence of the carious process in man, and in experimental animals, upon the presence of appropriate microorganisms in the oral cavity together with food residues for their metabolism, is generally accepted. Genetic and nutritional influences during or after tooth development have not, however, been as well established. After reviewing dietary influences on caries reported by many investigators, Bibby and Shaw find themselves in broad agreement with Tomes and Tomes (1873), who wrote, "caries is the effect of external causes in which co-called 'vital'

forces play no part . . . and when once the disintegration is established in some congenitally defective point the accumulation of food and secretions in the oral cavity so intensify the mischief by furnishing new supplies of acid."

Newbrun (146) in a report of 1969, titled "Sucrose, The Arch Criminal of Dental Caries", states that 'there has emerged recently a considerable body of laboratory evidence specifically implicating sucrose rather than any of the other carbohydrates as the 'arch criminal' of dental caries. The hypothesis that it is only sucrose which serves as a substrate for extracellular polysaccharide synthesis by cariogenic microorganisms is developed by the author on the basis of numerous investigative reports that are reviewed under headings: biochemical evidence; microbiological evidence; evidence from animal caries studies; evidence from studies on plaque formation; hereditary fructose intolerance; and case reports. In a paper given before the Division of Carbohydrate Chemistry, at the recent (1974) meeting of the American Chemical Society, Bowen (13) observed that plaque formation is found in primates in the presence of glucose or fructose alone, with substantial levels of extracellular polysaccharide and high acidogenic potential. From this, and dietary experimental data from rodents, Bowen has expressed doubts that a substitution of glucose or fructose for sucrose in human diets would lead to "a substantial reduction in the prevalence of dental caries".

An extensive review of the literature concerning investigations in experimental animals on the cariogenicity of diets containing sucrose and/or starch, by Grenby (68) also concludes that sucrose produced in rodents much more caries than other carbohydrates tested, among which were glucose, wheat starch (cooked or raw), white flour and wholemeal bread.

A most recent (1973) review of the literature concerning the role of sucrose and other sugars in the development of dental caries has been published by Maekinen (129) of Finland; an extensive literature-cited list is included. A tabular presentation of factors which can be considered as caries theories is shown below, followed by Maekinen's report summary:

TABLE 1

Factors which can be considered as caries theories. The ingestion of sucrose (and evidently some other sugars as well) may lead to caries through the factors listed

1. Enamel decalcification by the acids produced by some micro-organisms. Such acids were lactic acid, citric acid, and perhaps some others. Hamilton et al. (1972) suggested that the mechanism of acid attack upon human enamel is primarily by a penetration through the interface between the rod-core and the surrounding rod-sheath. The acid formed may also undergo further transformations. Hu and Sandham (1969) showed that under certain conditions plaque streptococci utilized lactic acid, converting it to acetic acid, ethanol, and CO_2. Komiyama et al. (1972) have demonstrated a pH rise factor which was considered to be a major regulator of the carbohydrate metabolism of the oral microorganisms. The factor was fractionated on Bio-Gel P-4 into components with intermediate molecular weight. The exclusion limit of P-4 is approximately 1600.
2. Hydrolysis of peptide bonds of the organic matrix of teeth by microbial enzymes. The hydrolysis of peptide bonds would involve the action of collagenases and more unspecific proteinases, as well as aminopeptidase and carboxypeptidases. A number of such enzymes have been demonstrated in dental plaque and carious dentine (*cf.* Makinen, 1966a,b, 1969, 1970).
3. Chelation of tooth calcium with products of microbial digestion or products of microbial fermentations. Such products would include amino acids liberated during peptide hydrolysis, and citric acid, lactic acid, and related acids. The complex-forming property of sucrose itself is to be noticed (the complexing non-acid theory; Schatz and Martin (1962)
4. The transfer of tooth phosphate and other material into bacterial cells of plaque at the enamel interface (*cf.* Luoma, 1972; Eggers-Lura, 1949, 1958, Keyes and Jordan, 1963; Konig, 1965). According to Howe et al. (1972) it is clear that tooth phosphate is utilized by *Streptococcus mutans* for growth. The ability of cariogenic organisms to utilize phosphate from the tooth may be in part responsible for their ability to colonize the tooth, resulting in decay.
5. Action of phosphatases and phosphoprotein phosphatases on the phosphate ester bonds of the tooth (Kreitzman et al., 1969; Paunio et al., 1968; Makinen and Paunio, 1969, 1970).
6. Action of all other enzymes and enzyme toxins on the tissue, which have not yet been discovered. Bacterial endotoxins have been demonstrated in plaque.
7. Increase of the solubility of human enamel and dentin by certain salts (for example, ammonium sulphate and related salts; *cf.* Paunio and Makinen, 1971).

1. It is possible to regard many oral micro-organism species as cariogenic, although it is evident that some species or strains on certain tooth surfaces may be more cariogenic than others. Anyhow, the disease can be regarded as a mixed infection.

2. Somewhat different and even controversial results have been obtained in animal experiments. This is most probably due to the difficulties in standardizing the methods. It is evident that in human experiments the difficulties will be even more pronounced.

3. It can be regarded as proved that sucrose is the most cariogenic common sugar. The cariogenicity is evidently mostly related to the rather high energy of hydrolysis of the covalent bond between the monosaccharide moieties. In this respect sucrose is in a special position among other disaccharides.

4. The energy content of sucrose is utilized by oral micro-organisms to synthesize, among other things, hydrophilic polysaccharides, which may form a bulk of the extracellular compartment of dental plaque. The polysaccharides (such as dextrans and levans) may be adsorbed on crystal surfaces in enamel, thereby partly promoting the growth and manifestation of cariogenic and other bacteria, creating diffusion barriers, acting as inflammatory agents, and providing strating material for the formation of simple cariogenic monosaccharides (glucose, fructose, etc.) and acid.

5. Although the role of polysaccharides in the etiology of caries is strongly emphasized at the present time, their significance should not be overestimated. Some polysaccharides may turn out to be more harmful than others and the ultimate effect also depends on which tooth surface is involved. The bacterial polysaccharides in plaque most probably represent a very heterogenous group of polymers which deserve intensive study.

6. The cariogenicity of some common sugars decreases roughly in the following order (on the basis of both animal and human studies): sucrose > glucose > maltose, lactose, fructose > sorbitol > xylitol. Invert sugar (fructose-glucose, 1:1) has not been found to be advantageous from the dental point of view. Certainly, other sugars or their derivatives will be found which may be effective in reducing caries. However, before becoming accepted, they should be shown to occur normally in usual natural products and as normal intermediates in the intermediary metabolism. Xylitol seems to fulfill these requirements, although controversial opinions exist concerning the insulin increasing effect of xylitol. The effect of xylitol on intestinal flora should be thoroughly studied. It is obvious that pentitols are more effective in reducing caries than hexitols.

7. Xylitol and other polyols act in certain fungi as hydrogen acceptors (i.e. the acceptor causes a metabolite to become oxidized; in many other cases molecular oxygen acts as a hydrogen acceptor). Although they may serve as the usual energy source in bacteria, they may also be shown to act as hydrogen acceptors in some bacteria.

8. Bacteria grown on polyols normally develop inducible polyol dehydrogenases and related enzymes which render the exploitation of these sugars possible as an energy source. This bacterial adaptation is a normal consequence of the presence of polyols in growth media. In effect, bacterial adaptation and enzyme induction are conditions for the evolutionary life of cells. Consequently, bacterial adaptation as regards polyols in the oral cavity is not a disadvantage, because polyols (particularly xylitol) do not promote the growth of plaque as effectively as sucrose, glucose, or fructose.

9. There are several interesting new findings which will receive attention in future, for example: a fructose lethality effect against an oral micro-organism strain, the utilization of lactic acid *in vivo* in plaque by certain bacteria, the catabolism of plaque polysaccharides to yield cariogenic sugars, the inducive formation of plaque invertase during high sucrose diet, the production of bacterial endotoxins in plaque, the proposed more cariogenic nature of levans when compared to dextrans, the utilization of tooth phosphorus by cariogenic streptococci, etc.

Numerous epidemiological studies of the dietary habits of children and caries incidence have been performed in school systems throughout the world; the results have generally lead to sweets as the villains in caries of epidemic proportions in school populations. A few such studies are included in the summary portion of this monograph, briefly; the papers may be found in the Documents Section.

Trithart and Weiss (203) reported results of several studies of five-year-old pre-school children and parents; interviews elicited information concerning caries and between-meal eating habits of the children as regards confections (candies, chewing gum, carbonated drinks, etc.). There appeared to be a direct relationship between the number of d.e.f. teeth found and the number of confections eaten regularly between meals. This study involved 1373 children. A second study of 1124 five-year-old children gathered data concerning the number of times or frequency of eating the various cariogenic items. The correlation between the average number of d.e.f. teeth and the frequency of between-meal snacks was significant.

Zita et al. (229) selected 200 children of ages 5 to 13 years, 106 boys and 94 girls. Dental examinations and 7-day diet surveys of mealtime and between-meal total sugar consumed revealed little relationship between dental caries experience and total sugar consumed. The between-meal sugar consumption, however, was positively correlated to the caries incidence. Frequency of eating between meals did not increase caries significantly, and no significant sex-related caries experience was observed.

Ninety healthy white children of ages 4-14 years, their parents and siblings were studied by Lauterstein and Massler (122) to investigate the relation between sugar ingestion of the child during his infancy and at present and of the mother during pregnancy and at present. The children with low caries experience generally had siblings with little or no caries; the converse was true. No relationship of children and parent caries experience was found. Neither the child's sugar ingestion during infancy to present nor that of the parent's during pregnancy and the same period, and the caries experience showed a significant relationship. A distinct relationship between clean teeth and low-caries experience was seen. The nature (adherent or detergent) of the food, tooth-brushing habits, or salivary volumes were unrelated to caries experience.

Glass and Hayden (61) compared caries prevalence in two groups of children 9-11 years of age; 132 children were of the Seventh-Day Adventist religion which proscribes certain items of food, such as sugar, sticky desserts etc.; and 158 children were of other faiths. All children were of similar socio-economic status and lived in communities with fluoridated drinking water. Significant differences between the Adventist and other children were observed with regard to delayed permanent tooth eruption in the Adventist group, and in lower caries prevalence. It was suggested that the limited intake of highly refined carbohydrates by Adventist children could be associated with their lower caries experience.

Papers by Fanning et al. (51) and Millican (137) have shown that dental caries in children attending schools with canteens or vending

machines, where sweets may be obtained, are more prevalent- and both reports question the practice. Millican asks the question "Are candy sales in schools justified?" It was noted that the problem was one principally of educating the children toward sound attitudes to nutritionally adequate foods. Unfortunately, children have acquired a "sweet tooth" well before reaching school age.

Rowe et al (165) recently (1974) reported on a study of 375 adolescent public school children who participated in a 3-year program of permissive breakfast consumption of ready-to-eat cereals, seven varieties of which four were sugar coated or presweetened. Other breakfast regimens were permitted, such as bacon and eggs, cooked cereals, etc. Complete dental examinations were performed at 0, 12, 24, and 36 months of the study. Records were kept of the cereal consumption which ranged from about 20 boxes to more than 600 for the 3-year period. Consumption of fewer than 28 boxes ranked the participant with the "noncereal eater" group; "heavy eaters" were those receiving more than 200 boxes. When analysed, the data revealed no difference in caries increments between cereal eaters and noneaters.

Perhaps the problem of sugar and dental caries will be resolved by socio-economic factors, such as that presented in the following clipping taken from the Washington Post, Sunday, November 17, 1974:

WASHINGTON POST
Sunday, Nov. 17, 1974

The Great Sugar Crisis, And What It Could Lead to

By Judith Martin

Look, Ma, no sugar!

As if dentists didn't have enough troubles these days, they may now have to face the fact that the high price and increasing scarcity of sugar could threaten the great American cavity industry. It used to be that a dentist could, in good conscience, advise children never to eat sweets—safe in the knowledge that a child who would obey a suggestion like that would probably also suck his thumb and need braces.

Incidentally, in case you thought that dentists didn't have troubles but only created them, the reference is to another case of big mouths. A psychiatrist informed the Ohio Dental Association recently that a lot which is diagnosed as dental disease is really depresssion or other emotional disease—for which the fees should go to guess whom? You don't catch those guys telling big, burly garage mechanics that cars only think they have dents in them.

But it's not only the poor dentist who will suffer if the cavity industry goes under. How will we develop a healthy sense of competition in our children if they can't count cavities? How will we teach them to count? How are we going to keep up the price of gold?

And what about the acne industry? That develops character in children, as we teach them that beauty is only skin deep and that we're all going to be sick if they don't stop picking.

It's acne which often prompts a child to take a good honest look at himself for the first time, and to develop that image of hopelessness which is to serve him in adult life. Without acne, young girls would not have the chance to learn how to smear skin-colored (orange) gook all over their faces.

But the greatest institution of all which is in danger of going under without the support of mountains of sugar is the fat industry. Where would we all be if we didn't eat too much sugar?

What would we talk about if nobody dieted? Who would buy wardrobe after wardrobe if people's size didn't change between Thanksgiving and Christmas every year? Who would support the skilled craftsmen who make those little charts showing everybody their ideal weight in a way that cleverly illustrates that everybody on earth has an ideal to strive for, and it's always 13 pounds away?

Common to all of these industries is sugar. They were created by the chocolate bar, the stick of chewing gum, the bottle of soda pop, the iced doughnut—all of which the acne, cavity and fat practitioners ungratefully designate as The Enemy.

Industry is fighting back, of course. Did you ever see an office or factory without vending machines selling — funny thing—candy bars, gum, soda and doughnuts? But what happens when these things become rare or unavailable, and Sugarless Saturday is followed by Sugarless Sunday?

Some of this has already happened. Try to follow the old American custom of borrowing a cup of sugar from the neighbor and see if he takes the chain down from his door. Ask a zoo animal when was the last time he saw an honest lump of sugar instead of a peanut some child didn't want.

And then go drown your feelings in Kool-Aid while you still have the chance.

The literature of dental caries is extensive, and can be only lightly considered here; animal and human studies are described, and some general considerations noted. The impact of sugar (refined carbohydrates) on the etiology of dental caries has been documented overwhelmingly; the solution of the problem, is - however, not easily attained.

Pigman (158), in 1957, reviewed some of the literature of the dental aspects of carbohydrates and discussed the subject under four headings: pre-eruptive effects; post-eruptive effects; the oral clearance factor; and saliva.

The pre-eruptive general metabolic effects of carbohydrates on teeth have not been well established; some indirect evidence, largely epidemiological, has been presented, but is inconclusive. Post-eruptive information comes principally from animal experiments; human nutritional studies and in vitro studies with extracted teeth have provided some information. The factors that must be considered in human nutritional studies are varied, and - again, epidemiological studies have not been conclusive in correlating diet with dental caries. The low rates of dental caries seen among primitive and isolated peoples such as Eskimos and African tribes, where diets may be low in carbohydrates, indicate some indictment of carbohydrates. Wartime diets, restricted in purified or refined carbohydrates, have also been accompanied by reduction by dental decay, but other factors may also have contributed to the phenomenon.

Rats and hamsters are most commonly used in animal experimentation for caries research; the rat lesions are dissimilar from human lesions in that the granular size of rat foods has considerable influence in cariogenesis. Hamster hereditary and nutritional factors have not been sufficiently established. Caries-resistant and caries-susceptible strains of rats have been established. Numerous investigators have shown a correlation between carbohydrate-rich diets and the incidence of dental decay.

The development of the "Artificial Mouth" and the use of extracted human teeth in in vitro studies have demonstrated two general types of cariogenic attacks on teeth, both of which are dependent upon the amount of available carbohydrate in the nutrient medium bathing the teeth, particularly with regard to glucose concentrations.

A marked correlation has been shown between the average reducing sugar level of saliva and the caries incidence. As yet, all factors involved in the clearance rates of dietary carbohydrates from the oral cavity have not been established. The consistency, adhesiveness and solubilities of materials comprising the oral environment surrounding the teeth are of obvious importance as are the microorganisms of the mouth. The composition of the saliva is not entirely known, but the carbohydrate material may be of considerable importance in determining the actual oral flora and subsequent caries production.

Animal Studies

The rat and the hamster have been principally used in studies designed to demonstrate relationships between dietary compositions, particularly carbohydrates - and dental caries. Other experimental animals have occasionally been used, such as mice and monkeys, but the rat and hamster show carious lesions similar to those found in humans and the literature abounds with reports of studies with these animals, particularly with the rat.

Many difficulties are observed in interpreting animal experimental data in terms of human caries: rodent teeth are smaller, with thin enamel; the rate of caries production is usually much higher than in humans; the animals do not engage in hygienic practice of brushing teeth; and physiological differences relative to salivary composition, oral flora, etc. plus many other factors may render animal data highly suspect as possessing value in resolving the problems of cariogenesis in humans.

We have included reports from the literature, which is extensive, that reflect the typical animal studies, but are not intended to be more than representative. The details from which the following brief descriptions are derived may be found by reference to the complete document included in that section of the monograph.

Rodents

Sognnaes (186) pointed out in 1948 the controversial nature of the significance of refined carbohydrates, especially sugar, and the incidence of dental caries. His report consists of two parts: the experimental approach, separating "internal" and "external" effects of a purified high carbohydrate ration in cariogenicity; and a "reinterpretation of observations in other animals."

Three species of rodents were studied, mouse, rat and hamster; two diets were used, a stock (Purina laboratory chow) diet and a purified ration differing principally in the carbohydrate granulated sugar, 67%, versus the natural carbohydrates of the stock diet. Pre-eruptive influence of the diets on hamster and rat, and the comparison of three species of post-eruptive influence as shown in the following tables:

Table 3.—Pre-eruptive influence of purified ration on the caries susceptibility of the teeth of Syrian hamsters

Group & no. of hamsters	Ration during dental development		Oral environment after eruption of the teeth					
	Prenatal life	Lactation (infancy)	Ration	Age period exposed* (months)	Carious molars	Carious cavities	Caries score	Score per months of oral exposure
1a 13	Stock	Stock	Stock	1st-5th (4)	0.8	0.8	0.8	0.2
1b 15	Stock	Stock	Stock	1st-8th (7)	1.0	1.2	1.2	0.2
1 28	(Average)			(4-7)	0.9	1.0	1.0	0.2
2a	Stock	Stock	Sucrose	4-8 (4)	2	2	2	0.5†
	Stock	Stock	Sucrose	4-8	2	3	3	0.8
	Stock	Stock	Sucrose	4-8	3	4	4	1.0
	Stock	Stock	Sucrose	3-8 (5)	5	5	5	1.3
	Stock	Stock	Sucrose	3-8	5	6	6	1.6
	Stock	Stock	Sucrose	3-8	6	6	7	1.8
	Stock	Stock	Sucrose	3-8	4	7	8	2.1
					3.8	4.7	5.0	1.3
2b	Stock	Stock	Sucrose	1-5 (4)	2	2	2	0.5
	Stock	Stock	Sucrose	1-5	4	6	6	1.6
	Stock	Stock	Sucrose	1-5	5	4	6	1.6
	Stock	Stock	Sucrose	1-5	5	5	7	1.8
	Stock	Stock	Sucrose	1-5	5	6	7	1.8
	Stock	Stock	Sucrose	1-5	7	9	9	2.4
					4.6	5.3	6.1	1.6
2c	Stock	Stock	Sucrose	1-8 (7)	10	14	18	2.4
	Stock	Stock	Sucrose	1-8	10	19	25	3.2‡
	Stock	Stock	Sucrose	1-8	10	19	31	4.0
	Stock	Stock	Sucrose	1-8	8	17	34	4.4
					9.5	17.3	27.0	3.5
2 17	(Average)			(4-7)	4.5	7.9	10.6	1.9
3a	Stock	Sucrose	Sucrose	1-3 (2)	4	5	9	4.5
	Stock	Sucrose	Sucrose	1-3	5	9	14	7.0
	Stock	Sucrose	Sucrose	1-3	7	13	17	8.5
	Stock	Sucrose	Sucrose	1-3	7	14	27	13.5
	Stock	Sucrose	Sucrose	1-3	8	23	35	17.5
					6.2	12.8	20.4	10.2
3b	Sucrose	Sucrose	Sucrose	1-3 (2)	8	27	40	20.0
	Sucrose	Sucrose	Sucrose	1-3	9	33	51	25.0
	Sucrose	Sucrose	Sucrose	1-3	10	35	53	26.5
					9	31.6	48.0	23.8
3 8	(Average)			(2)	7.3	20.0	31.0	15.3

*Figures in parentheses indicate the actual number of months during which the teeth were exposed to the given ration after eruption into the oral cavity

Table 4.—Pre-eruptive influence on the caries susceptibility of rats' molars

No. of rats	Ration during dental development		Oral environment after eruption of teeth					
	Prenatal life	Lactation	Ration	Length of exposure (months)	Dental effects: Carious molars	Dental effects: Carious cavities	Dental effects: Caries score	Score per months of exposure
15	Stock	Stock	Stock	7–9	0	0	0	0
2	Stock	Stock	Sucrose	3	0	0	0	
	Stock	Stock	Sucrose	3	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	7	0	0	0	
	Stock	Stock	Sucrose	7	0	0	0*	
12				3–7	0	0	0	0
3	Stock	Sucrose	Sucrose	4	1	1	1	
	Stock	Sucrose	Sucrose	4	1	1	1	
	Stock	Sucrose	Sucrose	4	2	2	2	
	Stock	Sucrose	Sucrose	4	3	3	3	
	Stock	Sucrose	Sucrose	4	3	3	4	
6				4	2.0	2.0	2.2	0.6
4	Sucrose	Sucrose	Sucrose	2	0	0	0	
	Sucrose	Sucrose	Sucrose	2	1	1	1	
	Sucrose	Sucrose	Sucrose	2	1	1	1	
	Sucrose	Sucrose	Sucrose	2	2	2	2	
	Sucrose	Sucrose	Sucrose	2	2	2	2	
	Sucrose	Sucrose	Sucrose	2	3	3	3	
	Sucrose	Sucrose	Sucrose	2	3	4	4	
	Sucrose	Sucrose	Sucrose	2	4	4	4	
	Sucrose	Sucrose	Sucrose	2	4	5	5	
	Sucrose	Sucrose	Sucrose	2	4	5	5	
10				2	2.4	2.7	2.7	1.4

Table 5.—Comparative increase in caries susceptibility of hamsters, rats and mice when "bred" on a high sucrose diet (Series A)

Species	Number of animals	Breeding diet	Posteruptive (experimental): Diet	Posteruptive (experimental): Period (months)	Average caries incidence: Molars affected	Average caries incidence: Areas affected	Average caries incidence: Caries score
Hamsters.....	6	Stock	Sucrose	4	4.6	5.3	6.1
Rats.....	12	Stock	Sucrose	4	0	0	0
Mice.....	15	Stock	Sucrose	4	0	0	0
Hamsters.....	3	Sucrose	Sucrose	2	9.0	31.6	48.0
Rats.....	10	Sucrose	Sucrose	2	2.4	2.7	2.7
Mice.....	21	Sucrose	Sucrose	2	0.4	0.5	0.5

A series of rat experiments with the two diets and their influence, prenatal and postnatal, on production of caries, yielded data summarized in the following table. The most significant aspect of the findings is the indication that a purified ration, complete in all known nutritional essentials but containing a large proportion of refined carbohydrates, is extremely conducive to caries, provided its ingestion is begun before and continued during tooth development.

Table [illegible]—Summary of effects of different carbohydrates on experimental rat caries (Series B)

Group	No. rats	Ration during dental development		Postcruptive experimental conditions and findings						
		Pregnancy	Lactation	Ration	Age at start (months)	Duration (months)	Carious molars	Carious cavities	Caries score	Score per month
A	5	Stock	Stock	Stock	1	7	0	0	0	0
B	7	Stock	Stock	Whole wheat	1	3	0	0	0	0
C	6	Stock	Stock	Dextrin	1	6	0	0	0	0
D	12	Stock	Stock	Sucrose	1–3	3–8	0	0	0	0
E	17	Stock	½ part sucrose	Sucrose	½	4–8	2.7	3.9	5.7	0.9
F	4	Sucrose*	Sucrose	Whole wheat	1	3	0	0	0	0
G	19	Sucrose*	Sucrose	Dextrin	1	3	0.7	0.8	0.8	0.3
H	8	Dextrin*	Dextrin	Dextrin	0	3	0.7	0.7	0.7	0.2
I	14	Dextrin*	Dextrin	Sucrose	1	3	3.0	5.0	7.0	2.3
J	10	Sucrose†	Sucrose	Sucrose	0	3	3.3	5.6	7.0	2.3
K	8	Sucrose*	Sucrose	Sucrose	0	3	5.1	8.6	13.7	4.5

*Mothers started on given ration four months preceding pregnancy.
†Mothers started on given ration two months preceding pregnancy.

Sognnaes discussed experimental data from guinea pig and monkey studies which indicated that diets which left adhesive masses of refined carbohydrates on the teeth for prolonged periods were not cariogenic, downgrading as it were the importance of the oral environment. Similar results have been reported for dogs and rats. The Syrian hamster and the cotton rat are particularly susceptible to experimentally produced caries.

The following three points summarizing the author's discussion are made:

1. The findings indicate that hamsters, rats and mice are susceptible to experimental caries when fed a purified, finely powdered ration, complete in known nutritional essentials and high in refined carbohydrates, particularly if its ingestion is begun during tooth formation.

2. The caries susceptibility appears to be influenced by an unrecognized mechanism, a nutritional factor or combination of factors operating before eruption of the teeth from conception to maturation of the offspring and their individual teeth.

3. It is possible that the long suspected relation between dental caries and the excessive consumption of refined carbohydrates may be partly in the nature of an unrecognized indirect influence on the quality of the developing teeth and that the effect may be accentuated through generations.

Rats

Cox et al. (34) reported the results of three experiments with albino rats given various diets of coarse corn meal with or without high levels of selected carbohydrates, and diets without the coarse corn but with a single carbohydrate. Maintenance diets without coarse corn, regardless of the carbohydrate content, produced caries only rarely in rats kept on the diet for as long as eleven months. Preliminary studies with albino rats kept for several generations on diets higher in starch, sucrose or glucose, and without cornmeal, showed virtually no caries. It was concluded that sugars have little or no effect in starting rat caries.

Summaries of the three experiments are shown below (the HWC diet is the coarse corn meal ration of Hoppert, Webber and Canniff who described the rat cariogenic diet in 1932):

Summary for Experiment No. 1.

1. Forty-eight rats were placed for six weeks on the Hoppert-Webber-Canniff diet, after which thirteen of the rats were given a diet made by adding an equal weight of powdered confectioner's sugar to the HWC diet. These diets were fed for the balance of the experiment of 104 days.

2. Although the experimental rats grew less rapidly than the control rats and laid down excessive amounts of peritoneal fat, there were no gross abnormalities in the organs of either group.

3. On the average, the experimental rats developed the same number of carious teeth and the same number of cusps missing per rat as did the control rats.

4. The experimental rats exhibited twice as many teeth totally destroyed as did the control rats. Apparently, excessive amounts of sugar do not initiate rat caries but existing cavities are rapidly extended.

Summary for Experiment No. 2.

1. One hundred twenty-two male and female rats were placed on HWC diets for a period of eight weeks. Twenty rats fed coarse corn and thirteen rats fed fine corn were sacrificed at that time: the average caries scores were 4.8 and 2.6, respectively.

2. The remaining rats were divided into groups of twelve to sixteen each, and they were fed diets containing (a) corn ground coarse or fine, and (b) low or high carbohydrate content, but without corn particles.

3. At the end of an additional eight-week period, the rats maintained on coarse corn had an average caries score of 7.5, whereas those on fine corn had an average score of 4.2. In both these groups, continuation on the diets produced increased caries scores.

4. The rats fed high carbohydrate diets in each instance had higher average caries scores than those maintained on the low carbohydrate diet.

5. When the caries scores of the rats fed continuation diets high in starch, sucrose or glucose, respectively, were compared, inconclusive differences were found.

Summary for Experiment No. 3.

1. One hundred eight rats were placed on a specially modified HWC diet in which a synthetic milk replaced the powdered milk usually included. The synthetic milk contained a single sugar, sucrose or glucose; in a control group, uncooked starch was added in an equivalent amount.

2. After eight weeks on these diets, the rats were given new diets in which no corn meal was present, but in which large amounts (about 65 per cent) of each diet was the same sugar or starch which had been in the initial diets.

3. After eight additional weeks on these continuation diets, on the basis of either whole mouth or tooth-by-tooth comparison, it was shown that rats fed uncooked corn starch had less progress of cavitation than rats fed fermentable sugars.

McClure (132) has described a diet which produces in rats caries involving lingual, buccal and proximal molar tooth surfaces similar to human dental caries. This basal cariogenic diet consists of: cerelose (commercial glucose) 18%; enriched rye bread, 25%; enriched white bread, 25%; cooked rolled oats, 15%; cooked yellow corn grits, 15%; and sodium chloride, 2%. Vitamin supplements are added to the diet.

Muller and Gortner (142) demonstrated in experiments with albino rats that the addition of 10% sucrose, lactose or glucose to a 0.055% phosphoric acid solution did not alter significantly the decalcification of rat molars beyond that produced by a similar saccharin-phosphoric acid solution.

Albino rats were used in the experiments which lasted a week, after which the animals were sacrificed and teeth scored for lesions. Sugar and phosphoric acid solutions, or sugar-free solutions of the acid with saccharin added, were given daily. Glucose or lactose were also substituted for the sucrose. With the sugar-acid solutions, etching of the molars was found; with saccharin, no effect whatever was noted. The pH of the sugar-acid solution was 2.5-2.6; the pH of the saccharin solution, greater than 4.0 (due to buffering of saccharin with sodium bicarbonate). Phosphoric acid solutions containing sucrose, glucose, lactose and/or pure saccharin produced no significant differences in decalcifying effects, as shown in the following table:

TABLE I

In Vivo Decalcifying Effects of Phosphoric Acid-Sugar and Phosphoric Acid-Saccharin Solutions at pH 2.5-2.6 on Rat Molar Teeth

Solution	No. of rats	Average molar scores		
		Lower jaw	Upper jaw	Whole head
Acid-sucrose	13	3.7	2.7	3.2
Acid-lactose	6	3.6	2.8	3.2
Acid-glucose	4	3.8	3.0	3.4
Acid-saccharin	10	3.2	2.9	3.1
Sucrose (controls)	6	0.0	0.0	0.0

The anomalous results variously obtained in similar experiments with buffered saccharin and with lactic acid in decalcification studies were explained by the authors as due to the fact that it is the titratable acidity and not the pH of the solution which determines the decalcification. The final pH of the solution, when in the mouth, determines the extent of enamel destruction, and this in turn is a function of salivary buffering and the properties of the acid being used.

Shaw et al. (177) reported that replacing sucrose in a cariogenic diet of rats by glucose, confectioner's sugar or lactose did not alter the cariogenicity. Maltose and flour substituted for sucrose did reduce the periodontal syndrome. Five separate experiments were performed: rice rats were used in two experiments in which sucrose was replaced in the diet by glucose, maltose, confectioners sugar, whole wheat or white flour, or half-replaced by lactose; three experiments used white rats with the dietary sucrose replaced by maltose, glucose, or half-replaced by lactose.

The first two experiments lasted 84 days and the next three were terminated after 60 days; all animals were sacrificed and the periodontal syndrome (lesions and alveolar bone resorption) in the rice rats and the carious lesions of the albino and Harvard caries-susceptible rats by the three last experiments were scored.

The results of the two rice rat experiments are shown below:

TABLE 2. INFLUENCE OF TYPE OF CARBOHYDRATE ON DEVELOPMENT OF PERIODONTAL SYNDROME IN THE RICE RAT

Group	Diet	No. of rats		Wt. gain (g)		Soft tissue lesions		Alveolar bone resorption	
		♂	♀	♂	♀	No. of areas	Ext. of areas	No. of areas	Ext. of areas
Experiment 1									
1	700	8	10	54·3 (4·2)	29·2 (2·4)	26·2 (0·9)	50·8+ (5·3+)	27·8 (0·1)	66·2+ (4·7+)
2	717	9	9	49·6 (2·8)	29·7 (2·0)	27·7 (0·2)	61·3+ (2·9+)	28·0 (0)	74·2+ (2·7+)
3	727	7	6	50·0 (4·9)	31·6 (3·6)	27·8 (0·2)	43·7+ (3·5+)	28·0 (0)	59·0+ (2·6+)
Experiment 2									
1	700	12	15	35·4 (2·7)	18·9 (2·0)	27·6 (0·3)	46·7+ (3·0+)	28·0 (0)	62·3+ (2·6+)
2	737	10	7	32·0 (3·2)	25·0† (2·0)	24·9† (1·1)	23·3+* (0·7+)	27·6† (0·2)	38·9+* (1·6+)
3	738	10	9	46·4† (3·2)	17·2 (1·3)	17·4* (2·1)	9·2+* (1·1+)	24·6* (0·7)	18·6+* (1·2+)
4	739	9	8	41·2 (3·4)	19·4 (1·8)	23·3* (1·0)	12·0+* (0·5+)	27·2† (0·3)	21·8+* (1·1+)
5	740	9	9	30·6 (2·8)	18·4 (2·7)	28·0 (0)	41·3+ (4·1+)	28·0 (0)	56·5+ (4·3+)

Values in parentheses are standard errors of means.
*1% level of significance.
†5% level of significance.

In the third experiment, significant reductions in carious lesions were observed in the animals receiving the diet with partial replacement of sucrose by lactose (Group 3) as shown in the following table; but the sucrose, glucose and maltose were indistinguishable in ability to promote dental caries:

TABLE 3. INFLUENCE OF TYPE OF SUGAR ON DEVELOPMENT OF CARIOUS LESIONS IN OCCLUSAL SULCI AND ON SMOOTH SURFACES IN THE MUTANT ALBINO RAT

		No. of rats		Wt. gain (g)		Carious lesions in occlusal sulci				Carious lesions on smooth surfaces			
Group	Diet	♂	♀	♂	♀	Caries-free rats (%)	No. of carious molars	No. of carious lesions	Ext. of carious lesions	Caries-free rats (%)	No. of carious molars	No. of carious lesions	Ext. of carious lesions
1	2700	12	12	181 (6)	118 (3)	0	6·7 (0·3)	14·0 (0·8)	50·2+ (3·1+)	4·2	4·4 (0·4)	4·5 (0·4)	11·5+ (0·9+)
2	2717	10	11	167 (8)	114 (3)	0	6·0 (0·6)	13·4 (0·9)	48·2+ (3·2+)	9·5	3·8 (0·4)	4·0 (0·5)	10·0+ (1·3+)
3	2727	10	9	159 (12)	107 (5)	0	5·8 (0·5)	10·6† (1·4)	39·0+ (6·1+)	42·1	2·2* (0·5)	2·2* (0·5)	5·8+† (1·5+)
4	2737	10	13	186 (6)	113 (3)	0	7·3 (0·3)	14·8 (0·7)	52·7+ (3·5+)	17·4	4·0 (0·5)	4·1 (0·5)	10·4+ (1·4+)

Values in parentheses are standard errors of means.

*1% level of significance.

†5% level of significance.

The results of the fourth experiment are shown below; no significant changes with the type of carbohydrate in the diets were observed except in Group 4, where maltose resulted in a modest increase in the number of molars affected, and the number and extent of carious lesions:

TABLE 5. INFLUENCE OF TYPE OF SUGAR ON DEVELOPMENT OF CARIOUS LESIONS IN OCCLUSAL SULCI OF HARVARD CARIES-SUSCEPTIBLE RATS FED DIETS OF THE 2700 SERIES

Group	Diet	No. of rats		Wt. gain (g)		Caries-free rats	No. of carious molars	No. of carious lesions	Ext. of carious lesions
		♂	♀	♂	♀				
1	2700	10	11	196 (5)	121 (5)	0	6·4 (0·3)	10·8 (0·8)	38·8+ (3·6+)
2	2717	12	12	186 (6)	129 (4)	0	4·9* (0·4)	8·8 (1·0)	27·0+† (3·7+)
3	2727	11	13	183 (4)	120 (4)	0	6·8 (0·3)	11·8 (0·9)	42·0+ (3·0+)
4	2737	12	12	196 (6)	122 (3)	0	7·4† (0·3)	13·0† (0·7)	42·3+ (2·8+)

Values in parentheses are standard errors of means.

*1% level of significance.

†5% level of significance.

Growth rates of the animals in the fifth experiment were slow on a diet from which sucrose was omitted, as well as glucose, lactose and maltose. In Group 4, using granulated sucrose versus powdered (confectioners) sucrose, reductions in the cariogenicity of the diet were observed.

The data secured from culturing oral swabbings and from other investigations indicates that the bacterial flora found in albino rats differs from that found in hamsters and humans, which may account for the diversity of findings resulting from similar experiments conducted with the different species.

Five experiments lasting 20 days, involving 214 weanling albino rats were performed by Green and Hartles (65), using a number of cariogenic diets differing only in the carbohydrate content. The nine different diets contained 72% total cargohydrate in each: sucrose; glucose; sucrose and glucose (36% each); fructose; glucose and fructose (36% each); and maltose. The number of animals used in the five experiments were: 1, 3 groups 14 each; 2, 4 groups 14 each; 3, 2 groups 14 each; 4, 4 groups 14 each; and 5, 2 groups 16 each.

Caries scores for the five experiments are shown in the following table:

TABLE 1. THE MEAN CARIES EXPERIENCE ARISING FROM THE CONSUMPTION OF DIETS CONTAINING DIFFERENT SUGARS, TOGETHER WITH THE AVERAGE DAILY GAIN IN WEIGHT

		Total lesions (cumulative-T)	Advanced lesions (cumulative-B)	Wt. gain/rat/day
Experiment 1	Sugar in diet			
RC 14	Sucrose	6·7±0·9†	1·7	3·5
RC 25	Glucose	4·5±0·8	1·6	3·3
RC 26	Sucrose + glucose	5·9±0·6	0·9	3·4
Experiment 2				
RC 14	Sucrose	6·6±0·6	1·6	3·5
RC 25	Glucose	6·8±0·6	2·7	3·5
RC 31	Fructose	6·3±0·5	3·3	3·1
RC 32	Glucose + fructose	6·4±0·6	2·4	3·2
Experiment 3				
RC 25	Glucose	5·1±0·7	1·6	3·3
RC 36	Maltose	4·6±0·2	0·6	2·8
Experiment 4				
RC 25	Glucose	6·9±0·5	1·6	3·4*
RC 33	Lactose	7·7±0·7	1·7	−0·2*
RC 34	Galactose	7·8±0·8	3·6	−0·3*
RC 35	Glucose + galactose	7·0±0·6	1·4	2·9*
Experiment 5				
RC 14	Sucrose	9·4±0·5	3·7	3·2
RC 25	Glucose	7·7±0·5	4·1	3·4

* Average exposure to diet 10 days.
† S.E.M.

Experiment 1. RC 15 and RC 26 were significantly more cariogenic than RC 25 ($P<0·05$) but not significantly different from each other.
Experiment 5. RC 15 and RC 25 were significantly different ($P<0·025$).

The rats receiving diets containing lactose or galactose lost weight; all others remained active and grew normally. The only significant effect of the diets was observed in 2 of the 3 containing sucrose which were more cariogenic than those with glucose only. The other sugars were not significantly different in cariogenicity than was glucose.

The authors, Green and Hartles (64) reported earlier in 1967 similar experiments in which a 72% sucrose diet was replaced entirely or for one half by uncooked wheat starch or by roll-dried wheat starch. The sucrose diet was significantly more cariogenic than the starch alone; roll-dried wheat starch was significantly more cariogenic than uncooked starch; and a mixture of uncooked wheat starch and sucrose was significantly more cariogenic than the mixture of roll-dried starch and sucrose.

Two high-sucrose diets used in rat studies, called the Emory and the Harvard diets, differed in some respects, but contained the same concentrations of sugar; the Harvard diet had been shown to be more cariogenic than the Emory diet in rat studies. Wynn and Haldi (218) observed that one factor distinguishing the two diets was the addition of small amounts of aluminum (Al) to the Emory diet only; a question arose whether the aluminum could be responsible for the low cariogenicity of the Emory diet.

Albino rats of the Wistar strain were placed on four diets differing only in the aluminum concentration: none; 0.16 ppm; 2.0 ppm; and 20 ppm. Two sets of experiments were performed: one on normal, non-desalivated animals; one on rats from which the major salivary glands were removed after 120 days on the various rations. The experiments lasted for 60 days longer, or for a total of 180 days.

The experimental results are shown in the following tables:

TABLE 1

Carious lesions and caries score of non-desalivated albino rats on rations containing various amounts of aluminum

ALUMINUM ADDED TO RATION	TOTAL NO. TEETH	TOTAL NO. CARIOUS TEETH	TOTAL NO. CARIOUS LESIONS	NO. OF LESIONS Grade 1	2	3	4	TOTAL SCORE	AV SCORE
				LOWER MOLARS					
None	84	32	38	21	15	2	0	57	0.68
0.16 p.p.m.	84	33	39	25	10	2	2	59	0.70
2.0 p.p.m.	84	31	39	24	10	3	2	61	0.73
20 p.p.m.	84	31	39	28	7	4	0	54	0.64
				UPPER MOLARS					
None	84	4	4	4	0	0	0	4	0.05
0.16 p.p.m.	84	5	6	6	0	0	0	6	0.07
2.0 p.p.m.	84	2	2	2	0	0	0	2	0.02
20 p.p.m.	84	3	5	5	0	0	0	5	0.06

TABLE 2

Carious lesions and caries score of desalivated albino rats on rations containing various amounts of aluminum

ALUMINUM ADDED TO RATION	TOTAL NO. TEETH	TOTAL NO. CARIOUS TEETH	TOTAL NO. CARIOUS LESIONS	NO. OF LESIONS Grade 1	2	3	4	TOTAL SCORE	AV SCORE
			LOWER MOLARS						
None	60	26	46	22	10	9	5	89	1.48
0.16 p.p.m.	60	30	46	19	14	11	2	88	1.47
2.0 p.p.m.	60	28	43	14	15	12	2	88	1.47
20 p.p.m.	60	25	45	13	19	12	1	91	1.52
			UPPER MOLARS						
None	60	10	14	12	2	0	0	16	0.27
0.16 p.p.m.	60	12	15	11	3	1	0	17	0.28
2.0 p.p.m.	60	13	20	15	5	0	0	20	0.33
20 p.p.m.	60	12	16	10	4	2	0	24	0.40

No significant differences were observed in either the number of carious lesions or their severity as a result of the aluminum in either desalivated or non-desalivated rats.

The authors (Wynn et al. (219)) at a later date conducted similar studies to test the significance of the pyridoxine concentration and the cariogenicity differences in the Harvard and Emory diets. The Harvard diet, more cariogenic, contains 3.5 ppm pyridoxine.

The Harvard diet, alone was used in the experiments; the pyridoxine was added 0, 1, 10 or 100 ppm. Two strains of albino rats, one caries-resistant and one caries-susceptible, were used; the resistant-strain (24 rats in 3 groups of 8 each) was desalivated (sialoadenectomized) and fed the pyridoxine diets for 70 days. The susceptible strain (60 rats in 3 groups 20 each) with salivary glands intact was fed for 120 days. All animals were sacrificed and scored for dental caries.

Pyridoxine in relatively large amounts, added to a cariogenic diet did not affect the number or severity of carious lesions in albino rats, either with or without salivary glands, or whether of a caries-resistant or caries-susceptible strain.

The role of the pH level on the cariogenicity of a high-sucrose diet in the rat was investigated by Wynn et al. (220) in 1962. Twenty littermate pairs of albino rats of the Emory-Wistar rats were sialo-adenectomized and placed on one of two diets: a highly cariogenic diet with pH of 6.0; and the same diet but with a pH of 6.9 produced by the addition of sodium carbonate. After 70 days on the diets, the animals were sacrificed and teeth examined for incidence and extent of caries. Additional experiments were performed with diets having pH 6.0, 8.9 and 4.7 by addition of sodium carbonate or lactic acid, the diets fed

normally; a third experiment treated the animals by feeding with stomach tubes to test the systemic influence of pH rather than the influence orally.

The addition of the sodium carbonate in the first experiment did not affect the incidence of caries when added as a 1.5% solution; when added as a 3% solution, the caries scores were significantly reduced. Lactic acid increased the caries score. Intubation of acid or base did not alter the incidence of caries; the cariogenicity was a local, oral action rather than a systemic one. Whether the reduction of the caries score by the sodium carbonate was due to the maintenance of an alkaline environment for the teeth, or to the increased solubility of dietary phosphates was not established.

Different strains of rats used in cariogenic studies have responded differently to experimental situations. Haldi and Wynn (83) observed that investigations of the prenatal influence of diet on caries susceptibility showed different results with different strains of rats. The Sherman strain, for example, showed no influence of a cariogenic diet given prenatally or at birth upon incidence or extent of dental caries; the Long-Evans strain, however, showed definite prenatal dietary influence.

Haldi and Wynn used Wistar rats in two experiments: one study of the relative cariogenicity of different diets; and a determination of a prenatal influence of these diets. Four diets were used: high sucrose; high protein; high fat; and stock. A total of 96 rats were divided into sets of 24 each and placed on the four varied diets for a period of 150 days; the animals were sacrificed and teeth examined microscopically for carious lesions. A similar group of rats were placed on the same diets for 100 days, after which brother-sister matings were performed. The offspring, or second generation from these matings were fed the same diets for 150 days after which they were sacrificed and the teeth examined. The results from the two experiments are shown in the following table:

TABLE 2

Dental caries in the albino rat (Wistar strain) on a high sucrose, a high protein, a high fat and a stock diet

DIET	NO. ANIMALS	ANIMALS WITH CARIES	TOTAL NO. OF CARIOUS MOLARS	TOTAL NO. OF CARIOUS LESIONS	GRADE OF LESION 1	2	3	4	5
					Number of lesions				
First generation									
High sucrose	24	12	22	24	14	8	2	0	0
High protein	24	2	6	7	5	2	0	0	0
High fat	24	4	8	8	8	0	0	0	0
Stock	24	4	6	6	5	1	0	0	0
Second generation									
High sucrose	24	14	29	29	20	9	0	0	0
High protein	24	1	1	1	1	0	0	0	0
High fat	24	3	4	4	4	0	0	0	0
Stock	24	2	3	3	3	0	0	0	0

The high protein and high fat diets produced some carious lesions in both first and second generation animals, but the presence in these two diets of 22-24% sugar produced essentially results not significantly different from stock diet. The high sugar diet, with 64% sucrose, produced a higher incidence of caries, which were also more extensive, but the numbers were small and not markedly significant. It was concluded that exposure of the rats to prenatal, high sucrose diets of parents did not increase the incidence of dental caries in the second-generation offspring.

Rosen (163), using germfree Sprague-Dawley, caries-resistant and caries-susceptible rats, performed a series of thirteen experiments with sucrose or glucose diets and various organisms used to innoculate the animals. The oral flora was thus controlled to compare the effects of diet on plaque formation and on dental caries. The organisms used were Lactobacillus casei and two species of Streptococcus, salivarius (H-1) and (ATCC 13419) from human and rat, and faecalis (ND 547). The experimental diets containing either 56% sucrose or a commercial glucose replacing sucrose were sterilized by gamma radiation; all animals were kept in plastic isolators throughout the approximate 100-day experimental period. Experiments and results are shown in the following table: jaws were removed from sacrificed animals and teeth scored for caries. Alveolar bone loss and dental plaque accumulations were also noted.

TABLE 1. EFFECT OF SUCROSE AND GLUCOSE ON CARIES INCIDENCE

		Sprague-Dawley				Resistant			
		Sucrose		*Glucose*		*Sucrose*		*Glucose*	
	Experiment No.	No. of rats	Mean caries score	No. of rats	Mean caries score	No. of rats	Mean caries score	No. of rats	Mean caries score
Lactobacillus casei ATCC 4646	1	4	4·8 (1–9)	—	—	3	1·0 (1–1)	—	—
	2	5	16·0 (12–19)	—	—	—	—	—	—
	3	9	15·1 (4–22)	—	—	—	—	—	—
	4	3	0·0	—	—	3	0·0	—	—
	5	5	1·6 (0–5)	5	2·0 (0–4)	—	—	—	—
	6	4	0·0	4	1·2 (0–4)	5	0·0	5	3·8 (0–10)
Streptococcus salivarius (H-1)	7	4	15·0 (12–17)	—	—	—	—	—	—
	8	3	0·0	3	18·0 (8–30)	—	—	—	—
	9	4	0·0	5	0·0	4	0·0	4	0·0
Streptococcus salivarius ATCC 13419	10	3	6·0 (0–17)	2	10·0 (9–11)	3	0·0	5	0·0
	11	5	0·0	6	0·0	5	0·0	4	0·0
FA-1	12	3	2·7 (0–4)	3	12·0 (0–18)	3	12·0 (11–13)	3	12·0 (8–14)
Streptococcus faecalis ND 547	13	4	0·0*	4	3·0* (0–8)	3	0·0	3	0·0

* Caries-susceptible instead of Sprague-Dawley rats were used.

Numbers in parentheses are ranges of mean values.

Sucrose did not induce a greater mean caries score than glucose in any of the experiments; caries developed in fissures and not on any lingual or buccal surfaces of molars. No alveolar bone loss was observed, nor were accumulations of gelatinous plaques seen. In half the comparisons of mean caries scores between littermates fed either sucrose or glucose, a higher score was obtained in the rats fed the glucose diet, from which it was suggested that in the genesis of fissrue caries, glucose is more effective than sucrose.

British investigators Hartles et al. (93) described the establishment of a caries-susceptible colony of rats from animals of the Harvard School of Dental Medicine Colony. Four pairs of Harvard rats were randomly mated to establish the caries-susceptible stock. A high-sugar diet (67% sucrose) with all solids finely powdered comprised the typical diet given the animals; the general health was excellent on this diet. Measurable dental caries did not appear until the animals were 149 days old, at which time the average number of carious teeth was 1.3. At 184 days, there was a considerable increase in caries, the average number of affected teeth being 2.2. After 204 days on the diet a further slight increase was observed, carious teeth numbering 2.4 per animal. The original Harvard colony show caries much sooner, at 3-4 months on a high-sugar diet; there was no obvious explanation offered to explain why 6 months were required for caries development in the British colony. The animals were sufficiently caries-susceptible, however, to make them useful in the study of dietary factors in relation to experimental rat caries.

Fructose, glucose and sucrose diets (33% sugar) were given to two strains of rats (Wistar and Osborne-Mendel) by Grenby and Hutchinson (169). Both rat strains are caries-susceptible; the experiments were performed to compare the three sugars for cariogenicity.

Preliminary tests with the two strains of rats given a diet with 66% sucrose, indicated that the Osborne-Mendel strain gained more weight and developed more caries than Wistar rats. In tests with the fructose, glucose or sucrose diets, the results suggested that fructose cariogenicity was lower than glucose which was lower than sucrose. The results after 5 weeks showed no significance differences in diets, but at 8 weeks, combined caries scores of fructose and glucose were significantly lower than the sucrose score; the following table shows the results:

TABLE 3. THE EFFECTS OF FRUCTOSE, GLUCOSE AND SUCROSE ON CARIES TESTED IN TWO STRAINS OF RATS. THE DIETS CONTAINED 33 PER CENT OF A SUGAR, 33 PER CENT WHITE FLOUR, 32 PER CENT SKIM MILK POWDER, AND 2 PER CENT LIVER POWDER

	Diets					
	Fructose		Glucose		Sucrose	
	Males	Females	Males	Females	Males	Females
(A) Results after 5 weeks						
Wistar strain						
Number of rats	6	5	6	4	4	5
Mean weight gain (g)	113	90	126	87	119	80
Mean caries scores (Males + Females)	6·6		10·8		18·6	
S.E. of mean caries scores (27 D.F.)	±3·5		±3·7		±3·8	
Difference between the two extreme means: N.S. (10% > P > 5%).						
Osborne–Mendel strain						
Number of rats	5	7	5	7	4	7
Mean weight gain (g)	149	115	155	111	172	107
Mean caries scores (Males + Females)	16·8		26·0		25·2	
S.E. of mean caries scores (26 D.F.)	±3·7		±3·7		±3·8	
Difference between means: N.S. (20% > P > 10%)						
(B) Results after 8 weeks						
Wistar strain						
Number of rats	6	3	5	4	4	5
Mean weight gain (g)	144	118	171	119	164	112
Mean caries scores (Males + Females)	12·4		13·7		20·9	
S.E. of mean caries scores (17 D.F.)	±2·9		±2·9		±2·9	
Difference between the means: N.S. (P = 10%)						
Difference between means of sucrose group and (fructose + glucose groups): significant (P < 5%)						
Osborne–Mendel strain						
Number of rats	5	6	5	5	4	7
Mean weight gain (g)	230	158	251	158	257	158
Mean caries scores (Males + Females)	33·8		41·1		50·0	
S.E. of mean caries scores (11 D.F.)	±3·4		±3·5		±3·4	
Difference between means: N.S. (10% > P > 5%)						
Difference between means of sucrose group and (fructose + glucose groups): significant (P < 5%)						

The overall results of these experiments did not establish a significant difference in the cariogenicities of fructose and glucose; both were, however, somewhat less cariogenic than sucrose.

Guggenheim et al. (77) tested low fat diets containing various carbohydrates on groups of rats with relative oral gnotobiosis preliminary to inoculation with a cariogenic streptococcus (OMZ-61). The animals (Osborne-Mendel rats) were fed a non-cariogenic diet and were given erythromycin in the drinking water to reduce indigenous Gram-positive flora; daily inoculations with OMZ-61 were done during the 23-day experimental test period on diets containing 25% of one of the following carbohydrates: sucrose, glucose, fructose, lactose, maltose or wheat starch (uncooked). After sacrifice, jaws were prepared for scoring carious lesions. Bacteriological checks had been made periodically; the streptococcus OMZ-61 became quantitatively established only in those animals receiving sucrose diets. Few streptococci were detected in rats on glucose, fructose, lactose and starch; none at all on the maltose diet.

Examination of the extracellular polysaccharides produced by OMZ-61 showed glucose to be the only constituent of these macromolecules.

The average incidence of various lesions of smooth surfaces and fissures is shown in the following table:

Table V Results of 23-day caries test in relative gnotobiosis of 10 groups ($N = 9$) of Osborne-Mendel rats fed on diets containing different carbohydrates. Average number per rat of dentinal fissures lesions and of teeth in which the shallow distal fissure was more severely decayed than the corresponding deep central one, in first and second lower molars; average area score E of smooth surface lesions; average weight gain during 23-day test period

Treatment, diet number	Cariogenic component, per cent	Depression with erythromycin	Inoculation cariogenic str. OMZ-61	Dentinal fissure lesions	Distal-lesion predominance	Buccal surface lesions	Lingual surface lesions	Weight gain (grams)
1 590	25 sucrose	+	—	2.7	0.0	0.7	0.0	105
2 590	25 sucrose	—	—	7.9	0.4	1.8	0.0	94
3 2000a	56 sucrose	+	+	11.7	4.0	19.2	12.9	66
4 580s	66 sucrose	+	+	10.0	3.8	17.3	7.0	67
5 590	25 sucrose	+	+	7.6	2.8	6.4	4.9	95
6 591	25 glucose	+	+	2.4	0.0	0.1	0.0	88
7 592	25 fructose	+	+	0.7	0.0	1.2	0.0	91
8 593	25 lactose	+	+	2.9	0.0	1.8	0.0	82
9 594	25 maltose	+	+	0.7	0.0	0.0	0.0	94
10 595	25 starch	+	+	1.1	0.0	0.7	0.0	103
Standard deviations								
1				2.1		–	–	14
2				3.3		3.9	–	12
3				0.7		7.2	5.4	8
4				3.5		7.9	5.7	12
5				4.7		5.8	5.5	16
6				2.9		–	–	20
7				1.4		3.3	–	17
8				2.1		2.5	–	9
9				1.0		–	–	20
10				1.1		0.9	–	19

It was found that caries incidence was highest on sucrose diets, and very low on diets containing fructose, maltose or starch; glucose was less cariogenic than sucrose.

Shaw and Griffiths (178), using Harvard caries-susceptible rats, found that neither sorbitol nor mannitol supported the initiation and progression of carious lesions at a rate comparable to sucrose or dextrin in the diet. Only about 20% of sorbitol or mannitol could be used as substitutes in the sucrose or dextrin diets due to the tendency of the former to produce diarrhea.

Shaw and Ivimey (179) found that cornstarch given as the only dietary carbohydrate for a number of generations of Wistar albino rats produced a greater degree of carious lesions than anticipated. From 5 to 47 rats representing 7 generations were given the Harvard cariogenic diet in which all sucrose was replaced by corn starch. Offspring of parents fed the starch diet grew slowly and were subject to neonatal mortality. The results of the starch diet on caries production are shown below:

TABLE
INFLUENCE OF STARCH DIET ON CARIES ACTIVITY

			Carious Lesions in Occlusal Sulci		Carious Lesions on Smooth Surfaces	
Generation	Age	No. of rats	Percentage of Cariesfree Rats	Extent of Carious Lesions	Percentage of Cariesfree Rats	Extent of Carious Lesions
1	84	8	37.5	12.4+ (5.1+)	12.5	10.1+ (2.6+)
2	84	5	60.0	10.6+ (3.0+)	0	5.9+ (1.5+)
3	84	6	66.7	2.2+ (1.5+)	50.0	0.7+ (0.4+)
4	84	12	33.3	10.4+ (3.3+)	33.3	2.1+ (0.6+)
5	150	26	73.1	5.5+ (2.4+)	46.2	3.5+ (1.0+)
6	150	27	22.2	12.0+ (4.8+)	18.5	8.3+ (1.4+)
7	150	47	38.3	16.7+ (2.9+)	25.5	5.7+ (0.8+)

Frostell and Baer (57) fed Sprague-Dawley rats diets containing 66% starch, amylose or amylopectin; twelve different types of starches were tested: unmodified, wheat, rice, arrowroot, tapioca, amioca, potato and cornstarch; pregelatinized - wheat, potato, tapioca and amioca. The remainder of each diet was of 32% skin milk and 2% liver powder. Eleven groups of 60 rats each were given the diets shown below, for 30 days:

Table II

Effect of various starches on calculus formation and dental caries, 30 day experimental period

Diet No	Diet Regimen (Starch)	Calculus scores N	$\bar{X}$	S.E.	Caries scores N	$\bar{X}$	S.E.	Vs Control t	P	Level of significance
S—1	Unmodified wheat	24	3.13	0.519	—	—				
Control	Cornstarch, regular	24	3.67	0.428	18	0.72	0.351			
S—2	Pregelatinized arrowroot	—	—	—	12	17.17	2.763			
S—3	Pregelatinized potato	24	1.63	0.300	12	34.67	5.855			
S—4	Unmodified rice	23	2.65	0.473	11	3.72	1.830	2.0211	0.10>P>0.05	-
S—5	Unmodified arrowroot	23	1.57	0.402	—	—	—			
S—6	Unmodified tapioca	23	2.70	0.442	—	—	—			
S—7	Pregelatinized amioca	22	0.77	0.130	10	25.00	3.952			
S—8	Pregelatinized tapioca	23	1.61	0.265	12	18.25	3.099			
S—9	Unmodified amioca	24	3.38	0.496	12	7.00	2.871	2.6564	0.05>P>0.01	*
S—10	Pregelatinized wheat	23	1.78	0.398	11	24.18	5.784			
S—11	Unmodified potato	15	0.47	0.369	9	3.67	1.607	2.4157	0.05>P>0.01	*

At the end of 30 days, 24 rats from each group were sacrificed and prepared for caries checks; no marked differences were obtained, but those animals on unmodified amioca and potato starch showed a tendency toward higher caries rates than on other diets. The remaining animals were kept on the various diets for an additional 30 days with similar results. A significant difference, however, was found in comparing all pregelatinized diets with unmodified starches or regular cornstarch; the number of carious enamel areas in the pregelatinized diets were more numerous, except for the wheat which also showed a strong cariogenic tendency.

In a second experiment, diets comprised of 55% regular cornstarch, high amylose cornstarch, corn amylose or corn amylopectin were compared for cariogenicity.

Table V

Effect of various starches and starch constituents on the caries experience of rats

Substance	Day	Unmodified caries-scores N	$\bar{X}$	S.E.	Amylose caries-scores N	$\bar{X}$	S.E.	Amylopectin caries-scores N	$\bar{X}$	S.E.
Cornstarch,	30				20	7.60	1.297	20	10.85	2.071
regular	60	20	3.10	0.767						
	90	40	5.50	0.914						
Cornstarch,	30	17	2.71	1.325						
high amylose	60	16	3.38	1.221						
Potato starch	30	19	5.78	1.420	20	12.45	1.991	19	30.00	4.447
	30	26	13.42	1.992						
	60	28	16.43	2.954	19	34.00	4.438	17	47.29	5.535

	Day	t	P	Level of significance
Corn starch				
Regular — corn starch, high amylose	60	0.2018	P>0.1	—
Amylose — corn starch, high amylose*)	30	2.6234	0.05>P>0.01	*
Amylopectin — corn starch, high amylose*)	30	3.1794	0.01>P>0.001	**
Amylopectin — amylose	30	1.3295	P>0.1	—
Potato starch				
Amylose — unmodified potato starch	30	2.7013	0.05>P>0.01	*
		0.3383	P>0.1	—
	60	3.4331	0.01>P>0.001	**
Amylopectin — unmodified potato starch	30	5.1830	P<0.001	***
		3.7255	P<0.001	***
	60	5.3788	P<0.001	***
Amylopectin — amylose	30	3.6591	P<0.001	***
	60	1.8885	0.10>P>0.05	—

*) If calculated on the number of lesions the difference was highly significant.

The corn amylose and amylopectin diets produced more carious enamel areas than the high amylose corn starch diet; corn amylopectin appeared to be more cariogenic than the corn amylose, but the difference was not significant. Potato amylopectin produced more carious enamel areas than potato amylose after 30 days; an additional 30 days of the diets reduced the difference to insignificance (statistical).

A third experiment was performed to determine a correlation between calculus formation and caries experience. Individual animal data were not available, but group means were used to establish a correlation diagram. The diagram is shown below:

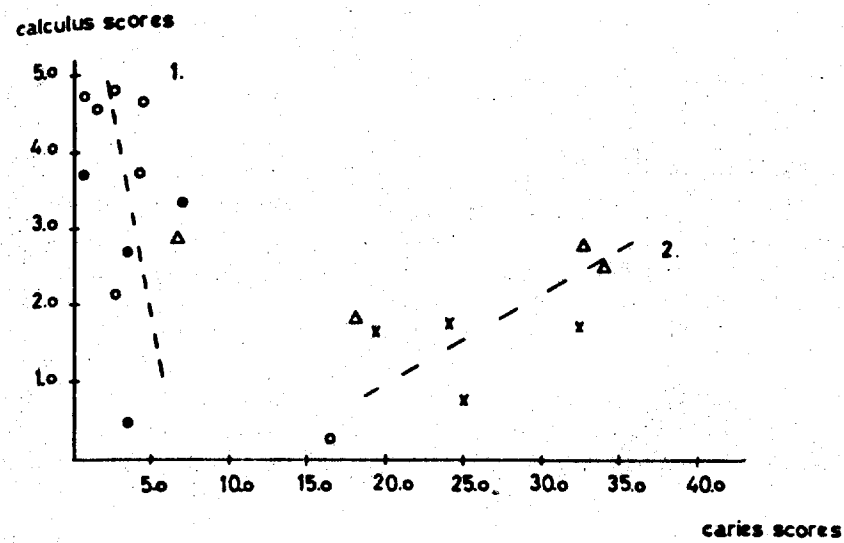

Fig. 3. Distribution of group means of calculus scores and caries scores.

1. Unmodified starches. 2. Pregelatinized starches.

O unmodified starches 30 days

● unmodified starches 60 days.

△ pregelatinized starches 30 days.

▲ pregelatinized starches 60 days.

No clear correlation was found between calculus formation and caries scores; generally, however, the unmodified starch diets produced very high calculus scores and low caries scores. A tendency was seen in pre-gelatinized diets for high caries scores to be accompanied by high calculus scores.

The overall results tended to show that the processing of starch increases its cariogenicity considerably, chiefly due to the amylopectin content and to a lesser degree, to its amylose content.

Frostell and Baer (58) also compared the cariogenicities of sucrose, unmodified potato starch, pregelatinized potato starch and a hydrogenated potato starch deriative in the diets of female Sprague-Dawley rats. The number of animals used, the diets and caries scores are shown in the table below:

Table I. *Number of carious enamel areas in the dietary groups*

Carbohydrate group	No. of animals	Days	CT	Bucco-lingual E.	Morsal	Sulcal E.	Proximal E.	Total no of carious areas	Percent vs control
Sucrose	20	30	5.2	8.6	0.2	3.2	2.6	14.6	100
	19	60	10.5	31.3	0.6	13.7	11.9	60.6	100
Pregelatinized potato starch	12	30	7.8	22.4	2.0	8.4	1.8	34.3	235
	23	60	9.5	13.2	3.8	14.7	2.2	32.9	54.3
Unmodified potato starch	22	60	6.4	6.6	0.2	9.6	1.2	17.6	29
Hydrogenated saccharides	26	30	0.2			0.3		0.3	2.1
Hydrogenated saccharides, $CaCO_3$	42	30	0.8			1.0		1.0	6.9

CT = carious teeth per animal
E. = number of enamel areas

The results indicated that even a comparatively mild processing of starch increases its cariogenicity. Since all diets contained fermentable lactose from the skim milk powder, it is possible that the sugar contributed to the caries development.

It has been suggested that "elimination of carbohydrates, particularly sucrose, from our diet would in all probability drastically reduce the incidence of carious lesions on the crowns of human teeth", even though the evidence is circumstantial, according to Grunberg et al. (76) in a recent (1973) report. Sugar alcohols might serve as substitutes for sucrose or glucose; xylitol possesses satisfactory sweetening properties and Grunberg et al. tested it, together with mannitol and sorbitol in various diets fed to male Swiss albino Wistar rats. Seven groups of 20 rats each were fed one of the following diets: a normal laboratory diet or a cariogenic diet either alone or supplemented with glucose, sucrose, mannitol, sorbitol or xylitol as from 5-10% of the total diet. The animals were examined at 3-week intervals for carious lesions throughout the 21 weeks of the experiment; teeth were evaluated for lesions which were scored from 0 (intact tooth) to 4 (entire tooth missing). The percentage of rats showing caries of the left lower molars for each diet is shown in the following table:

Tab. 2: Incidence of caries in rats fed normal or cariogenic diets supplemented with sugars or sugar alcohols

Group	Type of Diet	Percentage of Rats Showing Caries Left Lower Molars		
		1st	2nd	3rd
1	Normal	0	0	0
2	Cariogenic (C)	46	92	69
3	C + 10% Glucose	73	82	64
4	C + 10% Sucrose	50	63	38
5	C + 10% Mannitol	23	54	15
6	C + 10% Sorbitol	38	63	38
7	C + 10% Xylitol	0	14	0

The data indicate clearly that xylitol is far less cariogenic than either glucose or sucrose and is the least cariogenic of the sugar alcohols.

Esposito (49) has observed from studies with caries-susceptible rats that the amount of sodium chloride in the drinking water alters the cariogenic effect of sucrose in the diet. Eleven groups of 20 rats each were placed on diets containing 20, 40 or 60% sucrose; drinking water contained 0.5, 1.0 or 1.5% sodium chloride. The caries activities of combinations of sucrose and sodium chloride in terms of enamel units destroyed are shown below:

TABLE

INFLUENCE OF SODIUM CHLORIDE AND SUCROSE ON DENTAL CARIES

Diet	Group	Sodium Chloride (%)	Mean Enamel Units Destroyed	Changes in Enamel Units Destroyed (%)	t Values
20% Sucrose	1	0.5	7.8 ± 0.8*	+ 7.7	0.46
	4	1.0	8.4 ± 1.0		
	7	1.5	13.0 ± 1.1	+ 55.0	3.09†
				+ 62.7 (1 vs 7)	...
40% Sucrose	2	0.5	6.0 ± 1.5	− 16.6	0.55
	5	1.0	5.0 ± 1.0		
	8	1.5	11.3 ± 1.1	+ 126.0	4.23†
				+ 109.4 (1 vs 8)	2.84†
60% Sucrose	3	0.5	4.0 ± 0.9	− 45.0	1.82
	6	1.0	2.2 ± 0.4		
	9	1.5	2.7 ± 0.8	+ 22.7	0.56
				− 22.3 (3 vs 9)	...
Control	10	1.5	0	...	...
Controls without sucrose	11	(H O)	0	...	...

* Mean ± standard error
† Significant at the 0.01 level

At the 20 and 40% sucrose levels and 1.5% sodium chloride a significant increase in enamel units destroyed compared with other experimental groups. In general, increasing sodium chloride concentrations increased the destruction of enamel except at 60% sucrose levels. For one particular chloride concentration, however, increased sucrose decreases mean enamel units destroyed.

A study by Hess et al. (98) demonstrated that the amino acid composition of rat and human dentinal proteins is very similar. The objective of the experiment was, however, to determine the influence of varied casein diets given rats on the caries production and amino acid composition of the protein's isolated from incisor dentin. Chromatographic analysis of human and rat dentinal protein yielded results shown in the following tables:

TABLE 1*

AMINO ACID COMPOSITION OF HUMAN DENTINAL PROTEIN

	New (Per Cent)		Hess† (Per Cent)		Binkley‡ (Per Cent)
	Amino Acid (1)	N (2)	Amino Acid (3)	N (4)	Amino Acid (5)
Alanine	8.0	1.26	8.5	1.34	6.0
Aspartic	5.7	0.60	5.6	0.59	5.6
Arginine	7.8	2.50	7.4	2.38	7.5
Cystine	0.1	0.01	0.1	0.01	0.0
Glutamic	10.0	0.95	10.6	1.01	9.3
Glycine	22.4	4.18	22.5	4.20	20.2
Histidine	0.8	0.22	0.8	0.22	0.7
Hydroxylysine	1.3	0.14	1.0	0.11	2.7
Hydrexyproline	14.8	1.58	13.1	1.40	15.7
Isoleucine	1.4	0.15	1.3	0.13	1.1
Leucine	3.8	0.41	3.6	0.38	2.7
Lysine	3.4	0.65	3.3	0.64	2.6
Methionine	0.5	0.05	0.5	0.05	0.7
Phenylalanine	2.0	0.18	1.9	0.16	2.1
Proline	13.5	1.64	16.3	1.98	13.0
Serine	3.5	0.45	3.5	0.45	3.6
Threonine	2.1	0.25	3.1	0.36	1.5
Tyrosine	1.0	0.08	1.1	0.09	0.5
Tryptophan	0.0	0.0	0.0	0.0	0.0
Valine	2.2	0.26	3.0	0.36	2.3
Ammonia	0.5	0.41			0.6
Nitrogen	16.15	15.97	16.15	15.86	17.9
Per cent recovery of N		98.8		98.2	98.4

* The values in cols. 1 and 3 are expressed as grams of amino acid per 100 gm. protein. The values in cols. 2 and 4 are the amount of nitrogen in each amino acid per 100 gm. protein. The values in col. 5 are residue weight and need to be increased by 10-15 per cent to be comparable to those in cols. 1 and 3.

† See ref. 4 ‡ See ref. 5

TABLE 2*

AMINO ACID COMPOSITION OF RAT DENTINAL PROTEINS

	20P		30P		40P	
	Amino Acid (Per Cent) (1)	N (Per Cent) (2)	Amino Acid (Per Cent) (3)	N (Per Cent) (4)	Amino Acid (Per Cent) (5)	N (Per Cent) (6)
Alanine	5.9	0.92	6.4	1.01	5.9	0.92
Aspartic	5.8	0.61	5.4	0.57	5.4	0.57
Arginine	6.6	2.12	6.8	2.19	7.0	2.25
Cystine	0.1	0.01	0.1	0.01	0.1	0.01
Glutamic	8.6	0.82	8.2	0.78	8.2	0.78
Glycine	20.6	3.81	20.8	3.88	21.0	3.92
Histidine	0.9	0.24	1.0	0.27	0.7	0.19
Hydroxylysine	1.3	0.15	0.9	0.10	0.9	0.10
Hydroxyproline	14.3	1.53	14.0	1.50	13.2	1.41
Isoleucine	1.1	0.12	1.1	0.12	1.2	0.13
Leucine	4.0	0.43	3.7	0.40	3.6	0.38
Lysine	4.0	0.77	3.8	0.73	3.6	0.69
Methionine	0.5	0.05	0.5	0.05	0.5	0.05
Phenylalanine	2.3	0.20	1.8	0.15	2.0	0.17
Proline	13.3	1.62	12.8	1.56	12.8	1.56
Serine	3.0	0.40	3.2	0.43	3.7	0.49
Threonine	1.8	0.21	1.8	0.21	2.2	0.26
Tyrosine	1.0	0.08	1.0	0.08	1.0	0.08
Tryptophan	0.0	0.0	0.0	0.0	0.0	0.0
Valine	2.8	0.33	3.3	0.39	3.3	0.39
Ammonia	0.5	0.41	0.5	0.41	0.5	0.41
Nitrogen	15.1	14.86	15.6	14.84	15.4	14.76
Per cent of recovery of N	....	98.4	....	95.1	....	95.8

* The values in cols. 1, 3, and 5 are expressed as grams of amino acid per 100 gm. protein. The values in cols. 2, 4, and 6 are the amounts of nitrogen in each amino acid per 100 gm. protein.

No significant differences in the composition of the rat dentinal protein were found among the three dietary casein levels fed to rats, and the values were substantially the same as those found in human dentin.

Hamsters

The implantation of caries-inducing streptococci into the oral cavity of the hamster was found by Krasse (118) to depend for successful recovery of the organisms upon the composition of the diet. A substrain of the HSR-6 strain of streptococcus was inoculated into golden or albino hamsters; the diets differed with regard to the carbohydrate content, and all diets were finely powdered. After one to three weeks following implantation, samples of plaques and saliva were plated and incubated; colonies of cariogenic streptococci were identified and counted. Hamsters fed a standard lab chow yielded few recovered streptococci, whether the lab chow was ground or not. On the regular caries test diet, large numbers of the implanted streptococci were recovered. Since both lab chow and caries test diet were given in the same consistency, it appeared that composition and not consistency was the determining factor in the recovery of implanted streptococci. Implantation was less successful when glucose was substituted for lactose (from skim milk powder) in the diet. When sucrose was replaced by glucose in the diet, very few implanted streptococci were

recovered. Other investigators have observed that sucrose favors caries activity much more than glucose in hamsters and rats.

Krasse (119) followed the foregoing report with a study of the influence of sucrose or glucose diets upon the implantation of the substrain HSR-6 streptococcus in the albino hamster. The experimental techniques were those used before. The recovery of the implanted cultures in animals fed the glucose diet for 56 days was very low; recovery was very high in the infected animals on the sucrose diet.

Rat - Hamster

Frostell et al. (59) experimented with various dietary sugars and sugar substitutes to explore interactions between these ingredients and odontopathic bacteria and patterns of carious lesions in the molar teeth of hamsters and rats. Hamsters are particularly well-suited to studies of bacterial colonization on the teeth, smooth surface cavitation, and periodontal lesions; rats are similarly useful in studies of smooth-surface lesions; but particular suited to studies of proximal and crevicular lesions.

A caries-promoting diet was used as a control ration; the sucrose component was substituted for in experimental diets by fructose, dextrose, fructose and dextrose, maltose and several hydrogenated potato starches. All sugars were in powdered form. Four hamster experiments were performed; weanlings were inoculated intraorally 3 times with cultures of caries-conducive streptococci, and weekly (at 1, 2 and 5 weeks) samples of plaques and saliva were cultured for microorganism recovery. Coronal cavitation and bacterial plaques were periodically examined and evaluated.

Four rat experiments were similarly conducted, the inoculation was performed with fecal bacteria. The experiments lasted 56 days. In both hamster and rat experiments the data were summarized in the following tables:

TABLE 1

Summary of observations in hamsters fed a diet containing sucrose for 10 days and then fed a sucrose substitute for the remainder of the experimental period

Principal carbohydrate	No. of animals	Preassay-assay period [1]	Streptococcal recovery	Coronal plaque	Avg caries score [2]	Final wt
						g
			Experiment HA			
Sucrose	6	10+40	++++	extensive	37	77
Fructose	6	10 / 40	++ to ±	sparse	7	68
			Experiment HB			
Sucrose	4	10+45	++++	extensive	83	80
Fructose	4	10 / 45	+++ to ++++	sparse	5	73
Dextrose	4	10 / 45	++ to ++++	sparse	2	85
Fructose-dextrose	5	10 / 45	++ to ++++	sparse	10	75
Hydrogenated starch (6563)	4	10 / 45	+	sparse	<1	84
			Experiment HC			
Sucrose	5	10+73	++++	extensive	169	92
Dextrose	6	10 / 73	+ to −	sparse	4	98
Fructose-dextrose	6	10 / 73	± to −	sparse	10	89
Maltose	6	10 / 73	± to −	moderate	22	97
			Experiment HD			
Sucrose	6	10+50	+ to ++++ [3]	extensive	139/40	86
Hydrogenated starch (6563)	5	10 / 50	± to +	sparse	56/0	63

[1] The number to the left of the virgule refers to the number of days the animals were fed sucrose (diet 60); that to the right the days fed the test diet.
[2] The average values listed are for the entire dentition. In experiment HD the number to left of virgule is average score for dentition as a whole; that to the right is for third molars only; P. H. Keyes (9).
[3] No organisms were isolated from one animal.

TABLE 2

Comparison of various carbohydrates in caries test diets fed to Osborne-Mendel rats in experiments RA, RB, RC, and RD, showing the number of rats in each group and the number of areas of carious enamel on the bucco-lingual (BL), proximal (P), and sulcal (S) surfaces

Carbohydrate in diet	No. rats/ group	Exp. RA			No. rats/ group	Exp. RB			No. rats/ group	Exp. RC			No. rats/ group	Exp. RD		
		BL	P	S		BL	P	S		BL	P	S		BL	P	S
Sucrose	5	26.5	13.8	49.2	5	23.6	9.6	45.8	7	14.3	5.3	44.4	6	11.5	9.5	41.5
Dextrose	4	12.5	6.3	32.0									11	10.7	9.0	21.7
Fructose	4	6.0	7.5	28.3									4	9.8	11.5	22.1
Dextrose-fructose	2	8.5	5.0	20.0									12	8.6	8.0	24.6
Maltose	2	8.0	1.0	20.0	4	2.3	2.0	29.1								
Sucrose-potato starch	2	16.0	9.0	31.0	4	11.5	1.0	28.0	6	21.3	6.0	45.5				
Sorbitol starch					2	7.0	0.0	15.0	4	10.0	1.0	42.3	6	3.5	1.3	29.6
Potato starch					5	7.4	0.6	18.2					6	5.5	0.8	14.7
Hydrogenated starch (6563)	5	0.0	1.0	21.2 [1]					6	0.2	0.0	16.8	6	0.0	0.0	11.0
Hydrogenated starch + $CaCO_3$	5	0.2	0.0	15.8 [1]					6	0.0	0.0	23.6	6	0.0	0.0	6.0

[1] On experiment 63 days; all other groups assessed for 55 or 56 days.

Twenty-one hamsters fed 56% sucrose developed extensive plaques; massive cavitation of smooth coronal surfaces was observed and became more extensive with time. Less extensive deposits in rats given the sucrose were seen, but proximal and sulcal lesions were numerous.

Ten hamsters previously on a sucrose diet were transferred to a fructose diet, whereupon the plaque deposits, begun with sucrose, largely disappeared. No further progress in carious lesions was observed. In ten rats, the influence of fructose was also less than with sucrose. Much the same findings in both hamster and rat given dextrose as a substitute for sucrose were seen. A combination of fructose and dextrose paralleled the results found with the individual sugars. Six hamsters given maltose showed more noticeable plaque deposits and caries progress than with the simple sugars preceding, but the effect was less than with sucrose. Except for diarrhea, the animals fed hydrogenated potato starch differed little from controls or a sucrose-free diet.

Sucrose apparently provided a substrate especially conducive to plaque formation and smooth surface decay if polysaccharide-producing micro-organisms were present. Lesions in crevices apparently depend upon a variety of carbohydrates and broader spectrum of acidogenic bacteria.

Dalderup (39) in studies with albino Wistar rats found that both glucose and fructose were highly cariogenic in disagreement with Frostell et al., possibly due to a difference in oral flora. Lactose and starch produced low caries scores, but were also low in food efficiency.

Hamsters

Campbell and Zinner (20) have recently (1970) reported on the results of feeding hamsters a variety of dietary sugars, using human cariogenic streptococcal strain, AHT-type as an inoculant in a comparative cariogenic effect study. The sugars, glucose, fructose, sucrose and an equal mixture of glucose and fructose were added to the experimental, cariogenic diet. Seven groups of 20 hamsters each, 2 of which comprised the controls, were used in the experiments. At age 19 days, the test animals were inoculated with the AHT-type streptococcus and were placed on the experimental diets. When periodic inspection indicated the possible appearance of caries, the animal was sacrificed and teeth examined. Periodic sacrifice up to 100 days revealed the progressive development of caries. Two types of plaque were seen: a coronal plaque on the crown; and a gingival plaque in and above the gingival sulcus.

All of the experimental diets supported the formation of gingival plaque and were associated with periodontal lesions; the sucrose diet was, however, most effective and most rapid. The other sugar diets were slower to develop and progress in cariogenic action, but extrapolation within a hamster lifetime indicated considerable cariogenicity, implying

that "time is an important element in the interplay of factors involved in the development of dental caries."

Monkey

Caries-active monkeys (Macaca irus), fed for at least three years on a cariogenic diet with high sucrose content, displayed a reduced amount of dental plaque after being placed on a lower carbohydrate diet for at least 3 weeks. Bowen and Cormick (14) made tooth surface pH measurements in 4 monkeys by the use of micro-antimony electrodes attached to the teeth; measurements were made after a 17-hour fast, after application of 0.1 M sucrose and over the following 20 minutes. Before each pH measurement, plaque was removed from buccal surfaces adjacent to active occlusal carious lesions, and after dispersion and dilution, was cultured for enumeration of total streptococci and extracellular polysaccharide-forming organisms.

The plaque diminished progressively during the period of carbohydrate restriction, returning rapidly on the cariogenic diet. The pH values remained unchanged with change of diet, but after application of sugar solution, the pH was significantly lower while the animals were on the cariogenic diet. The total population of streptococci remained unchanged, but the proportion of organisms forming extracellular polysaccharide fell dramatically when the animals were fed the non-cariogenic diet.

The authors were convinced that the amount of plaque was drastically reduced when the monkeys were placed on the non-cariogenic, low carbohydrate diet.

Human Studies

The extreme variability found in human reactions to environmental conditions has plagued the efforts of investigators to determine the causes of dental caries. Some of the variability is genetically based or inherent, such as the developmental history of the tooth to its final size, shape and position in the mouth, or the composition of the saliva, the nature or integrity of the tissues surrounding the teeth, etc. Other factors, of environmental origin, as in variations of the oral microflora and the mechanical wearing away of enamel may be significant. Epidemiological or statistical treatments of the problem have served chiefly to simply emphasize the variations within human populations and have revealed the complexity of the problem.

Controlled dietary studies have indicated that of the various classes of foods, carbohydrates - particularly sugars - are most likely cariogenic, and especially so in a physical form which renders them sticky, or adherent to the teeth. Perhaps the carbohydrate should be qualified as of refined class to more readily be considered cariogenic; sucrose is labeled most frequently as the "villain".

Sugar substitutes for sweetening foodstuffs; the restriction in our diets of known or suspected cariogenic substances; the practice of good dental hygiene; and the reduction of, or the abstinence from - in-between-meal sweets are among the suggested approaches in combatting the epidemic caries experience.

As in the preface to animal studies, it is noted here that only selected representative studies of human caries have been abstracted; the complete document is included in that section of the monograph.

The search for the cause of the etiology of dental caries throughout the first thirty years of this century lead investigators to a number of inconsistent explanations. Jay (104) in 1940 reviewed briefly the various hypotheses proposed to explain caries: the action of acid-producing bacteria in food debris surrounding the tooth causing decalcification of the tooth; vitamins A and D together with a diet low in cereal reduced the incidence of caries; vitamin C was more important, and a pint of orange juice a day would reduce caries by 50%; phosphorus and calcium deficiencies were responsible; and caries could be controlled by a diet adequate for good health and development. In most instances, a diet low in carbohydrates has been recommended, not because of the importance of carbohydrates in caries production, but because carbohydrates compete with the more nutritionally important foods in the appetites of children. In addition to the correlation of high sugar diets and caries incidence, the presence of Lactobacillus acidophilus in the mouth has also been shown to be related to the incidence of caries. A reduction in carbohydrates in the diet reduces the Lactobacillus population and may possibly be related to a lowered

caries incidence. The problem is, however, confused by evidence of caries-susceptibility or resistance in certain individuals. It was concluded that dental caries are "not a manifestation of malnutrition" and that adding vitamins and minerals to high carbohydrate diets would not control caries; "control of carbohydrate consumption is the only dietary procedure thus far demonstrated by which the disease can be controlled."

Collins et al. (29) in 1942 discussed controversial attitudes regarding the role of dietary measures in the prevention and control of dental caries. Two "schools of thought", labelled the "Iowa school" and the "Michigan group" had expressed apparent divergent views in the 1940 animal meeting of the American Dental Association. Briefly, the "Iowa school" stressed the point of view that an optimium dietary program can prevent and control dietary caries; the Michigan group stressed the importance of oral Lactobacillus activity and sugar in the diet as caries related and suggested a reduced carbohydrate consumption as the proper approach to caries prevention.

A definition for "optimium diet" that is generally acceptable has not been attained, primarily because of the great differences in individual requirements. Racial, familial and climatic factors, tastes and digestive capacities are all highly variable in humans.

A large number of students were examined clinically and three groups were selected for comparative nutritional studies of caries-free and caries-active individuals, the latter of two degrees of caries activity, one slight and one with rampant caries. Evaluations of food intake, protein, minerals and vitamins for the students were quantized by use of a code based on an "arbitrary standard level." All three groups obtained comparable, satisfactory nutrition, but the caries-free groups remained caries-free and the caries-active groups continued caries activity. An "optimum" diet did not appear to be required for caries control. On the basis of sugar intake, the caries-susceptible group consumed about 7.7 teaspoonfuls daily than the immune group; however, some individuals in the caries-free group consumed more sugar daily than some in the caries-susceptible group. The most significant finding of the study was in the high degree of correlation between the presence orally of Lactobacillus acidophilus and caries activity. More than 80% of the completely caries-free individuals had no Lactobacillus count; nearly 90% of the individuals with rampant caries had very high Lactobacillus counts. Food essentials were not more deficient in the caries rampant individuals and it appeared doubtful that certain food elements advocated to control caries would favorably influence caries control or prevention.

A considerable amount of investigation into the solubility of human dental enamel as related to the dental caries process has been conducted over the period of the 1930's to 1950's. Blackwell and Fosdick (9) reported

on the effect of sucrose upon the solubility of human dental enamel: sucrose is accepted by most investigators as the principal substrate for acid formation in the mouth, playing an important role in dental caries. The authors included 75 references of studies related to the solubility problem; their own work indicated that sucrose has a retarding effect on the rate of enamel dissolution by lactate buffer solutions.

Powdered human dental enamel was tested by shaking samples in each of several solutions and after various time intervals, analysis of the solutions for calcium and for phosphate was performed. The solutions used were lactate buffer and lactate buffer with 0.5 M sucrose or 1.0 M sucrose; the lactate concentration was 1 mole per liter. Sodium hydroxide was added to produce the desired pH of the lactate solutions; pH's of 4.0, 4.5, 5.0, 5.5 and 6.0 were employed. The effect of sucrose alone upon enamel solubility was also tested in a 1 M sucrose solution.

The results of the lactate buffer solution rates of dissolution of dental enamel as shown in the following figure:

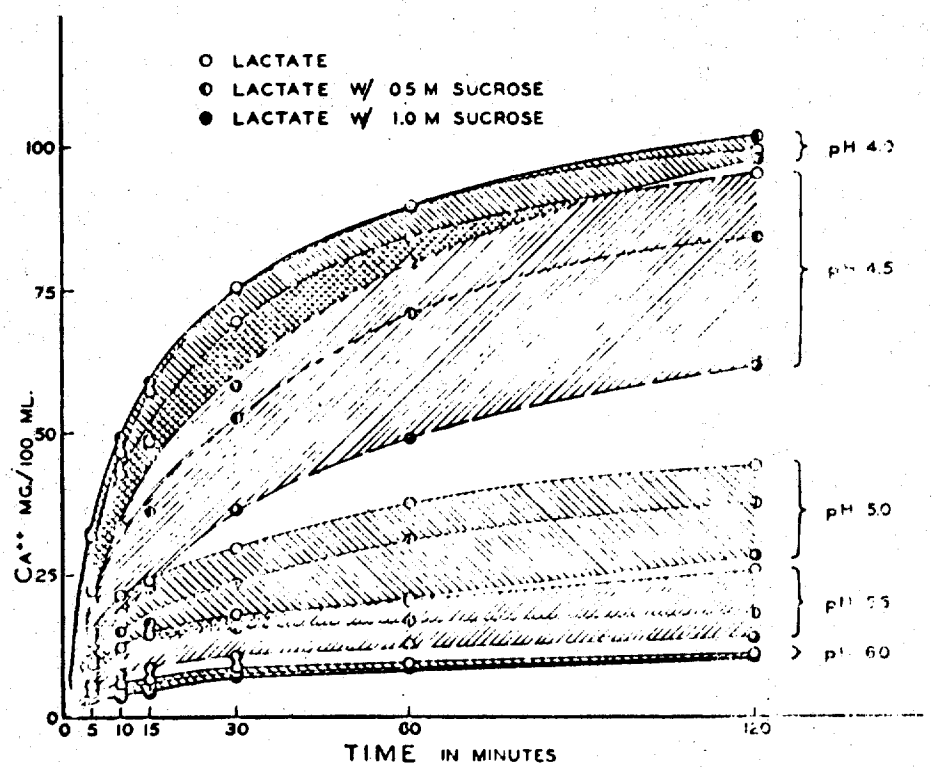

Fig. 1. -Rate of dissolution of powdered dental enamel in lactate [illegible] as measured by Ca^{++} content of the solutions. The three solutions at each pH [illegible] with cross hatched lines.

The curves all show a slowing rate of dissolution with time as a result of the increasing calcium and phosphate ions in the solution with time as well as the decrease in the enamel surface of the powdered enamel. The sucrose effect on the rate of enamel dissolution is least

pronounced at pH 4 and 6; in most instances, the presence of sucrose reduced the amount of calcium and phosphate going into solution.

In neutral aqueous solutions, sucrose alone, tends to increase enamel solubility. In the buffered solutions, the possible lowering of dissolution rates of enamel particles may be due to viscosity effects, coating effects, chelation or hydration.

In 1961, the authors Blackwell et al. (19) reported continued studies on the influence of sucrose in the etching or dissolution of dental enamel in lactic acid solutions. Whole enamel surfaces were utilized instead of powdered dental enamel; electron microscopy aided in determination of the etching of labial and buccal surfaces of central incisors and first molars. Half the surface of each tooth studies was covered, and half was subjected to a 15-minute treatment with test solutions. Test solutions included: a 0.1 M lactic acid buffer - 1 M sodium chloride solution, pH 4.5; 1 M sucrose solution, pH 7; 0.1 M lactic acid buffer - 1 M glucose solution, pH 4.5; and 1 M sucrose, pH adjusted to 11 with sodium hydroxide. Preceding these solutions, preliminary studies utilized similar solutions, all at pH 4.5 and 0.1 M with respect to lactate. Treated tooth surfaces were examined by electron microscopy; the degree of etching of the surface was determined by comparison of protected and exposed surfaces qualitatively.

It was found that the addition of sodium chloride to the lactate buffer did not alter the etching action of the lactate solution. Glucose retarded, the same degree, the etching action; sucrose to a much greater degree. The data from this series of experiments agreed with the findings of the earlier experiments with powdered enamel. The sucrose action is related to the pH of the solution; a 1 M sucrose solution at pH 7 had no noticeable effect, but at pH 11, a slight but detectable etching of the enamel was observed.

The results of these several studies indicate a protective action of sucrose as far as the tooth enamel is concerned. The authors have commented as follows regarding the studies:

"It is well to emphasize that the following results are not presented in an effort to minimize the deleterious effects of sugar as an agent in caries development. Instead, the results are presented for the information they provide. They serve to demonstrate once again the amazing complexity of factors involved in the process of caries formation."

The relationship of pH in dental plaques to caries in caries-susceptible and caries-free individuals was studied by Rosen and Weisenstein (164). Samples taken from both types of subjects, from gingival areas of noncarious buccal surfaces of upper molars, were measured for pH. An oral rinse of 20 ml 50% sucrose solution in water for 30 seconds was followed by periodic pH determinations of plaque material by a one-drop assembly-glass electrode. The mean plaque pH values for before and at 30 seconds,

5, 10 and 15 minutes after a sucrose rinse are shown in the following figure:

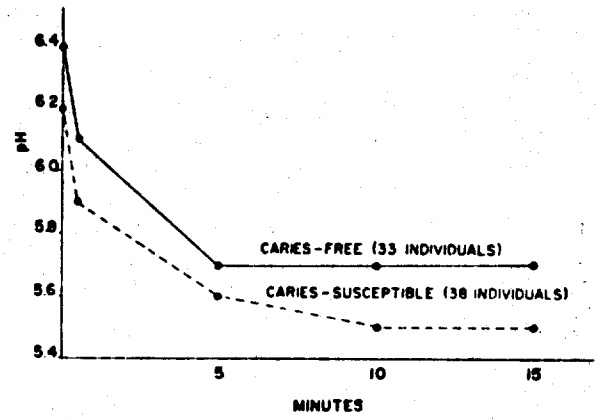

Fig. 1. Mean pH of dental plaque before and after a sucrose rinse

A statistical treatment of the data showed no significant differences between caries-susceptible and caries-free individuals for values before and at 30 seconds and 5 minutes after sucrose rinsing. The values for 10 and 15 minutes were significantly different. The pH of plaques is 0.1 to 0.2 higher in caries-free than in caries-susceptible individuals, both before and after the sugar rinse, generally.

Pigman et al. (159) described a study utilizing the artificial mouth which consists of a device exposing extracted human teeth to a mixed culture of oral microorganisms. The medium is dripped continuously on the tooth which is covered with a mixed bacterial plaque derived from oral microorganisms. The rate at which the enamel is softened is measured. Various sugars can be used in the medium and their cariogenicity determined.

The D-form of glucose, fructose, mannose and galactose were studied by the authors, as well as lactose, maltose and sucrose. Soluble starch was also studied. Using glucose as a comparison, fructose, mannose and sucrose rates of softening enamel were similar to that of glucose; galactose, maltose and lactose appeared to have rates lower than for glucose. Only for maltose was the difference from glucose significant. Soluble starch showed no change in the teeth during the experimental period of 8 hours. Presumably the ineffectiveness of starch is due to failure of the micro-organisms to break it down to fermentable sugars.

Kinoshita et al. (111) investigated the effects of frequent sucking

of sugar toffees on the rate of plaque formation on labial tooth surfaces. Ten male volunteers followed a regime consisting of a period of sucking 15 pieces of sugar toffee, one per hour, during the day for one week and for 3 days in a second trial. This was followed by chewing sugarless chewing gum, 10 pieces daily, each one for about 1.5 hours. Sandblasted standardized plastic foils were attached lingually to two thoroughly cleaned lower central incisors of each subject at the beginning of each experiment. Plaques formed on the foils and on the lower left lateral incisor were examined, the former chemically and the latter photographically.

In the 7-day experiments, significantly more plaque was formed when administering sucrose; less plaque when chewing gum. The 3-day experiment produced no significant results. Foil deposits were slightly less mineralized after sucrose treatment, and chewing gum inhibited the rate of plaque formation on labial tooth surfaces, but not on lingual deposits.

In 1940, Hopkins (99) asked the question: Has it been proved that sugar is a significant factor in dental caries? The author comments on the complexities and confusion regarding the presumed causes of dental caries and reviews the findings of several investigators (principally epidemiological). The summary indicates the status of knowledge concerning dental caries of more than 30 years ago:

1. A certain amount of sugar may be needed in the dietary.

2. Food habits of the nation, encouraged by advertising propaganda, make omission of the use of sugar impossible.

3. Good dental health and good general health are dependent on good nutrition. This involves not only eating habits, but also healthful living.

4. We do not know the cause of dental caries. Many conditions are responsible for its frequency.

5. That sugar is a significant factor in dental caries is a proved and accepted fact. Theories as to its action are still unproved. The consumption of sugar cannot as yet be identified as the real cause of dental caries.

More than twenty years ago the Council on Dental Health of the American Dental Association (33) published a comprehensive statement, documenting the known or potential hazards to dental health resulting from the frequent consumption of sweetened beverages and other sugar-containing substances. One hundred seventy-two references, covering the period of

1873 to 1953, on dental literature, are cited. A brief chronology of dental research "highlights" is followed by a discussion of five factors on conditions essential for dental caries: a caries-susceptible individual; the presence of acid-producing and acid-tolerant bacteria; an optimum bacterial enzyme system; an orally fermentable carbohydrate; and an adherent dental plaque.

The decalcifying and cariogenic properties of sweetened drinks are discussed. The summary and conclusions are presented here in their entirety; the details of the supporting arguments may be read in the document which is included in the document section of the monograph.

Summary

Because of the length of such a documented report, it may be helpful to provide a brief summary of its various areas in order to bring together the conclusions which reasonably may be achieved.

1. Five essential factors in the process of human caries have been reviewed and documented. They are (1) a caries-susceptible individual, (2) the presence of acid-producing and acid-tolerating microorganisms in the oral cavity, which are (3) capable of producing an optimum oral bacterial enzyme system for the prompt degradation of simple sugars to acid, (4) the availability of orally fermentable carbohydrates and (5) bacterial plaques adherent to the surfaces of the enamel of teeth.

2. Several additional factors which may contribute to the process of caries also have been listed without thorough documentation.

3. The behavior and essentiality of the dental bacterial plaque, through which sugar solutions must operate in caries, have been presented and documented in considerable detail.

4. The decalcifying effect of sweetened beverages and fruit juices has been examined in sufficient detail to warrant certain conclusions:

   (a) Research has shown that some sweetened drinks and some natural fruit juices decalcify teeth because of their acidity.

   (b) Decalcification has been observed when either are consumed in large amounts or at frequent intervals.

   (c) Decalcification probably should not be significant unless such solutions are consumed in large amounts or at frequent intervals.

   (d) Fruit juices provide some of the valuable food elements

of human diets, while prepared soft drinks provide nothing but calories.

5. The examination of the scientific literature on the cariogenic properties of soft drinks and fruit juices also justifies a number of conclusions:

   (a) Any sugar solutions, in order to stimulate caries, must be capable of diffusing into the adherent dental bacterial plaques on teeth.

   (b) Available evidence indicates that starchy carbohydrates are of minor importance in the process of caries since complex carbohydrates cannot diffuse into bacterial plaques and cannot be degraded promptly to simple sugars in most mouths.

   (c) Monosaccharides and disaccharides diffuse readily into bacterial plaques.

   (d) The retention time of carbohydrates in the saliva appears of much less importance in the caries process than the retention time of simple sugars in bacterial plaques and the inability of the saliva to contact rapidly and buffer the acid produced under plaques.

   (e) It has been demonstrated that solutions of simple sugars are degraded promptly to lactic acid in the bacterial plaques of caries-active individuals.

   (f) This production of acid from retained solutions of sugar may continue in plaques for one-half to three hours following a brief rinse of the patient's mouth with a 10 per cent solution.

   (g) The dental plaque appears to have the ability to store acid and to hinder the saliva from neutralizing it.

   (h) The pH of an individual's saliva can be too high for decalcification to take place while, at the same time, the pH in a bacterial plaque becomes so low that decalcification occurs rapidly.

   (i) Solutions of some natural sugars, such as lactose, and sorbitol are fermented with difficulty in the human mouth.

   (j) Data regarding the cariogenic potentialities of solutions of simple sugars that are derived from studies of laboratory rats must be appraised critically by dentists since great variations in susceptibility to caries exist for a number of strains of these animals and findings can be prejudiced merely by the selection of the strain of animal

and the physical qualities of the diet which he is fed in an experimental situation.

Conclusions

1. The dental profession and other interested agencies have a responsibility to warn the public of the cariogenic property of sugars and their solutions and to point out that many of these products contain no highly important nutritional factors.

2. Research should be encouraged on the part of producers of sweetened beverages and confections to support the development of new or improved procedures or agents for the prevention and control of dental caries which may result from the use of their products.

An early study (1937) by Osborn et al. (153) indicated that refined carbohydrates (sugar and flour) were more effective in producing decalcification of tooth enamel *in vitro* than were unrefined cane and whole wheat meal. Teeth from healthy human extractions were incubated in mixtures of saliva and sugar, or saliva and cereals; normal saline, crude sugar-cane juice, refined white sugar, and "porridges" made either from whole meal or from white flour provided the incubation media. The refined products produced decalcification more rapidly than the crude; the pH of the media was about the same for all, and it was suggested by the authors that "naturally occurring carbohydrate is accompanied, in sugar-cane and in cereals, by a protective factor which exerts its effect otherwise than by controlling pH." They were not prepared to speculate as to the identity of the "protective agent."

Hartles and Wasdell (92) measured the oxidation rates of four sugars, glucose, fructose, maltose and sucrose, added to human saliva taken from 20 volunteers with varying caries experience. The mean oxygen consumption, measured by Warburg manometers in vitro of the sugars is shown in the following table:

Table I. *Mean oxygen consumption of salivary samples due to the addition of different sugars*

(All values expressed as $\mu l.$ O_2/ml. saliva/hr. Final concn. of added sugar, 0·605 M. Temp. 38°.)

Subject	Teeth decayed, missing, or filled (DMF)	Glucose	Fructose	Maltose	Sucrose	*S.E.	No. of days
			Group I				
S.F.S.	4	91·7	87·1	98·5	91·1	4·0	12
J.M.	6	68·5	62·9	77·5	77·8	4·0	10
J.G.	8	63·7	59·6	74·7	63·0	3·1	12
J.R.D.	10	180·4	170·2	186·7	186·3	5·8	12
A.G.L.	12	143·0	132·4	149·8	140·5	5·7	11
Mean	8	109·4	102·4	117·4	111·7	—	—
Weighted mean		110·2	103·4	118·1	112·5	—	—
			Group II				
R.L.H.	17	397·1	334·8	390·2	411·5	14·7	13
M.R.W.	17	136·4	113·5	128·9	121·4	5·1	14
L.G.	18	108·0	77·5	103·1	110·8	5·1	10
I.A.R.	20	60·6	46·8	68·0	65·0	3·9	11
P.D.B.	23	134·6	115·5	138·5	135·6	5·2	12
Mean	19	167·3	137·6	165·7	168·9	—	—
Weighted mean		173·9	143·3	172·0	175·0	—	—

* S.E. is the standard error of the difference between any two means: thus, roughly speaking, a difference of more th twice this value between any two means in a given row is statistically significant.

There was no significant difference in the metabolic rates of glucose, maltose and sucrose; the metabolic rate of fructose was, however, significantly lower.

Waugh and Waugh (213) described a field study in 1940 by Eskimos in south western Alaska. Three native villages were surveyed over a period of about 5½ weeks during which time the inhabitants were examined for caries, and saliva tests were made for Lactobacilli. Each native subject was given a sweet daily consisting of dried fruits, maple syrup or honey as sources of natural sugars, or foods containing refined sugars, such as figs preserved in cane sugar, a chocolate candy bar, lollipop, or sugar cubes.

Practically all subjects free from caries in the beginning, and given natural sugars during the experimental period, remained free from caries and had reduced oral lactobacilli concentrations. Those with caries to start and given natural sweets, continued to develop caries; thus the natural sugars did not initiate caries or increase oral lactobacillus concentration.

Those subjects caries-free at the beginning, but given refined sugars, showed inception of caries, and those already with caries showed an increase in caries incidence. The initiation of growth or oral lactobacilli also accompanied the increases in caries development.

Ericsson et al. (47) in 1954 discussed the relationship between saliva and dental caries; investigations were conducted with human volunteers of two types: caries-resistant and high-caries, 31 subjects in the resistant group and 32 in the high-caries group. On the basis of the knowledge of salivary physiology and chemistry at that time, the following factors were considered of the foremost importance in the formation of acid and the dissolution of tooth enamel: amylose hydrolysis of starch into simple sugars and acid; retardation of the acidity by high pH values and strong buffer effect; presence of calcium and phosphate should counteract the dissolution of the calcium phosphate structure of the dental hard tissue; the rate of salivary secretion providing dilution and rinsing of substrate and products of acid-forming bacteria should be significant; and mucin creates a viscous saliva, retarding flow and diffusion.

Clinical examinations and case histories of the experimental subjects were taken; saliva was collected to determine rate of secretion and viscosity. The saliva amylase was estimated and fasting values of reducing substances (as mg glucose/100 ml saliva) and lactate were determined. White bread was eaten by each subject and the foregoing values determined; the caries-susceptible group was found to have higher average sugar and lactate levels in the saliva during the 30-minute period following the ingestion of bread. Significant positive correlations were found in both groups between glucose and lactate contents. Trends, not statistically significant, were observed towards higher values for secretion rate and flow for the resistant group. No difference between the groups in amylase activity was seen.

Shannon et al. (175) collected parotid fluid samples from more than 500 systemically healthy young adult males, recording flow rates, and analyzing for glucose concentration; blood samples were also taken and the serum glucose determined. Subjects were classified into 6 dental status groups based upon the sum of decayed, missing and filled tooth surfaces (DMFS).

The parotid fluid flow rate was independent of the DMFS status. Serum glucose concentrations were not significantly different among the DMFS groups; parotid fluid glucose was significantly lower in the DMFS group 41-50 than in three lower groups, but no pattern was discernible, and the observation may have been due to change.

The presence of glucose in parotid fluid was confirmed by this study; about 1% of the serum glucose mean appears in parotid fluid. It was concluded that salivary carbohydrates, because of the small amount present, probably play an insignificant role in acid formation in saliva or in plaque.

Volker (208) investigated the glucose content of human saliva; un-

important levels were detected. The salivary sugar content was observed to remain not elevated in diabetes. Oral clearance of glucose was dependent upon rate of saliva flow, the morphology of dental arches, and the action of mastication of the food. The physical form of the carbohydrate was a more significant factor than was quantity. One percent sucrose was rapidly converted to glucose by saliva (from 10 mg % to 70 mg % within 2 minutes). Raw and refined sugars all comparably lowered the pH of saliva in caries-active persons examined. Caldwell (19) studied the adhesive characteristics of 77 different foods to determine whether sticky foods were more cariogenic than non-sticky. An "adhesion tester" was devised and the effects of pellicle on the tooth surface as affecting adhesion of food was measured; neither the pellicle nor the surface roughness was a major factor in the adhesion of food. Mastication studies indicated that about the same amount of saliva was collected for the different foods tested. Cox et al. (34) had earlier observed in albino rat studies that a particulate diet was more significant in creating caries than was a high-carbohydrate diet alone. These investigators concluded that sugars, such as glucose and sucrose, do not initiate rat caries. Shockley et al. (180) tested sorbitol as a sweetner with possible application in oral mouthwashes or dentrifices; the emphasis was placed upon the fermentation of sorbitol with subsequent acid formation by Lactobacillus acidophilius and casei, and other oral microorganisms. The strains of Lactobacillus were identified as "smooth" or "rough"; glucose and sorbitol were fermented by the smooth strains, but sorbitol was little or not at all fermented by the rough. Cultures of the rough strain from oral bacteria taken from caries-immune individuals did not ferment sorbitol; acid from sorbitol was produced slowly and in small quantity.

The cariogenicity and anticariogenicity of different foods and beverages that people like and commonly consume was the subject of investigations by Stephan (191). Detailed clinical studies of over 80 patients with rampant dental caries could not explain the condition, but the most common factors associated with the caries development were a history of frequent or excessive eating of sweets during and between meals; extensive bacterial plaque formation; and lower pH levels in plaques and food material retained in carious areas than in mucous membranes or saliva. The extreme difficulties in securing scientifically adequate dietary controls for any given food material in human consumption leads necessarily to animal experiments. Carefully controlled experiments with Osborne-Mendel strain rats were described. The basic diets were a simple low-fat high-sugar fine particle cariogenic diet, and the same diet without the sucrose, essentially noncariogenic. Fifty-three representative foods and refreshments were tested for cariogenicity; the following table lists the foods:

TABLE 2

EXPERIMENTS 6350-98: LIST OF FOODS TESTED

Carbohydrates	*Candies*	*Cookies*	*Biscuits*
Sucrose	Caramels	Chocolate sandwich	Teething biscuit
Dextrose	Chocolate	Vanilla wafers	Melba toast
Starch	Marshmallows	Baby cookies	Soda crackers
Confectioners' sugar	Mints	Chocolate graham	Graham crackers
Sorbitol†	Chewing gum	Honey grahams	Dog biscuits*

Breads	*White Bread with Spreads*	*Soft Drinks*	*Vegetables*
White bread	Bread+raspberry jam	Cola	Carrots
Whole wheat	Bread+grape jelly	Orange drink	Lettuce
Rye	Bread+peanut butter	10% sucrose water	Cabbage
Raisin	Bread+peanut butter+grape jelly	10% dextrose water	Potato
Cracked wheat	Bread+butter	Whole milk	Spinach

Fresh Fruits	*Dried Fruits*	*Snacks*	*Experimental Diets* (24 hours)
Apples	Figs	Peanuts	580
Bananas	Apricots	Popcorn	581
Oranges	Dates	Potato chips	585
Lemons	Raisins	Corn chips	593
Grapes			1503

No supplements (Control I and Control II)

* Diet records showed that occasionally some children who have dogs will try to eat dog biscuits.
† Sorbitol is actually a hexitol or "sugar alcohol."

A preliminary experiment with 240 rats was performed, but the main experiments used 960 rats, with duplicate tests and control animals. Test foods were given for 28 days and the animals were sacrificed and heads prepared for oral examination: dental erosion, periodontal disease, dental calculus formation, adherent plaque, attrition, incisor pigmentation, alveolar bone derisity, and growth. Caries scores for the foods tested with both noncariogenic basic diet and with the cariogenic diet are shown in the following table:

TABLE 3

Comparison of Caries Scores Produced by Different Foods

Food Material Tested	Experiments with Noncariogenic Supplement (Basic Diet 581)		Experiments with Cariogenic Supplement (Basic Diet 580)	
	Mean	S.E.	Mean	S.E.
A:				
Dog biscuits	0.0	0.00	0.0	0.00
Popcorn	0.0	0.00	1.8	0.53
Sorbitol	0.0	0.00	1.0	1.00
Peanuts	0.0	0.00	2.3	1.61
Whole milk†	0.0	0.00	4.2	0.79
B:				
Lemons*	0.0	0.00	4.3	1.42
Corn chips	0.0	0.00	7.0	1.98
Cabbage	0.0	0.00	7.0	1.69
Lettuce	0.0	0.00	9.7	3.33
Control (basic diet only 2-hour supplement)	0.0	0.00	11.3	2.03
Dried apricots*	0.0	0.00	11.3	3.53
Oranges*	0.0	0.00	16.8	3.17
C:				
Soda crackers	0.3	0.24	11.6	2.81
Spinach	0.6	0.49	4.6	2.99
Cracked wheat bread	1.3	0.91	13.7	2.80
Potato chips	1.6	0.68	6.7	2.93
D:				
Whole wheat bread	2.0	0.13	17.7	2.01
Carrots†	2.1	0.05	21.1	1.42
Cornstarch†	3.3	0.49	10.2	2.28
White bread and butter	4.9	1.00	22.1	5.80
White bread and peanut butter	5.2	1.88	18.0	4.43
Graham crackers	8.7	1.81	22.7	3.98
Raisin bread	9.0	4.11	17.2	4.55
Melba toast	9.0	3.17	19.6	2.73
White bread	9.2	1.31	15.1	2.92
E:				
White bread and raspberry jam	10.2	1.63	33.0	4.81
Figs	10.3	1.92	29.0	6.06
Rye bread	12.7	2.98	29.0	6.30
Chewing gum	14.0	3.67	27.2	7.19
Caramels	16.0	6.21	50.4	9.42
Chocolate graham crackers	18.0	3.77	39.1	8.76
Baby cookies	18.5	1.25	22.0	6.27
Honey graham crackers	19.2	2.92	21.6	4.44
Apples*	19.4	8.01	81.7	7.17
Vanilla wafers	19.7	3.56	30.2	5.52
Bananas	21.0	6.06	73.7	7.60
Chocolate sandwich cookies	23.8	1.60	92.2	7.93
Grapes*	24.1	3.92	42.4	10.55
Candy mints	24.7	8.40	42.12	4.95
Teething biscuits	29.0	5.27	29.2	5.43
Cola*	29.6	8.46	40.2	9.59
Marshmallows	30.1	3.28	42.2	5.63
Dextrose	30.6	3.56	43.5	8.66
Raisins	30.9	5.32	61.6	7.78
Irish sucrose water	32.3	12.20	20.6	5.00
Dates	32.7	3.39	38.5	6.28
Milk chocolate	34.1	9.91	41.2	11.30
White bread and jelly	36.7	7.22	36.2	9.44
Confectioners' sugar	38.4	4.68	60.2	8.12
10% dextrose water	41.3	9.58	45.5	8.66
Orange drink*	43.5	8.96	26.1	10.27
Sucrose	62.1	8.57	51.9	7.10

* Product of dental erosion.

† Produced a higher than average caries score > 1.5

Only 7 of the foods tested produced dental erosion: cola, apples, orange and lemon slices, grapes, dried apricots and orange drink. The pH of these foods were all quite acid (pH below 4.5); the remaining 46 foods showed a pH above 4.5. Periodontal disease (alveolar bone destruction) was relatively slight; sucrose, dextrose, confectioners' sugar and cola beverage were most effective, but still not significantly. Calculus formation was not extensive, and bacterial plaque material was mostly lost during preparation of the heads for examination. Alveolar bone showed porosity from diets deficient in minerals and high in carbohydrates, in some animals; dog biscuit produced very dense alveolar bone formation.

In general, the data from these experiments support the conclusion that habitial and excessive between-meal consumption of candy, cookies and soft drinks may lead to the development of rampant caries in susceptible individuals.

Patients with average to high dental caries and a tendency to accumulate dental plaques were subjects of a study by Frostell (54) who measured the pH of plaque material after various solutions or foods were ingested. Groups of 10-18 patients were given sucrose solutions of concentrations of from 0.05% to 50% as 30-second rinses and the pH of dental plaques determined at 5 minute intervals for 30 minutes. The pH was lowered to a degree related to the sucrose concentration, except at the 0.05% level which showed a moderate pH increase.

Phosphate buffered 50% sucrose solutions were effective in preventing lowered pH values in the plaques only at pH of 7.8 (buffers tested were at pH of 3.5, 4.9, 6.5 and 7.8); buffer of pH 4.9 was also less depressive than pure sucrose solution. Three percent citric acid added to the 50% sucrose solution significantly lowered plaque pH at 2 minutes, but elevated pH at 10, 20 and 30 minutes after rinsing mouth. Sucrose-peanut butter mixtures tested showed that the presence of fat prevented a drop in pH as low as produced by sucrose alone. The addition to sucrose of flavors, menthol and eucalypthol, had similar results. Repeated sucrose rinses did not further decrease plaque pH below the initial values. Carbamide (urea) at 5% concentration prevented to a great extent the lowering of pH by sucrose. Substitution of sorbitol for fermentable sugars resulted in significant differences in usually increasing pH rather than lowering it. Substitution of hydrogenated dextrines for fermentable sugars in various sweets generally prevented a lowered pH. The actual cariogenicity of foodstuffs is, however, only partly related to the effect on pH; other factors must be considered.

In vitro studies of the effect of sucrose on the solubility of whole teeth, enamel and calcium salts in saliva supernatant and a range of buffers were reported in 1971 by Jenkins and Tatevossian (107). Calcium

phosphate and tooth enamel powder were unaffected by sucrose. Calcium saccharates were shown to be dissociated in solution; it was concluded that calcium saccharate formation was an unlikely factor in the initiation of dental caries, and that the cariogenicity of sucrose cannot be ascribed to a 'direct chemical interaction' with tooth enamel.

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Inferences from Natural Occurring Variations in Caries Prevalence

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If, as is surely true, all research on disease in man has its beginning and its end in observations on human subjects, it is appropriate to preface our consideration of the role of food in caries by making a general survey of food relationships to recognized variations in caries prevalence to see whether the natural experiments that conditions of life have been carrying out on man contribute any knowledge to the subject. If there were clear-cut and incontrovertible relationships between the use of certain foodstuffs and caries prevalence, a reexamination of the subject would not be necessary. However, since reviews, such as those of Toverud[1] and Finn,[2] have failed to establish any explicit correlations, another survey is justified, even though all that can be expected is that it will cast a little more light on the nature of the food variables that relate to known differences in caries prevalence that occur in relation to time, geography, social status, race, sex, or manner of life.

Time

The period in history in which people live has had a definite relationship to caries prevalence. There was little caries in prehistoric or ancient man and the tooth destruction that occurred seems to have been related to occlusal wear more than anything else.[2] The comparisons that have been made on the amount of caries occurring at different time periods in Great Britain, the Rhine Valley, or Greece, show that before the second millenium of the Christian era no more than 10% of skulls examined had caries.[3] The changes that occurred before that time, such as the reduction of caries in Great Britain from the Roman to Anglo-Saxon period or the doubling of caries in the early Christian era in Greece, have been attributed, respectively, to the deterioration of agriculture and the adoption of Roman practices of cooking foods. In all three countries the last 400 or 500 years saw the most dramatic increase in caries. This is the period in which transshipment of food, particularly sugar, increased tremendously. Hardwick[3] found that before the establishment of the sugar trade with Madeira and the Atlantic islands the cost of a pound of sugar was 50 times that of a pound of bread. As a result, appreciable amounts of sugar were used only by the wealthy, and decayed teeth became a matter of note. As the supply of sugar became more plentiful, Pierre Fauchard[4] and other dental pioneers began attributing dental decay to the excessive use of sugar.

Toward the end of the nineteenth century another factor was named[5] as a contribution to increased caries in the West. This factor was the increased refinement of flour, which resulted from the introduction of roller milling. Another suggestion that wheat might be implicated in caries dates back to ancient Egypt, where the teeth of fourth dynasty mummies showed much more decay than those of the third dynasty, when cereal is reported to have come into use.

Evidence from other parts of the world also suggests that caries has increased with the passage of time. For instance, Pedersen[6] reports that in Greenland a change occurred from 0.5% of people with caries in ancient times to 69% at the present, and Pickerill[7] reports that in New Zealand caries in the Maori increased from 2.9% to the 90% level of today.

Changes in caries prevalence that occurred in a narrower time span will be discussed later, but one which has particular relevance to United States should be noted here. Brekhus[8] found that in students enter-

ing the University of Minnesota in 1929, 1939, and 1949 there was a 14% increase in the number of decayed teeth. Since these observations cover the period of enthusiasm for vitamins and minerals in caries prevention and overlap into the era of fluoride therapy, it is reasonable to ask whether an implacable increase in the caries attack rate might not in the future surmount the presently known methods of caries prevention.

Geography

Broad variations in caries prevalence exist between different parts of the world. These variations probably result from the interaction of several factors, among which are race, climate, soil, water, culture, traditions, and food habits. It is difficult, therefore, to determine what part food plays in producing the observed variations in caries. Nevertheless, some inferences can be drawn. One inference is that poorly nourished populations in Asian and South American countries seem to have less caries than populations living under the more adequate nutritional conditions in the Western world.[9] There is no statistical evidence to show whether the nature of the basic cereal substrate (rice, corn, or wheat) in the diet is in any way related to the caries rates, but it is fairly obvious that caries is most rampant where highly refined wheat flour is used most. In an early consideration of this subject, Bibby[10] also noted the lack of correlation between caries and the nutritional adequacy of diets in population groups, and suggested that caries was more closely related to the frequency of eating. The higher prevalence of caries in temperate climates than in warmer climates is well documented by Dunning[11] and general observations and some diet analyses suggest that this may be associated with a greater consumption of bakery products and confections in the former regions. It is interesting to note that in the US, at least, there is no caries relationship to the consumption of carbonated beverages. Thus, although the evidence is fragmentary, it suggests that sweet foods in solid form make a greater contribution to caries causation than when used as liquids.

Toverud,[12] Hewat,[13] Baarregard,[14] Waugh and Waugh,[15] and Russell[9] have noted that in Norway, New Zealand, Greenland, Alaska, and Vietnam, less caries occurs in rural regions than in urban regions. Whether this is correlated to the mode of life or the mode of eating is not firmly established. However, the distance from the source of imported refined foods seems to have relevance in the Eskimo studies. Meeredy[16] found more caries in children who lived close to candy shops than in those who lived at some distance.

Cultural Status

It has already been implied that the changes in caries prevalence over the centuries were the results of cultural evolution that led to modern ways of living and eating. Such changes, however, do not always have to await the passage of time. They also result from the transplantation of modes of living into new territories or the movement of people from primitive societies into modern communities. There are numerous examples of the way in which changes of customs by people in primitive societies have led to dramatic increase in caries. Toverud[1] and others (Finn,[2] Staz[17] and Oranje, Noriskin, and Osborn[18]) have recorded what happened to the teeth of various African tribes, East Indians, Maoris, and Eskimos as they gave up their traditional manner of living and eating in favor of those of Western man. In the Eskimo and Maori, at least, the increases of caries occurred in a generation or two in people who suffered no genetic admixtures and made no change in geographic location, climate, or water, and little alteration in clothing or housing. What changed most was the food, and with it the teeth. In West Greenland, Baarregard[14] reported the percentage of Eskimos suffering from caries increased from 58 to 95% between 1940 and 1945. In Alaska, Waugh and Waugh[15] reported that the children of nomadic Eskimos had no decay, those of Eskimos who visited the trading posts had occasional caries, and almost 100% of the teeth of children living in the settlements were carious. This last group, for the most part, ate imported foods. Other than increased use by the native peoples of such manufactured foods, of which refined sugar and flour products make up a major part, associations between caries and specific types

of food are hard to find. Osborn, Noriskin, and Staz[19] offered some evidence of the importance of the refined foods in caries causation when they showed that the change from crude sugar and grain meal to more refined substitutes paralleled caries increases in South African natives. They also showed that the refined products produced more decalcification of teeth on incubation in saliva than did the crude sugar and meal.

Recent studies in Ghana[20] and in Sudan[21] revealed that dramatic increases in caries are taking place. This is happening particularly in the better paid, educated people who have begun to use Western foods, notably refined sugar and wheat flour. As millions of people in Africa and elsewhere adopt Western ways of living and eating the prospect of a caries explosion in the world is sufficiently alarming to cause concern among some cariologists.

The extent to which socioeconomic factors in Western society are related to caries prevalence is not clear. The weight of evidence indicates that the poorer inhabitants of the US are no more prone to dental decay than those living in more favored circumstances. If significant differences between the eating habits of the rich and the poor could be established, a sort of negative conclusion could be drawn that because the diet of the economically favored groups is nutritionally superior to that of the poor, the factor of nutritional adequacy cannot be a dominant one in determining the caries attack rate.

War

The changes in eating habits that result from wartime conditions are in a sense the reverse of what occurs in native peoples adopting Western ways. The changes have contributed to the information on the importance of specific dietary components in caries causation. War-associated changes in caries incidence were first noted in 1920, when Wheatley[22] reported that in England, since 1914, the percentage of caries-free 5-year-old children increased from 5% to 44%. Subsequently, similar information relating to both World War I and II came from Germany and the Scandinavian countries. The caries reductions occurring in the Scandinavian countries during the latter war have been subjected to much study. They have been interpreted both as the result of effects of the development of teeth with higher resistance to decay[23] and a reduction of the direct local effects of sugar and bolted flour[24] on the erupted teeth. However, critical examination of the data and analysis of the attack rates on individual teeth has produced general agreement[25–27] that the factor that determined the severity of the caries attack was the amount of sugar used during the posteruptive experience of the teeth. Toverud[1] points out that among the four Scandinavian countries there was least caries reduction in Sweden, where nutrition was best and sugar reduction least, and greatest caries reduction in Finland, where nutrition was worst and the sugar ration the lowest. Denmark, where the food supply suffered less than Norway, did not fare as well dentally.

Convincing evidence of the importance of posteruptive sugar has come from a study[28] of the relationship between first molar caries and the sugar consumption in Japan, which showed that the caries attack rate bore a direct statistical relationship to the posteruptive sugar intake, but not to the use of sugar during the period of tooth maturation.

In considering wartime changes in caries it should not be forgotten that the effect of food rationing on the frequency of eating may also have contributed to lowering the caries attack rate. Also the effect of wartime conditions on caries in communities using fluoride-containing water deserves mention. Weaver[29] found that from 1943 to 1949 the caries rate in 12-year-old children in "nonfluoride" North Shields fell to the prewar level of South Shields, which had 1.4 ppm fluoride in the water, and, further that during the same period the number of carious teeth in South Shields fell to about half of its previous level. Apparently modification of the diet gave as great a caries reduction in North Shields, as would have resulted from fluoridation of the water, and in South Shields the wartime diet almost doubled the existing preventive effect of fluoride. This could be interpreted to mean that fluoride plus proper food usage would produce twice the effect on caries

that would result from fluoridation alone, and that this would be particularly beneficial to important teeth such as the molars.

Race

Different races show wide variations in caries prevalence. Their dietary practices also vary widely. But, because geography, culture, and genetic factors that are related to tooth form and position or metabolic activity may also play a part in determining the caries attack, it cannot be assumed that food variables tell the whole story. However, the failure of family or twin studies to establish genetic factors leaves little doubt that cultural or dietary influences are more potent than genes in determining the onset of caries. The nonexistence of racial immunity to caries is implicit in the rapid changes in caries prevalence noted in the Eskimo and other primitive people when they adopt Western food practices. Hyde[30] drew a similar conclusion from his study of caries in American army personnel of different racial backgrounds. He found that deviations from the racial pattern of caries attack occurred in recruits who had married into different racial groups, and that statistically, the changes were in the direction of the wife's group caries level. These findings reinforce Pickerill's[7] aphorism that the main hereditary factor in dental caries is the inheritance of the family cookbook.

Sex

A higher caries attack rate in girls than boys is well established.[2] Whether this sex difference continues into adult life is less certain. Although some of it can be attributed to earlier tooth eruption in females, the possibility that some systemic or dietary factors are partly or largely responsible cannot be ignored. There does not seem to be enough knowledge on eating habits of girls and boys to justify attributing the sex differences in caries to any specific food usage. If pregnancy contributed to caries, as may be the case in primitive populations on Tristan da Cunha,[31] the use of frequent carbohydrate snacks to combat morning sickness could be suggested as a possible factor.

Other Groupings

Friel and Shaw[32] first recorded that children living in institutions have much less dental decay than children of corresponding age living with their families. This observation has been repeatedly confirmed[1] and is accepted by everyone who has had experience with institutionalized children. This low caries attack rate seems to exist regardless of whether the institutionalized children are using nutritionally adequate or inadequate diets, and Toverud[1] believes that it is related to the reduced availability of cookies and candies for between-meal eating.

Low caries attack rates have been reported in diabetic children who live on controlled diabetic diets. Although the relative absence of caries in these children has been attributed to unidentified metabolic changes, a more likely explanation is that it is due to the low sugar content of their food. Abstinence from sucrose will also account for the caries-free teeth in people with hereditary fructose intolerance.

In children in several tropical regions a unique pattern of extensive decay of the anterior deciduous teeth, followed by largely caries-free permanent teeth, has been described. This condition is sufficiently well documented to become named "odontoclasia." Whether it is related to some peculiar aspect of infant feeding is not satisfactorily established, but its similarity to what Curzon[33] found in his study of Eskimo children who lived on a feeding bottle for several years suggests this possibility.

The similar conditions of "bottle caries" described in American and British children has been reviewed by Finn,[34] who concluded that damage to the teeth is likely to occur only if sugar is added to milk or water in the feeding bottles or pacifiers used by infants. Whether foodstuffs without added sugar will produce similar tooth destruction, as in odontoclasia, has not been satisfactorily established.

In respect to the effect of duration of food contact with the teeth, it should be noted that if caries is compared in children grouped according to whether they do or do not eat candies or confections between meals, Weiss and Trithart,[35] Ludwig,[36] and Bradford and Crabb[37] have shown that those who eat less frequently have less caries.

The earliest comparisons of caries preva-

lence were not made between population groups, but between types of teeth. The variations noted in the caries attack on different teeth apparently convinced some early observers, including Aristotle, that dental decay was the result of sweet foods sticking to certain teeth. By updating such classical findings and correlating them with the life history of individual types of teeth, clear evidence can be adduced that the local effects of food on the tooth surface are considerably more important in caries causation than are systemic or metabolic factors. How else can we explain the finding in Rochester[38] of a tenfold greater caries attack rate in the first permanent molars than in the mandibular incisors? Both types of teeth were formed and erupted at the same time, and, both preeruptively and posteruptively, were under the same metabolic influences, and yet there is this dramatic difference in caries incidence. A similar question can be raised regarding the contrasting caries rates between the second premolars and canines. This subject can be brought into even more critical focus by comparing caries rates in individual surfaces of the same teeth, such as the buccal and lingual surfaces of the maxillary and mandibular first molars. Here fivefold or tenfold differences occur between the lingual and buccal surfaces of the same teeth and between the same surfaces of opposing teeth.

If such comparisons of intraoral caries incidence were carried to their logical conclusion, they could give useful information on the role of specific types of foodstuffs in producing different types of carious lesions. Some progress in this direction has already been made in studies with rats.

Conclusions

In almost all of the naturally constituted population groups between which comparisons of caries prevalence have been possible there is an association between high caries and high use of sugar. Although this association is suggestive, it does not justify concluding that sugar working alone is responsible for the extent of the caries attack. The lack of parallelism between the available figures on sugar consumption in various countries and their caries records indicates that other factors play a part. If the records on sugar use by various countries accurately reflected the amounts going into other than locally consumed foodstuffs, and also showed the amounts eaten in different forms of solid, semisolid, or liquid food mixtures, we could come closer to drawing a meaningful conclusion on the precise role that sugar plays. This, of course, would be true only if more reliable figures on caries prevalence were available for purposes of correlation. Obviously, the final word on the relationship of sugar or other foods to caries causation must await more detailed information on food use and more reliable data on caries prevalence in human population groups.

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DIET AND DENTAL CARIES

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Food has been implicated in the causation of dental caries since ancient times and early written records offer both local and systemic theories on how food causes destruction of the teeth. On one hand, Aristotle stated that dental decay resulted from sweet figs sticking to the teeth, and on the other, Galen claimed that lack of nutrition made the teeth weak and brittle and caused the teeth to die away, and enlarged cavities. These early hypotheses dealt with erupted teeth and gave little or no consideration to the possible role of food during the prolonged period of tooth development within the jaws when the systemic relationships to the developing structures are maximal.

The inflammatory or internal hypothesis on dental caries had numerous staunch followers among the early students of dental caries, but lost ground as the experiments of Robertson (1835) and others strengthened the concept of an external attack on the teeth. The classic experiments of Miller (1890) established beyond reasonable doubt the action of oral bacteria on carbohydrate foods as the activating factor in the caries attack. Recent and more erudite bacteriological and chemical investigations have led to the realization that the carious process in man, and in experimental animals, was absolutely dependent upon the presence in the oral cavity of appropriate microorganisms together with food residues to provide for their metabolism.

During the period when bacteriology was making its greatest contributions to medicine, the action of bacteria on carbohydrates was widely accepted as the sole and completely adequate explanation of caries causation. Indeed, some investigators still tend to adhere to this concept with little or no appreciation for the possible roles of nutritional or genetic influences during or after tooth development. Likewise, when and where the importance of nutrition occupied a central place in medical thought, many accepted inadequate nutrition as the complete explanation for caries susceptibility and believed that even after the teeth were fully formed a nutritionally adequate diet imparted a high resistance to caries independently of the amount or type of oral flora and food present on the teeth. As usual, the truth lies between the two extremes and no simple explanation adequately describes the multifaceted etiology of dental caries.

In this brief review, reference will be made only to a small fraction of the published work relating caries activity to food factors after tooth eruption. It will include evidence that the presence of food in the mouth is mandatory for the

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development of carious lesions and that nutritional and systemic influences play, at most, modifying roles. As far as possible, an evaluation will be made on the role that nutritional experience during tooth development has on the subsequent susceptibility of the teeth to any caries attack resulting from the presence of the required oral caries-producing factors.

EXTERNAL EFFECTS OF FOOD

Examinations of the pattern of caries development in the mouth, as well as histological studies of carious teeth, have convinced investigators that caries occurs at those sites where food residues are retained most readily and that destruction of the tooth proceeds inward from the external surface. Many in vitro studies and investigations with experimental animals under a wide variety of circumstances support these concepts.

Laboratory animals have been invaluable in appraising the factors in the oral environment which contribute to caries. In experiments, not yet possible in human subjects, microoorganisms have been demonstrated to be essential to the carious process in animals. The demonstration by Orland et al. (1954) of complete freedom from caries in germ-free rats while their normally contaminated counterparts developed caries was the first absolute, direct evidence that the initiation and progress of carious lesions was dependent upon microorganisms. Later studies by Keyes (1960) and Fitzgerald and Keyes (1960) demonstrated that under certain circumstances caries-producing microorganisms could be transmitted from caries-active rodents to caries-inactive rodents of the same species, where they caused major carious lesions. Caries-producing microorganisms for one species did not initiate caries in another species. A full study of the types of microorganisms capable of transmitting animal caries has yet to be made. Recent researches implicate varieties of streptococci rather than the lactobacilli as having major responsibility for caries production.

Animal studies with antibiotics have also provided much information on the microbial relationship to dental caries. When incorporated into the diet or the drinking water these agents, and particularly penicillin, greatly decreased experimental dental caries (Shaw, 1959). The use of penicillin in toothpaste and dentifrices has been evaluated in several clinical studies; in some trials, good levels of caries reduction were reported, but in others, usually where toothbrushing was not supervised, no benefit was observed (Shaw, 1959). Little information is available on the effect of prolonged systemic administration of antibiotics on dental caries in man. In two studies, children with rheumatic fever on prolonged penicillin administration did not show a lowered dental caries experience (Handelman and Hawes, 1962; Zackin et al., 1963), while in a third where the children had been hospitalized, modest reductions in caries were observed (Littleton and White, 1964).

Finally, animal experiments have answered the question of whether the increase of caries following the post-developmental feeding of carbohydrates is the result of nutritional changes operating through the bloodstream or the consequence of reactions on the tooth surface. When caries-producing diets

were fed to animals by means of stomach-tube, so that no food came in contact with the teeth, caries did not occur; when this same diet was eaten in the normal way and allowed to come in contact with the teeth, caries occurred to the expected extent (Kite et al., 1950). The same result was obtained when only the carbohydrate was taken orally and the remainder of the diet given by stomach tube (Haldi et al., 1953).

Although cumulative clinical observations in human mouths had led to the same conclusion regarding the importance of carbohydrate contact with the teeth in caries causation in man, the Vipeholm Study (Gustafson et al., 1954) in Sweden produced clear-cut evidence on this point. The amount of caries increase in the human subjects was more directly related to the duration of sugar in the mouth than to the amount consumed. Sugar in solution which cleared quickly from the mouth produced much less caries than smaller quantities of sugar in a sticky bread or caramels, chocolate or toffee, particularly when the latter items were eaten between meals.

NUTRITIONAL EFFECTS ON DEVELOPED TEETH

There is little clinical evidence to show that nutritional or systemic factors operating after tooth eruption have comparable importance to the external effects of food on the teeth in determining the onset or progress of dental decay. The various clinical studies conducted in the heyday of nutritional experimentation of this have been reviewed previously (Shaw, 1952).

Comparisons of caries activity in human population groups have, as a whole, failed to show any association with particular dietary deficiencies; indeed, in many cases, the poorly nourished populations of the world evidently have less caries than those with abundant food supplies. Epidemiological studies with population groups have often proved to be misleading because the many variables to be considered have been interpreted erroneously or incompletely. No justification exists for the frequent implication made from population studies that every deficiency of an individual nutrient or of a combination of nutrients can be expected to cause an increase in dental caries activity. For instance, for the people of India many single and multiple deficiencies of nutrients are prevalent throughout the nation and yet on the average the dental caries incidence is among the lowest in the world even though their carbohydrate intake is very high. Therefore, the assumption has been made that dental caries is not associated with particular nutritional deficiencies. However, additional facts need to be included. For example, first the people of India not only are deficient in many individual nutrients, but probably their most critical nutritional problem is an insufficiency of calories. In animal studies, two of the most straightforward ways to reduce caries experience are to restrict the caloric intake and to reduce the frequency of eating. Second, Indians in the lower income groups have little access to sugars and other readily fermentable and adherent sources of carbohydrates that are particularly responsible for a caries-producing oral environment. On this basis also they could be expected to have a low dental caries experience. A third and possibly the most important factor is the high fluoride content of Indian teeth, which would impart a high caries-resistance

(Shaw et al., 1956). Together these three variants could far outweigh any caries-producing effect that might result from the dietary deficiencies, poor oral hygiene, or insufficient dental care.

Obviously, it is an equal oversimplification to suggest that the high dietary carbohydrate content among the Chinese, Indians and Italians, who are known to have little caries, refutes the importance of carbohydrates in caries causation. Certainly, unless these peoples can be shown to be better nourished than Americans, their low caries rate cannot be used to support the significance of general nutrition on caries. In the absence of such evidence, it is more logical to seek an explanation among the variables in the post-developmental diet which may be related to a chemico-parasitic attack on the teeth. Such reasons can be found in the recognition of the central importance of the frequency of eating in caries causation; in this respect the low caries populations certainly differ from those of countries where food is more abundant. Differences in fermentability of carbohydrates by oral bacteria and the relatively low intake of monosaccharides in these low caries populations probably have significance. Other variables will be the adhesiveness of food and the effects its fluidity or content of oil or fat will have in reducing retention on the tooth surfaces. Carbohydrate-containing foods also differ in the types of acids produced on fermentation and also in presence or absence in them of factors which reduce the decalcifying effects of any produced (Andlaw et al., 1962; Jenkins et al., 1959). Incubation of unrefined cereals, for instance, will produce more acid, but will cause less enamel decalcification than their refined counterparts.

PHOSPHATES

In a wide variety of recent laboratory studies, the addition of relatively high amounts of phosphates to experimental diets after the teeth have erupted resulted in clearcut reductions in dental caries in rodents (McClure, 1963). Comparable amounts of phosphates fed by stomach tube were much less effective than in the diet. The mechanism by which phosphates produced beneficial effects is uncertain. Current evidence suggests that the increased dietary phosphate levels raise the phosphate content of the milieu of the tooth surface and increase the resistance to the decalcification of the carious process.

Phosphate supplementation to human studies has been undertaken; as yet the results have not been as encouraging as those in experimental animals. Several tests of feeding bone meal or other phosphate preparations were either inadequately controlled or gave indefinite results. Three recent controlled experiments using additions of dicalcium phosphate gave, in one instance, a reduction of decay (Strålfors, 1962), whereas two failed to show evidence of caries inhibition (Bibby and Averill, 1963; Ship, 1963). It is, therefore, premature to assume that phosphates will be effective in retarding dental decay in humans. However, the particular circumstances under which these clinical evaluations were made and the experimental designs may not have been optimal for the full expression of any phosphate influence. The greater effectiveness of phosphate against animal caries than human caries may be related to the fact that rat

saliva has a much lower phosphorus concentration than human saliva. For this reason, dietary phosphates dissolve in rodent saliva sufficiently to raise its phosphorus content to an effective level, whereas further increases in the phosphorus concentration in human saliva may have no benefit.

Whether the post-developmental influence of phosphates on rodent caries operated entirely through the oral environment and the tooth surface or partly through a systemic pathway cannot be stated with certainty. However, its effect on caries does permit the authors to state that phosphate, which is so important during tooth and bone development, and plays many roles in soft tissues, continues to have an important influence on caries activity after tooth development.

FLUORIDE

The continued increase in fluoride concentration in the surface enamel after eruption, even in areas where food and water are low in fluoride, has been unequivocally demonstrated by Brudevold and co-workers (1960). Fluoride increase in the surface enamel was observed up to the sixth decade of life and approximately doubled between the second and sixth decades. In areas where the water supply contained 1.0 ppm of fluoride, much more fluoride was present in the surface enamel at the time of tooth eruption than in the surface enamel of teeth in very low fluoride areas. In areas where the water supply contained 3 to 5 ppm of fluoride, the levels of fluoride in the surface enamel at eruption were appreciably higher and the plateau of post-eruptive increase was reached much earlier in life. This plateau for surface enamel in a high fluoride area was not a great deal higher than the plateau for surface enamel in an optimal fluoride area.

This post-eruptive surface incorporation of fluoride may explain why teeth which were fully developed at the time fluoridation in Grand Rapids, and elsewhere, was initiated, developed significantly less caries than would have been the case without the addition of fluorine to the water (Hayes et al., 1957). This mechanism may be responsible, in part, for the decreases in caries-susceptibility in human teeth with increasing age. This suggestion is also consistent with the observations of Sognnaes and Shaw (1952) and Sognnaes, Shaw and Bogoroch (1955) that a much higher turnover of radioisotopes occurred in enamel immediately after tooth eruption, the so-called maturation period, than at later post-eruptive stages.

Whether this reaction of fluoride with the external surface of the enamel can be regarded as a truly nutritional effect is largely a matter of semantics.

VITAMIN D

A variety of studies was conducted in the 1920's and 1930's concerning the influence of optimal Vitamin D metabolism in children on caries activity in erupted teeth (Shaw, 1952). In general, these studies indicated that caries-susceptibility was reduced in children with adequate levels of Vitamin D intake and normal calcium metabolism. However, it was always clear that this was a modest reduction in spite of generally inadequate consideration of modifying factors other than Vitamin D.

HORMONES

Apparent changes in caries activity in the hormonally-active adolescent years, in pregnancy or in debilitated states, have been cited to support claims that systemic and nutritional factors are more important in caries than food fermentation on the teeth. Therefore, although these factors are only indirectly nutritional, they deserve mention here. In the absence of evidence of changes in the saliva, or differences in the structure of the second bicuspid, or second molars, whose formation is completed during the adolescent years, it seems more logical to attribute the high caries activity between the ninth and sixteenth years to more direct causes. One of these may be found in change from parental, controlled dietary to independence in food selection, social eating and greater frequency of eating. Probably of greater importance is the eruption during adolescence of sixteen new caries-susceptible teeth with some of the most caries-susceptible areas of the entire dentition. The incompletely proven changes in caries activity which may occur in association with pregnancy or illness could parallel changes in nutritional status. However, in the absence of evidence of related changes in saliva or tooth structure, any effects that these conditions might have in increasing the caries attack can just as logically be attributed to changes in the dietary habits in debilitated or pregnant patients in the direction of the more frequent use of bland carbohydrate foods or the possible neglect of normal oral hygiene.

NUTRITIONAL RELATIONSHIPS DURING TOOTH DEVELOPMENT

VITAMINS

In the 1910's and 1920's the classical studies of Mellanby (1929) on Vitamin D deficiency and on calcium deficiency and of Wolbach and Howe (1925, 1933) on Vitamin A and Vitamin C deficiencies clearly indicated that abnormal histological structure could be produced by critical deficiencies during tooth development. None of these studies was extended sufficiently in experimental animal populations or in man to show whether or not prolonged deficiencies during tooth development resulted in increased caries susceptibility.

FLUORIDE

In recent years attention has turned more to abnormalities at the molecular level of tooth composition rather than on gross histological abnormalities. Undoubtedly, the nutritional entity best known to influence both chemical composition and caries-susceptibility is fluoride. Whether fluorine is provided in the water or in foodstuffs, as in Tristan da Cunha or in India, is really immaterial with respect to its incorporation into the developing enamel and dentin. Many studies have demonstrated that the fluoride level of enamel, dentin and bone increases in proportion to the amount of fluoride ingested during their

development. With low levels of fluoride intake, the average content of the total enamel is in the neighbourhood of 60 to 90 ppm of fluoride, a level associated with high caries activity when teeth are exposed to cariogenic oral environment. At optimal levels of fluoride ingestion (1 ppm in the water) the fluoride content of enamel is increased to 100 to 120 ppm at which level good caries resistance is observed (McClure and Likins, 1951).

One of the most challenging facts about fluoride metabolism is the high fluoride level in the surface enamel compared to that in the deep levels of enamel. This gradient occurs at low, moderate or high levels of fluoride intake. Brudevold and co-workers have reported that an approximately tenfold gradient exists by the time the crown of the tooth is half formed. The gradient persists through the remainder of the formative period and tooth eruption, and throughout the rest of life. The fact that fluoride in the enamel is concentrated in the surface layer during crown development seems to indicate that fluoride does not pass freely from the blood vascular bed in the enamel organ through the surface enamel during calcification to the deeper and earlier formed areas of enamel. The same is true after tooth eruption, causing continued increase in fluoride in the surface enamel with little or no change in the deep levels of enamel. Clearly fluoride deposition during tooth development fulfills in every detail the criteria necessary for a nutritional effect mediated by a completely systemic mechanism.

CALCIUM AND PHOSPHORUS IMBALANCE

Paynter and Grainger (1956) demonstrated in rats that either fluoride supplementation or an imbalanced calcium-phosphorus ratio during gestation and lactation resulted in offspring with smaller molar teeth than the offspring of control females. In addition, the cuspal patterns and general anatomical characteristics of the molars in the group with the altered calcium-phosphorus ratio were significantly different from the controls. Later, Sobel et al. (1960) demonstrated that altered calcium-phosphorus ratios during early stages of molar development in cotton rats resulted in alteration in tooth composition. With a calcium-phosphorus ratio of 3:1, the carbonate concentrations of the enamel and dentin were significantly higher than with calcium-phosphorus ratios of 1:3. In a caries assay, the cotton rats with the high carbonate concentration in their dental structures had a significantly higher caries incidence than those with the low carbonate concentration. While these latter data are suggestive of a relationship between tooth composition and caries-susceptibility, the results from this experiment are equivocal because the altered calcium-phosphorus ratios were continued after tooth development was completed. Hence a post-developmental phosphate effect may have been involved as well as the developmental influence.

PROTEIN DEFICIENCY

The imposition of a borderline protein deficiency on pregnant and lactating rats resulted in dramatic alterations in certain dental characteristics among the offspring as well as in striking reductions in body weight at weaning (Shaw and

Griffiths. 1963). The molar teeth were significantly smaller among the offspring of the protein-deficient females. The eruption time of the third molars was significantly delayed. Certain characteristic cusps of the molars did not develop and in two rats the third molars did not develop. In addition, caries-susceptibility was significantly increased among the offspring as a result of the protein deficiency in the mothers. Whether the offspring actually suffered from protein deficiency per se or from some secondary nutritional problem has not been shown. However, these findings offer clear evidence of the dramatic ways in which nutritional abnormalities can alter the expected genetic pattern.

CARIES ATTACK ON ADJACENT TEETH

The observation that caries occasionally progresses unequally on opposite sides of the same interproximal space has been offered as a reason for believing that nutritional factors so influenced tooth formation that one tooth would break down, where as the other would not under an attack which was supposedly equal on both of the approximating tooth surfaces. Since adjacent teeth are formed and erupt into the mouth at spaced intervals, variable pre-eruptive systematic influences with fluoride or other nutrients could give rise to different caries susceptibilities in adjacent teeth. However, the more that is learnt about the nature and variability of the caries attack the more likely it seems that variables in the attack could explain the observed differences, and the less mandatory it becomes to attribute them solely to nutritional influences during tooth formation.

SUMMARY

As a broad summary of the information selected for presentation in this paper, and that available elsewhere, the authors believe that food, its adherence to tooth surfaces and its metabolism there by oral microorganisms are essential to the initiation and progression of carious lesions. Further, nutritional influences on tooth structure during its development greatly modify the extent of damage due to this attack; after the teeth have erupted into the mouth systemic nutritional factors appear to have little significance, except as they might modify the saliva and the tooth environment. They find themselves in broad agreement with Tomes and Tomes (1873), who wrote, 'caries is the effect of external causes in which so-called 'vital' forces play no part . . . and when once the disintegration is established in some congenitally defective point the accumulation of food and secretions in the oral cavity so intensify the mischief by furnishing new supplies of acid.'

RÉGIME ET CARIE DENTAIRE
RÉSUMÉ

D'après un large résumé des informations recueillies et présentées dans cet article et d'autres obtenues par ailleurs, les auteurs pensent que la nourriture, son adhérence aux surfaces dentaires et son métabolisme par micro-organismes sont essentiels pour l'apparition et le développement des lésions de carie.

De plus, l'influence des aliments sur les structures dentaires en cours de développement peut modifier considérablement la salive et le milieu dentaire.

Ils sont pleinement d'accord avec Tomes et Tomes (1873) qui écrivait: 'la carie est l'effet de causes externes dans lesquelles ce qu'on appelle les 'forces vitales' ne jouent aucun rôle . . . lorsque la désintégration s'installe en un point congénitalement défectueux, l'accumulation d'aliments et de sécrétions dans la cavité buccale intensifie le mal en fournissant de nouvelles quantités d'acide'.

ERNÄHRUNG UND ZAHNKARIES
ZUSAMMENFASSUNG

Auf Grund der Informationen, die für die Aufnahme in diesem Artikel ausgewählt wurden oder an anderer Stelle vorliegen, glauben die Autoren, daß die Nahrung, ihre Haftfähigkeit an der Zahnfläche und der Stoffwechsel, der sich dort durch die Mikroorganismen des Mundes vollzieht, für das Auftreten und Fortschreiten der kariösen Läsionen wesentlich sind. Ferner werden Speichel und Mundmilieu durch nahrungsbedingte Einflüsse bei der Entwicklung der Zahnstruktur erheblich modifiziert. Die Autoren stimmen weitgehend mit Tomes und Tomes (1873) überein, der schreibt: 'Karies ist die Auswirkung äußerer Ursachen, bei denen die sogenannten 'Vitalkräfte' keine Rolle spielen . . . und wenn einmal die Auflösung an einigen kongenital schwachen Stellen eingesetzt hat, wird der Schaden durch die Ansammlung von Nahrung und Sekreten in der Mundhöhle ständig vergrößert, weil hierdurch die Säure immer wieder ergänzt wird.'

DIETA E CARIE DENTARIA
RIASSUNTO

Come largo riassunto delle conoscenze scelte per la presentazione in questo lavoro e di quelle reperibili altrove, gli AA. ritengono che il cibo, la sua aderenza alla superficie dentaria ed il suo metabolismo per opera dei microrganismi orali, siano fattori essenziali per l'inizio e lo sviluppo delle lesioni cariose. Inoltre, le influenze dei fattori alimentari sui tessuti dentari nel corso del loro sviluppo sono in grado di modificare profondamente la saliva e l'ambiente dentario. Pertanto gli AA. si trovano largamente d'accordo con Tomes e Tomes (1873) che scrisse: 'La carie è l'effetto di cause esterne, nelle quali le cosiddette 'forze vitali' non hanno alcun ruolo . . . ed una volta che la disintegrazione si è iniziata in qualche punto difettoso congenitamente, l'accumulo di alimenti e le secrezioni della cavità orale intensificano il processo fornendo nuove quantità di acidi.'

DIETA Y CARIES DENTAL
RESUMEN

Al igual que en el amplio resumen de reportes, seleccionados para la preparación de este trabajo, disponibles en otro lugar, los autores creen que los alimentos en adherencia a la superficie de los dientes y su metabolismo en esta parte, por los microbios orales, son esenciales para el comienzo y progresión de las lesiones de la caries. Además las influencias nutritivas sobre la estructura de los dientes durante su desarrollo, la saliva y el medio ambiente de los dientes. Los autores están en completo acuerdo con Tomes y Tomes (1873) el cual escribió:

'La caries es el resultado de causas externas en las cuales las llamadas fuerzas vitales no participan . . . y cuando la desintegración se ha producido, en algun defecto congénito, el acúmulo de alimentos y secreciones de la cavidad oral intensifican el daño con nuevas aportaciones de ácido.'

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STUDIES ON THE PHYSICOCHEMICAL PHENOMENA RELATED TO DENTAL CARIES

III. The Effect of Sucrose Upon the Solubility of Human Dental Enamel

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SOLUBILITY studies on human dental enamel have engaged the attention of many investigators.[1-75] While the methods used have varied widely the purpose of most of the studies was to learn about the caries process or how to prevent it. Many investigations have dealt with the dissolving effect of various solutions; most of the solutions used were acidic in nature. The belief that carbohydrates and, more specifically, products of carbohydrate degradation were involved in caries formation gradually gained prominence over a span of years. This idea stimulated further research involving the effect of carbohydrate fermentation upon enamel. In addition, many investigators have sought to treat enamel to render it less soluble in acidic solutions. The results of these efforts by many workers have been to establish that carbohydrates serve as the main source for acid formation by fermentation and that this acid is directly involved in the decalcification process. It also has been shown that many substances (the majority of which are fluoride compounds) when applied to the enamel surface diminish the acid solubility of that surface. In none of the many studies, however, has the possible effect of sucrose per se on the enamel solubility been emphasized.

The purpose of the present paper is to report a study which was designed to test the effect of sucrose on tooth enamel solubility in acid solutions.

Sugars are accepted by most investigators as the principal substrate for acid formation in the mouth and thus are seen to play a direct role in dental caries. It is possible that sugars may affect the dental caries process in other ways in addition to that of acting as a source of acid. Such a possibility has prompted or influenced several investigations. For example, the effect of sucrose and other sugars upon tooth permeability has been studied.[76-78] The effect of sucrose upon ion diffusion through semipermeable membranes also has been investigated.[79-81] The latter studies showed that sucrose changes the diffusion rates of hydrogen ions, calcium ions, and phosphate ions. This may possibly indicate that sucrose influences factors affecting the development of dental caries and therefore it is of interest to determine further influences of a physicochemical nature exerted by sucrose on the process of caries development. Preliminary studies in this laboratory[82] showed a definite

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influence of sucrose upon enamel solubility. The present work indicates that sucrose has a retarding effect on the rate of enamel dissolution by lactate buffer solutions.

EXPERIMENTAL METHODS AND MATERIALS

The effect of sucrose upon the solubility of powdered human dental enamel was tested by shaking samples of the powdered enamel in each of the several solutions for varied time intervals following which the solutions were tested for calcium (and, in some cases, for phosphate content). The amounts of calcium appearing in solution were taken as an index of the rate of enamel dissolution in the several solutions. In addition, phosphate determinations were made on the same solutions at 3 of the pH levels.

The dental enamel used in the present work was the homogeneous mixture of a pooled sample of ground enamel with a particle size ranging from 270 to 150 mesh. The enamel was obtained from noncarious, clean, adult human molars which were dried for 24 hours at 100° C. The teeth were cracked in a mortar and pestle. Those pieces of crown enamel apparently free of dentin were separated by inspection, ground in a mortar and pestle and sieved. The particles smaller than 150 mesh (the holes in 150 mesh screen are approximately 0.0041 inch diameter) and larger than 270 mesh (approximately 0.0021 inch diameter) were collected and washed with a 50-50 (volume) mixture of ethyl alcohol-ethyl ether and dried. The material was subjected to the Brekhus and Armstrong[53] technique for separation of dentin and enamel in order to remove any dentin particles. All samples for the individual solubility tests were taken from one pooled sample of powdered enamel treated in the manner just described (with the exception of the samples tested in the pH 5.5 solutions which were run with a separate sample of enamel prepared in identical manner).

The solutions used in the study were lactate buffer, lactate buffer with 0.5 M sucrose, and lactate buffer with 1.0 M sucrose. In all cases the total lactate present was 1 mole per liter. Sufficient NaOH was added to the 1 M lactic acid in the preparation of each solution to obtain the desired pH. All solutions were preserved by addition of a small amount of mercuric iodide and by storage in the refrigerator until used. The above 3 types of solutions were prepared at pH values of 4.0, 4.5, 5.0, 5.5, and 6.0.

The solubility tests were run by shaking individual 50 mg. samples of the powdered dental enamel with 20 ml. of the desired solution in screw-cap vials at 37° C. The vials were shaken in a thermostated water bath with constant agitation for varied time intervals of 5, 10, 15, 30, 60, 120, and 240 minutes. Separate vials of solution were used for each time interval and in most cases duplicate vials were used at each time interval. Duplicate analyses were made on the contents of each vial. All vials in a given run were started at the same time and at the appointed intervals vials were removed from the incubating bath and the contents immediately filtered with suction to separate the remaining undissolved enamel from the solution in order to stop the process of dissolution. Filtration was accomplished by suction with Buchner filter funnels.

Samples of the filtrate were taken for calcium and phosphorus analyses. The calcium determinations were made on all samples by the Clark-Collip[54] modification of the Kramer-Tisdall method which involves the precipitation of calcium as the oxalate followed by the titration of the oxalate with standardized potassium permanganate solution. The phosphorus determinations, which were made on the pH 5.0, 5.5, and 6.0 buffer solutions, were carried out with a modification of the colorimetric method of Fiske and Subbarow.[53]

In a separate experiment, soluble calcium ions in the presence of 0.5 M and 1.0 M sucrose were determined with the above-mentioned method in order to ascertain that the observed differences in calcium content in the enamel solutions were real and not artifacts. That is, it was necessary to rule out the possibility that sucrose was affecting the rate or degree of precipitation of calcium oxalate. For this purpose calcium chloride solutions were prepared with calcium concentrations of 10.0 and 50.0 mg. per 100 ml. of solution. Such solutions were made with no sucrose as well as with 0.5 M and 1.0 M sucrose. Upon addition of ammonium oxalate to the various solutions it was apparent that the rate of precipitation of calcium oxalate was slightly slower in the presence of 0.5 M sucrose and slower in the presence of 1.0 M sucrose. However, the precipitation was complete in all cases in less than 1 hour. Samples standing for 1 hour and those standing overnight showed the same results. The viscosity and density of the 1.0 M sucrose solutions were noticeably greater than in the other solutions so that mixing of the ammonium oxalate solution and the sucrose solution was slower. This could have resulted in low calcium results if the 2 solutions were not mixed and if they were not allowed to stand for precipitation for at least 1 hour. All of the solutions in the experimental runs with dental enamel were mixed with the ammonium oxalate solution when the latter was added and all of the solutions were allowed to precipitate well over 1 hour (many of them stood overnight before the supernatant fluid was removed following centrifugation). Thus it can be stated definitely that the differences in calcium content of the solutions following incubation with enamel were real differences and not due to incomplete precipitation of calcium oxalate in the presence of sucrose. This is further supported by the fact that the amount of phosphate ion going into solution paralleled the calcium.

Finally, the effect of sucrose alone upon enamel solubility was tested. Human dental enamel samples prepared as described above but of smaller particle size (270 mesh and finer) were shaken for 24 hours with distilled water and with 1 M sucrose. One gram samples of the enamel were shaken at room temperature (27 to 29° C.) with 100 ml. of distilled water and with 100 ml. of 1 M sucrose. Both solutions were adjusted to pH 7.0 (by addition of minute amounts of NaOH) before the enamel was added. After 24 hours the pH of both solutions remained essentially unchanged (water, pH 6.92; sucrose, 6.95). Both solutions were centrifuged (20,000 rpm, 20 min.) to remove

suspended particles of enamel and aliquots of solution taken for calcium determination as before. Little enamel was dissolved by either solution; however, the amount dissolved by the sugar solution was slightly higher.

RESULTS

The numerical results are presented in the tables. Fig. 1 shows graphically the effect of time on the solubility of human dental enamel in the various buffer solutions (the content of calcium ion in solution at the given time being taken as the index of solubility). In the figure the sets of 3 times for each pH level are joined by hatch lines. (The 4-hour figures given in Table I were not plotted in the graph in order to show the first 2-hour results in more detail.)

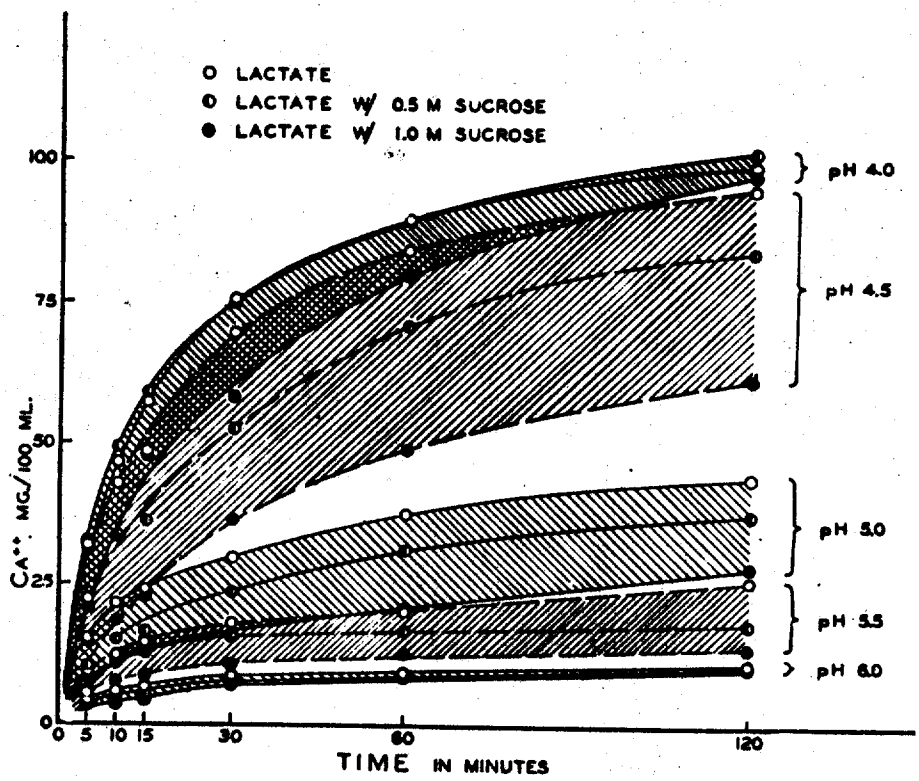

Fig. 1.—Rate of dissolution of powdered dental enamel in lactate buffer solutions as measured by Ca++ content of the solutions. The three solutions at each pH level are connected with cross-hatched lines.

The curves all show a deceleration of the dissolution rate with time as noted in other studies.[30, 66] This is due to the retarding effect of the increasing amounts of calcium and phosphate ions in solution with time as well as to the rapid dissolution of the smaller particles with the resulting decrease in total enamel surface. The effect of the sugar on the rate of enamel dissolution is less marked in the pH 4 and pH 6 solutions than in the rest. In all of the

pH 4 solutions the rate of dissolution is quite high while at pH 6 the rate is low. In the pH 4 solutions most of the enamel was dissolved by the end of 1 hour. In almost all cases it can be seen that the presence of sugar in the buffer solution reduced the amount of calcium (and phosphate) going into solution. From the standpoint of comparison of the present results with possible in vivo conditions of enamel decalcification the most important solutions are those of pH 4.5, 5.0, and 5.5 since a pH of 4.0 on the tooth surface would not be expected to occur very frequently and at a pH of 6.0 the rate of decalcification is expected to be almost negligible. The magnitude of the sugar effect on the dissolution rate can be assessed by reference to Table I. For example, at pH 4.5, the amount of calcium in solution after 5 minutes in the lactate buffer (32.0 mg./100 ml.) is approximately equal to that found after 10 minutes in the lactate buffer containing 0.5 M sucrose (33.4) and after 30 minutes the amount dissolved in the presence of 1.0 M sucrose is only slightly more (36.4). Likewise the amounts dissolved in lactate buffer, 0.5 sucrose +

TABLE I
ENAMEL DISSOLVING EFFECT OF BUFFER SOLUTIONS WITH AND WITHOUT SUCROSE AS MEASURED BY DISSOLVED CALCIUM (MG. CA/100 ML. SOLUTION)

pH	SOLUTION	TIME IN MINUTES						
		5	10	15	30	60	120	240
4.0	Lactate	31.3	46.3	57.2	75.6	89.8	99.6	111
	0.5 M Sucrose in Lactate	32.6	49.5	58.9	74.9	89.9	102	111
	1.0 M Sucrose in Lactate	20.5	33.2	47.8	58.2	79.9	98.0	115
4.5	Lactate	32.0	42.8	48.6	69.7	84.5	95.4	108
	0.5 M Sucrose in Lactate	22.3	33.4	36.2	52.7	71.2	84.4	94.6
	1.0 M Sucrose in Lactate	15.0	18.3	22.7	36.4	49.1	61.6	80.5
5.0	Lactate	15.2	21.4	23.8	29.7	37.9	44.4	50.8
	0.5 M Sucrose in Lactate	10.0	15.0	16.6	23.5	31.2	38.0	42.5
	1.0 M Sucrose in Lactate	8.2	10.7	12.6	16.0	20.9	28.5	33.8
5.5	Lactate	9.6	12.2	14.7	18.0	20.2	26.0	31.2
	0.5 M Sucrose in Lactate	9.8	12.1	14.4	15.4	16.8	18.0	18.8
	1.0 M Sucrose in Lactate	5.3	7.5	8.6	10.6	12.8	13.9	16.9
6.0	Lactate	5.6	5.8	6.6	8.9	9.4	11.4	12.3
	0.5 M Sucrose in Lactate	4.6	5.0	5.2	7.1	8.6	10.6	11.6
	1.0 M Sucrose in Lactate	3.4	3.9	4.1	7.4	8.4	10.9	12.4

TABLE II
ENAMEL DISSOLVING EFFECT OF BUFFER SOLUTIONS WITH AND WITHOUT SUCROSE AS MEASURED BY DISSOLVED PHOSPHORUS (MG. P/100 ML. SOLUTION)

pH	SOLUTION	TIME IN MINUTES						
		5	10	15	30	60	120	240
5.0	Lactate	6.0	9.0	9.7	12.8	16.1	19.0	21.4
	0.5 M Sucrose in Lactate	3.3	5.3	6.6	9.8	12.6	15.3	17.6
	1.0 M Sucrose in Lactate	2.3	2.9	3.1	4.7	7.6	10.2	12.3
5.5	Lactate	3.9	5.1	6.0	7.2	9.2	10.4	11.7
	0.5 M Sucrose in Lactate	3.6	4.7	5.2	5.9	6.7	7.2	7.5
	1.0 M Sucrose in Lactate	2.1	2.6	4.1	4.8	5.7	6.4	7.2
6.0	Lactate	1.5	1.8	2.2	2.4	3.3	3.7	4.1
	0.5 M Sucrose in Lactate	1.6	2.0	2.0	2.8	2.9	3.7	4.4
	1.0 M Sucrose in Lactate	1.2	1.5	2.0	2.4	3.0	4.0	4.6

lactate buffer, and 1.0 M sucrose + lactate buffer are about equal at 15 minutes (48.6), 30 minutes (52.7) and 60 minutes (49.1), respectively. These examples are sufficient to show the effect of the presence of sucrose on the dissolving action of the buffer solutions. The results on phosphate determinations parallel reasonably well those of the calcium; however, there was little difference found between the 3 solutions at pH 6.0.

The calcium/phosphorus weight ratios were found to vary considerably but in no particular pattern. The extreme values were 2.0 and 4.1, the mean value was 2.70 with a standard deviation of 0.4. These values are above the Ca/P weight ratio consistently found in this laboratory for pooled human dental enamel which approximate 2.0 (e.g., Suess,[64] 2.01; and Young,[32] 1.96). The generally accepted literature values approximate 2.0 also as shown by the work of Armstrong and Brekhus[66] who found the ratio on carefully purified enamel to be 2.03.

TABLE III
CA/P WEIGHT RATIO

pH	SOLUTION	TIME IN MINUTES						
		5	10	15	30	60	120	240
5.0	Lactate	2.5	2.4	2.5	2.3	2.4	2.3	2.4
	0.5 M Sucrose in Lactate	3.0	2.8	2.4	2.4	2.5	2.5	2.4
	1.0 M Sucrose in Lactate	3.6	3.7	4.1	3.4	2.8	2.8	2.7
5.5	Lactate	2.5	2.4	2.4	2.5	2.2	2.5	2.7
	0.5 M Sucrose in Lactate	2.7	2.6	2.8	2.6	2.4	2.5	2.5
	1.0 M Sucrose in Lactate	2.5	2.9	2.1	2.2	2.3	2.2	2.2
6.0	Lactate	3.7	3.6	3.0	3.7	2.8	3.1	3.0
	0.5 M Sucrose in Lactate	2.9	2.5	2.6	2.5	3.0	2.9	2.6
	1.0 M Sucrose in Lactate	2.8	2.6	2.0	3.1	2.8	2.7	2.7

Sucrose alone, in neutral aqueous solution, tends to increase enamel solubility as shown by the results obtained when enamel was shaken with water and with 1 M sucrose. The amounts of calcium in solution at the end of 24-hour exposure of the finely powdered enamel (<270 mesh) to the solutions were 1.67 ± 0.20 mg. Ca^{++}/100 ml. of 1 M sucrose and 1.19 ± 0.15 mg. Ca^{++}/100 ml. of distilled water. The figures ±0.20 and ±0.15 represent standard deviations from the mean of 4 determinations each and reflect the variation encountered with low amounts of calcium. The probability of the 2 average figures, 1.67 and 1.19, occurring by chance alone is roughly 1 in 20.

DISCUSSION

Several possible explanations exist for the observed effect of sucrose in lowering the dissolution rate of enamel particles in buffered acid solutions. These can be classified as: (1) viscosity effects, (2) coating effect, (3) chelation or complexing effect, and (4) hydration effects.

The viscosities of the buffer solutions containing 0.5 M sucrose and 1.0 M sucrose are considerably greater than the viscosities of the solutions with no sugar. The increased viscosity would be expected to diminish the rates of diffusion of the various ions present. As shown by Strålfors,[65] increased vis-

cosity in the surrounding solution results in a lower rate of dissolution of enamel due to the increase in amount of saturated solution surrounding the enamel surface (i.e., saturated with respect to calcium and phosphate ions). Both the rate of diffusion of calcium and phosphate ions away from the enamel surface and the diffusion rate of hydrated protons toward the surface are diminished in the more viscous sugar solutions. This effect should operate in an identical fashion with intact or with powdered enamel of equal total surface area. It is of interest in this connection to recall that Forscher[79-81] found that the rate of ion diffusion through a semipermeable membrane was diminished in the presence of sucrose. In another respect, however, the results obtained with powdered enamel are not directly comparable with in vivo conditions. The dissolution rate of the enamel particles depends, as discussed above, upon the rate of diffusion of ions to and from the particle surface; this rate will be affected by relative movement of the enamel particles and the surrounding solution. The increased viscosity in the presence of sucrose could materially reduce this rate of mixing. The reduction in solubility due to this effect, the magnitude of which is unknown, would not be expected to operate in vivo.

Another mechanical or physical action by which sucrose would be expected to reduce the rate of enamel dissolution is the coating or layering of the enamel particles by the sugar, or hydrated sugar, molecules. The magnitude of this effect is difficult to assess but it might be expected to be reasonably small since the sugar molecule in acid solutions would undergo only an infinitesimal amount of ionization and then would have practically no ionic charge. The sugar molecules through their many –OH groups could be attracted by van der Waal's forces to the ions in the enamel surface either directly or through water molecules. That such a phenomenon does exist seems probable in light of the effect shown by sucrose (vide supra) in slowing the rate of precipitation of calcium oxalate. In vivo the coating effect of sucrose might be expected to be less due to the presence on the tooth surface of many molecular and ionic species which would leave less room to be occupied by sucrose molecules.

Sucrose forms compounds with calcium ions in basic solution.[87] The exact nature of the compounds is unknown; they appear to vary with the relative amounts of calcium ions and sucrose present.[87] These compounds, which are soluble, must be complexes and perhaps they are chelates. Calcium oxide and calcium hydroxide are dissolved by sucrose solutions; use has been made of this in analytical procedures[88-90] in which these materials are extracted away from calcium carbonate (which is not soluble in the sucrose solution) or from magnesium compounds. The extent of compound formation between calcium ions and sucrose may depend upon the ionization of sucrose as an acid; if this be true it appears likely that such compound formation would be extremely limited in acid solution due to the suppression of sucrose ionization. It was noted previously that at neutrality a sucrose solution, of 1 M concentration, dissolved powdered enamel slightly more than did dis-

tilled water. This is due, most likely, to the slight tendency of the sucrose to form calcium complexes at pH 7. Calcium ions chelate with a number of organic compounds[91] including lactic acid[92-94]; sucrose may exert an effect on the calcium lactate chelate by combination as suggested for the interaction of cupric chelates with sugars.[95] Such a combination, depending upon its nature, could decrease or increase the rate of enamel dissolution.

Sucrose molecules should, by binding water molecules through hydrogen-bonding, reduce the number of free water molecules and increase thereby the effective concentration of hydrogen ion in a given solution.[96] This effect is not significant in the present experiment since the buffer solutions were adjusted to the proper pH after sucrose was added. The effect is of significance in vivo but its magnitude is unknown.

The significance of the relatively high Ca/P ratio in the buffer solutions is unknown. Swartz and Phillips[67] found variable Ca/P ratios in solutions resulting from the acid dissolution of enamel from restricted areas on intact tooth surfaces. On the same area from 20 teeth immersed in acetic acid the decalcification produced solutions with Ca/P ratios ranging from 1.99 to 2.82 with an average of 2.38. Kanthak[30] found the Ca/P ratios in solution to parallel closely the ratio found in the enamel.

From the discussion of the present work it would appear that sucrose exerts a retarding effect upon the rate of enamel dissolution in lactic acid solutions which may indicate a possible influence on the rate of caries formation. Experiments on whole enamel are indicated as the next logical step in approaching more nearly in vivo conditions.

SUMMARY

The presence of sucrose in lactate buffer solutions was shown to decrease the solubility of powdered human dental enamel. It is suggested that the picture of caries development may be influenced by the presence of sucrose in plaque material acting in the dual role of (1) the main substrate for degradation to acid to cause decalcification, and (2) a retarding influence on the rate of enamel dissolution.

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Sucrose Retardation of Acid Etching in Dental Enamel: An Electron-Microscopic Study

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In a previous communication from this laboratory,[1] data were presented to show that the solubility of powdered human dental enamel in acid solutions was reduced in the presence of sucrose. The present communication deals with the logical extension of that work from powdered enamel to whole enamel surfaces. It will be shown that sucrose diminishes the rate of etching of tooth surfaces by lactic acid solutions. This effect, demonstrated in the present work by means of the electron microscope, is in agreement with the chemical studies reported earlier.[1]

It is well to emphasize that the following results are not presented in an effort to minimize the deleterious effects of sugar as an agent in caries development. Instead, the results are presented for the information they provide. They serve to demonstrate once again the amazing complexity of factors involved in the process of caries formation.

EXPERIMENTAL MATERIALS AND METHODS

The human teeth used for the present study were central incisors and first molars obtained from the Great Lakes Dental Research Facility of the U.S. Navy. The labial surface of the incisors and the buccal surface of the molars were used for the experimental surfaces.

The plane areas required for electron microscopy were prepared on the tooth surfaces by polishing and buffing according to a standardized procedure used in this laboratory.[2] Because it is a common procedure, it will be described only briefly. The tooth was imbedded in a cold-setting acrylic resin and polished with successively finer grades of levigated alumina followed by buffing with Sturge's heavy calcium carbonate. The polishing and buffing were carried out on a Buehler style R-1138 polishing wheel. The calcium carbonate buffing produces a reasonably standard type of plane surface in which the ends of the enamel rods are visible. Following the buffing operation the plane enamel surface was rinsed well with distilled water, wiped with lens paper, and soaked briefly in 95 per cent ethyl alcohol. The surface now was ready for examination by replication with polyvinyl alcohol (PVA) film. This film was used for the initial replicas, which were examined by means of the light microscope to establish the suitability of the surface. The PVA film also served to strip the surface of any lightly bound solid debris. In some cases the surfaces required additional buffing or were found to be unsuitable and discarded. The suitable surfaces were ready for the etching studies. In certain cases the tooth surface, after the initial use, was ground down, a new surface prepared, and the etching experiment repeated.

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The solutions used for the main phases of this study, all at pH 4.5, were as follows: (1) 0.1 M lactic acid buffer; (2) 0.1 M lactic acid buffer–1 M sucrose solution; (3) 0.1 M lactic acid buffer–0.5 M sucrose solution; and (4) 0.1 M lactic acid buffer–0.1 M sucrose solution. Each of the above solutions were 0.1 molar with respect to lactate. To prepare them, the required amount of freshly boiled 85–90 per cent lactic acid solution was added to the volumetric flask along with some water; the required amount of sugar was added, followed by more water to bring the solution nearly to the desired volume. Next, sufficient NaOH was added to bring the pH up to 4.5, after which the final small amount of water was added to fill the volumetric flask to the proper level.

After the above solutions were tested for their action on the enamel surfaces and the results were considered, other solutions were tested to determine the degree of specificity of the sucrose action. These solutions included (1) 0.1 M lactic acid buffer–1 M sodium chloride solution, pH 4.5; (2) 0.1 M lactic acid buffer–1 M glucose solution, pH 4.5; (3) 1 M sucrose solution, pH 7; (4) 1 M sucrose, pH adjusted to 11 with sodium hydroxide.

Over a period of several years, in pursuit of other problems, numerous combinations of pH, nature of the acid etching solution, and time intervals have been tried in this laboratory for their effectiveness in etching enamel surfaces. A considerable degree of variation in rate of etching has been found to exist among teeth. That is, several teeth subjected to the same etching conditions will show differences in their degree of etching. However, a generally useful procedure which produces reasonably marked etching of prepared enamel surfaces consists of the application of 0.1 M lactate buffer, pH 4.5 for 15 minutes. All the etching studies reported here, therefore, were done with solutions left on the prepared enamel surfaces for 15 minutes. Because of the above-mentioned natural variations in etching rates of teeth, it was considered desirable to use each tooth surface as its own control for comparison of treatments. This was done with variations in other work as well as in the present problem. In the present case the technique was to keep half of the prepared planar tooth surface covered with pressure-sensitive electrician's tape, while the other half was subjected to the 15-minute treatment with the desired solution. After water rinsing and careful blotting, the treated half was covered with tape, and the other half uncovered and treated with a second solution. In this manner, pairs of solutions were compared on individual tooth surfaces for their effect. In all cases in the present report the first half of the surface to be treated was subjected to the pure buffer solution while the adjoining half of the surface was covered with tape. After that initial treatment, the tape was removed from the untreated half-surface, while tape was applied to the previously treated surface. Next, the second half-surface was treated with the buffer-sugar or buffer-salt solution. In this manner both surfaces were covered with electrician's tape during one part of the process. However, the pure buffer-treated surface was covered after buffer treatment, whereas the buffer-sugar–treated surface was covered with tape prior to treatment. A commonly used technique to remove loose surface debris from an etched surface prior to replication is the application of a PVA film or cellulose tape to the surface. By stripping the surface, the operator can obtain in subsequent replicas a reproduction of the remaining tightly bound surface structure. In an unetched standard tooth surface prepared as described above and used in the present study, such stripping technique produces no noticeable difference in the surface, as shown by replica-

tion before and after such stripping. On the other hand, such a standard surface after a 15-minute treatment with 0.1 M lactate buffer, pH 4.5, is deeply etched. Such a surface appears quite rough upon replication either with or without stripping.

By the order in which the surfaces were treated in the present study it is apparent that the pure buffer-treated surface was stripped after treatment while the buffer-sugar–treated surface was not. Furthermore, it was conceivable that the prepared tooth surface would react differently to a solution after having been covered previously with the tape. For example, a water-repellent film might be left adhering to the surface and tend to protect the surface from etching by the solution. Therefore, any differences seen between degree of etching of the two surfaces might result from the biased treatment. Preliminary tests showed, however, that such was not the case. The effect was independent of the order. Accordingly, the standard procedure as described was adopted. In the initial experiments the 0.1 M lactate buffer, pH 4.5, was compared with the pH 4.5 lactate-sucrose solutions. In subsequent experiments the other solutions were compared with the pH 4.5 0.1 M lactate-buffer solution and with each other.

Finally, the treated surfaces were examined by electron microscopy of surface replicas. Polyvinyl alcohol (PVA) "negative" replicas, made by direct application to the tooth surface, were treated with Formvar to form the "positive" replica, after which the PVA "negative" was dissolved off the positive by water flotation. The resulting free Formvar replica was shadowed with chromium and examined in the RCA electron microscope. The degree of etching of the surface was determined simply by inspection and comparison of the two portions of each tooth surface. No attempt was made to quantitate the amount of etching. The results obtained are given below.

RESULTS

Approximately twenty tooth surfaces were studied, some of which were used two or three times (by rebuffing as described above). It was found invariably that the degree of etching caused by the pH 4.5 lactate buffer was reduced in the presence of sucrose. The portion of the enamel surface subjected to the lactate-buffer solution always showed a greater degree of etching than the adjoining portion treated with the sucrose-lactate-buffer solution. As mentioned earlier, some variation is generally found among individual teeth in the degree of etching produced by a given acid treatment. As anticipated, therefore, in the present experiments some tooth surfaces were more susceptible to etching than others. Those teeth showing somewhat less etching accordingly showed less difference between the lactate surface and the lactate-sucrose surface. That is, for the full effectiveness of the sucrose retarding action to be demonstrated, it was necessary for the tooth to be etched appreciably in the 15-minute period. In most cases the lactate-sucrose–treated surfaces showed minimal amounts of etching, while the corresponding lactate-treated surfaces showed quite appreciable etching. Although no actual quantitative means of assessing the etching was used, the 0.5 M sucrose appeared to be as effective as 1.0 M sucrose in depressing the etching, while the 0.1 M sucrose appeared to be slightly less effective.

An illustration of the typical difference between lactate-buffer and lactate-buffer sucrose solution is provided in Figure 1. In this illustration the boundary region where the two separately treated areas of the tooth surface meet is shown. Electron-micro-

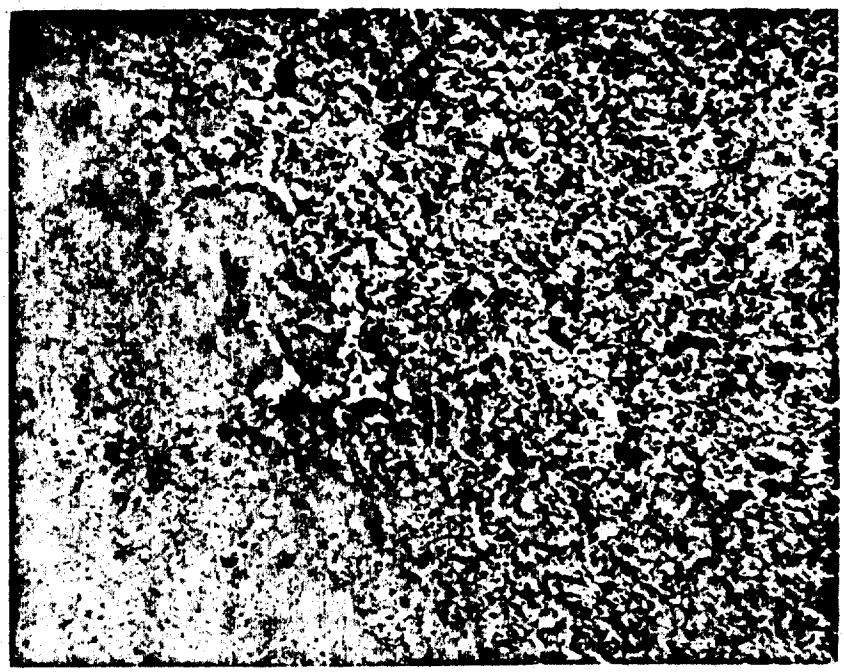

FIG. 1.—Tooth 4. Standard surface, produced as described in text. Boundary region. Surface in lower-left-hand corner treated for 15 minutes with 0.1 M lactate buffer–1 M sucrose, pH 4.5. Surface on other side treated for 15 minutes with 0.1 M lactate buffer, pH 4.5. (Approx. mag. ×1330.)

FIG. 2.—Tooth 4. Standard surface, produced as described in text. Another view of the same tooth surface shown in Figures 1 and 3. In this picture the entire area in view was treated for 15 minutes with 0.1 M lactate buffer, pH 4.5. (Approx. mag. ×3380.)

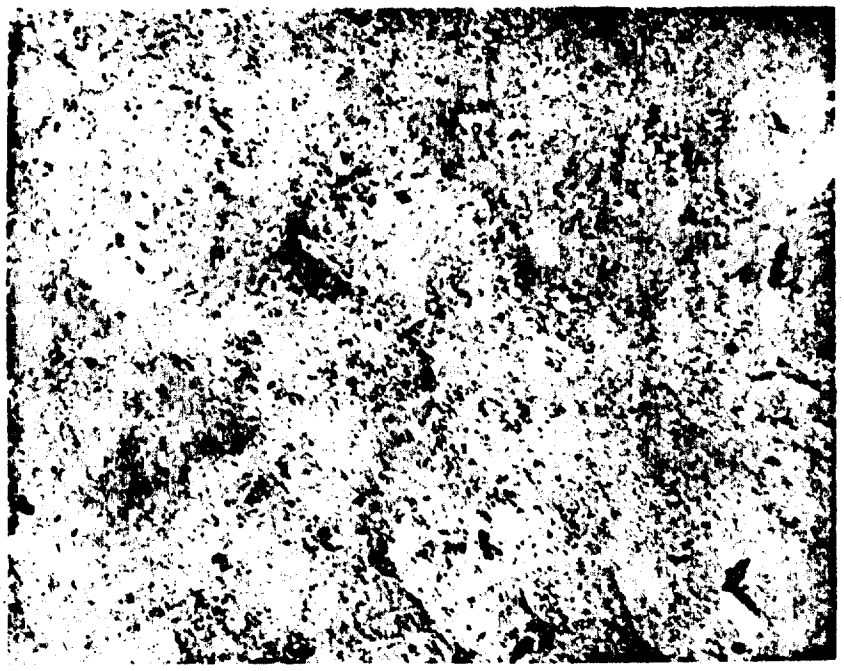

FIG. 3.—Tooth 4. Standard surface, produced as described in text. Another view of the same tooth surface shown in Figures 1 and 2. Here the entire area in view was treated for 15 minutes with 0.1 M lactate buffer-1 M sucrose, pH 4.5. (Approx. mag. ×2330.)

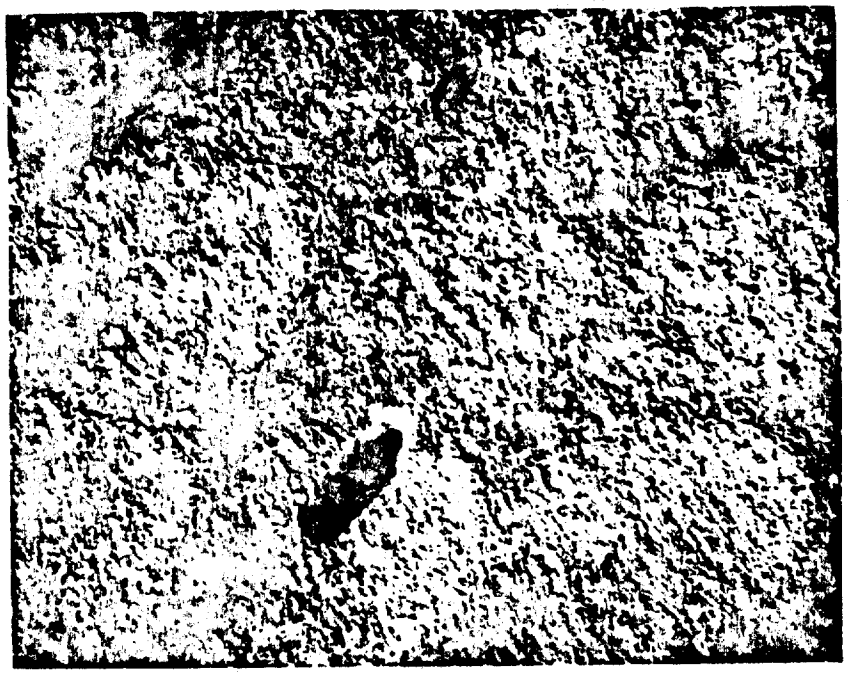

FIG. 4.—Tooth 11. Standard surface, produced as described in text, treated with 1 M sucrose solution adjusted to pH 11 with sodium hydroxide. (Approx. mag. ×3380.)

scope examination of this particular replica, for example, showed the smooth regular appearance typical of an unetched surface on one side of the boundary and the rough irregular appearance typical of etching on the other side. Figures 2 and 3 provide additional views of the same replica. In Figure 2 the entire area in view was in the region treated with the lactate buffer, whereas the area shown in Figure 3 had been treated with the lactate-buffer-sucrose solution. These figures show that, although both solutions had a pH 4.5, their actions were not equal. The same result was obtained in other teeth; nothing would be gained by additional illustrations of the results obtained in other teeth by the same treatment.

When the etching actions of 0.1 M lactate buffer, pH 4.5, and pH 4.5 lactate buffer containing 1 M sodium chloride were compared, no difference was found. Both portions of the enamel surface exhibited the etched appearance of the type shown in Figure 2. Sodium chloride, therefore, exerts no retarding effect on the etching of enamel by lactate buffer. The use of glucose in place of sucrose results in some retardation of acid etching. Thus when 0.1 M lactate buffer-1 M glucose solution, pH 4.5, is compared with the regular 0.1 M lactate buffer, pH 4.5, the glucose-containing solution causes less etching. The difference appears to be much less than that shown by sucrose.

Neutral sucrose solutions caused no noticeable change in the enamel surface in the relatively short period of 15 minutes; however, at pH 11 sucrose did attack the enamel surface. That is, 1 M sucrose solution adjusted to pH 7 with a very small amount of sodium hydroxide had no noticeable effect on the tooth surface, whereas when the sucrose solution was raised to pH 11 with sodium hydroxide the resulting solution produced a slight but detectable change in the enamel surface in 15 minutes. The surface was roughened slightly, as shown in Figure 4. Additional work is required to investigate the nature of this action of sucrose on enamel in basic solutions.

DISCUSSION

When it was found previously that sucrose lowered the dissolution rate of powdered enamel particles in lactate-buffer solution,[1] several possible explanations were offered. They included viscosity effects, coating effects, chelation or complexing effects, and adsorption effects. Although the present work provides no actual direct proof that any of these effects is operating, the fact that the present results do agree with those from the initial study increases the possibility that such effects do operate. For example, in the original discussion[1] it was suggested that at least part of the observed sucrose reduction of enamel solubility in acid might be due to the nature of the test. That is, small enamel particles agitated in a container might possibly act differently from intact enamel surfaces treated with the same solutions. As it developed, the effect is also observed in the intact enamel surface. The discussion offered in the previous article,[1] therefore, applies to the present work.

Although more work with other solutes substituted for sucrose will be required to delineate it, the etch-depressant action of sucrose appears to have some specificity. Certainly, the action is not unique, as shown by the slight effect found for glucose. That more is required, however, than the simple presence of a 1 M concentration of solute is indicated by the inactivity of sodium chloride. Purely from the practical standpoint of most direct concern in dental caries, of course, it would not matter if

other substances were found to be equally as effective as sucrose. The important point is that sucrose is frequently present in the plaque on the tooth surfaces in reasonably high concentrations and thus is available to exert its effect. Furthermore, because it is an important substrate for acid formation, sucrose is in the plaque at the time when acid concentration becomes appreciable. From a theoretical standpoint it is important to determine whether other substances may also exert actions similar to that of sucrose, even though those substances are in fact never found on the tooth surface in vivo. More work along this avenue may provide information sufficient to establish the mode of action of sucrose.

Additional work is also required to determine the nature of the action of sucrose on enamel in basic solutions. It appears reasonable to assume that sucrose-calcium chelation compounds will prove to be of importance in this case. It also appears possible that differential etching of the enamel surface by basic sucrose solutions may provide an additional approach to the study of enamel structure.

Finally, it will be of interest to determine (1) whether teeth from caries-active individuals etch more rapidly in lactate buffer than do teeth from individuals with no caries and (2) whether sucrose depresses the lactate enamel-etching rate more in one group than in the other.

SUMMARY

In confirmation of earlier chemical studies, sucrose has been shown by electron microscopy to exert a retarding action on the rate at which pH 4.5 lactate buffer etches human dental enamel surfaces. Further work is necessary to ascertain the nature of the action.

These results should not be construed as an argument against the accepted role of sucrose as a fermentation substrate for acid formation on the tooth surface. Instead, they should be regarded as additional evidence for the existence of multiple factors involved in caries development.

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Effects of Carbohydrate Restriction in Monkeys (M. irus) with Active Caries

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Abstract.—The amount of plaque on the tooth surfaces of caries-active monkeys (*Macaca irus*) was reduced when the animals were fed a diet low in fermentable carbohydrate instead of a cariogenic diet. Although the total streptococcal population, and the numbers of intracellular polysaccharide forming organisms remained constant, the population of extracellular polysaccharide forming bacteria fell when the monkeys consumed the low carbohydrate diet. The acid-producing potential of plaque, as measured by the fall in pH value over 20 min following the application of sucrose solution, was also reduced. The amount of plaque and the population of extracellular polysaccharide-forming bacteria rapidly increased when the animals were again fed the cariogenic diet.

It is widely accepted that dental caries can be controlled by a reduction in the frequency of intake of fermentable carbohydrate. However, it is not known whether this effect is attributable to diminished frequency of acid production or whether it results from a fall in population of the microorganisms responsible for producing acid. Previous investigations have shown that the composition of the salivary flora can be altered following changes in diet. The numbers of lactobacilli in saliva are greatly reduced following restriction in the consumption of carbohydrate (JAY 1947); and FEATHERSTONE (1960) has demonstrated that the species of lactobacilli which predominates in saliva is influenced by the proportions of carbohydrate, fat, and salt ingested. Specific carbohydrates can effect the flora of the mouth. The establishment of implanted cariogenic streptococci in the mouths of humans and experimental animals is greatly facilitated by the frequent intake of sucrose (KRASSE 1965).

In the present investigation an attempt has been made to determine the effects of restricting the intake of fermentable carbohydrate on (a) acid production in the plaque in response to a defined application of sucrose solution, and (b) the numbers of extracellular (EPF) and intracellular (IPF) polysaccharide-forming microorganisms in plaque.

MATERIALS AND METHODS

Animals. Caries-active monkeys (BOWEN 1965) of the species *Macaca irus* were used in this investigation. All the animals had permanent teeth only, and were aged between 5 and 6 years approximately. The monkeys were caged singly during the experimental period; cages were made of expanded aluminium mesh. The animals had 17 h of light daily throughout the experiment and were maintained at a temperature between 68 and 72 °F.

Diets. Two diets were used in this investigation. The first was a cariogenic diet similar to that used by COHEN and BOWEN (1966), but having a higher sucrose content; the amount of sucrose was increased by adding 10 parts of icing sugar to every 90 parts of Complan (Glaxo Laboratories Ltd.) and in the late afternoon each animal received a sugar lump which it rapidly consumed.

In the second diet readily fermentable carbohydrate was eliminated as far as possible. The animals received one half of a hard-boiled egg (shelled) in the morning and evening instead of a sweet biscuit and sugar lump. Jam was omitted from bread sandwiches and sucrose was not added to Complan. Complan however, normally contains 5.5% sucrose. Tap water (F content < 0.1 ppm) was provided *ad libitum* during the entire experimental period.

Experimental Procedures. The monkeys which had been fed a cariogenic diet for at least 3 years were placed on the lower carbohydrate diet for at least 3 weeks, at the end of which period they returned to the cariogenic diet. Food was offered with the same frequency throughout both experimental periods (COHEN and BOWEN 1966).

The monkeys were fasted for 17 h, and anaesthetized, prior to pH measurements being carried out. pH values were estimated weekly

Fig. 1 Anaesthetized monkey showing electrodes attached to teeth

on at least 6 occasions, in each of 4 animals, the time and day being kept constant. 4 teeth were investigated in each animal.

The pH was measured by means of microantimony electrodes prepared by electrodeposition of antimony on fine tinned copper wire. These electrodes gave accurate readings in buffers, and are so designed that it is possible to attach 4 to teeth in a monkey's mouth and record pH changes from these surfaces in rapid sequence (Fig. 1).

pH was recorded on an EIL direct reading pH meter (model 23A). The standardization of the electrode system and reference electrode has been described previously (Bowen, Eastoe and Cock 1966).

Following the measurement of the fasting pH on the tooth surface, 0.1 molar sucrose was applied and the pH values on the tooth recorded over a 20-min period.

Bacteriology. To determine the effect of dietary changes on the plaque flora, plaque was removed from buccal surfaces adjacent to "active" occlusal carious lesions before pH measurements were carried out (Fig. 2). An additional animal was included in this part of the investigation. Plaque was taken from the same site on each occasion. In some instances, however, when the animal was being fed a diet low in carbohydrate it was necessary to collect samples from more than 1 tooth, although in each instance the tooth used showed evidence of carious attack. Approximately 2 mg of plaque was obtained on each occasion; the sample was placed in a tared sterile stoppered test tube and weighed to the nearest 0.1 mg. Sterile brain heart infusion broth (Oxoid) was added to the tube in the ratio of 1 ml per mg of plaque and suspension achieved by shaking vigorously for 2 min; 6 sterile glass beads were present in the tube to facilitate thorough dispersion of the plaque. Serial tenfold dilutions up to 10^8 were then prepared from the suspension.

For the enumeration of total streptococci and extracellular polysaccharide-forming organisms 3 plates of mitis-salivarius agar (Oxoid), without added tellurite, were inoculated with 0.1 ml of each dilution of plaque suspension. Intracellular polysaccharide-forming microorganisms were counted on a medium composed of peptone 1%; tryptone 1%; yeast extract 0.1%; glucose 6%; and ionagar 1.2%. The pH was adjusted to a value of 7.2 before autoclaving. All plates were incubated in an atmosphere of 95% nitrogen and 5% carbon dioxide. Following incubation for 48 h, the mitis-salivarius plates were examined with the aid of a binocular microscope at 15× magnification and a combination of transmitted and reflected light. Extracellular polysaccharide-forming (EPF) colonies were readily identified. The plates used for estimating the intracellular polysaccharide organisms were incubated for 96 h. Colonies turning a deep brown following the flooding of plates with Lugol's iodine (Gibbons and Socransky 1962) were counted as intracellular polysaccharide-forming (IPF) colonies. Care was, however, taken to distinguish between IPF colonies and yeasts, which take up the iodine stain in their cell walls.

Results

All animals consumed their diets readily. More plaque was present in animals fed the

Fig. 2 Plaque being removed from caries-active tooth

Table I The effect of sucrose solutions on the pH of plaque, when the monkeys were fed high and low carbohydrate diets

	Initial pH		*4 min after sucrose solution*		*20 min after sucrose solution*	
	High carbohydrate	Low carbohydrate	High carbohydrate	Low carbohydrate	High carbohydrate	Low carbohydrate
Mean	6.94	6.93	6.37	6.33	5.17	5.35
Range	8.10–5.30	8.70–5.22	7.35–5.19	8.05–5.25	6.75–4.11	6.95–4.45
	not sig.		not sig.		$p < 0.05$	

Table II The percentage distribution of pH values found in plaque following application of sucrose solutions

High carbohydrate			Low carbohydrate		
pH 4–5	pH 5–6	pH 6–7	pH 4–5	pH 5–6	pH 6–7
53.4	33.3	13.3	39.2	39.2	21.6

cariogenic diet than in those given the low sugar diet. Indeed, the amount of plaque diminished progressively during the period of carbohydrate restriction and showed a rapid and substantial increase with the return to the cariogenic diet. The changes in diet influenced neither the initial pH values, nor the values after 4 min following the application of sugar solution. However, pH values on tooth surfaces 20 min after the application of sucrose were significantly lower when the animals were fed the cariogenic diet (Table I). Furthermore, the pH on more than 50% of tooth surfaces dropped to a value of less than 5 in monkeys fed the cariogenic diet; fewer that 40% did so in animals fed the non-cariogenic diet (Table II).

Although the accumulation of plaque was greatly reduced in monkeys on the low sucrose diet no significant difference was detected in the total population of streptococci as estimated on the mitis-salivarius plates (Table III). Higher total counts of microorganisms were obtained on this medium than on the IPF medium. However, the proportion of organisms forming extracellular polysaccharide fell dramatically when the animals were fed the non-cariogenic diet. The difference detected was highly significant (Table III). When the monkeys were restored to the high sucrose diet the percentage of EPF organisms in the total population rose rapidly.

Changes in composition of the diet did not influence the proportion of the total count

Table III The effects of dietary changes on the microbial population in plaque

	Total streptococci (mg wet wt of plaque)		*% Extracellular PS forming colonies*		*% Intracellular PS forming colonies*	
	High carbohydrate	Low carbohydrate	High carbohydrate	Low carbohydrate	High carbohydrate	Low carbohydrate
Mean	93.5×10^7	73.6×10^7	52.5	34.2	70.1	64.9
Range	19–300	21–200	27.1–93.9	2.6–69.4	35.7–96	0.6–98.2
	not sig.		$p < 0.01$		not sig.	

Estimated on same medium

comprising IPF organisms (Table III); the percentage remained fairly constant throughout the experimental periods.

Discussion

These results show that the amount of plaque on tooth surfaces in caries-active animals can be influenced by the composition of the diet, which supports the findings previously reported (Carlsson and Egelberg 1965) to occur in man. It would seem that the constituents of the carbohydrate diet, in addition to the appropriate microorganisms, provide the necessary circumstances for the formation of cariogenic plaque. However, it must be remembered that once plaque has accumulated on the tooth surface, acid is rapidly formed following the ingestion of glucose or fructose (Bowen, Eastoe and Cock 1966). It is, therefore, probably unrealistic to refer to any fermentable carbohydrate as non-cariogenic, when an animal or human is consuming a diet containing a mixture of carbohydrates. If the diet is such as to promote the development of plaque, a milieu has been created for subsequent caries activity following the ingestion of any fermentable carbohydrate.

It is interesting to observe that the reduction in the amount of plaque parallels the fall in the EPF population. A substantial proportion of plaque matrix is comprised of levan and dextran (McDougall 1964), both of which substances are synthesized from sucrose by "cariogenic" streptococci (Wood and Critchley 1966 and Guggenheim, König, Herzog and Mühlemann 1966) and perhaps by other micro-organisms as well. It seems probable that a fall in the number of EPF bacteria would lead to diminished plaque formation.

A reduction in the acid producing potential of plaque, as measured by the fall in pH over 20 min following applications of sucrose, was also found to accompany the reduction in number of EPF organisms. Nevertheless, a substantial drop in pH did occur which strongly suggests that other microorganisms make significant contributions to the acid formation when in the plaque. Indeed Sims (1965) has shown that certain oral strains of *Streptococcus viridans* and lactobacilli can effect a rapid drop in pH of sugar solutions *in vitro*. However, pH measurements do not indicate the type of acid being formed, some of which can decalcify enamel more rapidly than others. Furthermore, saliva can buffer acid formed in thin plaque more effectively than that formed in thick plaque (Graf and Mühlemann 1966).

A failure to demonstrate any difference in either the initial pH or the proportions of IPF organisms in plaque following the dietary changes is, at first sight, puzzling. However, it is possible that the two observations are related. All the animals were fasted for 17 h before either plaque was collected, or pH measurements were carried out. This long fast would allow saliva to neutralize any acid formed following the last ingestion of fermentable carbohydrate and thereby mask any differences that might otherwise have been observed. Furthermore, Gibbons and Socransky (1962) have suggested that the resting pH in plaques might be determined by the proportion of intracellular polysaccharide-forming organisms in plaque; microorganisms storing intracellu-

polysaccharide can form acid from their ed carbohydrate. It is probable that such organisms are able to synthesize glycogenolypectin from carbohydrates other than which would explain the failure in this stimulation to induce any changes in their population.

Although this investigation continued for a short period only, the results indicate that changes in the bacterial composition of plaque occur rapidly following alterations in diet, and furthermore they emphasize the necessity of adhering to well-controlled conditions when studying the microbial flora of the mouth. It was the incontrovertible impression of both authors that the amount of plaque was drastically reduced in the animals under carbohydrate restriction. Photographic illustration of this feature is unsatisfactory unless dyes are used for disclosing the deposits, but this procedure was contraindicated because of the effects that would be produced upon the bacterial population. Furthermore, the evidence adduced here demonstrates that acid producing potential in plaque can be diminished by certain changes in diet thereby offering additional explanations for reduced caries activity following restricted consumption of fermentable carbohydrate.

Zusammenfassung. Die Plaquemenge auf den Zahnoberflächen kariesaktiver Affen (Macaca irus) wurde verringert, wenn die Tiere statt einer kariogenen eine Diät mit niedrigem Gehalt vergärbarer Kohlenhydrate erhielten. Während die totale Streptokokkenzahl und die Zahl der intrazelluläre Polysaccharide synthetisierenden Mikroorganismen konstant blieb, ging die Anzahl der Bakterien, die extrazelluläre Polysaccharide bilden, auf der kohlenhydratarmen Diät zurück. Die Säurebildungsfähigkeit der Plaque, gemessen am pH-Abfall über 20 min nach Applikation einer Saccharoselösung, war ebenfalls reduziert. Belagmenge und Zahl der Bakterien, die extrazelluläre Polysaccharide bilden, stiegen rasch wieder an, wenn die Tiere wieder auf kariogene Diät gesetzt wurden.

Résumé. Cette expérience, faite sur des singes prédisposés à la carie (Macaca irus), a montré que la quantité de plaque déposée sur les surfaces dentaires peut être réduite en donnant à ces animaux une nourriture pauvre en hydrates de carbone fermentescibles en place d'une diète cariogène. Alors que le nombre total des streptocoques et celui des microorganismes synthétisant des polysaccharides intracellulaires reste constant, le nombre de bactéries produisant des polysaccharides extracellulaires diminue sous l'effet de la diète pauvre en hydrates de carbone, de même que le pouvoir acidifiant de la plaque. L'inverse se produit si l'on redonne à ces animaux une nourriture cariogène.

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Adhesion of Foods to Teeth

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The Vipeholm Dental Caries Study[1] clearly established the relationship between the physical form of the diet and dental caries activity. In their report on that study, Gustafsson, Quensel, Swenander-Lanke, Lundqvist, Grahnen, Bonow, and Krasse stated (p. 361): ". . . The risk of sugar increasing caries activity is great if the sugar is consumed in a form with a strong tendency to be retained on the surfaces of the teeth." This fact had been suspected for many years, and the great importance of adhesion of the diet to teeth demonstrated in the Vipeholm study has helped to call attention to the lack of basic knowledge of such matters in dentistry.

A preliminary investigation of the adhesion of foods to teeth has been made,[2] and recently Beck and Bibby[3] reported on a centrifugal method of measuring food retention on teeth. This present study was an evaluation of the adhesion of over seventy foods to teeth. It provided data that, it is hoped, might be helpful in understanding the properties of foods that are involved in intraoral food retention. Such data might also be used in designing caries-reducing diets.

EXPERIMENTAL METHODS

A variety of foods was selected for study to make possible comparisons between different general types—such as vegetables, cereals, candies, baked goods, fruits, nuts, dairy products, and meat and fish products. Within each group several representative foods were tested, and in some cases the same food was tested after it was prepared in different ways. For example, potatoes were tested when boiled, mashed, French-fried, and as potato chips and sticks.

In determining the adhesiveness of the foods, it was decided to study conditions under which a foodstuff was mixed with saliva and forced into a narrow space, such as between the walls of a tooth's occlusal fissure or interproximally between teeth. It is pertinent that dental caries most frequently begins in such areas. Under these conditions the adhesiveness of the food-saliva mixture probably assumes its greatest importance because of the slower dissolving or washing effect of the saliva than that occurring in more accessible areas of the tooth surface. Consequently, an adhesion testing procedure was devised to measure the adhesiveness of food-saliva mixtures squeezed into a space between two enamel surfaces.

Apparatus.—The apparatus had to be specially designed and constructed because no conventional adhesion tester was found entirely suitable. In a previous study[2] a commercial adhesion tester was used to establish that differences in food adhesion to teeth

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could be measured, but it was apparent that neither that machine nor others readily available were suitable for forming adhesion bonds under the pressures typically encountered in chewing (1 kg/mm²)[4] while having the accuracy to record the relatively low tensile strength of food to tooth-enamel bonds. The apparatus used in this study satisfied these criteria and was similar in principle to the one used earlier,[2] but it was better suited for recording the wide range of values encountered.

The main part of the adhesion tester was Beam *A* (Fig. 1*A*). Two flattened tooth-enamel surfaces were prepared as previously described,[2] and one was attached to the underside of Beam *A* and the other to Pedestal *B*. The two tooth enamel surfaces *C* fitted together accurately (Fig. 1*B*), and the food-saliva mixture to be tested was placed between them. As the tooth surfaces came together, the distance between them was recorded on Dial Indicator *D*, which had 0.002-mm. graduations, with a range of 0.200 mm.

On the right of Beam *A* (Fig. 1*A*) was Traveling Weight *E*, which was driven by Motor *G*. When the strength of the food-saliva mixture bond to the enamel surfaces was to be measured, the motor was started with Hand Switch *H*. Traveling Weight *E* then moved along the beam toward the end, thereby producing a steadily increasing separating force on the bond between the tooth surfaces at *C*. When the bond broke, the traveling weight was stopped automatically, and the distance traveled was read from a scale on the beam and a vernier on the traveling weight. These were calibrated so that loads from 0.0 to 540.0 gm. could be measured to 0.1 gm. with the vernier. With Weight *E* at zero on the scale and the tooth surface attached on the underside of Beam *A*, balancing of the beam was achieved by small lead washers at *J* prior to performing the test (Fig. 1*B*). The area of the tooth surfaces in contact was determined by weighing a piece of paper exactly trimmed to the same area as the contacting surfaces and comparing this with the weight of a piece of the same paper cut to a known measured area. The area was measured several times, and the average was obtained.

The adhesion values were recorded in grams per square centimeter, the weight in grams required to break the bond being read directly from the scale and vernier, the area of the tooth surfaces in contact having previously been measured as described above.

For forming the bond of the desired thickness, Weights *K* were hung on the end of Beam *L*. By lowering this beam as Screw Support *M* was turned, a food-saliva mixture could be squeezed between Enamel Surfaces *C* to the desired thickness, with a force sufficient to simulate biting pressures. Excess food was removed, and the bond strength was ready to be measured. Hanging Weight *K* was then removed, and Beam *L* was swung up away from Main Beam *A* and held up by Support Arm *N* (Fig. 1*C*). By depressing the button on Hand Switch *H*, the test was started. On completion, the separation force was read from the scale and the vernier.

Test conditions.—Saliva from 2 subjects chewing paraffin was collected into test tubes packed in ice. (The same two subjects were used throughout the study.) Equal volumes of the salivas were then pooled and mixed with the test food by mortar and pestle. The test was then performed immediately, to minimize physical and chemical changes in the mixture due to enzyme action or further denaturation of the salivary proteins.

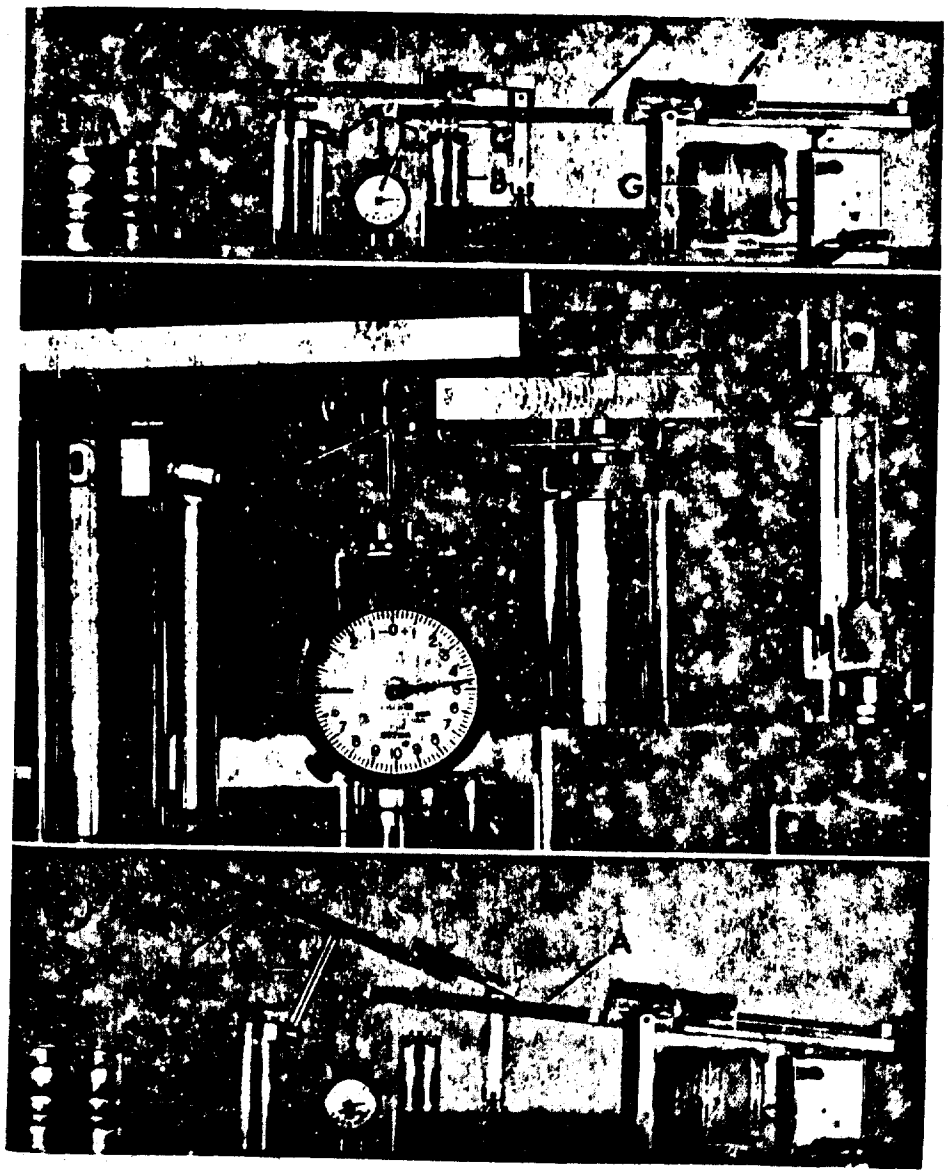

FIG. 1*A*.—Adhesion tester, showing the following parts: *A* = main beam, *B* = pedestal supporting lower tooth surface, *C* = position of upper and lower tooth surface, *D* = dial indicator, *E* = traveling weight, *G* = electric motor, *H* = switch for starting motor, *J* = lead washers for balancing the main beam; *K* = weights for hanging on beam to press the upper tooth surface against the lower, *L* = beam for exerting vertical pressure on tooth surfaces, *M* = screw support for lowering beam, and *N* = support that rotates to hold up Beam *L*. Tester is shown with Beam *L* in position to exert pressure on tooth surfaces at *C*.

FIG. 1*B*.—Closeup of tooth surfaces *C*.

FIG. 1*C*.—Adhesion tester with Beam *L* held up by Support Arm *N*.

It was obvious that the quantity of saliva added to the food would influence the adhesiveness of the mixture. It was considered that the proper proportions of saliva to food could best be determined by measuring the amount of saliva found in samples of each test food recovered from the mouth after chewing. Accordingly, a preliminary study was made of the amount of saliva in food particles found adhering to tooth surfaces after chewing and swallowing had been completed. Food particles found on the soft tissues were also collected for comparison. Four dental students with full dentitions and good occlusions were used in the preliminary study, and subsequently one of the students chewed each food that was to be tested later in the adhesion study. The foods were chewed on two separate occasions, and samples found adhering to the teeth after swallowing were removed, weighed moist, and then dried to constant weight at 55° C. Samples of the unchewed food and samples found on the soft tissues of the mouth were also weighed and dried in the same way. The volume of saliva incorporated in the food during chewing was calculated by difference from the moisture content of the food before chewing on the basis of saliva having the same density as water (the maximum error introduced was approximately 1.5 per cent).

Another consideration in this test was the thickness of the layer of food and saliva between the enamel surfaces. Results vary with different thicknesses of bond for each food-saliva mixture. It would not necessarily be correct to compare different foods at the same bond thickness. In this experiment each mixture was tested at various bond thicknesses, and the bond that gave the maximum reproducible values for each food was used. An average of 10 measurements at this optimum bond thickness was then used as the value of adhesion. The tests were conducted at room temperature (20–24° C.) rather than at 37°, partly for convenience and also because it is questionable whether 37° C. was the temperature of all foods adhering to the tooth surface soon after eating.

Effect of pellicle and surface roughness on adhesion.—The rationale involved in using flat, polished enamel surfaces for testing food adhesiveness has been discussed previously.[2] However, it was apparent that, in vivo, the presence of an organic pellicle on the intact enamel surface might modify the adhesion of a food to the tooth. To evaluate the effect of such pellicles on adhesion, teeth were selected that microscopically had a clear or stained film of firmly attached organic material, which could be scratched with a dental instrument. Such teeth were mounted in the adhesion tester, so that a smooth surface could be repeatedly pressed into a food saliva mixture to the same depth each time. Adhesion measurements were made with the pellicle intact on the tooth, and then the pellicle was removed by gently cleansing with a paste of pumice flour on a rotating brush. Measurements were then obtained for the adhesion of the test food to the pellicle-free tooth surface.

To evaluate the effect of surface roughness, the pellicle-free teeth were sandblasted to give a surface of different roughness, and measurements were repeated. Finally, the enamel surface was scratched with rough sandpaper and values recorded again.

RESULTS

Amount of saliva in food adhering to teeth.—Table 1 shows how 4 different subjects chewed various foods with respect to the total number of chewing strokes before swallowing, the duration of chewing, and rate of chewing. Also included in Table 1

are the average moisture contents of the food samples before eating, of samples found adhering to the teeth, and of samples found on the soft tissues after eating.

No remarkable results were obtained except that Subject 4 (D. S.), who was selected to chew all the foods to be used in the adhesion study, consistently chewed each food less than the other three subjects, although his chewing rate was similar to the others. An important factor in this study was that the amount of saliva in food recovered from the teeth of Subject 4 was representative of the results for the group as a whole. It should be noted that each of the values in Table 1 is the average of 6 separate observations. During the work, it was observed that daily variations in the amount of moisture in food recovered from the teeth of Subject 4 were similar to the daily variations in water content of the foodstuffs before eating.

An additional observation concerning the amount of saliva in foods adhering to the teeth was that if the foods were listed in order of increasing amount of saliva found in samples on the teeth, then it made little difference from which subject the samples were collected (Table 2). This was because caramel, corn flakes, and one of the other three foods fall in the same order and have similar moisture contents after chewing in the

TABLE 1*

COMPARISON OF AMOUNTS OF MOISTURE IN VARIOUS FOODSTUFFS BEFORE AND AFTER CHEWING

Foodstuff	Subject	Average No. of Chews	Average Chewing Time (Sec.)	Average Chewing Rate (Chews/Sec)	Per Cent Moisture in Food Samples		
					Before Chewing	After Chewing	
					Control	From Soft Tissues	From Teeth
Chocolate caramel	1. (J. M.)	46	39	1.1	4.9	10.2	9.5
	2. (D. P.)	55	42	1.3	3.9	10.1	9.7
	3. (S. K.)	60	43	1.4	4.4	9.4	9.3
	4. (D. S.)	31	24	1.3	4.5	8.5	9.5
Corn flakes	1. (J. M.)	20	15	1.7	2.8	67.9	40.3
	2. (D. P.)	27	19	1.1	2.9	61.9	54.7
	3. (S. K.)	30	19	1.6	3.0	56.4	40.5
	4. (D. S.)	13	12	1.1	2.4	58.0	46.4
Corn flakes and milk	1. (J. M.)	19	14	1.3	47.2	62.2	54.7
	2. (D. P.)	18	11	1.5	57.7	62.4	60.1
	3. (S. K.)	23	13	1.4	62.5	60.2	58.5
	4. (D. S.)	10	7	1.4	50.3	55.7	54.2
White bread	1. (J. M.)	31	23	1.3	27.1	61.8	58.0
	2. (D. P.)	29	19	1.6	32.3	67.5	59.4
	3. (S. K.)	44	27	1.6	30.7	58.3	57.4
	4. (D. S.)	21	15	1.4	26.8	64.3	64.0
White bread and margarine	1. (J. M.)	25	19	1.3	27.1	60.9	54.3
	2. (D. P.)	27	18	1.5	38.2	66.6	59.1
	3. (S. K.)	34	20	1.7	31.9	61.1	60.1
	4. (D. S.)	15	10	1.4	32.3	62.4	58.1

* Each value is the average of six measurements.

case of each subject. There was some variation among the subjects in the case of the other three foods (corn flakes with milk, bread, bread and margarine). This did not seem surprising, in view of the slight difference in the average moisture-content values that these three foods had after chewing. It was interesting that values for Subject 4 could be arranged in exactly the same order as the average for the other three subjects. For these reasons and those previously stated, it was determined to be satisfactory to use Subject 4 alone and to obtain duplicate results from him. Accordingly, the values for saliva incorporated in each food reported in Tables 3–9 refer to data obtained from a single subject (Subject 4, D. S.).

Adhesiveness of foods.—Tables 3–9 include data on the adhesiveness of 77 foods commonly found in the diet. An attempt was made to describe the foods accurately,

TABLE 2*

FOODS ARRANGED IN ORDER OF INCREASING MOISTURE CONTENT OF SAMPLES RECOVERED FROM TOOTH SURFACES OF DIFFERENT SUBJECTS

Foodstuff	Relative Order of Foodstuffs and Per Cent of Moisture Content				
	Subject				Average of Subjects Nos. 1, 2, 3
	1 (J.M.)	2 (D.P.)	3 (S.K.)	4 (D.S.)	
Chocolate caramel	1 (9.5)	1 (9.7)	1 (9.3)	1 (9.5)	1 (9.5)
Corn flakes	2 (40.3)	2 (54.7)	2 (40.5)	2 (46.4)	2 (45.2)
Corn flakes and milk	4 (54.7)	5 (60.1)	4 (58.3)	3 (54.2)	3 (57.7)
White bread and margarine	3 (54.3)	3 (59.1)	5 (60.1)	4 (58.1)	4 (57.8)
White bread	5 (58.0)	4 (59.4)	3 (57.4)	5 (64.0)	5 (58.3)

* Each value for moisture content is the average of six measurements.

TABLE 3

ADHESIVENESS OF VEGETABLE PRODUCTS TO TOOTH SURFACES

Foodstuff	Per Cent Moisture Content		Adhesiveness (Gm. Cm²)	Relative Adhesiveness (0–12)
	Before Chewing	After Chewing		
Lima beans	71.9	71.2	97	0
Potato (mashed)	70.9	76.5	418	4
Cheese corn [illegible]	0.1	47.0	432	4
Potato chip (crisp)	1.5	35.0	507	5
Potato stick	1.9	47.0	551	5
Black-eyed peas	72.0	77.0	606	6
Baked beans	66.0	70.0	672	6
Canned whole kernel corn	73.0	75.0	717	7
Green peas	82.8	78.1	741	7
Sweet potato	71.0	34.0	766	7
Spaghetti	80.1	78.0	791	7
Carrot (boiled)	91.0	92.0	804	8
Potato (French fried)	66.5	72.8	829	8
Turnip greens	90.6	90.5	1055	10
Potato (boiled)	87.0	87.1	1100	11

TABLE 4

ADHESIVENESS OF BREAKFAST CEREALS TO TOOTH SURFACES

FOODSTUFF	PER CENT MOISTURE CONTENT		ADHESIVENESS (GM/CM²)	RELATIVE ADHESIVENESS (0-12)
	Before Chewing	After Chewing		
Raisin bran	2.3	56.0	111	1
40 per cent bran flakes	2.0	52.0	163	1
Grape Nuts	2.0	47.0	189	1
Shredded Wheat	3.0	50.0	424	4
Rice Krispies	3.3	57.0	498	4
Corn flakes	2.4	46.4	574	5
Corn flakes and milk	50.3	54.2	745	7
Sugar-coated corn flakes	2.0	50.0	754	7

TABLE 5

ADHESIVENESS OF CANDIES AND DESSERTS TO TOOTH SURFACES

FOODSTUFF	PER CENT MOISTURE CONTENT		ADHESIVENESS (GM/CM²)	RELATIVE ADHESIVENESS (0-26)
	Before Chewing	After Chewing		
Gelatin dessert	79.0	83.0	58	0
Chocolate-coated coconut bar	7.5	24.6	94	0
Milk chocolate	0.5	9.2	507	5
Semisweet chocolate	1.8	7.8	627	6
Chocolate caramel	4.5	9.5	1010	10
Toffee	4.9	9.5	2020	20
Caramel	5.3	11.2	2630	26

TABLE 6

ADHESIVENESS OF BAKED GOODS TO TOOTH SURFACES

FOODSTUFF	PER CENT MOISTURE CONTENT		ADHESIVENESS (GM/CM²)	RELATIVE ADHESIVENESS (0-12)
	Before Chewing	After Chewing		
Chocolate snap cookie	4.8	29.5	160	1
Peanut-butter cookie	2.0	43.0	165	1
Wheat bread	30.6	53.7	207	2
Apple pie	44.2	34.8	224	2
Cracker (oil-sprayed)	2.0	40.0	232	2
Vanilla wafer	12.2	34.7	329	3
Soda cracker	1.0	52.0	334	3
Cheese cracker	4.4	37.3	392	3
Rye bread	30.0	60.0	445	4
Rye bread and margarine	25.0	58.0	456	4
Doughnut	11.0	48.0	557	5
White bread and margarine	32.3	58.1	595	5
White bread	26.8	64.0	610	6
French bread and margarine	20.0	59.0	762	7
French bread	18.0	63.0	811	8
Chocolate cake	19.9	30.3	823	8
Cup cake	5.5	32.1	1086	10
Plain cake	17.0	34.1	1070	10

TABLE 7

Adhesiveness of Fruits and Nuts to Tooth Surfaces

Foodstuff	Per Cent Moisture Content		Adhesiveness (Gm/Cm²)	Relative Adhesiveness (0–12)
	Before Chewing	After Chewing		
Banana	76.5	77.0	174	1
Olive	69.5	59.8	278	2
Peanut	0.8	28.5	413	4
Raisin	13.0	30.9	427	4
Peanut butter	0.6	27.2	436	4
Peach	76.8	75.4	491	4
Orange	88.2	83.8	502	5
Black walnut	2.9	34.7	537	5
Date	26.0	41.8	614	6
Apple	80.5	84.7	619	6
Cherry (Maraschino)	35.0	62.8	735	7
Tomato (canned)	92.0	90.0	753	7

TABLE 8

Adhesiveness of Cheese and Eggs to Tooth Surfaces

Foodstuff	Per Cent Moisture Content		Adhesiveness (Gm/Cm²)	Relative Adhesiveness (0–12)
	Before Chewing	After Chewing		
Cheese (American)	35.0	49.7	650	6
Egg (fried)	54.1	80.0	850	8
Egg (hard-boiled)	70.0	71.0	948	9
Egg yolk (hard-boiled)	47.0	64.0	958	9
Egg (scrambled)	72.2	53.4	1115	11

TABLE 9

Adhesiveness of Meat and Fish Products to Tooth Surfaces

Foodstuff	Per Cent Moisture Content		Adhesiveness (Gm/Cm²)	Relative Adhesiveness (0–12)
	Before Chewing	After Chewing		
Deviled ham	48.1	48.7	232	2
Potted meat	66.3	69.0	291	2
Vienna sausage	60.8	62.0	378	3
Liver sausage	48.0	63.0	385	3
Meat loaf	74.0	79.0	388	3
Sliced "dried" beef	51.4	71.5	438	4
Chicken spread	58.0	73.0	477	4
Sardine	49.3	62.3	480	4
Ground beef (broiled)	43.9	69.4	521	5
Sandwich meat (slice)	61.0	73.0	646	6
Bologna	50.1	68.2	658	6
Ham (boiled)	65.0	78.0	1012	10

ather than by using local or trade names. Even so, in some cases the reader (particularly one from a country other than the United States) may have difficulty in relating the food described to one that is eaten in his locality. For instance, certain "cookies" are sometimes referred to as "biscuits" in other English-speaking countries, and such items as "black-eyed peas" or "turnip greens" are restricted mainly to the diet of the southern United States. Also included in Tables 3–9 are data for the food's moisture content before chewing and that of samples found adhering to the teeth after chewing.

The data in Tables 3–9 are fairly self-explanatory, and it can be seen that there was a considerable range of adhesiveness. The difference observed between a food with a value of less than 100 gm/cm^2 and one with a value greater than 1,000 gm/cm^2 was very marked. Since the purpose of this study was mainly to determine the relative adhesiveness of foods rather than the exact adhesion under a certain set of conditions, a column is included on the right of the table assigning a value between 0 and 12 for each foodstuff. The scale of 0–12 was chosen because it was wide enough to enable comparisons to be made between each of the foods except toffee and caramel. These two candies were so much more adhesive than other foods that they were not included in the same scale of relative adhesiveness (0–12) as were the other foods. If the scale chosen were 0–26, then all the foods would be included, but variations between 0–12 would seem to be less important than, in all probability, they are.

Tables 3–7 include data for carbohydrate foodstuffs, and such values probably have particular significance for dental caries. Tables 8 and 9 reveal that other foods commonly eaten are as adhesive as many of the carbohydrate foods; such values may have importance in periodontal conditions arising from food retention around or under the free margin of the gingiva.

Effect of pellicle and surface roughness on adhesion.—The adhesion of food-saliva mixtures to the tooth surface was usually affected more by the degree of roughness of the surface than by the presence or absence of pellicle. Roughening the surface by sandblasting followed by sandpaper increased adhesion by about 20 per cent, but the effect of pellicle was difficult to assess. This was because the change in adhesion caused by removal of the pellicle was not greater than could be accounted for by changes in surface roughness. Thus it was not possible to separate the effect of pellicle removal from the change in surface roughness produced by the pumicing necessary to remove the pellicle.

Table 10 shows food adhesiveness to intact tooth surfaces before and after pellicle removal. Generally, the procedure of removing the pellicle brought about an apparent increase in the adhesion of food to the surface, e.g., chocolate snap cookie (+9 per cent), corn-flake cereal (+4 per cent), soda cracker (+22 per cent), and vanilla wafer (+9 per cent). With cholocate caramel the adhesion change due to pellicle removal ranged from an 18 per cent decrease to a 15 per cent increase in adhesion.

DISCUSSION

It is tempting to compile a table comparing these results with data from other studies. For instance, Lundqvist[5] has data on oral sugar clearance; Bibby, Goldberg, and Chen[6] have data on intraoral food retention; and Beck and Bibby[3] have data on a "centrifugal retention" technique.

The first problem in making comparisons is that, in each case, different units of measurements were used. It is difficult, if not impossible, to relate percentage concentration of sugar in saliva, to milligrams of food, to "centrifugal retention index," and to grams per square centimeter. Also, as Beck and Bibby[3] point out, a lack of correlation between intraoral tests and the centrifugal retention test (and presumably in this case the adhesion measurements) may be because each procedure measures different properties of foods. For these reasons, it does not seem to this author that, at the present time, there is sufficient information available to predict or explain intraoral food retention in terms of food properties. As yet there is no information on the solubility of foods in saliva, and it is likely that this is of major importance in the length of time that a food will be retained in the mouth. It is hoped that studies such as the present investigation, in which an attempt is made to measure physical properties individually, may have significance in better understanding intraoral food retention. However, the relationship of intraoral food retention to the initiation or progress of oral disease also needs further clarification.

TABLE 10

EFFECT OF PELLICLE ON FOOD ADHESION TO TEETH

Test Food	Food Adhesiveness*		Percentage Change	
	Tooth Surface with Pellicle	Tooth Surface without Pellicle	Average	Range
Chocolate caramel	284.6	281.9	− 1	−18–+15
Chocolate cookie	32.8	35.8	+ 9	+ 1–+16
Corn flake	68.0	70.4	+ 4	+ 1–+ 7
Soda cracker	39.2	47.9	+22	+ 9–+42
Vanilla wafer	43.2	49.1	+ 9	+ 1–+13

* Average number of grams required to break adhesion bond between food-saliva mixture and the tooth surface.

On first inspecting the results, some foods seemed surprisingly adhesive. Subjectively, it appeared that chocolate snap cookie and peanut-butter cookie would be more adhesive than fresh apple or cheese. Instead, the reverse was actually observed. However, when a food is squeezed with a biting pressure of 1 kg/mm^2 into a narrow space, like a fissure in a tooth or between tightly contacting teeth, then, possibly, the impressions regarding adhesiveness are in error. Since the present test procedures simulated such intraoral conditions, the values are reported with some confidence. In this connection, it has recently been shown by Beck and Bibby[3] that apple has a fairly high retention value by their centrifugal method of measuring food retention. They also observed the phenomenon, seen in this investigation, that adding butter or margarine to bread has practically no effect on the adhesion recorded.

Cohesion was not measured in this study. In cases where the present results differ from Beck and Bibby's, this may be because in their study there occurred (page 158) ". . . cohesive failure within the food, rather than adhesive failure between the food and tooth."[3] Cohesive failure of bonds was occasionally noticed in the present investigation, and, in such instances, testing of that particular food was discontinued, so that

no values for these foods are reported here. In addition, a few foods, such as fig cookies, figs, or raspberries, could not be tested because of the difficulty in removing the seeds that prevented a thin adhesion bond from being formed between the teeth.

There were a few foods that had relatively high values for adhesion and that have been shown to possess low intraoral food-retention values.[6] For instance, boiled carrots, corn flakes, or apples are apparently more adhesive than one would suspect from intraoral food-retention measurements. Such examples illustrate that more is involved in the oral retention of these foods than simply food adhesiveness. However, limitations involved in day-to-day variation and intersubject variation in intraoral food-retention data make direct comparisons difficult.

In the Vipeholm Study[1] a major conclusion was that foods with a strong tendency to be retained on the teeth heighten the risk of increasing caries activity. Although the study suggested (p. 358) that Lundqvist's[5] values for "sugar time" of the saliva might not always reflect actual conditions on the dental surfaces, the results of the present study for carbohydrate foods seem to indicate that this may often be the case. In general, where Lundqvist observed long retention times, the same type of carbohydrate food in the present study showed high values of adhesiveness to tooth surfaces. There are, of course, exceptions, and adhesive foods, such as ham or eggs, have low values in Lundqvist's oral sugar clearance tables because of their low sugar content. Nevertheless, such foods may have importance in caries activity, as they modify the composition and metabolic activity of the oral flora. In addition to their possible effect on caries activity, such foods may be of importance in the initiation of periodontal tissue inflammation, if their high adhesiveness promotes long retention around or under the marginal gingivae.

Tackiness is a property of food that may be important but was not measured in this study. For the purpose of this discussion, tackiness can be considered as the ability of a food to stick to the tooth surface when only minimal force is involved in making the contact between the food and tooth. This property may be important in the retention of food on the buccal, labial, and lingual surfaces, which are not directly in the turmoil of mastication, as is the occlusal surface. However, tacky foods may not necessarily be those that will adhere firmly if a contact is made between the food and tooth surface under biting forces. In planning this study, it was considered that the most important type of adhesion measurement would be one in which the food is firmly lodged in fissures or interproximally, where dental caries usually begins. The adhesion measurements reported in this paper, therefore, cannot be considered a measurement of tackiness.

It does not appear that food adhesion to a tooth surface is markedly altered by the presence or absence of a pellicle on the surface. Likewise, the surface roughness is not a major factor in food adhesion to teeth, excluding, of course, surface deposits, such as calculus, or such frank defects in the surface as carious lesions.

SUMMARY

The adhesiveness of 77 foods to teeth was measured in an adhesion tester specially designed and constructed for this purpose. The adhesion test involved measurement of the tensile force required to break a bond between food-saliva mixtures and the enamel surface of teeth. It was observed that the effects of surface roughness and organic

pellicles on the adhesion of foods to teeth were relatively minor in comparison with the wide range of adhesion for different foods. The volume of saliva incorporated during chewing was fairly constant for each food, even though samples were collected from 4 different subjects, who chewed for widely varying lengths of time and at different chewing rates.

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The adhesion tester used in the study was designed with the assistance of Mr. D. B. Darden, of Southern Research Institute, Birmingham, Alabama, and was constructed at that Institute.

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Effect of Certain Dietary Sugars on Hamster Caries [1,2]

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ABSTRACT The comparative effect of various dietary sugars on hamster dental caries was studied. A strain of hamster which does not normally present caries unless infected with a cariogenic organism was inoculated with AHT-type human cariogenic streptococcus and fed diets high in either sucrose, fructose, glucose, lactose or a glucose-fructose mixture. All of the sugars tested supported caries, but the total caries scores varied from a statistical zero at 100 days on the basal diet (no added carbohydrate) to a high score on sucrose. Sucrose was the most cariogenic sugar tested — or at least supported the most rapidly progressive carious process. Given sufficient time fructose, lactose and glucose — in decreasing order of activity — also supported dental destruction under this experimental regime. High dietary sucrose was not essential for the development of periodontal lesions or of microbial deposits along the gingival margin of the tooth (gingival plaque). Microbial deposits on the tooth crown (coronal-plaque), however, formed primarily on the sucrose diet. The formation of large amounts of coronal plaque was not essential to carious attack upon the smooth surfaces of the tooth. Apparently sugars other than sucrose were also capable of supporting smooth surface caries.

Certain rodent and human strains of oral streptococci have been shown to induce dental caries in hamsters and rats fed a high sucrose diet (1–4). Many investigators have studied the cariogenic and plaque producing potential of other sugars as compared to sucrose in the cariogenic diet (5–11). There are, however, conflicting reports among their publications. This variability may be correlated with the choice of laboratory animal, test organism, if any, and the length of the experimental period as well as with the individual sugars tested. For this reason the present study was conducted in a strain of hamster which does not normally present more than minimal background caries unless infected with a cariogenic organism. Through methods reported earlier (3–4) a series of eight cariogenic human streptococcal strains was isolated. These strains were capable of producing rampant dental caries in hamsters fed a diet high in sucrose (4). The most virulent of these human cariogenic streptococcal strains, AHT-type (4), was used as the inoculant in the present study. A comparison was made of the effects on hamster dental plaque and caries of various sugars (glucose, fructose, lactose, a 1:1 mixture of glucose and fructose, and sucrose) added to the experimental diet.

EXPERIMENTAL METHODS

Animals and microbiological techniques. Nineteen-day-old Syrian hamsters were inoculated with a strain of human cariogenic streptococcus, AHT-type, and placed on the experimental regime. The inoculation procedures used were essentially those described by Keyes (12) and Fitzgerald and Keyes (1). In order to show that AHT strains were implanted in the experimental animals and that no similar organisms were present in the uninoculated control group, scrapings were taken from the upper molar teeth of each animal with a sterile 26-gauge hypodermic needle on the day it was killed. The needle and adherent material were incubated anaerobi-

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[2] Reported in part at the 46th Annual Meeting of the International Association for Dental Research and the North American Division of IADR, San Francisco, California, March 21–24, 1968. Campbell, R. G. and D. D. Zinner 1968 The dental caries effect of substituting various sugars for sucrose in the diet. IADR Abstracts, 46: 127 (abstract).

[3] From a thesis by R. G. Campbell, submitted in partial fulfillment of the requirements for the Master of Science Degree, University of Miami, Coral Gables, Florida.

cally overnight in modified Todd-Hewitt broth (3) at 37°. The cultures were examined by the fluorescent-antibody technique for the presence of human organisms of strain AHT, BHT and HHT (13) in all of the uninoculated control animals, and for AHT in all of the inoculated experimental animals. The methods of slide preparation and examination are those previously described by the U. S. Public Health Service (14) and Zinner et al. (15). The number of fluorescent microorganisms on the slide was expressed as follows: none, −; 1 or 2 chains, ±; 3 to 5 chains, 1+; 6 to 10 chains, 2+; chains distributed throughout the slide, 3+; many chains distributed throughout the slide, 4+.

Diets. Since previous publications indicated that hamsters did not survive well on high levels of lactose (16, 6) or fructose (6), a pilot study was conducted to determine the highest dietary level of the sugars that would allow good growth and reasonable health of the animals throughout the experimental period. On the basis of these results the final experimental regime was planned. The basal diet of the final study was derived from the original cariogenic diet of Keyes (12) and is described in table 1. The basal diet was then supplemented with carbohydrate in the form of the various sugars studied.

The pilot study suggested that a period of time was necessary for the metabolism of the animal to adjust to high levels of sugar. It was decided, therefore, that the experimental animals would be fed according to the following schedule:

Day 1 and day 2 — 2 parts (by weight) carbohydrate to 8 parts basal diet (20% sugar).

TABLE 1

Composition of the basal diet [1]

	% by wt
Skim milk powder	65.9
Whole wheat flour	14.6
Alfalfa powder	7.3
Liver concentrate	4.9
Whole liver powder	4.9
Modified Osborne-Mendel salt mixture	2.4

[1] The original cariogenic diet of Keyes (12), modified by the removal of 59% sucrose and recalculation of the percentages (by weight) of the remaining ingredients.

Day 3 to day 100 — 4 parts carbohydrate to 6 parts basal diet (40% sugar).

There were seven experimental groups of 20 animals each. Two different types of controls were included: 1) uninoculated animals fed a 40% sucrose diet (hereafter referred to as control animals), and 2) AHT-inoculated animals fed the basal diet. These animals were referred to as basal animals. In addition to the two control groups, five AHT-inoculated test groups were fed experimental diets containing 1) 40% fructose, 2) 40% lactose, 3) 40% glucose, 4) 40% of a 1:1 mixture by weight of fructose and glucose (hereafter referred to as glucose-fructose animals), or 5) 40% sucrose.

Observations. In order to study the time of caries onset and the rate of caries progression, the following procedures were implemented: When periodic inspection indicated that caries might be present, the experimental animal was killed and the teeth were examined for stereoscopic evidence of caries. By this method it was possible to establish the approximate date of caries onset within each group. From this point the progress of the carious lesions was followed by periodically killing a hamster up to and including 100 days. (Animals from the pilot study group were included in the lactose progression data.) Sufficient animals were maintained within each group to determine the total caries production with statistical validity after 100 days. Immediately after the animal was killed its weight was recorded, and an oral culture was taken as previously described. The teeth were examined for plaque and the jaws for periodontal lesions by stereomicroscopy at a magnification of 10 diameters. Two different kinds of plaque were observed in the experimental animals: one, occurring on the crown of the tooth, was a thick, transparent gelatinous mass, "coronal plaque"; and one, occurring in and above the gingival sulcus, was white, opaque and cheesy, "gingival plaque." The latter type was believed to be the plaque described by Keyes and Jordan (17) as associated primarily with periodontal lesions and root caries; it was recorded as + or −. Coronal plaque deposits were recorded as "heavy," "medium"

or "negligible"; periodontal pockets were recorded simply as + or −. The animals were then decapitated and the jaws were examined for dental destruction. The severity of caries was scored by the method of Keyes (18). Three caries scores were recorded for each animal: 1) a smooth surface score (S); 2) an occlusal pit and buccal-lingual fissure score (P); and 3) their summation, the total caries score (T).

Statistical treatment of data. The average terminal weights of the groups were tested for significance by a multivariate generalization of Student's *t* test adjusted for unequal variances and group sizes (19).

The total caries scores (T-scores), and the smooth surface scores (S-scores), were tested for significance between the experimental groups by repeated utilization of a nonparametric sum-of-ranks procedure for relative spread in unpaired samples, Wilcoxon's two-sample statistic (19).

RESULTS

The human cariogenic streptococcus, AHT-type, was recovered from all of the animals inoculated except two of the lactose animals. In no case was this organism recovered from a control animal, nor was there any recovery of human strains BHT or HHT from the control animals.

Lactose animals did not grow as well as the other groups and their terminal weight was significantly lower ($P < 0.01$). There was no significant difference in the terminal weights of the other groups. These data concur with the results reported by Gustafson et al. (6).

Only in sucrose and sucrose control groups was coronal plaque observed in more than negligible amounts. All of the experimental groups presented microscopic periodontal lesions and deposits of gingival plaque (table 2).

Caries progression study. The basal animals did not present clinical caries until day 100 at which time the mean Keyes caries score of 10 animals was 0.3 ± 0.4 out of a total possible score of 282. Progression curves of the caries scores of the other experimental groups are plotted in figure 1. The progression curves were drawn between the last zero Keyes caries score and the average caries score of the remaining animals (mean T-score) obtained at 100 days (demonstrated by vertical lines). Each point on the curve represents one animal unless a SD is indicated. The curves appear to fall into three definite sets or groupings.

The control and glucose progression curves are essentially similar. Caries onset was early — 28 and 22 days, respectively, but the caries score did not progress rapidly, nor was the final T-score high. The slopes of these curves indicate a slower rate of caries progression than the other groups.

Glucose-fructose, lactose and fructose groups also present comparable progression curves. Caries onset differed among

TABLE 2

Effect of various dietary sugars on growth, plaque formation, soft tissue lesions and streptococcal implantation in the Syrian hamster at 100 days

Group	No. of hamsters	Terminal wt (mean ± SD)	Gingival[1] plaque and soft tissue lesions	Coronal plaque	Recovery of streptococci	
					Contaminant[2]	AHT
		g				
Basal	10	116.3 ± 20.2	10	Negligible	—	+ to 4+
Control	13	127.1 ± 14.8	13	Medium	—	−
Glucose	13	128.5 ± 16.9	13	Negligible	—	± to 4+
Glucose-fructose	11	130.6 ± 26.7	10	Negligible[3]	—	± to 4+
Fructose	13	130.9 ± 11.8	12	Negligible	—	± to 4+
Lactose	18	94.7 ± 7.6[4]	18	Negligible	—	− to 3+[5]
Sucrose	9	126.7 ± 6.5	9	Heavy	—	3+ to 4+

[1] This column tabulates the number of animals within each group which presented gingival plaque or more than one periodontal lesion, or both.
[2] Animals were examined by the fluorescent-antibody technique for the presence of strains AHT, BHT and HHT.
[3] One animal within this group presented medium plaque deposits.
[4] The mean weight of this group was significantly different from all other groups ($P < 0.01$).
[5] AHT could not be recovered from two of the lactose animals.

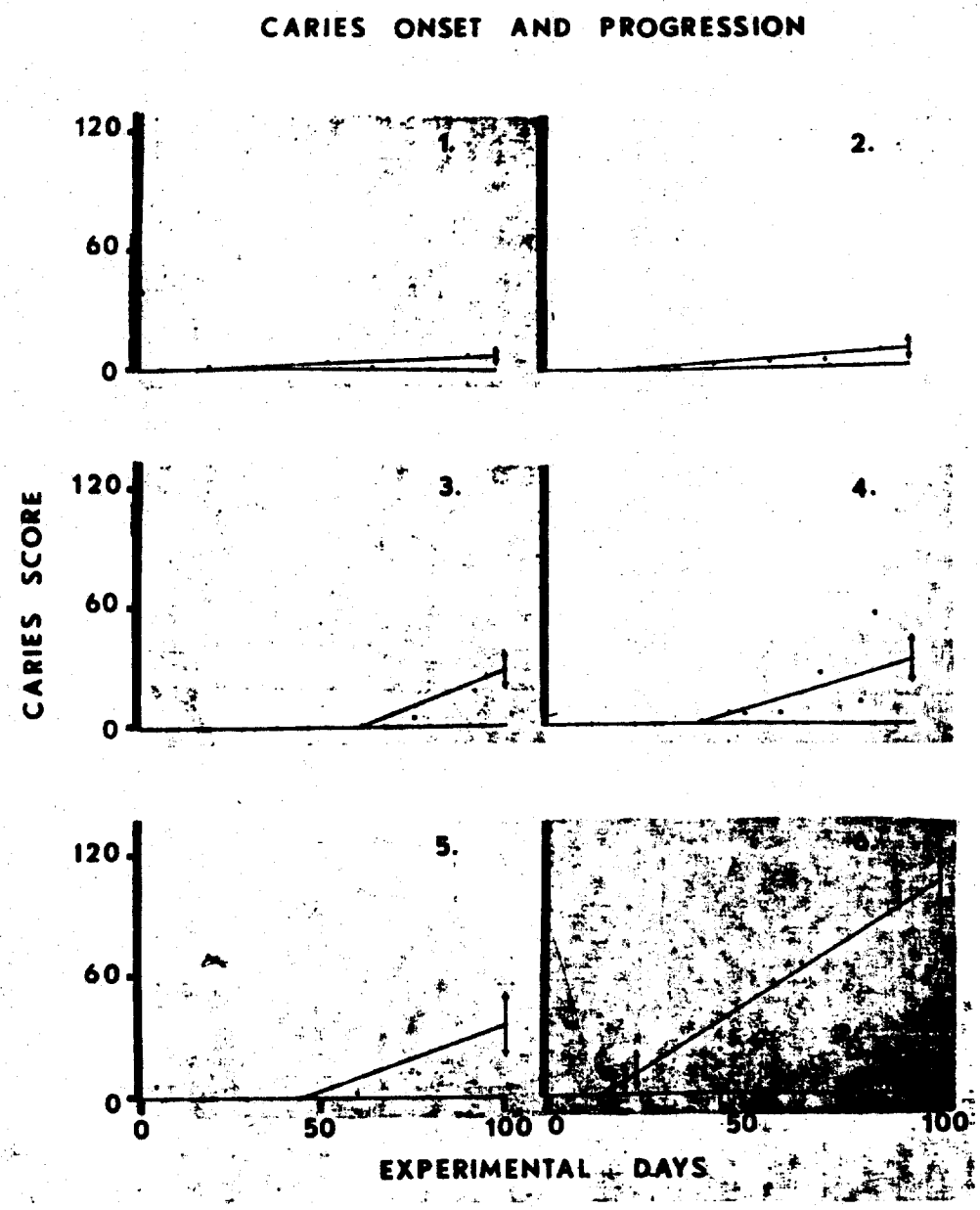

Fig. 1 Effect of the various dietary and microbial treatments on caries onset and progression in the Syrian hamster: 1) Sucrose control. 2) Glucose + AHT. 3) Glucose-fructose + AHT. 4) Lactase + AHT. 5) Fructose + AHT. 6) Sucrose + AHT.

these groups — days 62, 47 and 48, respectively, but the slopes of the curves are similar. The slope of the glucose-fructose curve, representing animals whose diet contained 20% glucose and 20% fructose, falls between the curves of the 40% glucose and the 40% fructose groups. This result should be expected if the curves represent a true comparison of caries progression.

The earliest onset — day 15, the most rapid progression and the highest total caries scores were obtained with sucrose plus AHT.

Smooth surface score (S-score) and total caries score (T-score). The mean S-scores and T-scores fell into the same four sets as the progression curves: a) basal; b) control and glucose; c) glucose-fructose, lactose and fructose; and d) sucrose (table 3, figs. 2, 3). These sets were significantly different from each other ($P \leq 0.002$), but the S-scores and the T-scores of the sugars within the sets were not statistically different.

DISCUSSION

Streptococcal recovery. Human cariogenic streptococcal strain AHT was recovered on day 100 from all of the experimental groups inoculated — including the basal group. This observation is noteworthy because Krasse (20) found that cariogenic streptococci implanted more easily on the Keyes cariogenic diet than on variations in which the lactose or sucrose portions had been replaced by glucose. In Krasse's study implantation ability was judged by the number of organisms recovered after swabbing the teeth with a cotton-tipped applicator stick. It should be mechanically easier to pick up the organisms when voluminous plaque is present (as in the sucrose animals) than when it is not (as in the case of the other diets). During sampling for the present study, chunks of plaque could be dislodged only from the sucrose animals — a fact that may account for their high AHT recovery rate. This observation suggests that the number of microbes counted on the slide may not be a quantitative measure of the actual numbers present in the mouth. In a recent paper Frostell et al. (10), using a similar recovery method, also suggest that present recovery methods are adequate to reveal the presence of cariogenic streptococci, but apparently are not adequate to quantify the bacterial population. The present data suggest that once AHT was placed in the mouth, it remained there in recoverable numbers regardless of the dietary carbohydrate administered.

Periodontal lesions, gingival and coronal plaque. All of the experimental diets fed in this study supported the formation of gingival plaque and all were associated with periodontal lesions — irrespective of whether or not sucrose was present. These data agree with those of Shaw et al. (11) who studied the results of 67% sucrose, glucose or a 1:1 mix of sucrose and lactose on the severity of periodontal lesions in the rice rat and found no essential differ-

TABLE 3

Effect of various dietary and microbial treatments on the total caries score (T-score) and the smooth surface score (S-score) in the Syrian hamster at 100 days

Group	No. of animals	S-score [1,2]	T-score [1,2]
		Keyes caries score units	
Basal	10	<0.2 [a]	0.3 ± 0.4 [a]
Control (uninoculated)	13	5.8 ± 2.0 [b]	6.9 ± 4.6 [b]
Glucose	13	6.1 ± 4.6 [b]	8.4 ± 5.7 [b]
Glucose-fructose	11	16.3 ± 5.0 [c]	29.5 ± 9.4 [c]
Lactose	18	22.0 ± 8.0 [c]	31.9 ± 11.6 [c]
Fructose	13	26.5 ± 10.2 [c]	37.3 ± 16.3 [c]
Sucrose	9	57.7 ± 13.8 [3,d]	106.8 ± 23.0 [d]

[1] Mean ± SD.

[2] Values in the same column with different letter superscripts differ significantly from each other, $P \leq 0.002$; values within the same superscript group, however, do not differ significantly from each other.

[3] The average sucrose animal presented approximately 40% of the total possible caries score at 100 days. Due to this massive destruction it was not possible to separate adequately the lesions which had originated on the smooth surfaces from those that had begun in the pits and fissures of the tooth; therefore, nearly equal values had to be assigned to the two areas.

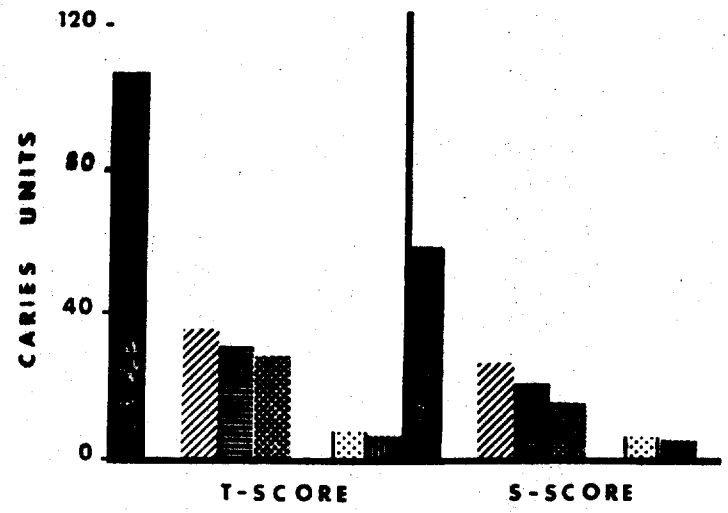

Fig. 2 Influence of various dietary and microbial treatments on the total caries score (T-score), and on the smooth surface score (S-score), in the Syrian hamster at 100 days.

Sucrose + AHT
Fructose + AHT
Lactose + AHT
Glucose-fructose + AHT
Glucose + AHT
Sucrose control

ence after an experimental period of 84 days.

Carlsson and Egelberg (7) conducted a study of coronal plaque formation in human subjects who ingested protein-fat diets with or without sucrose, fructose or glucose. They found that plaque was formed in every case, but that it varied as to pattern of development and amount. Glucose, fructose and the protein-fat diet alone supported ". . . thin and structureless plaque" over the entire buccal surface. A different type of plaque was observed with sucrose; one which ". . . had a striking resemblance to the firm, transparent, almost cartilaginous colonies formed by some strains of *Streptococcus salivarius* on sucrose agar media." This plaque was ". . . voluminous and turgid." Voluminous coronal plaque was observed in the study reported in this paper only in those animals which had been fed sucrose diets: data which also agree with those of Guggenheim et al. (9) and Frostell et al. (10). In summary: high dietary sucrose does not appear necessary to the development of gingival plaque or periodontal lesions, but transparent, gelatinous coronal plaque forms mainly on sucrose.

Smooth surface scores (S-scores). Guggenheim et al. (9) tested different dietary carbohydrates in rats during a 23-day experimental period. The authors reported that smooth surface lesions (as opposed to lesions originating in pits and fissures) occurred only in animals fed the sucrose diets and suggested that this might have been the result of the failure of other sugars to support the formation of coronal

Fig. 3 Photographs of the maxillae of representative animals within each set taken at a magnification of 10 diameters. Loss of the lingual alveolar bone can be seen in each photograph. The amount and severity of the visible carious lesions increase from no detectable caries in the basal + AHT group representative (photo 1) to the complete destruction of the second molar of the sucrose + AHT animal (photo 4). Between these two extremes are found photographs of the sucrose control animal (photo 2) presenting slight caries, and the fructose + AHT animal (photo 3) showing moderate caries.

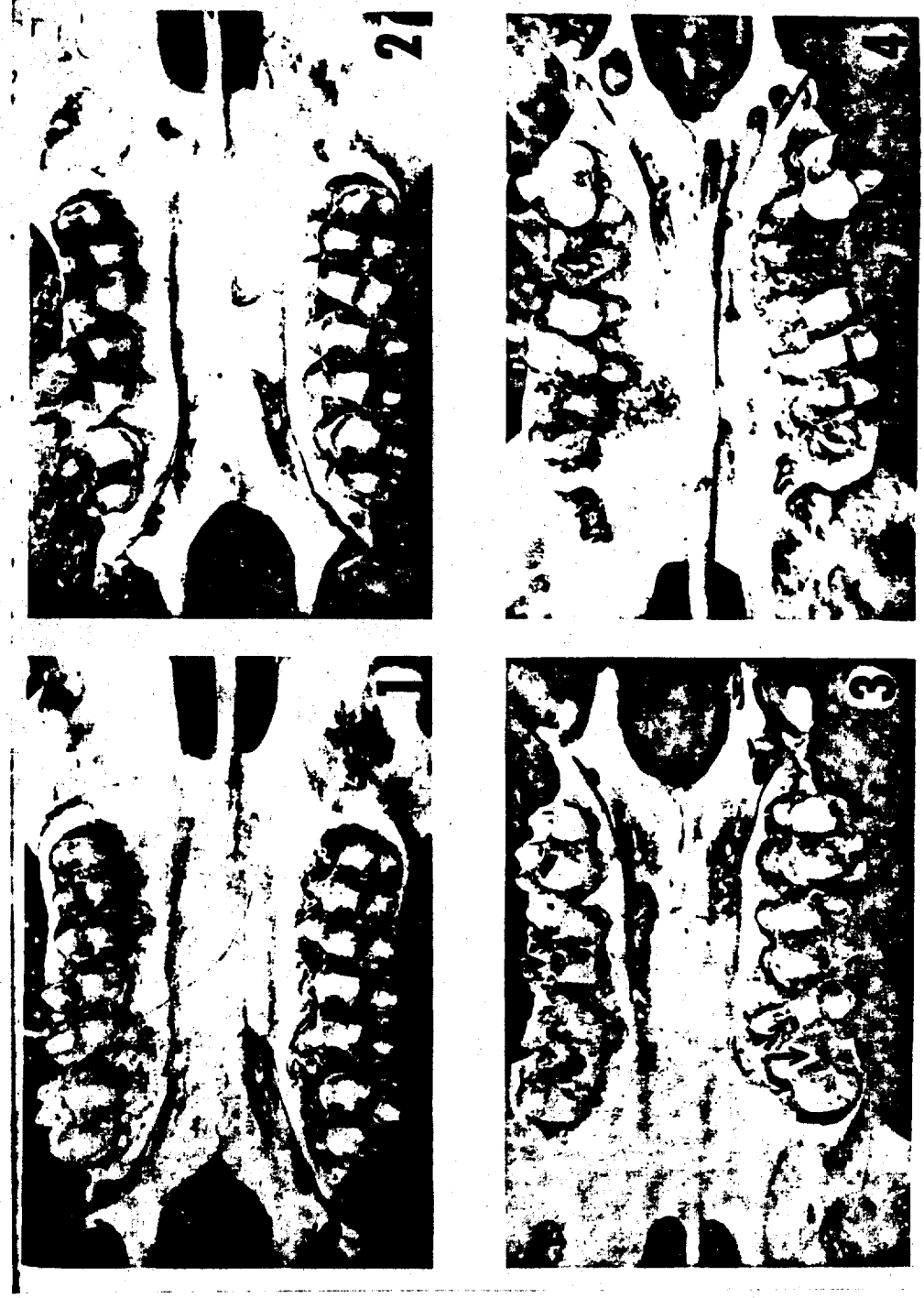

Figure 3

plaque. It is also possible that no smooth surface lesions were observed on sugars other than sucrose because the experimental period was not long enough for such caries to develop. Shaw et al. (11) reported the development of smooth surface lesions on sugars other than sucrose, but they also reported that the incidence of such lesions was higher on the sucrose diet than on the other experimental diets. In the present study the technique of recording three scores (smooth surface score, occusal pit and buccal-lingual fissure score and their summation, the total caries score) revealed the presence of smooth surface lesions in the animals of each group, regardless of the dietary sugar administered. The highest incidence of such lesions, however, was recorded in the animals fed the sucrose diet — the same animals which presented the greatest coronal plaque production. These data indicate that the formation of large amounts of coronal plaque is not essential to carious attack upon the smooth surfaces of the tooth, although it probably accelerates the process.

Caries progression and T-scores. Schweigert et al. (5) studied the effect of different dietary carbohydrates on the extent of dental caries in the cotton rat; the experimental period was 98 days. In their study no essential difference was found between the rats on sucrose glucose, fructose, maltose or lactose-sucrose diets, all of which supported a "very high incidence and extent of carious lesions . . ." A similar study conducted in Syrian hamsters harboring a cariogenic indigenous flora by Gustafson et al. (6), however, provided different results when 65% glucose, sucrose and lactose were each tested for experimental periods of 70, 110 and 150 days. These investigators found varying onset and progression curves among the sugars. Sucrose was accompanied by the earliest onset and highest terminal score at 150 days; glucose and lactose by very low scores at 70 days, but at 150 days caries scores had progressed to levels nearly half as high as those obtained from the sucrose animals. In a second study the investigators tested 65% glucose, fructose and sucrose for 150 days, and this time reported the same score for fructose as for sucrose; these authors concluded that fructose was as cariogenic as sucrose. In general, the data reported in this paper are consistent with the findings of Gustafson et al. Sucrose was the most cariogenic and had the most rapid progression; fructose was the second most cariogenic, but it did not attain a terminal score as high as that of sucrose. This discrepancy between our findings and those of Gustafson et al. may be due to the fact that in the present study the animals were fed a lower carbohydrate level or that the experimental period was only two-thirds as long, or both.

Krasse (8) studied the effect of the cariogenic streptococcal strain 3720 in hamsters fed diets in which the sucrose portion had been replaced by glucose. The experimental period was 56 days. He reported identical low caries scores for sucrose controls, glucose controls (i.e., uninoculated) and glucose inoculated animals, but reported a high caries score for the inoculated sucrose group. Strain 3720 is a substrain of HSR-6 (1) which is serologically related to AHT. In the present study low caries scores also were obtained with both the uninoculated sucrose controls and the glucose animals inoculated with AHT. The fact that Krasse had, in addition, a glucose control group with the same low caries score as his other two groups points up the possibility that AHT and related organisms are not cariogenic on a diet of glucose whereas other organisms of the indigenous flora are. The present study demonstrated that the uninoculated sucrose control group and the glucose group had similar onset dates, progression curves, S-scores and T-scores. A remarkable similarity in the lesion pattern of the two groups was noted during the scoring. Since the control group caries score must represent "background" caries produced by the indigenous flora (no AHT was ever recovered), the curves may be similar because the same indigenous flora, and not AHT, was responsible for both the glucose and the control lesions. This would explain why early experimenters, such as Schweigert et al. (5) and Gustafson et al. (6), noted higher caries scores with glucose than were observed here or in Krasse's study.

Cariogenicity of different dietary carbohydrates was tested in rats inoculated with

a cariogenic streptococcus, OMZ-61, by Guggenheim et al. (9). Sucrose was found to be significantly more cariogenic than fructose or glucose in this study; however, the length of the experimental period in their study was only 23 days. According to Gustafson et al. (6) and the present data, fructose and glucose would not be expected to support significant caries in such a short time.

In a study by Shaw et al. (11) sucrose, glucose and maltose diets fed to mutant rats for 60 days were indistinguishable in their cariogenicity; and a lactose-sucrose diet was less cariogenic. These investigators also reported that Harvard caries-susceptible rats on the same experimental regime had less caries with glucose and more with maltose. The authors suggested that these discrepancies within their own findings and those in the literature are based upon the interplay of at least three factors: the strain of laboratory animal, the type of diet and the resident oral flora. AHT, the human cariogenic streptococcus used as the inoculant, did not appear to be cariogenic with a glucose diet, but caused rampant caries with a sucrose diet. The interaction here of two of the factors — diet and oral flora — is apparent.

In the present experiment the most cariogenic sugar tested — or at least that which supported the most rapidly progressive process — was sucrose. However, dental destruction also developed in animals fed such carbohydrate substrates as fructose, lactose and glucose. Although the initial onset was slower and the progress of the disease less acute, extrapolation from progression curve scores indicates that considerable dental caries could be sustained within the lifetime of the hamster. This fact leads to the conclusion that time is also an important element in the interplay of factors involved in the development of dental caries.

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STUDY OF CARIES-FREE INDIVIDUALS: II. IS AN OPTIMUM DIET OR A REDUCED CARBOHYDRATE INTAKE REQUIRED TO ARREST DENTAL CARIES?

By Russel O. Collins,* D.D.S.; Arthur L. Jensen, D.D.S., and Hermann Becks, Dr. med., Dr. med. dent., D.D.S., San Francisco, Calif.

INTRODUCTION

At the meeting of the American Dental Association in Cleveland, July 1940, two views were expressed[1, 2] with regard to the prevention and control of dental caries by dietary measures. While the Iowa school stresses the point of view that "dental caries can be prevented and controlled in the majority of cases if the child or individual is in a normal physiologic state and if the person in question adheres to a complete and optimum dietary program," the Michigan group believes that "the oral lactobacillus count is not reduced and caries activity is not controlled by supplementing the diet with vitamin D and minerals unless the sugar content of the diet is reduced"; and "when the sugar in the diet is restricted, the lactobacillus counts are reduced and caries activity becomes retarded with or without other dietary supplements." It was furthermore reported by the Michigan school that an unusually low caries incidence was found among groups of children on low sugar diets which were deficient in vitamins A, C and D and calcium and phosphorus.

On the surface, there seems to be an extreme divergence between these two beliefs, one that an "optimum diet" and the other that "low carbohydrate consumption" is the deciding factor. However, there may likely be little difference between these two conditions. The elimination of sweets may bring about an increased use of other foods, and, again, the inclusion of a variety of whole foods may tend to decrease the use of sweets. In fact, if one analyzes the dietary program of the Iowa school it is evident that their "optimum diet" is substantially a sugar and starch free diet, which meets the specifications of the Michigan group. It appears to us that the only difference between the two schools is in the "labeling," in that one uses the label "sugar-and-starch-free," while the other uses "optimum"; and thus they are not necessarily in conflict. In order to obtain additional data on this rather controversial subject, it was believed that a detailed qualitative and quantitative analysis of the dietary intake of a group of caries-free individuals and a contrasting group with active caries was highly desirable.

WHAT IS AN OPTIMUM DIET?

Search for a clear definition of optimum diet met with difficulties. The current

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*Deceased.

literature fails to supply either a concise definition of the term or specifications for its fulfilment. It was, therefore, decided to obtain as much information as possible from some of the outstanding nutritionists, physiologists, biologists and clinicians concerning their concept of an optimum diet. The twenty-six answers received in response to our request were at great variance. It was not surprising to learn that a large number of those replying believed that because of the incompleteness of the present knowledge of nutritional requirements, a specific formula could not be written, and even if we should arrive at a compromise suitable to all at the present time, such a conclusion could be of only temporary value and would have to be revised from time to time to accommodate current findings.

TABLE 1.—LACTOBACILLUS ACIDOPHILUS STUDIES OF 366 STUDENTS OF THE UNIVERSITY OF CALIFORNIA

Group	Lactobacillus Acidophilus Index	Number of Cases	Percentage
1. Caries-free	High	23	18.9
	Zero	99	81.1
2. Active or inactive	High	50	40.9
	Zero	72	59.0
3. Rampant caries	High	107	87.7
	Zero	15	12.3

Blayney, Bogert, Davis, Fosdick, Howe, Jay, McCollum, Price, Sherman, Sure and Walsh expressed the general point of view as to the definition and requirements of an optimum diet as follows:

An optimum diet may be defined as one upon which an organism may function with maximum biologic efficiency. However, since the term "optimum" expresses an ideal rather than anything that can yet be put into the form of a prescription, it should be used only to refer literally to the best and should not be applied to diets which are merely better than adequate. Furthermore, the concept of what constitutes an optimum diet must be ever-changing, in accordance with new discoveries in the field of nutrition and with the wide zone which exists between a state of nutrition that is merely adequate and one that is desirable or optimum. It is doubtful that a reasonable generalization can be arrived at owing to the bizarre differences in individual requirements. Nearly all discussions assume that human beings are comparable in the matter of their susceptibility to dental caries. On this basis, a nutrition that would be adequate for one would be applicable for all; but it is not so simple as this, since the diet that is adequate for one individual may not be for another. While it is known that a liberal supply of all essential factors represents a favorable condition, it is not yet possible to state for most nutritive essentials just what level of intake gives maximum health benefits, further increases possibly being needless or even detrimental. It will be apparent that recommendations for an optimum diet are merely generalizations which will vary with the observer's interpretation as to what the evidence now in hand indicates with relation to where the optimum level of life may lie for the individual in question; and since the term optimum diet carries an expression of finality, there can never be such a thing as the optimum diet and it cannot be defined now or at any other time as a diet consisting of 1 quart of milk, glass of orange juice, etc.

The difficulty in speaking of an optimum diet without full qualification of the term is expressed in a statement by Francis Scott Smyth, head of the pediatrics department of the University of California Medical School: "If human beings were as uniform in familial and inheritance characteristics as a colony of rats, an optimum diet could be reliably

appraised. As this is not the case, we are faced with a multitude of racial, hereditary, familial and climatic factors which introduce important variables; for example, the optimum diet of the Eskimo is quite unlike that of the negro in the bayous of Louisiana. While these are extreme illustrations, one may find a myriad of examples of varying tastes and digestive capacities which probably affect the utilization of diet." Thus it can be seen that, with regard to a clear definition of what constitutes an optimum diet for our own people and specifications for its fulfilment, there is considerable variation of opinion among leading authorities in the field of nutrition, physiology and biochemistry as to our ability to define the term comprehensively, much less to speak of its specific requirements.

TABLE 2.—EVALUATION OF FOOD INTAKE OF 366 STUDENTS

	Group 1 122 Individuals Caries-Free					Group 2 122 Individuals Clinically Caries-Free X-Rays Showing 4-28 Caries Areas					Group 3 122 Individuals Five or More Open Caries Areas				
	1+	2+	3+	4+	Adequacy Per Cent	1+	2+	3+	4+	Adequacy Per Cent	1+	2+	3+	4+	Adequacy Per Cent
Protein		5	15	102	94.9			6	116	98.8		1	7	114	98.2
Calcium		14	17	91	90.8		17	9	96	91.2	2	15	21	84	88.3
Phosphorus		6	14	102	94.7			8	114	98.4		2	6	114	98.0
Carotene	1	8	112	1	73.2		30	84	8	70.5		19	77	26	76.4
Thiamine	2	119	1		49.8		105	16	1	53.7		111	11		52.3
Ascorbic acid	1	12	37	72	86.9	3	27	51	41	76.6	1	15	44	62	84.2
Vitamin D			31	91	93.6		3	38	81	91.0		3	45	74	89.5
Concentrated sweets	Teaspoonfuls of sugar: 10.4					Teaspoonfuls of sugar: 11.6					Teaspoonfuls of sugar: 17.9				

IS A REDUCED CARBOHYDRATE INTAKE PERMISSIBLE FROM A STANDPOINT OF GENERAL HEALTH?

It has been stated repeatedly by the Michigan school that the prevention and control of dental caries by a reduction of *Lactobacillus acidophilus* in the oral cavity are largely dependent on a reduction of sugar alone or sugar and starch. In limiting these easily available and economical sources of energy, it is, of course, important that we be familiar with the nutritional requirements of carbohydrates, which, according to McCollum,[3] "vary according to the activity of the individual." He continues: "It is believed that the carbohydrates furnish about 60 per cent of the fuel supply of the average normal adult. The minimum amount of carbohydrates necessary for maintenance of life, and the prevention of ketosis, is from 10 to 15 per cent of the total energy requirement." This means that the average carbohydrate intake today may often be several hundred grams per day. In fact, many persons use several hundred grams each day of refined carbohydrates alone. The popular lay concept is to the effect that this amount is essential for energy and health. This view obviously is inconsistent with

the observation of patients on diabetic and ketogenic diets, whose carbohydrate intake is limited drastically without impairment of physiologic well-being. A typical diabetic diet often permits the intake of 100 gm. or less of carbohydrates per day, and such diets have long been recognized as desirable and healthful.

In exploring the still lower limits of carbohydrate exclusion possible without jeopardizing physiologic well-being, it is found that the epileptic diet may be even more extreme. Carbohydrates are often limited to 50 gm. daily and frequently to as little as from 25 to 30 gm. daily, a definite ketosis resulting. Considering that such diets are continued for months at a time with resultant good health, it must be appreciated that limitation of carbohydrates, generally speaking, is permissible.

Therefore, since it appears possible, from a medical point of view, to experience favorable health on a low carbohydrate intake, and, furthermore, since persons on a low carbohydrate intake, as, for instance, in diabetes, are observed to have a reduced caries frequency,[4] the necessity of a comparative study of the nutritional status of the caries-free and the caries-active who were comparable in other respects has become urgent.

TABLE 3.—EVALUATION OF FOOD INTAKE OF CARIES-IMMUNE AND CARIES-SUSCEPTIBLE

	A Caries-Immune L. Acidophilus Negative 99 Individuals					B Caries-Susceptible L. Acidophilus Positive 107 Individuals				
	1+	2+	3+	4+	Per Cent	1+	2+	3+	4+	Per Cent
Protein	—	1	6	92	98	—	4	13	90	95.0
Calcium	1	14	13	71	89	—	11	14	82	91.6
Phosphorus	—	2	5	92	97.7	—	5	12	90	94.8
Carotene	—	18	60	21	75.7	1	6	99	1	73.3
Thiamine	—	91	8	—	52.	2	104	1	—	49.8
Ascorbic acid	1	12	37	49	84.8	1	12	33	61	86.0
Vitamin D	—	2	38	59	89.5	—	—	25	82	94.1
Concentrated sweets	10.5 teaspoonfuls					18.2 teaspoonfuls				

COMPARATIVE NUTRITIONAL STUDIES OF THE CARIES-FREE AND THE CARIES-ACTIVE

1. *Selection of Material.*—For this investigation, an attempt was made to obtain large numbers of adults with or without dental caries who were comparable as to age, sex, social and educational status, etc. In many previous investigations, persons who, at the time of the clinical examination, showed no evidence of caries, have been designated as caries-immune, even though caries immunity cannot be determined by clinical examination alone. Again, in others, caries activity has been determined on the basis of the unrepaired results of destructive processes in enamel and dentin, even though, at the time of examination, the caries process might have been inactive. In order to obtain a sufficiently large number of the completely caries-free, all students who entered the University of California in Berkeley in August 1939 and 1940 were examined clinically, and those found to be caries-free

roentgenographed in order to be sure that no hidden cavities were overlooked. It may be interesting to note that of the large number of students who were caries-free by clinical examination, only about one-fifth were completely free according to the dental roentgenogram. By this means, 122 caries-free were obtained for this study. However, to be certain that there was no caries activity present which the foregoing examinations did not reveal, a determination of *L. acidophilus* index was made on two successive days. It was found that ninety-nine of 122 had a negative *L. acidophilus* index, while twenty-three were positive. In percentage figures, 81.1 per cent were negative and 18.9 per cent were positive. This group is designated as Group I. (Table 1.)

A second group of 122 persons (Group 2) consisted of students who had no caries on clinical examination, but showed definite evidence of enamel defects and sometimes involvement of dentin in dental roentgenograms, having from four to twenty-eight areas per person. A large number of these defects were small nicks in the enamel on the proximal surfaces, while others showed definite involvement extending well into the dentin. It was found that seventy-two students, or 59 per cent of this group, had no *L. acidophilus*, which suggests that these were caries-inactive at the time, although, at some previous time, caries must have been sufficiently active to afford roentgenographic evidence of defects. The fact that 40.9 per cent were *L. acidophilus* positive suggests an active state of caries at the time.

Finally, a third group was selected, here referred to as Group 3, which again consisted of 122 individuals who had rampant caries, with five or more open cavities evident on clinical examination. Here the *L. acidophilus* examination (Table 1) revealed the antithesis of the condition of Group I. While 12.3 per cent had negative counts, 87.7 per cent had positive.

These data, i.e., the large percentage of negative counts in the caries-free group (Group 1) and the high percentage of positive counts in the rampant caries group (Group 3), support the concept of the Michigan school and our concept that the majority of caries-free persons have no *L. acidophilus* or have insignificant counts, while the counts of persons having rampant caries are consistently high. Thus it must be recognized that the *L. acidophilus* test of saliva is important, reliable and valuable for the determination of the current dental caries status.

2. *Procedure in Evaluating Food Intake.*—It is generally recognized that the evaluation of individual food habits in the past and present by taking dietary histories is obviously subject to a wide range of error, primarily due to variation in the accuracy of recollection, integrity and honesty of the patient as well as the thoroughness and experience of the recorder, and the question arises, "Can we be sure, on the basis of present dietary histories, as extensive as they may be, that our recording and evaluating of food intake are correct?" We must recognize that we are badly in need of analytic methods of determining whether intake standards, for instance calcium and phosphorus, iron, carotene, thiamine, ascorbic acid* and vitamin D, are being met individually and whether these nutrients are actually present in the body (Christie). However, until such standards have been established, we shall have to rely more than ever upon more accurate dietary analyses, even though these have to give way to biochemical and physical methods for determining validity.

However, in view of the fact that pre-

*The terminology for the vitamins now preferred is: thiamine for B_1 and ascorbic acid for C. Carotene is mentioned as provitamin A as well as vitamin A (Morgan).

cisely objective methods were not available, a uniform procedure was developed and used in this investigation having as its aim determination of the quality and quantity of diets with regard to an "arbitrary standard level," as well as the amount of carbohydrate intake. Emphasis was placed on a personal interview with each student which was conducted by two examiners, who recorded the intake of the following popular foods:

Meat: Kind and number of servings (weight in ounces) per day or week.

Eggs: Number per day or week as such or in cooking.

Milk: Amount in glasses; also milk products, such as cheese.

Vegetables: Kind and amount, expressed in number of average servings, cooked or raw.

Fruit: Kind and amount, also considering fruit juice.

Cereal: Kind and amount, also considering cereal products.

Bread: Kind and amount, in slices per day.

Butter: Amount daily as such and in cooking.

Dessert: Kind and number of average servings per day or week.

Jam, jelly, syrup, honey: Amount in teaspoons per day or week.

Dates, figs, raisins, currants, prunes: Amounts.

Candy: Number of chocolate bars or equivalent in other candies per day or week.

Chewing gum: Sticks per day, week or month.

Soft drinks and other confections: Kind and amount per day, week or month.

Sugar: Number of teaspoons in or on food per day, not listed above.

Cod liver oil: Amount and duration of use; and duration, frequency and extent of sun exposure.

From this recording of food intake, qualitative and quantitative evaluations were made of carbohydrate as well as of other nutrients.

EVALUATION OF CARBOHYDRATE INTAKE

In the evaluation of carbohydrate intake, the teaspoon of granulated sugar was used as the unit of measure by which to express the amount contained in the diet. The approximate refined carbohydrate content of popular foods was expressed in amounts equivalent to teaspoonfuls of sugar. These values were computed from gram expressions of carbohydrate content of foods in "Food Values of Portions Commonly Used," by Bowes and Church,[5] as well as other publications giving values of popular foods.[6, 7, 8]

100 gm. sugar—20 teaspoonfuls—one-half cup—3½ ounces—400 calories

Candy: From 75 to 85 per cent sugar. Popular candy bars are likely to weigh from 1 to 5 ounces and may contain from 5 to 20 teaspoonfuls of sugar.

Hershey bar: 61 gm. in 5-cent size	7*
Chocolate creams: 13 gm. (35 to 1 pound)	2
Chocolate fudge: 30 gm. (1½ inch square, 15 to 1 pound)	4
Butterscotch: 5 gm. (1 by 1 by ¼ inch)	1
Chocolate mints: 22 gm. (1 medium, 20 to 1 pound)	3
Life Saver: 1 usual size	½
Chewing gum: 1-cent stick	½
Chocolate sauce: 30 gm. (1 heaping tablespoonful, thick)	4½
Marshmallow: 7.6 gm. (1 average, 60 to 1 pound)	1¼
Jam: 20 gm. (1 tablespoonful, level, or 1 heaping teaspoonful)	3
Jelly: 20 gm. (1 tablespoonful, level, or 1 heaping teaspoonful)	2½
Marmalade: 20 gm. (1 tablespoonful, level, or 1 heaping teaspoonful)	3
Syrup, maple: 20 gm. (1 tablespoonful, level, or 1 heaping teaspoonful)	2½
Honey: 20 gm. (1 tablespoonful, level, or 1 heaping teaspoonful)	3
Angel food cake: 45 gm. (1 piece, ½ of large cake)	6
Chocolate cake: 100 gm. (2 layer icing, ½ cake)	15
Sponge cake: 50 gm. (1/8 of average cake)	6
Cream puff (iced): 80 gm. (1 average custard-filled)	5
Doughnut (plain): 40 gm. (3 inches in	

*Figures in this column indicate number of teaspoonfuls of sugar.

diameter) 4
Macaroons: 25 gm. (1 large or 2 small) 3
Gingersnaps: 6 gm. (1 medium) 1
Molasses cookies: 15 gm. (3½ inches in diameter) 2
Brownies: 30 gm. (2 by 2 by ¾ inch) ... 3
Custard, baked: (½ cup) 1
Brown Betty: 130 gm. (½ cup) 9
Gelatin: (½ cup) 1
Junket: (⅛ quart) 3
Icecream: (⅛ quart) 5-6
Water ice: (⅛ quart) 6-8
Apple pie: ⅙ medium size pie 12
Cherry pie: ⅙ medium size pie 14
Custard, cocoanut pie: ⅙ medium size pie 10
Raisin pie: ⅙ medium size pie 13
Pumpkin pie: ⅙ medium size pie 10
Chocolate, all milk: (1 cup, 5 ounces of milk) 6
Cocoa, all milk: (1 cup, 5 ounces of milk) 4
Cocomalt, all milk: (1 glass, 8 ounces of milk) 4
Eggnog, all milk: (1 glass, 8 ounces of milk) 4½
Coca Cola: 180 gm. (1 bottle, 6 ounces) 4⅓
Gingerale: 180 gm. (6-ounce glass) 3⅓
Peaches, canned in syrup: 100 gm. (2 halves, 1 tablespoonful of juice) 3½
Sweet cider: 180 gm. (6-ounces glass) .. 4½
Rhubarb, stewed: 100 gm. (½ cup, sweetened) 8
Apple sauce (no sugar): 100 gm. (½ cup, scant) 2
Apple sauce (sweetened): 100 gm. (½ cup, scant) 4
Prunes, stewed, sweetened: 100 gm. (4 to 5 medium size, 2 tablespoonfuls of juice) 8
Apricots, dried: 30 gm. (4 to 6 halves) .. 4
Dates, dried: 30 gm. (3 to 4, stoned) ... 4½
Figs, dried: 30 gm. (1½ to 2, small) 4
Raisins: 30 gm. (¼ cup) 4
Currants, dried: 30 gm. (2 tablespoonfuls) 4
Prunes, dried: 30 gm. (3 to 4, medium size) 4
Persimmons, fresh: 100 gm. (½ cup) 7
Orange juice: 100 gm. (½ cup, scant) 2
Fruit cocktail: 120 gm. (½ cup, scant) .. 5
Pineapple juice, unsweetened: 100 gm. (½ cup, scant) 2⅗
Grapefruit juice, unsweetened: 100 gm. (½ cup, scant) 2⅓
Grape juice, commercial: 100 gm. (½ scant) 3⅓

EVALUATION OF PROTEIN, MINERALS AND VITAMINS

For the purpose of qualitative and quantitative comparison of nutritional intakes, it is, of course, necessary to have some working hypothesis. Therefore, the following nutrients were considered in this investigation on the premise that if adequate quantities were supplied from natural food sources, other nutrients would probably be present in like amounts: protein, calcium, phosphorus, carotene, thiamine, ascorbic acid and vitamin D.

A quantitative evaluation of these factors in each diet was made, using the following arbitrary standard, which was taken from the 1941 adult needs (arbitrary standards) set up by the Food and Nutrition Committee of the National Research Council:

Protein: 60-70 gm.
Calcium: 0.8 gm.
Phosphorus: 1.2 gm.
Carotene: 5,000 I.U.
Thiamine: 1.2-2.3 mg.
Ascorbic acid: 70-75 mg.
Vitamin D: When not available from sunshine, probably up to 400 I.U.

The estimate of vitamin D was based on habits of sunshine exposure as well as supplements of D concentrates, since little or none was contributed by routine food intake.

Other vitamins of the B complex than thiamine which are now well understood and for which dietary estimates may henceforth be made are riboflavin (formerly called G or B_2) and nicotinic acid. Almost as well established are pyridoxine (formerly called B_6) and pantothenic acid. From experimental work in progress, it seems that one or more of these is far more likely to be concerned in caries than any of those for which analysis is offered in this paper. This provides an interesting prospect for further investigation.

The method used to express quantitative evaluations of the nutrients in each diet is as follows:

+, up to 25 per cent of amount expressed in the foregoing standard.
+ +, up to 50 per cent of amount expressed in the foregoing standard.
+ + +, up to 75 per cent of amount expressed in the foregoing standard.
+ + + +, up to 100 per cent of amount expressed in the foregoing standard.

Thus, a very simple summary may express an evaluation of the adequacy of each diet in supplying various nutrients as compared with a stated standard, as well as an evaluation of sweets indicated in units of measure equivalent to teaspoonfuls of sugar. Though mathematical, factual or analytical accuracy is not claimed for these evaluations, it is believed that they have comparative value since all cases here reported were recorded by the same method and evaluated qualitatively and quantitatively on the same basis.

RESULTS

With the above-mentioned shortcomings of any recording of food intake in mind, painstaking efforts were made to maintain comparative values on the data in the three contrasting groups listed in Table 1. A summary of results is given in Table 2.

In comparing the percentage adequacies of food intake of Groups 2 and 3, it becomes evident from Table 2 that there are only very small variations among the three groups for the various items protein, calcium, phosphorus, carotene, thiamine, ascorbic acid and vitamin D. In fact, the differences are so small that they can be ignored for practical purposes. No severe inadequacies in any of these important food elements appear. All three groups are comparable in satisfying nutritive requirements, which fails to explain why Group 1 remained caries-free, while Groups 2 and 3 developed caries. It appears, therefore, that the findings of the Iowa school, which led to the conclusion that an optimum diet is required for the control of dental caries, cannot be confirmed by this investigation.

It is therefore imperative to take into consideration other possible factors, in particular the carbohydrate intake of these individuals. A comparison of carbohydrates derived from such foods as vegetables, fruits and cereal products revealed no significant difference among the three contrasting groups. However, an analysis of the sugar intake as such and the sugar content of sweet foods, such as candy, desserts, jam, jelly, syrup, honey, dates, figs, raisins, currants, prunes, pastries, soft drinks and other confections, gave the following surprising results (Table 2):

Group 1: Average 10.4 teaspoonfuls of sugar daily.
Group 2: Average 11.6 teaspoonfuls of sugar daily.
Group 3: Average 17.9 teaspoonfuls of sugar daily.

This contrast between Groups 1 and 3 seems to be of significance because the individuals in the latter group consume an average of 7½ teaspoonfuls more of sugar per day than those in Group 1.

Since there was such a striking confirmation of the association of *L. acidophilus* with dental caries in that ninety-nine of 122 caries-free individuals (Group 1) had no *L. acidophilus* and 107 of 122 caries-active individuals (Group 3) had high *L. acidophilus* counts, these ninety-nine and 107 students were accepted as truly caries-immune and caries-susceptible, respectively, and were revaluated as Group A and Group B in Table 3 with regard to their dietary régime.

Here, the results of adequacy values and carbohydrate intake are similar to those of the entire group. Expressed in teaspoonfuls of sugar, the caries-suscept-

ible group (B) consumed an average of 7.7 more per day than the caries-immune group (A). Again, this appears to be the only significant difference in the food intake of these two groups.

It is interesting to observe that even though Group A was completely caries-free, a number of individuals consumed more than 10.5 teaspoonfuls per day. On the other hand, there were a number of caries-susceptible students in Group B who had less than 10.5 teaspoonfuls and developed rampant caries. The sources of these sweets were similarly distributed, among sugar, sweet desserts, chewing gum, ice cream, soft drinks, life savers and other confections. This strongly suggests that each individual may have a carbohydrate limit within which he continues free of dental caries and which, when exceeded, exposes him to the ravages of this increasingly prevalent disease.

SUMMARY AND CONCLUSIONS

Because of the great variance in concepts regarding the etiology of dental caries, especially as to the influence of nutritional conditions, a survey was undertaken to study the character of food intake of a large number of persons completely free from tooth decay in comparison with a like number having rampant caries.

For this study, 366 students of the University of California of comparable age, sex, race, environment and occupation were selected on the basis of (1) clinical dental examination with mirror and explorer, (2) roentgenographic examination of all teeth and (3) *L. acidophilus* studies of the oral cavity.

The group was divided into three groups of 122 each as to caries absence, questionable caries activity and definite caries.

By a uniform procedure, detailed dietary histories were taken of each individual at personal interviews and the adequacy of dietary intake was evaluated against a stated "arbitrary standard."

Because of the fact that definition of an optimum diet as well as specifications for its fulfilment presented great difficulties, owing to the variation in individual requirements and in concept and to incompleteness of knowledge, it is suggested that this confusing term be abandoned.

The results of this investigation can be summarized as follows:

1. *L. acidophilus* studies of the three distinct groups of 122 students each revealed that:

(a) In the completely caries-free group (Group 1), ninety-nine, or 81.1 per cent, had no *L. acidophilus* (Group 2 [C]).

(b) In 107 students, or 87.7 per cent, of the rampant caries group (Group 3), there were very high *L. acidophilus* counts.

(c) In Group 2, which included students with questionable caries activity, seventy-two, or 59.0 per cent, had no *L. acidophilus*.

2. The findings under paragraph 1 show a striking correlation between the presence and absence of *L. acidophilus* in saliva with caries activity and with freedom from caries respectively.

3. Analysis of food intake with regard to protein, calcium, phosphorus, carotene, thiamine, ascorbic acid and vitamin D of all 366 students showed clearly that these food essentials were not more deficient in the rampant caries group than in the caries-free group and not more adequate in the caries-free group than in the caries-active group. Therefore, on the basis of nutritional adequacy, or any concept of a so-called optimum diet, no one group was superior to the other, and yet one developed rampant caries, while the other remained caries-free. It appears doubtful, from these studies, that the caries incidence can be favorably influenced by increasing certain food elements so vigorously advocated currently.

4. While carbohydrates derived from

vegetables and cereal products were similar in amounts for all three groups, the refined sugars and concentrated sweets averaged for Group 1, 10.2 teaspoonfuls of sugar daily; Group 2, 11.6, and Group 3, 17.9. These values present the only striking differences in the food intake of the three contrasting groups, especially between Groups 1 and 3.

5. Because the caries-free group (Group 1) included twenty-three individuals with high *L. acidophilus* counts, and the rampant caries group (Group 3), fifteen with no *L. acidophilus*, which does not conform with our concept of caries immunity and caries susceptibility, respectively, for the purpose of this study these were eliminated from the two groups and the remainders again statistically evaluated.

The new Group A consisted of ninety-nine students who clinically and roentgenographically were found to have no caries and no *L. acidophilus*. Group B consisted of 107 students with rampant caries (clinically and roentgenographically) and high *L. acidophilus* counts. Their dietary evaluations revealed the same lack of difference, except in sugars and concentrated sweets. Here, the difference was even more pronounced. Caries-immune Group A averaged 10.5 teaspoonfuls of refined sugar and concentrated sweets, and caries-susceptible Group B, almost twice the amount, or 18.2 teaspoonfuls.

6. A number of individuals in the caries-immune group had extremely high, and a number in the caries-susceptible group very low, concentrated sweets and sugar intake. This suggests that the carbohydrate consumption alone does not explain the absence or presence of dental caries in these cases, and that other etiologic factors must be sought.

7. The similarity in the degree of adequacy of nutritional intake of these two contrasting groups fails to support the concept that a so-called optimum diet is required for the prevention of dental caries.

8. The higher concentrated sweets intake in the caries-active group, associated with a high *L. acidophilus* count and high caries incidence, indicates a direct relationship among these three factors: concentrated sweets; *L. acidophilus*; caries. Therefore, a reduced carbohydrate intake seems to be at least one means of decreasing the caries incidence and comes closer to the solution of the problem of dental caries prevention than present expressions of nutritional adequacy.

It must be realized that this investigation is based on a study of a highly selected group of American youth which constitutes approximately 1 per cent of all students examined. It is not to be overlooked that such factors as heredity, familial tendencies, environment, geographic location and possibly dietary essentials not assessed in this study may play a rôle in conferring upon this small percentage of persons an unusual resistance to dental caries. Further evaluation of these caries-free cases will be reported separately.

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COMMITTEE ON MEDICAL ASPECTS OF FOOD POLICY

Panel on Cariogenic Foods: First Report

1. Terms of Reference

'To consider the cariogenic action of foods with particular reference in the first place to vitamin preparations as used in babies' comforters, and to make recommendations.'

Membership

Chairman:

Dr. T. E. Oppé, M.B., B.S., F.R.C.P., D.C.H., Director Paediatric Unit, St. Mary's Hospital Medical School.

Members:

Dr. M. J. Barnes, M.A., Ph.D., member of Research Staff Dunn Nutritional Laboratory, M.R.C. and University of Cambridge.

Dr. V. F. C. Berry, M.A., M.D., D.T.M. & H., Principal Medical Officer, Ministry of Health.

Dr. W. H. F. Bowen, B.D.S., M.S.D., Ph.D., F.F.D., Research Fellow, Department of Dental Science, Royal College of Surgeons of England.

Professor R. Hartles, Ph.D., D.Sc., Professor of Dental Science, University of Liverpool.

Professor P. M. C. James, M.D.S., L.D.S., D.P.D., Professor of Dental Health, University of Birmingham.

Professor G. L. Slack, *O.B.E.*, *F.D.*, D.D.S., F.D.S., Dip. Bact., Dean of Dental Studies, London Hospital Medical College.

Professor G. B. Winter, M.B., B.S., B.D.S., F.D.S., D.C.H., Professor and Head of Department of Children's Dentistry, Institute of Dental Surgery, Eastman Dental Hospital.

Secretaries:

J. Rodgers, *D.F.M.*, L.D.S., Ministry of Health,

Joan M. Firth, Ph.D., Ministry of Health.

2. Definitions and Descriptions

2.1. The following definition was accepted by the panel:

Rampant caries is a lesion of acute onset involving many or all of the erupted teeth, rapidly destroying coronal tissue, often on surfaces normally resistant to decay and leading to the early involvement of the dental pulp and when involving the deciduous dentition occurs typically in very young children soon after their teeth have erupted.

2.1.1. The panel believe that rampant caries is harmful to children afflicted by the disease because they do not reach that state of well being to which they are entitled and to which they would normally attain.

2.2. Certain other terms used in this report may require explanation.

Erosion refers to a generalised dissolution of the enamel surface of the tooth; it should not be confused with caries which is initiated in localised positions, particularly less self-cleansing areas where bacterial invasion follows.

The Reservoir Feeder is the name given to a feeding bottle of very small capacity. The essential difference between reservoir and ordinary feeding bottles is that the former, being lighter, can be retained longer in a baby's mouth; they are intended to act as comforters rather than as means of nourishment. The essential distinction between a reservoir feeder and a dummy is that the latter contains no fluid; if sweet fluids or other foods such as sugar are used at all with dummies it is by dipping the dummy into them.

3. Nature of Evidence

3.1. Published and unpublished material relating to

(*a*) the prevalence of rampant caries;

(*b*) the association between dummies, reservoir feeders and dental caries;

(*c*) the association between caries and the use of vitamin supplements;

(*d*) the association between erosion and the use of vitamin supplements.

4. Scope of Report

4.1. This report deals only with rampant caries as defined.

4.1.1. Rampant caries is not a new phenomenon. Pitts (1927), James *et al.* (1957) and Winter *et al.* (1966) have described it in England and Beltrami and Romieu (1939), Syrrist and Selander (1953) have described it abroad. It is encountered wherever bags of honey or other sweeteners have been used frequently to comfort babies. What is new is the belief of dentists that there has been a substantial increase in rampant caries since the war. James *et al.* (1957) who referred to it as labial caries and Winter *et al.* (1966) have commented on the frequency with which they encountered severe cases. We therefore examined the evidence on prevalence, both to try to ascertain the magnitude of the problem and to discern whether or not the incidence of the disease had increased. We also examined the causes of the disease, past and present, and concluded by suggesting possible ways to prevent, delay, or mitigate it.

5. The Prevalence

5.1. In establishing prevalence use has been made of information obtained from fluoridation studies in different areas within the U.K. These studies were carried out in certain places only and the findings do not necessarily apply to the country as a whole. All caries was recorded, no specific distinction being made between rampant and other caries. We have taken as an index of the former the incidence of young children with ten or more carious teeth (Appendix A, Table I). The relevant figures have been taken from the report on fluorida-

tion studies in the United Kingdom (Ministry of Health, 1962) together with unpublished data from current studies. Bransby and Heasman (1967) in an unpublished paper entitled 'A study of the association between dental caries, the taking of vitamin supplements, eating between meals and the use of a dummy' found considerably less rampant caries than that recorded in the fluoridation studies (Appendix A, Table IV). They studied children from Birmingham, Middlesex, and Northumberland and found that differences of habit and even heredity might account for variations (Appendix A, Table II).

Variations in the standard of recording may lead to errors in dental survey work and statistics must be critically examined lest this should be the case. In a survey by Goose and Gittus (1967) over 5,500 children were examined and the prevalence of incisor caries found to be 5·9 per cent (Appendix A, Table III). In this survey health visitors examined the infants and an underestimate of prevalence is a possibility. In a small survey in the London Borough of Tower Hamlets in 1965 the dental condition of day nursery school children was recorded; the percentage of incisor teeth affected and the percentage of children with rampant caries is given (Appendix A, Table V).

5.1.1. No absolute criteria have been laid down for the identification of rampant caries; in the present study we have used two different standards to identify children suffering from the condition. (*a*) It was considered that children aged 3 and 4 years suffered from rampant caries if they had ten or more carious teeth. (*b*) It was also considered that children aged between 1 and 2 years (and in one study children aged 3 to 3½) who exhibited incisor caries might well develop rampant caries. The evidence is tabled below and it can be seen that the incidence showed wide geographical variation; nevertheless, a considerable percentage of children from all centres but one had rampant caries.

6. Causes

6.1. Although carious lesions, are primarily of environmental origin, their onset and rate of progression may be influenced in greater or lesser degree by the physical and chemical structure of the teeth. Dental caries is a disease which starts at the surface of the tooth, it penetrates the enamel and dentine, and if unchecked will eventually reach the dental pulp destroying the tooth. The onset of the initial lesion is due to demineralisation by the acid secretions of micro-organisms on the enamel surface of the tooth in a structure known as the dental plaque. Incontrovertible evidence exists that the micro-organisms of the plaque can convert fermentable carbohydrates into organic acids and it is these acids which lower the pH locally to a point where dissolution of the highly mineralised enamel cover occurs, causing the initial break on the surface of the enamel.

An important cause of the disease in young children is a cariogenic diet, i.e. one which provides a suitable substrate for the development and metabolism of the micro-organisms of the plaque. The accumulation of acid within the plaque varies with different substrates, but there is a body of evidence implicating sucrose as the sugar most likely to promote caries; not only is it rapidly degraded by micro-organisms into acids but it is also synthesised by bacteria into the extracellular polysaccharides levan and dextran. These sugars provide an improved medium for the growth of the micro-organisms of dental decay and a reserve of substrate for their use during times of scarcity.

6.1.1. There is evidence that erosion can be caused by the ingestion of fluids and syrups of high acidity. It differs from caries in that the whole tooth surface is affected and bacterial invasion does not occur; although circumstances which favour erosion probably also favour caries.

6.1.2. Though much still remains to be learnt about the exact way in which caries arises, it is reasonable on current evidence to conclude that, if situations arise in which sugar-containing solutions (particularly concentrated ones) are in contact with teeth for sufficiently long, caries is likely to result.

7. The Evidence from Field Studies

7.1. James *et al.* (1957) found that the occurrence of early labial caries of the deciduous incisors is strongly associated with the retention of sweet sticky and acid substances on the labial enamel surfaces. The most important vehicles were found to be sweetened comforters.

7.2. Winter *et al.* (1966) found that the most important factor controlling the incidence of rampant caries 'is due to changes in the oral environment brought about by the frequent and prolonged ingestion of milk, fruit juices or syrups which have been sweetened by the addition of sugar. The mode of ingestion of these substances appears to be causally related to the prevalence of caries and in this respect the prolonged sucking of sweetened comforter feeding bottles, hollow feeders (reservoir feeders) and dummies seem to be extremely significant.'

7.3. Goose and Gittus (1967) found that 16·9 per cent of children had been given 'propped' bottles and there appeared to be a significant cause of caries; that the proportion of children having infant feeders (reservoir feeders) was 6·1 per cent and again this appeared to be a significant cause of caries and that children who had dummies dipped in vitamin syrup had significantly more caries than

those who had plain dummies or no dummies at all.

7.4. Bransby and Heasman (1967 unpublished) found no evidence that taking vitamin supplements in the recommended way was associated with an increased incidence of dental caries, but that if a sticky substance including such a supplement was taken on a dummy or dinky feeder then dental decay was more frequent. Goose and Gittus (1967) stated that 'undesirable habits increased with descending social groups and this was paralleled by the caries incidence'; Bransby and Heasman confirmed that caries occurs much more often in the lower social groups.

8. Conclusions of Panel

8.1. In the light of this evidence the panel concludes that reservoir feeders, dummies, and feeding bottles, when filled with or dipped in sugary and/or acid solutions including fruit juice preparations, are conducive to rampant caries. It has no clear evidence that the incidence of rampant caries has been increasing, nor objective evidence that fruit juice preparations are more frequently consumed on dummies and in feeders during the early period when teeth are more vulnerable. Nevertheless the impression remains that there has probably been an increase both in caries and in the consumption of fruit juice preparations on dummies and in feeders.

8.1.1. The next step is to consider which, if any, of the various agents conducive to rampant caries is really necessary.

9. The Use of 'Comforters'

9.1. Despite the disapproval of doctors and nurses, the use of comforters, at least by certain classes of mothers, has persisted over many generations. Presumably they are considered effective for making babies more content and, indeed, it has been argued that for the child who is denied frequent breast feeding the comforter acts in some measure as a substitute. Moreover, with improvements in hygienic standards, the comforter which falls from the mouth and is replaced after perfunctory cleaning is less hazardous to health than formerly. For our part we conclude that the limited use of a dummy does not lead to permanent deformity of the jaw or permanent displacement of the teeth: there are situations where for better or for worse the comforter has a well established place, and it is sensible to accept this fact and to plan in the light of it.

10. Fruit Juices and Vitamin Preparations

10.1. Fruit juice preparations such as welfare orange juice, rose hip syrup, blackcurrant juice provide a rich source of vitamin C and at the same time, when diluted, make an acceptable drink for a child. The popularity of these juices rests in the combination of these two factors. The need for an adequate supply of vitamin C is beyond question, although the optimum intake is a matter of some difficulty and not readily defined. However, in view of the association between rampant caries and the use of these fruit juices, particularly in their concentrated form, it is reasonable to consider whether the child's natural diet provides an adequate supply of vitamin C without such additions.

10.2. In 1957 the Joint Advisory Sub-Committee on Welfare Foods reported (para. 80) 'The clinical evidence provides strong grounds for concluding that a source of vitamin C should be provided for children up to the age of two and we recommend that the provision of welfare orange juice should be continued at the present level to this age group' (Ministry of Health, 1957). The main plank of this evidence is (para. 35) 'Clearly, scurvy does occur sporadically in children throughout Great Britain. The fact that most cases occur about the end of the first year may be explained by the finding of the 1951 survey that up to the age of one, in children on 'low' intakes, the sole important dietary source of vitamin C is milk, a food subject to destructive processing, particularly in the home. In older children some protection is provided by the greater variety of the diet, and scurvy is virtually non-existent, even though, as evidence has shown, many do not take welfare orange juice, other vitamin preparations, or appreciable amounts of citrus fruit.'

10.3. The Joint Advisory Sub-Committee felt no need to examine other possible ways of providing vitamin C for this age group. It was not until 1957 that rampant caries was reported to result from the misuse of fruit juice preparations and the Sub-Committee was not, by its terms of reference, concerned with vehicles for vitamin C other than welfare orange juice.

10.4. The members of the Sub-Committee disagreed about the needs of older children. The majority held 'there is no firm evidence from any source to show that in man there exists a wide zone of 'subclinical deficiency' of vitamin C. On the contrary, where investigations on man have been adequately controlled . . .' no benefit had been observed from increased amounts of vitamin C. 'Two of our members . . . hold . . . that there may be impaired growth and development or other ill effects associated with low vitamin C intake . . .' The majority advised that welfare orange juice was unnecessary for older children.

10.5. Nevertheless many nutritionists continued to advocate fruit or fruit juice preparations for older children, and the Ministry of Health's 1963 Nutrition Survey of pre-school children indicates the current situation (Ministry of Health, 1968).

Appendix B taken from that survey shows a wide range of intakes from foods. Besides this, fruit juice or medicinal supplements were consumed by about one-third of the children (about one-third of these consumed welfare orange juice) the average contribution from which being of the order of 30 mg. daily per child that did take such supplements (Appendix C). It was the impression of those who made the survey that in general the children who took supplements were least in need of them, though their vitamin C intake was not ascertained. Those on the lowest intakes from food would probably be unaffected if all fruit juice preparations were to be abolished. The Ministry of Health further reported: 'The recorded average daily intakes of vitamin C including supplements of (the only) 6 children whose gums bled on pressure and/or who had gingivitis were 16·3, 40·3, 11·3, 65·8, 21·5 and 42·8 mg. It seems unlikely that a scorbutic element existed.' Positive correlations between vitamin C intake and height and weight were found, but similar relationships existed with other nutrients (which in their turn were related one to another).

10.6. It is not for us to interpret this or other evidence bearing upon human needs for vitamin C. There is an expert panel of the Committee on Medical Aspects of Food Policy currently considering this question (and the question of other nutrients). At present we are content merely to remark that more rampant caries occurs among those who take vitamin preparations and fruit juices in feeders or bottles or on dummies than among those who do not. Should other measures fail to control rampant caries then the question whether supplementary vitamin C-containing preparations are necessary may have to be re-opened. In the meantime the practical approach is to examine the means by which the bad effects of fruit juice preparations might be reduced without prejudice to the vitamin C supply.

11. Preventive and Ameliorative Measures

11.1. If as seems likely some mothers will continue to provide comforters for their babies it is necessary to decide which type is the least harmful and what is the least harmful way of using it, and to advise mothers which is the method of choice. If some source of vitamin C additional to that in the diet is desirable, then it is sensible to consider how it may best be provided.

11.2. Dummies, Reservoir Feeders and Bottles

11.2.1. The dummy is only harmful if dipped in cariogenic fluids. Even when used in this way the cariogenic effect may not be so severe as with other appliances because less cariogenic fluid is taken over a much shorter period. The dummy has no function except to 'comfort' but it does not have to be used in a cariogenic way.

11.2.2. Unless some non-cariogenic fluid can be devised for use in reservoir feeders (and we consider this later in this report, in effect to dismiss it) then the reservoir feeder is unlikely to be used except in ways that cause caries. Moreover though it can be used as a means of giving the vitamin preparation which we accept as desirable, there are other ways of doing this.

11.2.3. The principal function of the feeding bottle is to give the child its milk. It is however often used to keep the child quiet and may be propped on the chest in order to give vitamin preparations. The feeding bottle is heavier than the reservoir feeder, and more likely, even though propped on the baby's chest, to remain in its mouth for shorter periods, and this helps to curtail its cariogenic action. There are however other ways of giving vitamin preparations. Nevertheless because they are used more often, feeding bottles probably cause rampant caries more often than any other appliance.

11.2.4. Neither dummies nor reservoir feeders have any function that cannot be achieved in other ways, but an important difference between them is that while dummies can be used in such a way as not to cause caries, reservoirs are unlikely to be so used.

Feeding bottles have one essential function, at least during the first 6 months or so of life. Few if any of a baby's teeth have erupted before then and if the use of feeding bottles for administering fruit juices were discontinued when the teeth began to arrive there would be little risk of rampant caries from this cause.

11.2.5. If it is considered unprofitable to advise against all forms of comforter, the right course is to suggest a dummy not dipped in sweetened fluids or sugar. This is a matter of education and of correct and adequate labelling instructions.

11.2.6. Reservoir feeders are more effective comforters than dummies and so long as they remain on the market mothers may turn a deaf ear to advice. As they have no function that cannot be replaced, the most effective measure would be to abolish them.

11.2.7. Feeding bottles cannot be abolished, but it might be possible to avoid using them for fruit juices after the teeth begin to arrive and this question will be examined in the succeeding section.

11.3. Vitamin Preparations and Sweetened Fluids

If all dried and evaporated milks intended for babies were fortified with ascorbic acid there would be no need for separate vitamin preparations during the first six months of life. A sufficiency for the child's daily needs may be equated with the amounts in breast milk plus a small surplus to allow for possible diminution in supply during the stage when the child is changing from a predominantly fortified

milk. If welfare orange juice were provided only to children over 6 months of age, if all vitamin C preparations were appropriately labelled, and if mothers were suitably instructed, the practice of feeding fruit juices and vitamin preparations in feeding bottles need never be established, at least for significant numbers of children.

11.3.1. We doubt whether it would be acceptable to carry this approach to the point of advising the fortification of liquid welfare milk with ascorbic acid in order that fruit juice preparations should not be needed until the age of five. Nevertheless, manufacturers of vitamin C preparations should appreciate the desirability of ensuring, by appropriate labelling and propaganda, that their preparations are used in non-cariogenic ways.

11.3.2. Despite all efforts, the milk preparations used in the first six months of life might still be used in later months particularly as a means of comforting the child. Therefore they should, if possible, be rendered less cariogenic. There appears to be no current work on the cariogenicity of lactose compared with sucrose. But since it is present in milk it might usefully replace sucrose in 'ready-made-up' milk powders. An additional advantage in this would be the later age at which children would develop a sweet tooth.

11.3.3. Finally, we have to consider the possibilities of reducing the cariogenicity of the vitamin preparations used in reservoir feeding bottles. We accept the view that non-nutritive sweeteners in vitamin preparations which may be consumed by babies should be kept to a minimum. This leaves, as possible alternatives to sucrose, such substances as polyhydric alcohols, glucose, fructose and the sugars in 'liquid glucose', or substances such as calcium sucrose phosphate, which may prevent caries (perhaps by combating erosion).

11.3.4. It has been reported by Schäfer (1949) and Gustaffson *et al.* (1955) that some of the simplest sugars are less cariogenic than sucrose. They are also less sweet and if monosaccharides were only moderately less sweet than disaccharides the need to use more of the former might cancel out any benefit they might have. There are here fields for exploration. We hope that the producers of vitamin preparations will actively consider this problem.

11.3.5. Besides this, the erosive property of the supplement might be diminished by:

(*a*) using a fruit juice rich in vitamin C which has little free acid available for demineralisation. One manufacturer claims his product to be less cariogenic for this reason and this claim deserves critical examination. In the case of welfare orange juice the products of some countries contain less free acid than others and this might be the juice of choice. We hesitate to suggest that a largely synthetic juice be produced containing added ascorbic acid as one of the objects of using fruit juice preparations is to encourage the consumption of fruit.

(*b*) A suitable buffering agent which is palatable, harmless and reduces erosion and concomitant caries may be found; several possible agents have been discussed by the panel.

11.4 Summary and Conclusions

The panel believes that rampant caries is more prevalent now than in previous years. They further believe that an association exists between vitamin supplements, appliances which contain them and rampant caries and that as far as possible the disease should be prevented.

They therefore recommend the following measures:

(*a*) That reservoir feeders be abolished.

(*b*) That additional sucrose should not be added to welfare orange juice.

(*c*) That mothers be advised to use only plain dummies for comforting their babies.

(*d*) That all vitamin supplements should carry cautions as to their use as comforting agents, as indeed some do now.

(*e*) That milk based preparations intended for babies should be reinforced with vitamin C so that no other supplement of this vitamin is required in the first six months of childhood and that the preparations be labelled and mothers advised accordingly.

(*f*) That the possible advantages of substituting lactose or other sugars for sucrose in such preparations be investigated.

(*g*) That all possible methods of rendering fruit juice preparations less cariogenic and erosive be tested.

(*h*) That consideration be given to the possibility of research into the effect of juices and supplements on primates, to be followed by further studies on children in the light of the knowledge so acquired.

(*i*) That the attention of the Health Education Council be drawn to this Report.

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TABLE I.—EVIDENCE FROM FLUORIDATION STUDIES IN THE U.K.

	Holyhead		Gwalchmai		Bodafon		Sutton		Watford		Ayr	Kilmarnock
	Per cent	*No. exam*	*Per cent*	*No. exam*	*Per cent*	*No. exam*	*Per cent*	*No. exam*	*Per cent*	*No. exam*	*Per cent*	*Per cent*
3 years old												
Per cent with 10 or more carious teeth 55/56	12·5	149	12·5	161	11	146	4·5	44	9·4	43	27·1	22·7
Per cent with 10 or more carious teeth 61	—	—	—	—	20	85	2·6	114	—	—	16·9	—
Per cent with 10 or more carious teeth 64/65	—	—	—	—	6	105	1	144	—	—	—	—
4 years old												
Per cent with 10 or more carious teeth 55/56	21·3	186	23·8	227	19·0	210	4·2	47	9·1	66	33·8	31·3
Per cent with 10 or more carious teeth 61	—	—	—	—	21	86	6·5	77	—	—	29·5	—
Per cent with 10 or more carious teeth 64/65	—	—	—	—	15	122	4	162	—	—	—	—
Per cent with 10 or more carious teeth 66	—	—	—	—	12	68	—	—	—	—	—	—

TABLE II.—EVIDENCE FROM AN UNPUBLISHED PAPER (HEASMAN AND BRANSBY, 1967)

	Birmingham		Middlesex		Northumberland	
3–3½ years old	*Per cent*	*No. exam*	*Per cent*	*No. exam*	*Per cent*	*No. exam*
Per cent with 10 or more carious teeth	7	363	7	354	3	200

TABLE III.—EVIDENCE INDICATING EARLY INCISOR CARIES

Mean age (Chester and Leicester) 21 months (Goose and Gittus, 1967)
309 children examined: mean age 21 months . . 6·8 per cent show incisor caries.

Mean age (72 authorities) 23·8 months
5,549 children examined: mean age 23·8 months . . 5·9 per cent showed incisor caries.

TABLE IV.—HEASMAN AND BRANSBY—1967 STUDY

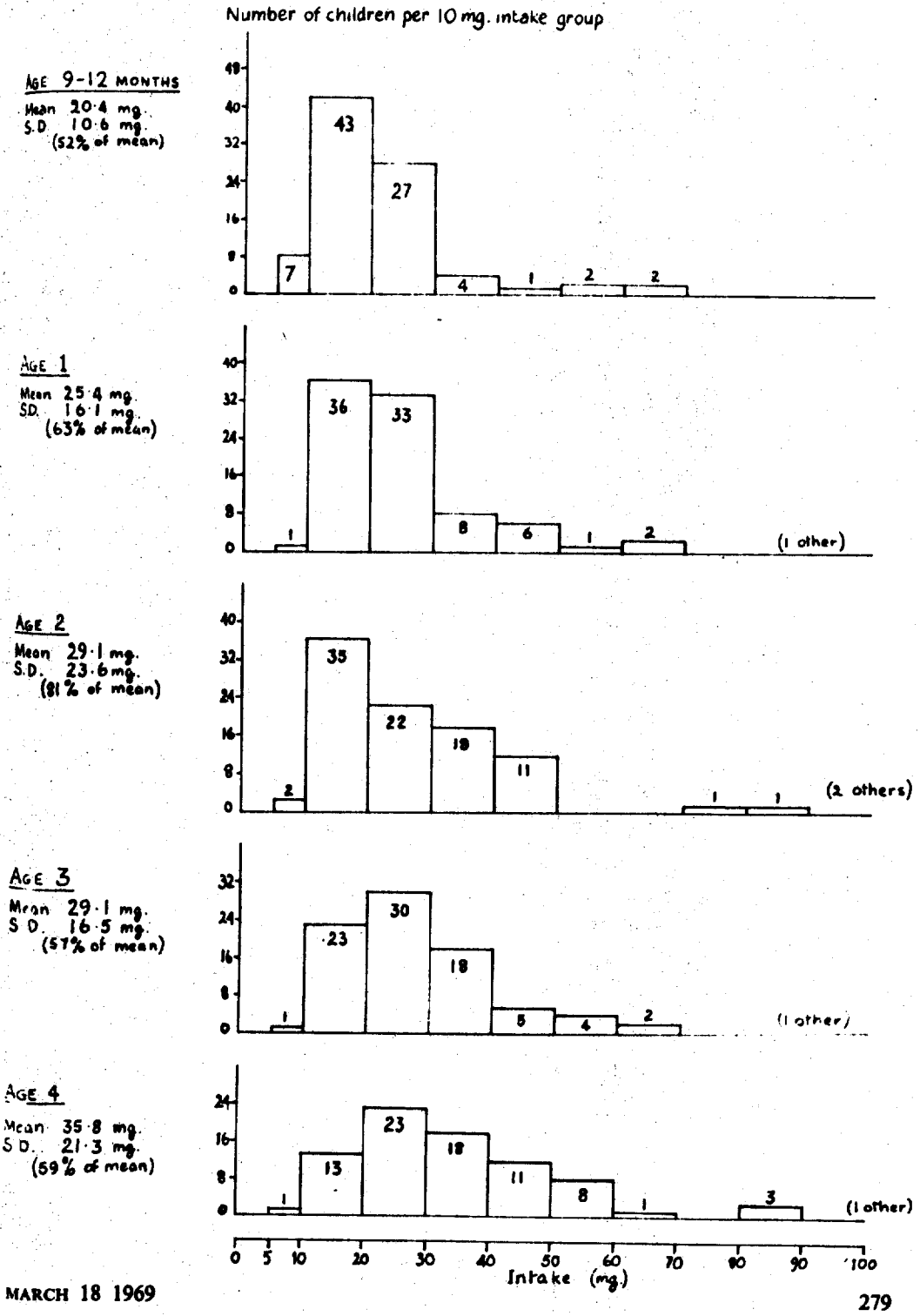
3-3½ years old	*No. of children examined* (1)	*Children with d.m.f. teeth*		*Children with incisor caries*	
Area		*No.*	*per cent of* (1)	*No.*	*per cent of* (1)
Birmingham	363	221	61	116	32
Middlesex	354	162	46	59	17
Northumberland	200	96	48	36	18

TABLE V.—TOWER HAMLETS DAY NURSERY SURVEY 1965

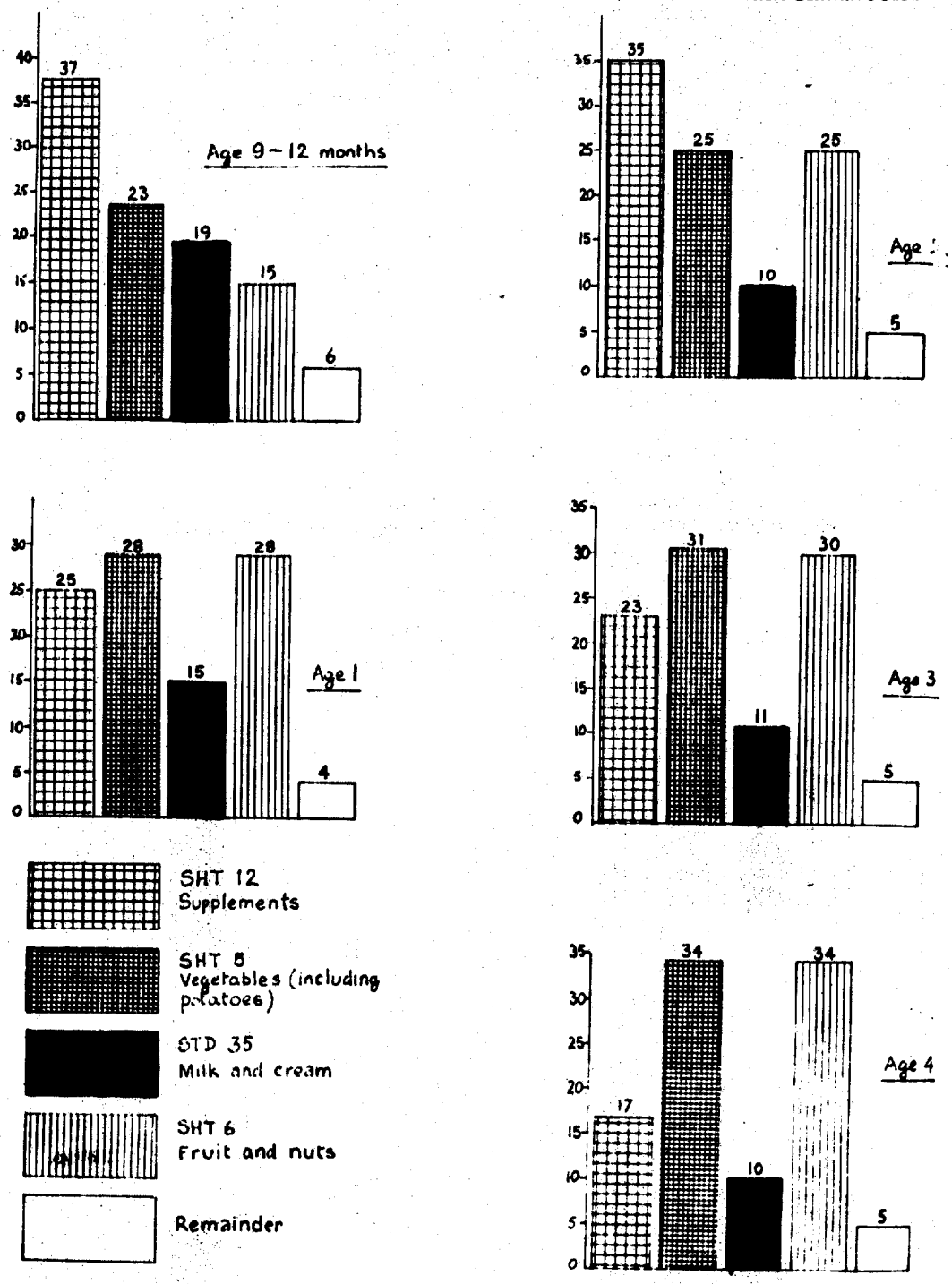
Percentage of anterior teeth affected (d/f) in 235 children

	Less than 2 years per cent	*Less than 3 years per cent*	*Less than 4 years per cent*	*Less than 5 years per cent*
Boys	2	6	11	16
Girls	0	5	11	15

PRE-SCHOOL CHILDREN'S PILOT NUTRITION SURVEY VITAMIN C INTAKE DAY (NO. SUPPS).



PRE-SCHOOL CHILDREN'S PILOT NUTRITION SURVEY PER CENT OF VITAMIN C DERIVED FROM CERTAIN FOODS



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Sugar and dental caries: the effect on the teeth of sweetened beverages and other sugar-containing substances

Previous studies of the Council on Dental Health of the American Dental Association have resulted in statements calling attention to the adverse effects of sugar on the teeth and in recommendations for reducing sugar consumption as a caries-preventive measure. The recommendations are based, primarily, on two substantiated facts: (1) that the consumption of sugar by a caries-susceptible person stimulates tooth decay and (2) that sugar in the diet frequently becomes a substitute for foods of higher nutritive value.

The recommendations of the Council have been challenged principally by manufacturers of products containing large amounts of sugar, such as soft drinks and confections. Because of the Association's responsibility for safeguarding the dental health of the American public, the Council developed the attached statement, documenting the known or potential hazards to dental health resulting from the frequent consumption of sweetened beverages and other sugar-containing substances.

A committee of the Council on Dental Health prepared the initial statement. After a critical review by members of the Council on Dental Health and the Council on Dental Therapeutics and by the research consultants of the Association, many suggestions were incorporated, and the statement was adopted by the two Councils as a joint report.

PURPOSE OF SCIENTIFIC APPRAISAL

In order to achieve a factual scientific appraisal of the effect of sweetened beverages on teeth, it appears appropriate (1) to review in some detail the essential etiologic factors in the process of caries, (2) to discuss in still greater detail the behavior of the dental bacterial plaque through which the sugar must operate in order for a beverage to become cariogenic, and (3) to submit the available evidence concerning (a) the decalcifying and (b) the cariogenic properties of such a beverage.

ESSENTIAL ETIOLOGIC FACTORS IN DENTAL CARIES

The patient researches of many investigators in many parts of the world have accumulated highly convincing evidence since 1867 regarding the process of dental caries and the factors essential for this process. The chronological road markers in the accumulation of this body of scientific information will be pointed out briefly.

1867 • Leber and Rottenstein[1] outlined most plausibly the thesis that intraoral, bacterial fermentation of carbohydrates produces dental caries; the inadequate bacteriologic technics of that period, however, prevented the submission of proof.

1881 • Underwood and Miles,[2] using aniline dyes as stains, demonstrated bacteria in the enlarged tubuli of carious dentin.

1879-90 • Miller,[3] working with Koch, determined that caries of a tooth is of external origin and, initially, is a decalcification of a limited area of enamel by localized acid derived from bacterial fermentation of carbohydrates.

1895 • Black[4] recognized the importance of and the specific distribution of bacterial plaques and developed the concept of extension of the margins of restorations to areas of the crowns of teeth least susceptible to caries.

1897 • Williams[5] demonstrated microscopically that bacterial masses (plaques) invariably were in contact with the surfaces of enamel experiencing beginning decalcification.

1903 • Goadby[6] pointed out that an acid-producing bacillus appeared essential for caries.

1915 • Kligler[7] determined that a group of lactobacilli was most responsible for the acids that produce caries.

1922 • McIntosh, James and Lazarus-Barlow[8] found that lactobacilli always were present in instances of active caries.

1922 • Rodriguez[9] demonstrated that only a few bacteria such as lactobacilli are sufficiently aciduric in nature to live in the degree of acidity necessary for decalcification of a tooth.

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1925 • Bunting[10] and co-workers showed that lactobacilli were absent from the salivas of individuals completely free from caries.

1926 • Bunting, Nickerson and Hard[11] produced areas of decalcification under clasps *in vivo* by cultures of lactobacilli.

1927 • Jay and Voorhees[12] reported that lactobacilli could be isolated from the mouth several months before the detection of cavities in the patient previously free from caries.

1932 • Dobbs[13] completed laboratory studies on dental plaques which showed that these bacterial masses behave similarly to semipermeable membranes.

1932-33 • Jay and others[14,15] carried on studies which determined some of the conditions in patients that are essential for immunity to dental caries.

1933 • Hadley[16] developed a technic for counting the number of lactobacilli per milliliter of saliva, making available a means to evaluate promptly a variety of experimental procedures.

1933 • Jay and co-workers[15] found that cultures of lactobacilli fed to immune individuals are eliminated rapidly from their salivas.

1934 • Koehne, Bunting and Morrell[17] completed studies on 33 hospitalized girls and showed conclusively that new cavities develop in children on adequate diets when sugar is added to such diets.

1936 • By adding candy to the diets, Jay and co-workers[18] produced caries in institutionalized children who had experienced very little caries on an inadequate but low-sugar diet.

1939 • Fosdick[19] pointed out that a series of enzymes is essential to reduce carbohydrates in the mouth to organic acids.

1940 • Stephan[20] measured the acidity developed in the plaques of susceptible patients when 10 per cent glucose solutions were taken into the mouth.

1943 • Fosdick and Burrill[21] determined that pure sugar solutions increase the acidity of carious lesions.

1943 • Dietz[22] produced caries *in vitro* under conditions simulating those of the oral cavity, and studied microscopically the entire development of a cavity under a bacterial plaque.

1944 • Hunt, Hoppert and Erwin[23] reported the breeding of susceptible and of immune strains of rats.

In 1943, reporting on his ingenious laboratory study of the process of caries, Dietz[22] pointed out the conditions essential for dental caries. They are (1) a caries-susceptible individual; (2) the

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presence of acid-producing and acid-tolerating bacteria which are capable of producing (3) an optimum bacterial enzyme system; (4) the presence of orally fermentable carbohydrate; (5) an adherent bacterial plaque. Some other oral conditions may modify the activity of caries. These conditions include types of proteolytic bacteria; flow, consistency, neutralizing power and antibacterial action of saliva; irregularity of teeth or tooth surfaces which contribute to the ready formation of a plaque; the presence or absence of certain amino acids and vitamin fractions, and the rate of solubility of enamel in organic acids. Practically all of these additional conditions, however, have to operate through a bacterial plaque, so only the five highly essential factors will be documented. Jay in 1951[24] stated these essential factors succinctly and listed four methods for preventing dental caries:

1. The complete restriction in the diet of the substances from which acids are produced.
2. The inhibition of the enzyme activity which is responsible for the acid production.
3. The elimination from the oral flora of the bacteria which produce the enzymes.
4. The reduction of the solubility of the tooth surfaces.

FACTOR 1. CARIES-SUSCEPTIBLE INDIVIDUAL

It was stated previously in this report that a caries-susceptible individual was one of the essential conditions for caries. Although much must be learned from a variety of research activities before physiologic immunity to dental caries can be induced in human beings, enough information already has been gleaned to know that there are persons in whose mouths caries cannot be produced by any means. Sweetened beverages apparently cannot serve as a source of cariogenic activity in those somewhat rare individuals who are truly immune. At this point, therefore, some of the characteristics of a caries-immune person should be reviewed.

Heredity is a characteristic that has been studied sufficiently to show that inheritance appears to be a distinct factor in immunity in animals.[23,25,26] Klein and associates[27,28] suggest the same possibilities in humans. Haldi and Wynn[29] did not find that high sucrose diets, fed to a strain of white Wistar mother rats during the prenatal period of their young, produced young rats highly susceptible to caries as did Sognnaes[30,31] experimenting with a strain of Norway rats. McClure[32] found that groups of rats of caries-susceptible strains fed on coarse synthetic diets containing excessive quantities of sucrose and glucose developed significant caries and later[33] could not repeat the findings on rats of caries-resistant strains. The ingestion of refined carbohydrates does not produce a significant drop in pH in an immune person's mouth[34,17] and lactobacilli are not found in the oral cavity and gastrointestinal tract of such a person.[15,35-39] Evidence

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has been submitted that lactobacilli cannot be implanted in the oral cavity of an immune individual.[15] One investigation, at least, has shown that phagocytosis of acid-producing organisms is stimulated in this person;[40] evidence has been published which indicates that the skin of this individual fails to react to an unpurified filtrate from a solution of heat-killed lactobacilli;[14] he fails, also, according to published evidence, to respond with a violent reaction to a vaccine of heat-killed lactobacilli, as does the susceptible person,[14,15] and, further, an increased agglutinin titer for lactobacilli is experienced by his blood.[14,15,41-44] Changes in susceptibility to caries have been reported for age and sex.[45]

FACTOR 2. ACID-PRODUCING AND ACID-TOLERATING BACTERIA

The presence of acidogenic microorganisms and, for long-range activity, aciduric organisms on the surface of a tooth has been listed as the second essential factor for the initiation of caries. For the destruction of the organic portion of enamel, proteolytic bacteria also must accompany or follow the decalcifying organisms, but, as Stephan[46] has pointed out, "It is quite significant, however, that in caries of the dentin the organic matrix remains after decalcification produced by the caries process has occurred. . . ." Burnett and Scherp[47] recently completed research which permitted them to conclude that:

> . . . proteolytic bacteria from dentinal caries are able to produce only minimal changes in intact dentin, although they readily digest dentinal protein made accessible by acid decalcification.

The evidence submitted to date regarding the role of acid-producing bacteria in dental caries is scientifically impressive, and some of it now will be reviewed.

Kligler,[7] it may be recalled, focused attention as early as 1915 on the frequency of growth of lactobacilli in the teeth of individuals experiencing active caries and the infrequency of growth of these organisms when no active caries existed. Bunting and Palmerlee[10] concluded in 1925 that aciduric organisms from caries-active mouths produced carieslike lesions in six to eight days. Enright, Friesell and Trescher[48] reviewed the earlier literature on the microorganisms associated with caries and then summarized their own observations in 1932. They pointed out that the only organism found in food debris in direct contact with enamel as it is being attacked by progressive caries is a lactobacillus. Further, they pointed out, the lactobacillus is the only organism which can tolerate and produce additional acid below a pH of 5.0.

Many additional details regarding the essential oral flora were accumulated during the 1940's. Volker[49] in 1940 demonstrated some variations in the solubility of enamel from deciduous teeth,

36. Snyder, M. L. The occurrence of lactobacilli and other acidogenic organisms in the salivas of selected caries-free children. J. D. Res. 18:497 Dec. 1939.

37. Henningsen, M. G. Caries-free individuals; a report on dentomedical investigations. J. California D. A. 17:1 Jan.-Feb. 1941.

38. Hill, T. J. The influence of immunologic factors on dental caries. J.A.D.A. 28:109 Jan. 1941.

39. Hill, T. J. A salivary factor which influences the growth of L. acidophilus and is an expression of susceptibility or resistance to dental caries. J.A.D.A. 26: 239 Feb. 1939.

40. Hammond, Carolyn W., and Weinmann, J. P. Opsonin in the saliva of caries-positive patients. J. D. Res. 21:509 Dec. 1942.

41. Canby, C. P., and Bernier, J. L. Bacteriologic and immunologic studies in dental caries: a preliminary report. J.A.D.A. 29:606 April 1942.

42. Williams, N. B. Salivary counts in humans following vaccination with oral lactobacilli. (Abst.) J. D. Res. 23:196 June 1944.

43. Williams, N. B. Immunization of human beings with oral lactobacilli. J. D. Res. 23:403 Dec. 1944.

44. Williams, N. B. Immunologic reactions of lactobacilli. J.A.D.A. 37:403 Oct. 1948.

45. Burrill, D. Y. Changes in caries susceptibility with age and sex. J. D. Res. 22:367 Oct. 1943.

46. Stephan, R. M. Some local factors in the development of cavities: plaques, acidity, aciduric bacteria, proteolytic bacteria. J. D. Res. 27:96 Feb. 1948.

47. Burnett, G. W., and Scherp, H. W. The effect on dentin of proteolytic and acidogenic bacteria isolated from the carious lesion. J. D. Res. 32:46 Feb. 1953.

48. Enright, J. J.; Friesell, H. E., and Trescher, M. O. Studies of the cause and nature of dental caries. J. D. Res. 12:759 Oct. 1932.

49. Volker, J. F. Studies on the acid solubility of human enamel. J. D. Res. 19:35 Feb. 1940.

young permanent and old permanent teeth when exposed to weak organic acid. Bibby, Volker and Van Kesteren[50] in 1942 found, in a study of the reaction of salivas, that streptococci formed acid most rapidly in incubated glucose-saliva mixtures, that actinomyces ranked second in rapidity and that lactobacilli were less rapid in the production of acidity. The same year, Florestano,[51] having studied the behavior of cultures procured from salivas, reported that 121 strains of lactobacilli produced a pH which varied from 4.9 to 3.6 while 14 strains carried the acidity no farther than a pH of 5.25, that 39 of 68 strains of streptococci produced a pH of 4.0 to 3.4, that 25 strains of staphylococci produced an acidity of 4.0 to 3.7, and that the lowest pH yielded by 14 yeasts was 5.17.

Canby and Bernier[41] in 1942 concluded that the *Lactobacillus acidophilus* had an important etiologic role in the caries process since carious dentin developed such a high H-ion concentration as to exclude ordinary oral streptococci, staphylococci and some other organisms which do not possess marked aciduric powers.

Dietz, in his continuous study of the production *in vitro* of plaques and caries, reported in 1943[22] that lactobacilli and streptococci invariably were recovered from the surfaces of carious lesions, and that occasionally the yeasts and staphylococci recovered throve on a tomato-agar medium.

At this point the question arises, "What is the critical pH for the initiation of decalcification?" In 1925, Bunting and Palmerlee[10] were able to decalcify teeth slowly in lactic acid at a pH of 5.0; McClelland[52] in 1926 produced distinct losses of weight in pieces of submerged enamel kept for 12 hours at a pH of 4.5; Enright, Friesell and Trescher[48] in 1932 produced "characteristic carious lesions" by lactic acid, pH 4.6, in 14 days; and Bibby, Volker and Van Kesteren[50] in 1942 found the critical pH of cultures containing enamel and dentin to be 5.0. Stephan,[20, 46, 53, 54] who contributed so much to the knowledge of plaques during the period 1938 to 1948, has established the critical pH under plaques at 5.0 or below.

In 1942 Becks[55] associated high counts of lactobacilli with a high incidence of carious lesions, and Stralfors[56] in 1948 noted that the higher the pH (nearer alkalinity) that is established in dental plaques, the lower the number of lactobacilli present. Jay[57-59] in 1944, 1947 and 1948 reported in detail on how to rid mouths of lactobacilli by a regime which consisted of low carbohydrate diets, and how, thereby, to halt cavitation. Kitchin and Permar[60] confirmed the results of Jay's technic in a 1948 report.

For more than 10 years, Blayney, of the Zoller Clinic, and his associates made a thorough study of the bacteria found in plaques, and this group has made a large contribution to the knowledge of the bacteriology of the dental plaque. In 1940, Bradel and Blayney[61] determined that lactobacilli, in 392 (82 per cent) of

50. Bibby, B. G.; Volker, J. F., and Van Kesteren, M. Acid production and tooth decalcification by oral bacteria. J. D. Res. 21:61 Feb. 1942.

51. Florestano, H. J. Acidogenic properties of certain oral microorganisms. J. D. Res. 21:263 June 1942.

52. McClelland, J. R. The decalcification of human tooth enamel. D. Cosmos. 68:127 Feb. 1926.

53. Stephan, R. M. Hydrogen-ion concentration of the dental plaque. J. D. Res. 17:251 June 1938.

54. Stephan, R. M., and Miller, B. F. A quantitative method for evaluating physical and chemical agents which modify production of acids in bacterial plaques of human teeth. J. D. Res. 22:45 Feb. 1943.

55. Becks, Hermann. Salivary and bacteriological considerations in the control of dental caries. J. Am. Col. Den. 9:184 June 1942.

56. Stralfors, Allan. Studies of microbiology of caries. II. The acid fermentation in the dental plaques in situ compared with lactobacillus count. J. D. Res. 27:576 Oct. 1948.

57. Jay, Philip. The reduction of oral Lactobacillus acidophilus counts by the periodic restriction of carbohydrates. Am. J. Orthodont. & Oral Surg. (Oral Surg. Sect.) 33:162 March 1947.

58. Jay, Philip. The effect of substrate on the oral flora. J.A.D.A. 37:416 Oct. 1948.

59. Jay, Philip. Nutrition and dental caries. J. New Jersey D. Soc. 15:20 Jan. 1944.

60. Kitchin, P. C., and Permar, Dorothy. Results of a three-year study of the effectiveness of carbohydrate restriction in reducing salivary lactobacillus counts. J. D. Res. 27:316 June 1948.

61. Bradel, S. F., and Blayney, J. R. Clinical and bacteriologic studies on dental caries. J.A.D.A. 27:1601 Oct. 1940.

477 patients studied, were present in 85.4 per cent of samples. In 1942 Blayney and co-workers[62] reported further details of the five-year continuous study of plaques removed from beginning cavities on the proximal surfaces of the young bicuspids of these 477 patients from which 3,290 specimens were obtained. Only 25 per cent of 333 specimens from the 56 caries-free patients studied yielded positive cultures of lactobacilli. Harrison[63] in 1940 reported that streptococci were more numerous than lactobacilli in the mouths of rats on cariogenic diets and, when the caries activity was inhibited by the addition to the ration of fluoride or iodoacetic acid, there was a significant numerical reduction of the lactobacilli while the number of streptococci remained the same. In 1941, Hemmens, Blayney and Harrison[64] published a fairly detailed report of the bacteria in the plaques which had been removed from teeth. The most active producers of acid were found to be cocci and streptococci which developed a pH of 4.0 in 24 to 36 hours. Some of the diphtheroids and fusiform bacilli developed more slowly a pH of 5.0. None of these acidogenic organisms, however, survived in an acid medium of their own ultimate pH. The percentage prevalence of the aciduric organisms isolated in this study was streptococci, 15 per cent; lactobacilli, 41 per cent; micrococci, 15 per cent, and yeasts, 10 per cent. Hemmens and co-workers,[65] in a 1946 report, stated it was true that diphtheroids and aciduric streptococci most commonly were isolated from the material of plaques but did not increase during the initial stages of decay, while hemolytic streptococci and lactobacilli did show a progressive increase during the onset of the lesion. Since the hemolytic streptococci were extremely limited in numbers, they appeared to have little or no role in caries. In 1947, Stephan and Hemmens[66] stated, from further studies, that lactobacilli, diphtheroids, some sarcinae and some streptococci were the only organisms capable of producing a sufficiently low pH rapidly enough to decalcify a tooth. Still later, in 1948, Harrison,[67] from a longitudinal study of over 600 plaques taken from 87 proximal areas of the teeth of 44 children, made a number of observations pertinent to the present report: (1) acid-producing and acid-tolerating streptococci both were found associated with dental decay; (2) aciduric streptococci were associated more persistently with advanced caries of the dentin while lactobacilli were found more likely to be associated with the initiation of the lesion in the enamel; (3) streptococci of the viridans type began to disappear in plaques as early as 42 weeks before the first appearance of cavitation and disappeared rapidly with the formation of carious lesions; (4) hemolytic streptococci, found infrequently, showed a slight increase as lesions developed, and (5) yeasts were found in comparatively few cases and did not change with carious progress. Harrison, thus, presents an excellent summary of the knowledge of the bacteriology of dental plaques.

FACTOR 3. ORALLY FERMENTABLE CARBOHYDRATE

One reason that bacteria die or thrive is that they, just like any organic cell, are

62. Blayney, J. R., and others. Continuous clinical and bacteriologic study of proximal surfaces of premolar teeth before and after onset of caries. J.A.D.A. 29:1645 Sept. 1, 1942.

63. Harrison, R. W. Bacteriologic studies in experimental dental caries in the rat. II. Changes in tooth surface flora associated with development and inhibition of caries. J. Infect. Dis. 67:97 Sept.-Oct. 1940.

64. Hemmens, Elizabeth S.; Blayney, J. R., and Harrison, R. W. The microbic flora of bacterial plaques removed from carious and noncarious dental enamel. J. D. Res. 20:29 Feb. 1941.

65. Hemmens, Elizabeth S., and others. The microbic flora of the dental plaque in relation to the beginning of caries. J. D. Res. 25:195 Aug. 1946.

66. Stephan, R. M., and Hemmens, Elizabeth S. Studies of changes in pH produced by pure cultures of oral microorganisms. I. Effects of varying the microbial cell concentration. II. Comparison of different microorganisms and different substrates. III. Some effects of mixing certain microorganisms. J. D. Res. 26:15 Feb. 1947.

67. Harrison, R. W. Lactobacilli versus streptococci in the etiology of dental caries. J.A.D.A. 37:391 Oct. 1948.

dependent on the availability of a suitable substrate. As Fosdick and Burrill[21] pointed out in 1943, chemical considerations show that the only substrates from which acids can be formed are the carbohydrates in general and that, in all probability, the fermentable carbohydrates in particular are the ones most likely to be converted to various acids under oral conditions. A number of research people have investigated the role of carbohydrates in the process of caries; their findings warrant a brief review.

Fosdick, Campaigne and Fancher[68] in 1941 reported,

> At the present time, most of the evidence indicates that free sugar is the predominant source of acid and that ingested starches do not often influence the susceptibility to decay. . . .

Galin[69] in 1941 demonstrated a sharp rise in the production of acid in patients' salivas immediately after they had eaten a lump of sugar. In 1942 Bibby, Volker and Van Kesteren[50] concluded,

> Since the action of acid is the only known mechanism by which human enamel can be destroyed and since the bacterial fermentation of carbohydrates is the only established source of mouth acids, the ability of various microorganisms to produce acids under mouth conditions becomes the first measure of their probable significance in the causation of dental caries. . . .

Also in 1942, the Council on Foods and Nutrition of the American Medical Association[70] submitted a report on the consumption of sugar:

> The per capita gross consumption of sugar in the United States increased steadily from about 10 pounds (4.5 kg.) in 1821 to 108 pounds (49 kg.) in 1931. . . . Sugar, as consumed in recent years, whether it originates from sugar cane or sugar beets is for the most part highly refined sugar. . . .
>
> Accurate information is not available as to what proportion of the total use of sugar is for use with other foods. However, much sugar is consumed in candies, some prepared desserts and sweetened beverages, which carry nothing of nutritional significance except sugar. Estimates of the consumption of candy show that it may be as great as 16 pounds (7.3 kg.) per person each year. . . . Figures are available on sales of sweetened beverages. They indicate that manufacturers of such beverages produced over eighteen billion 6-ounce bottles of soft drinks in 1939. It is also admitted that, since 1939, the consumption of soft drinks has increased by from 20 to 30 per cent. From such data it appears that the per capita consumption of soft drinks may be in the neighborhood of more than three bottles per week per capita. . . . It seems obvious that, regardless of the method used to estimate the amount of sugar consumed as soft drinks, one obtained a result that is definitely undesirable from the standpoint of the nation's nutritional status.

Another report on nutrition by the American Medical Association[71] in 1942 may be quoted:

> Sugar is not among the recommended foods. Its recent rationing will not provoke a hardship, for sugar supplies nothing in nutrition but calories, and the vitamins provided by other foods are sapped by sugar to liberate these calories. One of the worst of the many bad food habits that Americans have acquired is their use of sweetened carbonated beverages. Many persons take such beverages by the half pint many times a day with a resultant excessive consumption of sugar.

Becks,[55] also in 1942, reported a dietary analysis of 99 caries-free and 107 caries-active individuals. The only difference in the dietary intake of the two groups was in consumption of carbohydrates, especially in regard to refined sugars. The caries-active group consumed an average of 7.7 teaspoonfuls more of refined sugars per day than the caries-free group. Jay[59] in 1944 observed that nobody has reported a successful control of human dental caries by dietary means without

68. Fosdick, L. S.; Campaigne, E. E., and Fancher, O. E. Rate of acid formation in carious areas: the etiology of dental caries. Illinois D. J. 10:85 March 1941.

69. Galin, Nathan. Studies of acid production in the mouth. Mich. Univ. School Dent. Alumni Bul. 43:16 Oct. 1941.

70. American Medical Association, Council on Foods and Nutrition. Some nutritional aspects of sugar, candy and sweetened carbonated beverages. J.A.M.A. 120: 763 Nov. 7, 1942.

71. Wilder, R. M., and Keys, T. E. Handbook of nutrition: XIV. Unusual foods of high nutritive value. J.A.M.A. 120:529 Oct. 17, 1942.

restricting sugar. McClure[33] in 1945 reported that susceptible white rats, fed on a diet containing 66 per cent of course ground dextrins and on a diet containing 65.5 per cent of starch, developed no cavities, while rats, fed on diets containing 70 per cent of either sucrose or glucose, developed numerous cavities. Nonsusceptible rats developed cavities on none of these high carbohydrate diets, however. Lipner[72] in 1947 concluded that an essential condition for caries in man is fulfilled if carbohydrates in the form of monosaccharides or disaccharides are supplied in the mouth, or if salivary amylase is available in sufficient concentration to break down more complex carbohydrates rapidly into forms more utilizable by oral bacteria.

The federal Bureau of Agricultural Statistics[73] in 1949 submitted a report on the consumption of refined sugars in the United States. By 1948, per capita consumption of cane and beet sugar had risen to 95.9 pounds from 73.1 pounds in 1909. During the peak year, 1942, per capita consumption was 103.6 pounds. During 1948, in addition to the 93.5 pounds of refined sugar, per capita consumption of sugar included 1.6 pounds of cane sirup, 0.8 pounds of sorgo sirup, 0.2 pounds of edible cane molasses, 0.2 pounds of maple sugar and maple sirup, 0.3 pounds of refiner's sirup, 1.3 pounds of honey and 8.2 pounds of corn sirup, making an additional 12.6 pounds of highly concentrated sugar foods. A 1952 report of the Bureau of Agriculture[74] estimated civilian consumption of refined sugar per person for that year at a level not to exceed 96.3 pounds.

The policy of the American Dietetic Association[75] concerning candy and sweetened beverages, prepared late in 1952, is of interest. The American Dietetic Association does not accept advertising or exhibits for candy or soft drinks or carbonated beverages. As a professional organization, the American Dietetic Association feels a responsibility to the public in the solution of problems connected with foods and nutrition, particularly with regard to the nutrition of children. It cannot, therefore, accept foods and drinks which may contribute to the nutritional injury of children.

In view of the interest in carbohydrates, from the standpoint of cariogenic property and ability to develop a dietary imbalance, it would appear that a short review of carbohydrates, their types and their metabolism, should be pertinent to the evaluation of the effect of sweetened beverages on teeth.

West and Todd's textbook[76] may be utilized for a limited review of the types of carbohydrates and the manner of their metabolism in the oral cavity. Common practice classifies the carbohydrates in the main as monosaccharides, disaccharides and polysaccharides. The common monosaccharides are glucose, fructose and galactose, while the common disaccharides are sucrose (glucose combined with fructose), maltose (glucose plus glucose) and lactose (glucose plus galactose). Granulated sugar, which is obtained from either beets or cane, is sucrose. The common polysaccharides are (1) dextrins, (2) starches, (3) glycogen and (4) celluloses.

Dextrins represent products of the partial hydrolysis of starch by acids or amylase (ptyalin) and they occur naturally in the leaves of starch-producing plants and, to some extent, as constituents of such foods as honey or corn sirup. They are soluble in water; they cannot

72. Lipner, H. J. Physiological considerations of the relative specificity of dental caries in man. J. D. Res. 26:319 Aug. 1947.

73. Consumption of food in the United States, 1909-48. U. S. Department of Agriculture, Bureau of Agricultural Statistics. Washington, D. C., Government Printing Office, 1949, p. 119.

74. Sugar. Nat. Food Situation, N. S. 62:16 Oct.-Dec. 1952. Bureau Agricultural Economics, U. S. Department of Agriculture.

75. American Dietetic Association. Specific policy regarding candy and carbonated beverages or soft drinks, Dec. 15, 1952.

76. West, E. S., and Todd, W. R. Textbook of biochemistry. New York, Macmillan, 1951, p. 293-308.

dialyze through a semipermeable membrane.

Starch is a plant carbohydrate with a huge molecule and is insoluble although it may be dispersed in water. It does not diffuse through a selective membrane such as a dental bacterial plaque. With the aid of the salivary enzyme, amylase, starch may be hydrolyzed to maltose to a limited and variable extent in the mouth and in the stomach after being swallowed. The enzyme, maltose (very small amounts of which are found in the saliva, although yeasts are a prolific source) then is required to split maltose into glucose. In all, 14 reactions probably are required to break starch down into lactic acid, and 12 of the steps require an enzyme or source of energy.

Glycogen, which has been called "animal starch," is the form in which carbohydrate is stored in the human liver and muscles. As metabolized in muscle to lactic acid, it appears to require 13 steps or reactions, making its metabolism quite similar to that of starch.

Celluloses, the fibers of plant life, are insoluble in water and are nonfermentable in the mouth.

A number of individuals have discussed this basic information in contributions to dental literature. Of these individuals, Stephan[77] in 1948 pointed out that, of the carbohydrates which people commonly consume, the monosaccharides and disaccharides generally are fermentable by acidogenic oral microorganisms, they rapidly lower the pH in plaques and carious lesions and they produce caries in rats and hamsters under suitable experimental conditions, whereas the polysaccharides, starch and dextrins, are not fermentable directly by acidogenic oral microorganisms. From laboratory studies of plaque material taken from susceptible individuals, the plaque appears to be able to produce lactic acid promptly from dextrose, maltose and sucrose, but less readily from lactose and starch. Neuwirth and Summerson[78] in 1951 noted experimentally that lactic acid is produced within a few minutes after contact between microorgansims and glucose solutions, and it continues to be produced at the initial rate until all or nearly all of the glucose is metabolized. Neuwirth and Summerson were using a concentration of approximately 0.13 per cent glucose, about that of blood, and much lower than that found in carbohydrate-rich foods. Pigman and Reid[79] in 1952 pointed out that starch components (amylose and amylopectin) are large molecules containing several hundred to several thousand units of glucose which are broken down by amylase quite promptly to short dextrins containing six to eight units of glucose; that is, hexasaccharides, septasaccharides and octasaccharides. These dextrins are hydrolyzed very slowly, however, to monosaccharides, disaccharides and trisaccharides.

In view of the difficulty experienced in breaking down complex carbohydrates, it would appear helpful to the present evaluation if the various types of carbohydrate fractions in a variety of foods could be ascertained. Such information should help assess the possibilities possessed by a carbohydrate to diffuse into a bacterial plaque and to break down into an organic acid promptly. Some information is available. Chatfield and Adams[80] in 1940, McCance and Widdowson[81] the same year, Collins, Jensen and Becks[82] in 1942, the Iowa Dental Bul-

77. Stephan, R. M. Relative importance of polysaccharides, disaccharides, and monosaccharides in the production of caries. J.A.D.A. 37:530 Nov. 1948.

78. Neuwirth, Isaac, and Summerson, W. H. The production of acids from glucose by oral microorganisms: lactic and pyruvic acids. J. D. Res. 30:100 Feb. 1951.

79. Pigman, Ward, and Reid, A. Jane. The organic compounds and enzymes of human saliva. J.A.D.A. 45:325 Sept. 1952.

80. Chatfield, Charlotte, and Adams, Georgian. Proximate composition of American food materials. U. S. Department of Agriculture Circular No. 549. Washington, Government Printing Office, 1940.

81. McCance, R. A., and Widdowson, E. M. The composition of foods. London, His Majesty's Stationery Office, 1940.

82. Collins, R. O.; Jensen, A. L., and Becks, Hermann. Studies of caries-free individuals: II. Is an optimum diet or a reduced carbohydrate intake required to arrest dental caries? J.A.D.A. 29:1169 July 1, 1942.

letin[83] in 1947, Wooster and Blanck[84] in 1950, Watt and Merrill[85] in 1950, Bibby, Goldberg and Chen[86] in 1951, and Lundqvist[87] in 1952, all have studied the percentages of carbohydrates in foods. A composite table of some of these findings would appear to be valuable as a reference (Table 1).

Since some suggestions have been made that naturally concentrated sugars are less cariogenic than refined sugars, it is of interest to examine four findings that have been reported. Steggerda and Hill[88] in 1936 found that the Maya Indians, on a high cereal diet, and the Navajo Indians, on a high protein diet, exhibited a very low attack rate for dental caries (more than 50 per cent of both groups being caries-free until the age of 20 years). At the same time, Jamaicans (colored), who commonly chew sugar cane, experienced a high caries-attack rate on the smooth surfaces of their teeth. Osborn, Noriskin and Staz[89] in 1937 reported observations on extracted teeth incubated in salivas to which various carbohydrates had been added. These investigators observed that refined sugar produced decalcification in the highest percentage of teeth, that both whole wheat and refined flour produced a high percentage of decalcification and that crude cane juice produced the least. Dreizen and Spies,[90] however, in 1950, found an opportunity to study 147 people from Cuban families who had lived on a diet composed principally of unrefined carbohydrates but who, as cultivators of sugar cane, were habitual cane chewers. In the 137 persons who had complete or partial dentitions, the average caries prevalence was 48.5 surfaces and 15.1 DMF teeth.

Sorbitol, which was obtained originally from the berries of mountain ash and which now can be produced by the electrolytic reduction of glucose, is about one-half as sweet as sucrose. Grubb[91] in 1945 found that it was fermented very slowly by lactobacilli.

Bibby, Goldberg and Chen[86] (Table 1) developed an ingenious technic to study the cariogenic property of foodstuffs. They devised an index of decalcification potential based on the food retained in the mouth after swallowing (removed by brushing or spray from an atomizer) and the acid produced during four hours by food suspensions in saliva. Since decalcification of teeth *in vivo* has to take place under plaques through which large molecules of carbohydrates appear unable to diffuse promptly, and since a comprehensive system of bacterial enzymes must be available in the individual's mouth for the time-consuming process of breaking polysaccharides down to simple sugars that can be utilized rapidly in plaques, these investigators were loath to suggest that this index be used diagnostically until further work with it had been completed.

Lundqvist[87] in 1952 reported on a long study being carried on in Lund, Sweden, of the time required for the clearance of sugar from the saliva, a study which he utilized to develop an index of caries potentiality for a large group of foods. Briefly, he found that deproteinized fasting saliva was free of sugar in normal individuals and only showed the

83. Carbohydrate evaluation. Iowa D. Bul. 33:53 April 1947.

84. Wooster, H. A., Jr., and Blanck, F. C. Nutritional data. Pittsburgh, H. J. Heinz Co., 1950.

85. Watt, Bernice K., and Merrill, Anabel L. Composition of foods—raw, processed, prepared. United States Department of Agriculture, Agriculture Handbook No. 8. Washington, Government Printing Office. 1950.

86. Bibby, B. G.; Goldberg, H. J. V., and Chen, Eugene. Evaluation of caries-producing potentialities of various foodstuffs. J.A.D.A. 42:491 May 1951.

87. Lundqvist, Claes. Transl. by Evans, C. M. Oral sugar clearance; its influence on dental caries activity. Lund, Sweden, CWK Gleerup, 1952.

88. Steggerda, Morris, and Hill, T. J. Incidence of dental caries among Maya and Navajo Indians. J. D. Res. 15:233 Sept. 1936.

89. Osborn, T. W. B.; Noriskin, J. N., and Staz, J. A comparison of crude and refined sugar and cereals in their ability to produce in vitro decalcification of teeth. J. D. Res. 16:165 June 1937.

90. Dreizen, Samuel, and Spies, T. D. The incidence of dental caries in habitual sugar cane chewers. J.A.D.A. 45:193 Aug. 1952.

91. Grubb, T. C. Studies on the fermentation of sorbitol by oral microorganisms. J. D. Res. 24:31 Feb. 1945.

Table 1 • Percentages of carbohydrates in foods

Foods	Total carbohydrate (%)	Fiber (%)	Sugar (%)	Starch (%)	Acids (%)	Total carbohydrate (%)	Fiber (%)	Starch-dextrins—sugar (%)	Unavailable carbohydrate (%)	Sugar (%)	Dextrins-starch (%)	Total carbohydrate (%)	Soluble carbohydrate (%)	Retained carbohydrate (mg.)	0.1 (N) acid in 4 hours (ml.)	ml. of acid, after 4 hrs., multiplied by 4-hr. acid x mg. retained carbohydrate = decalcification potential.
	Chatfield and Adams[80]					Watt and Merrill[85]			McCance and Widdowson[81]			Bibby, Goldberg and Chen[86]				
Almonds (dry)	19.6	2.7	4.4	—	—	19.6	2.7	16.9								
Apples (fresh)	14.9	1.0	11.1	—	M-0.47	14.9	1.0	13.9	1.7	12.2	Trace	13.2	11.0	3	1.4	4
Apple juice	12.5	—	10.5	—	M-0.52	13.8	—	13.8								
Bananas	23.0	0.6	19.2	—	M-0.39	23.0	0.6	22.4	3.4	16.2	3.0	20.2	13.4	100	1.8	180
Beans-green or wax (canned)	7.7	1.4	0.4	2.2	—	7.7	1.4	6.3								
Bread (white)	52.3	.3	—	—	—	51.8	0.2	51.6		Trace	63.8	50.6	13.0	91	1.8	164
Cake (angel food)	58.7	0.0	—	—	—	58.7	0.0	58.7	—	29.8	24.5	66.6	25.8	125	1.8	225
Caramel candy	78.0	—	—	—	—	77.5	0.0	72.0				56.8	43.0	219	1.8	394
Choc. cream candy	72.0	—	—	—	—	72.0	0.0	72.0								
Fudge candy	88.0	—	—	—	—	81.3	0.3	81.0								
Hard candies	99.0	—	—	—	—	99.0	0.0	99.0								
Carrots (raw)	9.3	1.1	7.5	—	—	9.3	1.1	8.2				8.0	5.4	3	1.4	4
Chocolate milk (sweetened)	54.0	0.5	49.5	—	—	55.7	0.5	55.2	—	42.3	10.0	50.0	39.0	197	1.6	315
Cola drink	12.0	—	—	—	—	12.0	—	12.0?								
Corn flakes	80.3	0.5	—	—	—	85.0	0.6	84.4		10.2	73.8	84.6	14.6	21	0.7	15
Crackers (graham)	74.3	0.8	—	—	—	74.3	0.8	73.5				71.6	21.6	226	1.0	226
Cranberry (sauce)						84.3	8.7	75.6								
Dates (dried)	75.4	2.4	61.2	—	—	75.4	2.4	73.0	8.7	63.9	0.0	77.5	33.8	297	1.7	505
Figs (dried)	68.4	5.8	55.0	—	M-0.60	68.4	5.8	62.6	18.5	52.9	0.0	73.0	31.2	350	1.9	665
Flour (patent)	75.9	0.3	—	—	—	76.1	0.3	75.8		Trace	80.0					
Ginger ale	9.0	—	—	—	—	9.0	—	9.0?								
Grape juice	18.5	—	16.8	—	C-0.20	14.9	0.5	14.4								
Grapefruit juice	8.1	—	6.7	—	C-1.42	9.2	0.1	9.1								
Honey (strained)	79.5	—	76.0	—	—	79.5	—	79.5?	—	76.4						
Ice cream	20.3	—	—	—	—	20.6	0.0	20.6	—	17.5	0.0	20.7	20.0	196	1.3	255
Lemon juice	8.3	—	2.3	—	C-5.96	7.7	0.0	7.7	0.0	1.6	0.0					
Lime juice	8.3	—	1.4	—	C-6.90	8.3	0.0	8.3	5.2	3.2	0.0					
Molasses (cane)	65.0	—	65.0	—	—	65.0	—	65.0?	—	67.2	0.0					
Molasses (sorghum)	67.0	—	67.0	—	—											
Noodles (egg)	70.6	0.3	—	—	—	73.2	0.4	72.8				71.3	9.0	16	1.1	18
Oatmeal (cooked)	11.0	0.2	—	—	—	11.0	0.2	10.8		Trace	8.2					
Orange juice	10.1	—	9.0	—	C-1.16	11.0	0.1	10.9	0.0	9.4	0.0					
Orange soda												9.5	9.5	22	1.9	42
Peanuts (dry) (Virginia)	16.5	2.5	3.5	1.9	—	23.6	2.4	21.2	8.1	3.1	3.8					
Pop corn	77.8	1.7	—	—	—	76.7	2.2	74.5				74.1	12.9	32	1.5	48
Potatoes (boiled)	19.1	0.4	0.9	14.7	—	19.1	0.4	18.7	1.0	0.4	19.3	18.2	4.0	128	2.4	307
Potatoes (mashed)						17.0	0.3	16.7	0.9	0.6	17.4	22.4	3.6	74	3.3	244
Prunes (dried)	71.0	1.6	41.5	—	M-1.70	33.0	0.8	32.2	16.1	40.3	0.0	70.0	28.0	265	1.9	504
Prune juice	19.3	—	13.0	—	C-0.20	19.3	—	19.3?								
Raisins (dried)	71.2	—	63.0	—	M-1.80	71.2	—	71.2?	6.8	64.4	0.0					
Sugar (granulated)	99.5	—	99.5	—	—	99.5	0.0	99.5	—	99.9	—					
Sugar (maple)	90.0	—	90.0			90.0	—	90.0?								
Sugar (brown)	95.5	—	95.5	—	—	95.5	—	95.5?								
Tomato juice	4.3	0.2	3.4	—	C-0.40	4.3	0.2	4.1	1.5	2.8	Trace					
Zwieback	74.3	0.3	—	—	—							71.3	23.4	29	1.2	35

presence of sugar when carbohydrates dissolved in it during their passage through the oral cavity. He was able to associate increased caries activity with a high content of sugar in the saliva and a prolonged time for clearance of this sugar. On the basis of his index for caries potentiality, he classified such foods as candy, honey and sweetened bread as of highest potentiality. Recently (May 16, 1953)[92] a brief report from the same study at Lund states that "sugar in the form of toffee is more dangerous than sugar in chocolate." It is true that Teuscher and Fosdick[93] in 1937 observed in a study of 85 boys that sugar was retained longer after meals or consumption of candy in the salivas of those boys most susceptible to caries. Volker and Pinkerton[94] in 1942 stated a clearance time slowest after eating sticky candy and fastest after chewing gum. None of these studies, however, provided any information about the clearance time of sugar from bacterial plaques. Hence, the same criticism of the usefulness of the Lundqvist index, for example, may be expressed as for that of Bibby, Goldberg and Chen.

Such observations as those cited point, at least, to the desirability of a review of the essential oral enzymes as the next step in an evaluation of the cariogenic properties of sweetened beverages.

FATOR 4. APPROPRIATE BACTERIAL ENZYME SYSTEM

An appropriate bacterial enzyme system has been listed as an essential factor in the oral degradation of carbohydrates, inasmuch as amylase (ptyalin) is the only member of this complex system of enzymes found secreted naturally in significant amounts by the salivary glands. A serious interference with this enzyme system, at any level of activity prior to the production of acid, can serve to arrest the process of caries and can provide a technic for the control of caries. Some of the information which demonstrates the essentiality of such an oral enzyme system next will be examined.

Fosdick,[19] as early as 1939, decided, from two weeks of incubation of glucose with tribasic calcium phosphate and saliva at body temperature, that the enzymatic fermentation of glucose produced small amounts of phosphoglyceric, pyruvic, butyric and lactic acids which probably took part in dental caries. In 1940[25] he reported that the process of degradation of carbohydrates in the mouth was the same or similar to that in muscle tissue during muscular activity and that, in the mouth, the series of enzymes probably was derived from bacteria. He pointed out in cases of rampant caries that a pH as low as 4.0 can be attained in as short a time as three minutes, and that the lactic acid in the material of plaques may increase as much as 200 per cent in the first ten-minute interval following the ingestion of a sugar solution. In 1941,[68] with Campaigne and Fancher, he noted that amylase converts starch to maltose rather promptly but, for a number of reasons, little of the action takes place in the mouth. With Burrill,[21] in 1943, he pointed out that most concentrated sugar solutions produced an immediate drop in pH in caries lesions (927 patients studied) and that 10 per cent solutions of the monosaccharides, fructose and glucose, were the two sugar solutions which produced an initial drop of appreciable magnitude at this level of concentration. In 1948[96] he stated that 13 chemical reactions were necessary to carry sucrose down to organic acid.

92. Foreign letters. Research on dental caries. J.A.M.A. 152:264 May 16, 1953.

93. Teuscher, G. W., and Fosdick, L. S. Retention of reducing sugars in saliva after mastication of carbohydrates. (Abst.) J. D. Res. 16:354 Aug. 1937.

94. Volker, J. F., and Pinkerton, D. M. Acid production in saliva-carbohydrate mixtures. J. D. Res. 26:229 June 1947.

95. Fosdick, L. S. Etiology of dental caries. Illinois D. J. 9:248 July 1940.

96. Fosdick, L. S. The mechanism of caries control. Fort. Rev. Chicago D. Soc. 15:5 Feb. 15, 1948.

Florestano, Faber and James[97] in 1941 detected an increased diastatic activity of saliva from individuals with carious teeth or with teeth which later became carious. Hubbell[98] in 1933, however, reported no difference in diastatic activity for caries-free and caries-active children. At any rate, diastatic activity may not be truly a part of fermentation.

Barron[99] in 1945 may be quoted:

> In summary, the series of eleven enzyme systems (the 8th and 10th steps are nonenzymatic) of the first phase of fermentation is made up mostly of specific metalloproteins with, in instances, the addition of adenylic acid or its polyphosphates.

Lipner,[72] in 1947, it may be restated, designated, as an essential factor for caries in man, the presence of a monosaccharide or a disaccharide or of salivary amylase available to break complex carbohydrates down rapidly to a form utilizable by resident oral bacteria. Pfeffer[100] in 1948 pointed out that the splitting of the six-carbon chain, once a monosaccharide is achieved, still is a most complicated process, the first step of which involves adenosine triphosphate (ATP) for energy and hexokinase as a catalyst. The resulting products are adenosine diphosphate (ADP) and glucose phosphate. Splitting another section from another ATP molecule and utilizing a different catalyst converts glucose phosphate to fructose diphosphate. Continuing the process to lactic acid, Pfeffer enumerated 12 enzymes altogether which were involved in the fermentation of glucose.

Aitken[101] in 1949 sealed monosaccharides under amalgam in prepared cavities for his study of the process of caries and reached the gloomy conclusion that:

> . . . as long as the human race will persist in eating carbohydrates, as long as these carbohydrates are capable of being hydrolyzed to the monosaccharide state when present in the saliva, and as long as the tooth tissue remains permeable to these substances, we shall have dental caries.

Kite, Shaw and Sognnaes[102] in 1950 fed 13 rats a caries-producing diet for 16 to 25 weeks and fed 13 other comparable rats through a tube, thus by-passing the oral cavity. They found that none of the tube-fed rats developed carious lesions and all but one of the 13 controls did. From the results of experiments reported in 1950, Sreebny, Kirch and Kesel[103] concluded that "the acids formed by the action of saliva on suitable substrate occur as a result of microbial fermentation" and that their results "tend to lend credence to the fact that the enzyme systems involved are intracellular," hence, developed within the microorganisms. Weisberger,[104] also in 1950, found that intact human teeth, incubated in a solution containing glucose and mixed organisms from the oral flora, developed a pigmented carious lesion on the exposed surface of the enamel which penetrated the underlying dentin. Calandra and Adams[105] in 1951 postulated that immunity to caries in those who can ingest large amounts of sugar is due to an enzyme system capable of oxidizing some of the intermediate compounds in the degradation of glucose to lactic acid or even of lactic acid itself. *Micrococcus*

97. Florestano, H. J.; Faber, J. E., and James, L. H. Studies on the relationship between diastatic activity of saliva and incidence of dental caries. J.A.D.A. 28: 1799 Nov. 1941.

98. Hubbell, Rebecca B. The chemical composition of saliva and blood serum of children in relation to dental caries. Am. J. Physiol. 105:436 Aug. 1933.

99. Barron, E. S. G. Mechanisms of carbohydrate metabolism. An essay on comparative biochemistry. In Advances in enzymology and related subjects of biochemistry, Nord, F. F., and Workman, C. H., editors. New York, Interscience Publishers, 1945, p. 149-189.

100. Pfeffer, J. E. Enzymes. Scient. Am. 179:29 Dec. 1948.

101. Aitken, H. N. An investigation into the pathology of dental caries. New Zealand D. J. 45:75 April 1949.

102. Kite, O. W.; Shaw, J. H., and Sognnaes, R. F. An influence on dental caries experience produced in rats by tube feeding. (Abst.) J. D. Res. 29:668 Oct. 1950.

103. Sreebny, L. M.; Kirch, E. R., and Kesel, R. G. The location of the glycolytic enzymes in saliva. J. D. Res. 29:506 Aug. 1950.

104. Weisberger, David. Role of glucose in the production of artificial caries. J. D. Res. 29:14 Feb. 1950.

105. Calandra, J. C., and Adams, E. C., Jr. Oxidation of glucose degradation products in the presence of saliva and possible relation to caries immunity. J. D. Res. 30:229 April 1951.

lactilyticus, they found, was capable of oxidizing lactate rapidly and also pyruvate, acetate and propionate slightly. Pigman and Reid[79] in 1952 pointed out that starch substances (amylose, amylopectins, glycogen) were hydrolyzed relatively rapidly to dextrins (six, seven and eight molecules of glucose) but that these saccharides very slowly are reduced to the simpler monosaccharides, disaccharides or trisaccharides such as glucose, maltose and maltotriose.

In view of the preceding survey of a number of reports which deal with the degradation of carbohydrates, it should be of interest now to summarize the details of this complex process. West and Todd's[76] textbook will be used as the source of this summary of the degradation of starches.

THE DEGRADATION OF STARCH*

(From West and Todd,[76] p. 982)

1. Starch (amylase) = maltose.
2. Maltose (maltase) = glucose.
3. Glucose + adenosine triphosphate (i.e. ATP) (hexokinase) = glucose-6-phosphate and adenosine diphosphate (i.e. ADP).
4. Glucose-6-phosphate (phosphohexose isomerase) = fructose-6-phosphate.
5. Fructose-6-phosphate + ATP (phosphohexokinase) = fructose-1-6-phosphate + ADP.
6. Fructose-1-6-phosphate (aldolase) = dihydroxyacetone phosphate and 3-phosphoglyceric aldehyde (isomerase).

Note: With isomerase, dihydroxyacetone phosphate and 3-phosphoglyceric aldehyde are in equilibrium.

7. 3-phosphoglyceric aldehyde + H_3PO_4 = 1-3-diphosphoglyceric aldehyde.
8. 1-3-diphosphoglyceric aldehyde + diphosphopyridine nucleotide (i.e., DPN) (dehydrogenase) = 1-3-diphosphoglyceric acid + DPN • 2H.
9. 1-3-diphosphoglyceric acid + ADP (phosphokinase) = 3-phosphoglyceric acid + ATP.
10. 3-phosphoglyceric acid (phosphoglyceromutase) = 2-phosphoglyceric acid.
11. 2-phosphoglyceric acid—H_2O (enolase, Mg^{++}) = 2-phosphopyruvic acid (enol form).
12. 2-phosphopyruvic acid-enol form + ADP (phosphokinase, Mg^{++}, K^{+}) = enolpyruvic acid + ATP.
13. Enolpyruvic acid (spontaneous) = ketopyruvic acid.
14. Ketopyruvic acid + DPN•2H (dehydrogenase) = *lactic acid* + DPN.

Note: 14 steps in all.

*The parenthetic terms are the names of the enzymes and coenzymes which catalyze the reactions.

THE DEGRADATION OF FRUCTOSE

1. Fructose + ATP (hexokinase) = fructose-6-phosphate.
2. Steps 5 through 14 of starch degradation follow, utilizing 12 steps in all.

THE DEGRADATION OF GLYCOGEN

1. Glycogen (phosphorylase, adenylic acid, Mg^{++}) = glucose-1-phosphate.
2. Glucose-1-phosphate (phosphoglucomutase) = glucose-6-phosphate.
3. Steps 4 through 14 of starch degradation follow, utilizing 13 steps in all.

SUMMARY OF ENZYMES UTILIZED IN DEGRADATION OF STARCH TO LACTIC ACID

Step	*Enzyme*	*Step*	*Enzyme*
1.	Amylase	8.	Dehydrogenase
2.	Maltase	9.	Phosphokinase
3.	Hexokinase	10.	Phosphoglyceromutase
4.	Phosphohexose isomerase	11.	Enolase, Mg^{++}
5.	Phosphohexokinase	12.	Phosphokinase, Mg^{++}, K^{+}
6.	Aldolase	14.	Dehydrogenase

The necessity for ten enzymes (and a number of coenzymes) in order to initiate the activity at the various levels by which the process of degradation of glucose to lactic acid takes place points to at least ten stages in which enzymatic inhibitors might prove effective for interfering with dental caries. A substantial amount of research has been devoted to the development of such inhibitors as agents in the control of caries. Should some of them prove effective, the finding would lend further stature to the previously stated essentiality of an appropriate enzyme system in the process of caries.

The researches of Calandra, Fancher and Fosdick[106] in 1944, Burrill and others[107] in 1945, and Calandra and Adams[108] in 1950, indicate that enzymatic activity of saliva is blocked in the laboratory or mouth by various forms of the napthoquinones. In 1943, Stephan,[109] in 1942 and 1943, Hine and O'Donnell,[110,111] in 1946, Kesel and others,[112] and, in 1952, Rae[113] reported separately interference with enzymatic activity by carbamide (urea), urease-producing bacteria, and carbamide-quinine. Weisberger[114] in 1946 and Dreizen, Green and Spies[115] in 1947 found that the absence of the vitamin B fractions, thiamin and nicotinic acid, appeared to interfere with salivary production of acid. Dreizen and others[116] in 1947 completed research which indicated that sodium bisulfite interfered with salivary production of acid. In 1947, Calandra and Fosdick,[117] Kesel and others,[118] Mann and others[119] and Turner and Crowell[120] and, in 1948, Dreizen and Spies,[121] and, in 1950, Koser and Fisher[122] submitted reports of research with amino acids which showed that the presence of certain of these protein fractions in saliva reduced the production of acid. Fosdick and Calandra[123] in 1947 completed some promising laboratory studies with organic peroxides and glyceric aldehyde which indicated that glyceric aldehyde should be an active inhibitor of an enzyme involved in the degradation of sugars. Shaw,[124] however, in 1950, found it ineffective in the reduction of caries in rats. In 1950, Forbes, Cox and Smith[125] noted that hendecynoic acids and their ammonium salts reduced the production of acids in glucose saliva mixtures. In 1949, Hill and Kniesner[126] reported no

106. Calandra, J. C.; Fancher, O. E., and Fosdick, L. S. The effect of synthetic vitamin K and related compounds on the rate of acid formation in the saliva. J. D. Res. 23:31 Feb. 1944.

107. Burrill, D. Y., and others. The effect of 2-methyl-1, 4-naphthoquinone on the incidence of dental caries. J. D. Res. 24:273 Dec. 1945.

108. Calandra, J. C., and Adams, E. C., Jr. The inhibition of acid formation in the mouth by amino derivatives of 2,3-dichloro-1, 4-naphthoquinone. J. D. Res. 29:593 Oct. 1950.

109. Stephan, R. M. The effect of urea in counteracting the influence of carbohydrates on the pH of dental plaques. J. D. Res. 22:63 Feb. 1943.

110. Hine, M. K., and O'Donnell, J. F. Incidence of urease producing bacteria in saliva. J. D. Res. 22:103 April 1943.

111. Wach, E. C.; O'Donnell, J. F., and Hine, M. K. Effects of a mouth rinse on oral acidogenic bacteria. J.A.D.A. 29:61 Jan. 1942.

112. Kesel, R. G., and others. Biological production and therapeutic use of ammonia in the oral cavity in relation to dental caries prevention. J.A.D.A. 33: 695 June 1, 1946.

113. Rae, J. J. Some aspects of the chemical mechanisms involved in dental caries. J. Canad. D. A. 19: 249 May 1952.

114. Weisberger, David. Whole saliva as a source of certain growth factors for an oral strain of lactobacillus. J. D. Res. 25:83 April 1946.

115. Dreizen, Samuel; Green, H. I., and Spies, T. D. The utilization of cereals in various stages of refinement by an oral strain of Lactobacillus acidophilus. J. D. Res. 39:307 June 1950.

116. Dreizen, Samuel, and others. The influence of sodium bisulphite on acid production in saliva. J. D. Res. 26:93 April 1947.

117. Calandra, J. C., and Fosdick, L. S. The effect of amino acids on the rate of acid formation in the saliva. J. D. Res. 26:303 Aug. 1947.

118. Kesel, R. G., and others. The amino acids and their deaminating systems present in human saliva. Am. J. Orthodont. & Oral Surg. (Oral Surg. Sect.) 33: 68 Feb. 1947.

119. Mann, A. W., and others. A comparison of dental caries activity in malnourished and well-nourished children. J.A.D.A. 34:244 Feb. 15, 1947.

120. Turner, Naomi, C., and Crowell, G. E. Dental caries and tryptophane deficiency. J. D. Res. 26: 99 April 1947.

121. Dreizen, Samuel, and Spies, T. D. Further studies on the association between the products of protein putrefaction and dental caries activity. J. D. Res. 27: 305 June 1948.

122. Koser, S. A., and Fisher, Barbara J. Vitamin requirements of oral lactobacilli. J. D. Res. 29:760 Dec. 1950.

123. Fosdick, L. S., and Calandra, J. C. The effect of some chemicals on the rate of acid formation in the saliva. J. D. Res. 26:309 Aug. 1947.

124. Shaw, J. H. Studies on the effect of di-glyceric aldehyde on the dental caries activity in white rats and cotton rats. J. D. Res. 29:23 Feb. 1950.

125. Forbes J. C.; Cox, C. D., and Smith, J. D. Effect of certain hendecynoic acids and their ammonium salts on acid production in saliva containing glucose. J. D. Res. 29:583 Oct. 1950.

126. Hill, T. J., and Kniesner, A. H. Penicillin dentifrice and dental caries experience in children. J. D. Res. 28:263 June 1949.

significant reduction in caries activity by the use of a penicillin dentifrice. In 1950, Zander,[127] as well as Fitzgerald, Zander and Jordan,[128] found that penicillin reduced the development of new cavities in study groups of patients. Ludwick, Fosdick and Schantz[129] in 1951, experimenting with enzymatic inhibitors in dentifrices, found 0.07 per cent penicillin the most effective of the agents studied. Hill, Sims and Newman[130] in 1952 found no significant changes in caries activity as the result of the use of a penicillin dentifrice when an experimental group of boys was compared with a control group.

Obviously, the possibilities of the practical utilization of enzymatic inhibitors appear promising but their usefulness in the control of human dental caries is yet to be demonstrated.[130a] It may be that Fosdick, Ludwick and Shantz,[131] in 1951, while they were studying the extent of retention of these inhibitors in bacterial plaques, pointed out the reason for limited successes *in vivo* to date. Presumably, the inhibiting agent not only should diffuse into the bacterial plaque but should be retained there for an appreciable period of time in order to serve as the basis for a practical technic of caries control. This finding, at least, highlights the importance of a thorough presentation of the evidence for the essentiality of the bacterial plaque during an appraisal of the effect of sweetened beverages on teeth.

FACTOR 5. BACTERIAL PLAQUE

Williams[5] and Black,[4] it will be recalled, pointed out the importance of the bacterial plaque in the process of caries in the early 1890's; Williams, in fact, in a study of over 400 cases, found that, invariably, caries resulted from acids generated in the mouth under a feltlike mass of microorganisms. Miller[132] in 1902 reported that "films and caries must occur together." Bunting and Rickert's[133] extensive study of oral bacterial plaques in 1914 led to the conclusion that:

> . . . films or colloidal coatings are found upon all teeth: and that they play an important part in the chain of factors which, by their balance, determine the process of caries.

Since these early observations of the essentiality of an adherent bacterial plaque as one of the factors in the process of caries, many investigators have added information about the behavior of dental plaques. Inasmuch as the dental plaque appears to be such an important mechanism in the process of caries, since the bacteria and acids involved have to be protected by it and since a number of salivary agents which modify caries have to penetrate this barrier, information about the oral bacterial plaque will be reviewed in regard to (1) structure, (2) bacteriology, (3) behavior as a selective membrane and (4) acidity.

Structure of the Dental Plaque • Of the earlier investigators, Williams,[5] Miller[132] and Bunting and Rickert,[133] all pointed out that the long, threadlike actinomycetes, leptothrix and cladothrix appeared to serve as the basis of the plaque, that these threadlike forms entrapped oral debris and provided a shelter for a number of smaller organisms in the

127. Zander, H. A. Effect of a penicillin dentifrice on caries incidence in school children. J.A.D.A. 40:569 May 1950.

128. Fitzgerald, R. J.; Zander, H. A., and Jordan, H. V. The effects of penicillin dentifrice on oral lactobacilli. J.A.D.A. 41:62 July 1950.

129. Ludwick, W. E.; Fosdick, L. S., and Schantz, C. W. Effect of dentifrices on lactobacillus counts: antibiotics and enzyme inhibitors. J.A.D.A. 43:285 Sept. 1951.

130. Hill, T. J.; Sims, John, and Newman, Millicent. The effect of penicillin dentifrice on the control of dental caries and the production of resistant organisms. (Abst.) J. D. Res. 31:461 Aug. 1952.

130a. Fosdick, L. S.; Calandra, J. C.; Blackwell, R. Q., and Burrill, J. H. A new approach to the problem of dental caries control. J. D. Res. 32:486 Aug. 1953.

131. Fosdick, L. S.; Ludwick, W. E., and Schantz, C. W. The absorption of enzyme inhibitors and antibiotics in dental plaques. J.A.D.A. 43:26 July 1951.

132. Miller, W. D. The presence of bacterial plaques on the surface of the teeth, and their significance. D. Cosmos 44:425 May 1902.

133. Bunting, R. W., and Rickert, U. C. Dental caries. N. D. A. Bulletin 1:16 Oct. 1914.

mouth. Bunting and Rickert pointed out that there was considerable difference in their rate of attachment but, in many cases, a thick, adherent growth would develop in 24 hours.

Bradel and Blayney[61] in 1940, studying ground sections of enamel, reported that the plaque grossly was a "felt-like mass with long filamentous forms protruding." Miller, Muntz and Bradel,[134] the same year, stated that the dental bacterial plaque was a thin film which adhered tenaciously to a tooth and could not be removed by a stream of water or by ordinary swabbing or brushing; that, microscopically, it appeared as a mass of small bacteria embedded in a matrix of filamentous microorganisms, the bacteria supplying perhaps half of the total bulk. Dietz[22] in 1943 watched microscopically the development of the plaque. He found that leptothrichoid and oidiumlike organisms served as the base of the plaque, becoming a thin plaque in one day under which initial etching of the enamel would take place one-half hour after the proper carbohydrate was applied. In 1948 Ennever, Robinson and Kitchin[34] procured bacterial plaques on celloidin pontics inserted in the human mouth and found that new plaques contained a multitude of filamentous organisms, parallel to each other and attached to the surface roughly at right angles. Bruckner[135] studied plaques in 1948 which he obtained by suspending sheets of cellophane in a saliva-glucose mixture for three weeks. He noted the matrix of threadlike organisms and summarized briefly current knowledge of the structural characteristics of the dental plaque about the same as Miller, Muntz and Bradel had done earlier. The plaque is:

> . . . a thin, adherent film, white and cloudy in appearance, present on improperly cleansed tooth surfaces, and consisting of microorganisms and debris that is primarily of organic nature. It cannot be removed from the tooth surface by a stream of water or by gentle swabbing, but can be removed by vigorous brushing, especially with the aid of a mild abrasive.

Bacteriology of the Dental Plaque • The bacteriology of the dental plaque was reviewed earlier in connection with Factor 2, acid-producing bacteria. It appears significant to recall at this point, however, that Hemmens, Blayney and Harrison[64] in 1941 and Hemmens and others[65] in 1946 found lactobacilli present in high percentages during the period of initiation of caries and that only lactobacilli and a few hemolytic streptococci survive in their own lowered pH. Stralfors[56] in 1948, it should be noted, found a statistically significant relationship between a minimum pH and the number of lactobacilli.

Behavior of the Dental Plaque as a Selective Membrane • In 1932 Dobbs[13] made a thorough study of the behavior of plaques removed from teeth by moistening with 5 per cent hydrochloric acid. With the plaque sealed to a diffusion apparatus he found that the salivary buffers (carbonates and phosphates) and the viscous, amphoteric, salivary mucin did not pass such a membrane but that soluble carbohydrates did pass through rapidly at the rate of 3.2 mg. per day. Pincus,[136] working with a somewhat similar membrane obtained from the surfaces of newly erupted teeth, reported in 1937 that the enamel cuticle (Nasmyth's membrane), when placed over windows in glass slides, permitted the dialysis of crystalloids but not of colloids. One, 5 and 10 per cent lactic acid, cystine, sucrose and sodium chloride passed through but starch, gums, dextrins and proteins, which do not crystallize, did not dialyze through the membrane.

134. Miller, B. F.; Muntz, J. A., and Bradel, Sigmund. Decomposition of carbohydrate substrates by dental plaque material. J. D. Res. 19:473 Oct. 1940.

135. Bruckner, R. J. In vitro studies of sodium bicarbonate in relation to certain factors concerned with dental caries. J. D. Res. 27:128 April 1948.

136. Pincus, Paul. Nasmyth's membrane: structure and permeability. Brit. D. J. 63:139 Aug. 2, 1937.

Dietz[22] in 1943 noted that the buffer capacity of saliva was no lower in the mouths of caries-active than in the mouths of caries-free individuals, and hence that some form of protective covering would have to be present on the surface of an affected tooth. Others then demonstrated the selective permeability of the dental plaque to a number of substances. Stephan and Miller[54] in 1943 demonstrated that glucose penetrates such a plaque promptly; the same year,[137] they showed that Zephiran and Phemerol penetrated plaques and interfered in the expected drop of pH in an inverse relationship to the thickness of the plaque. In 1943 Stephan[109] also showed that urea in concentrations of 1 to 50 per cent would penetrate the dental plaque readily; again in 1944 Stephan and Miller[138] demonstrated that Zephiran and saturated solutions of urea would penetrate plaques; in 1943 Muntz and Miller[139] also showed that glucose and urea penetrated the dental plaque; in 1948 Bruckner,[135] reporting on the selectivity of bacterial plaques developed on cellophane sealed over 6.9 mm. glass tubes, stated that the plaque permitted the passage of sodium bicarbonate at a reduced rate; and Fosdick, Ludwick and Schantz[131] in 1951 reported the passage and prolonged retention of penicillin in the plaque.

Acidity of the Dental Plaque • Research reported by Etherington and Trimble[140] in 1934 indicated that "in every instance the plaque interior was more acid than the corresponding saliva." In 1938 Stephan,[58] it may be repeated, determined that the pH values of 211 plaques varied from 4.6 to 7.0, while in 1939 Miller and Muntz[141] reported that the amount of acid in 15 human carious lesions varied with the soluble calcium and phosphorus present. Again, too, it may be repeated that Fosdick and Starke[142] pointed out in 1939 that the pH of an individual's saliva can be too high for decalcification to occur while, at the same time, the pH in a bacterial plaque becomes so low that decalcification takes place.

Stephan[20] in 1940 reported in detail the effects of 10 per cent glucose rinses on bacterial plaques when measured in patients' mouths. The average maximum variation found was 2.0 pH units. In 18 minutes after the rinse, all plaques measured showed a pH of 5.0 or less and, about two hours later, approached their original pH. A second glucose rinse repeated the same finding. Since the greatest intensity of reduction of the pH in plaques was reached during the first 30 minutes and was gradually overcome during the next two to three hours, it appears that the repeated renewal of a carbohydrate which can diffuse into the plaque is important in the development of a cavity. In 1940 Miller, Muntz and Bradel[134] demonstrated that plaque material removed from teeth produced lactic acid readily from sucrose and maltose but to a much lesser degree from lactose, indicating that milk, from a cariogenic as well as a nutritional standpoint, appears to be a more desirable beverage than a sweetened drink for a school lunch.

The work of Muntz[143] in 1943 confirmed that a maximum acidity is produced in plaques by dilute glucose solutions in 30 minutes, but that acidity in incubated saliva probably is buffered within 30 to 90 minutes. Stephan[46] in

137. Stephan, R. M., and Miller, B. F. The effect of synthetic detergents on pH changes in dental plaques. J. D. Res. 22:53 Feb. 1943.

138. Stephan, R. M., and Miller, B. F. Effectiveness of urea and synthetic detergents in reducing activity of human dental caries. Proc. Soc. Exper. Biol. & Med. 55:101 Feb. 1944.

139. Muntz, J. A., and Miller, B. F. Factors influencing penetration of synthetic detergents and certain compounds into dental plaque material. J. D. Res. 22:73 Feb. 1943.

140. Etherington, J. W., and Trimble, H. C. Acidity in plaques. (Abst.) J. D. Res. 14:218 June 1934.

141. Miller, B. F., and Muntz, J. A. The quantity of lactic acid in carious lesions of human teeth and its relationship to the soluble calcium and phosphate in these lesions. (Abst.) J. D. Res. 18:259 June 1939.

142. Fosdick, L. S., and Starke, A. C., Jr. Solubility of tooth enamel in saliva at various pH levels. J. D. Res. 18:417 Oct. 1939.

143. Muntz, J. A. Production of acids from glucose by dental plaque material. J. Biol. Chem. 148:225 April 1943.

1948 found that a 10 per cent glucose solution introduced into the mouths of caries-free patients produced a drop in the pH of their bacterial plaques but never below 5.0, while a drop to 4.0 occurred promptly in the caries-active person's mouth. Stralfors[144] in 1948 observed that the plaque appears to have the ability to store acid and to hinder the saliva from neutralizing it. McGlynn[145] in 1949 reported that only eight hours was required for lactic acid, in concentrations detected in dental plaques, to penetrate enamel. Neuwirth[146] in 1950 found that citric acid was not produced from the action of oral microorganisms on glucose. Recently (1952), Fosdick[147] has published a stimulating review of the behavior of dental bacterial plaques, and has listed speculations which might explain the variance in pH determinations reported from studies which have been pursued.

The work reported by Stephan[148] in 1944 seems so important to an understanding of the role of the bacterial plaque and so important to a proper appraisal of the cariogenic properties of sweetened beverages that some of his report is repeated at this point:

> Since most of the work which has been reported on acid production by mouth bacteria has not simulated conditions in the mouth, it is worth pointing out some essential differences between such studies and the experiments described here. The bacteria growing on tooth surfaces exist in concentrated masses or "plaques" which possess a maximum concentration of enzymes and coenzymes in a minimum space with minimum buffering capacity. The acid production by such bacterial masses is very rapid when carbohydrate is available and, since little initial buffering capacity must be overcome, the drop in pH is likewise very rapid. . . .
>
> The subjects (65 in number) were seated in a semi-reclining position in a chair equipped with a headrest. They were instructed not to talk during the test. The labial surfaces of the upper and lower anterior teeth were generally chosen for the tests because of their accessibility. In some of the caries-active individuals, particularly those in Group V (8 in number), the enamel was etched or cavities existed under the plaques on these tooth surfaces. Direct pH determinations were made on the tooth surfaces with a polished-stick antimony electrode using a vacuum tube potentiometer. . . . The mouth was rinsed with 25 c.c. of a 10 per cent aqueous solution of glucose for two minutes following initial pH determinations. The pH determinations were repeated two minutes after the glucose rinse and at 10-minute intervals thereafter until the pH had returned to approximately its original value.
>
> Before the application of glucose, the pH values of most areas were around neutrality except in the cases of extreme caries activity, in which pH values were generally considerably lower. In all cases there was a sharp drop in the pH of bacterial material on teeth following rinsing the mouth with the glucose solution. The drop in pH was greatest and lasted the longest time in the caries-active cases.
>
> . . . Only in the caries-active cases were pH values below 5.0 consistently produced by the glucose.

Recently a report by John Haldi and Winfrey Wynn[148a] has been prepared for publication. On the basis of their study, findings were presented which indicated that drinking (5 unscreened subjects) or rinsing the mouth (8 subjects screened for rampant caries) with a 10 per cent solution of sucrose did not produce a sufficient drop in pH to initiate decalcification of the surface of a tooth—a finding quite opposite to Stephan's observations with 65 carefully screened subjects (4 categories).

It is of interest to note that Haldi and Wynn report no significant differences in the pH developed from drinking or from rinsing out the mouth with a 10 per cent solution of sucrose.

144. Stralfors, Allan. Studies of the microbiology of caries. III. The buffer capacity of the dental plaques. J. D. Res. 27:587 Oct. 1948.

145. McGlynn, R. P. The effect of organic acids on human tooth enamel. D. Record 69:60 March 1949.

146. Neuwirth, Isaac. The production of acids from glucose by oral microorganisms: citric acid. J. D. Res. 29:589 Oct. 1950.

147. Fosdick, L. S. The effect of sugar on the dental plaque. J. South. California D. A. 20:16 Nov. 1952.

148. Stephan, R. M. Intra-oral hydrogen-ion concentrations associated with dental caries activity. J. D. Res. 23:257 Aug. 1944.

148a. Haldi, John, and Wynn, Winfrey. Changes in the hydrogen ion concentration on the surface of the teeth, the gingiva, oral mucosa and of saliva after drinking an aqueous solution of sucrose. To be published.

DECALCIFYING AND CARIOGENIC PROPERTIES OF SWEETENED DRINKS

It was suggested at the beginning of this report, once the primary etiologic factors in dental caries were established and documented and once the importance of the dental bacterial plaque was discussed thoroughly, that the role which sweetened beverages may play either in decalcification or caries of the teeth should be explored. The possibility of decalcification of the teeth will be examined first.

Sweetened Beverages and Decalcification • It would appear that the pH of sweetened drinks and the pH which they can induce in saliva should have an important bearing on an appraisal of the effects of these solutions. Their acidity, therefore, will receive first consideration.

Restarski, Gortner and McCay[149] in 1945 and McCay and Will[150] in 1949 reported the analysis of a cola drink, for example, at the Naval Medical Research Institute in Bethesda, Md. It was "found to contain 10 per cent by weight of sucrose and 0.55 per cent of phosphoric acid (H_3PO_4). Electrometric pH measurements showed it to have an acidity equal to pH 2.6." In view of this reported analysis which may serve as a pattern, it appears advisable to present information about some of the prepared drinks and the natural fruit juices (their pH, percentage of acid and percentage of sugar) prior to any attempt to assess their potentialities for damage to human teeth. Bridges and Mattice[151] in 1939, Chatfield and Adams[80] in 1940, McClure[32] in 1943, Haggard and Greenberg[152] in 1951, and Miller[153] in 1952 contribute some of this information (Table 2).

Some of the comment on the decalcifying effect of acid beverages now may be presented chronologically. McClelland,[52] commenting on the dissolution of enamel in 1926, presented evidence that a definite loss of weight in pieces of enamel submerged in a solution with a pH under 4.5 takes place within 12 hours, and that decalcification occurs very rapidly in ranges of pH 1.0 to 2.5 (Bridges and Mattice[151] determined the range for a cola beverage as pH 2.4 to 2.65). McClelland concluded from his study:

> Certainly the presence of a reaction of 3.5 and below, even if existing for only a few minutes, is a potential source of damage to teeth.

Miller and Neuwirth[154] in 1935 reported their observation of cough drops (80 per cent sucrose), flavored disks (96 per cent sucrose) and flavored hard candies (99 per cent sucrose) held in the mouths of five adult patients daily until dissolved in the saliva. All experienced extravagant decalcification of their teeth.

West and Judy[155] in 1938 reported a somewhat similar study. They stated:

> When an individual places a piece of ordinary acidified candy in his mouth and allows it to dissolve slowly against his teeth, the concentration of the solution at the surface of the candy (and in contact with tooth enamel) will be very high with a probable pH of 3.4. . . .

They reported further that 10, 20, 40 and 60 per cent water solutions of orange, lemon and lime drops provided an initial pH of 2.5 to 2.8 (slightly less acid than the cola beverage) and that 40 per cent solutions in saliva developed a pH of 3.4

149. Restarski, J. S.; Gortner, R. A., Jr., and McCay, C. M. Effect of acid beverages containing fluorides upon the teeth of rats and puppies. J.A.D.A. 32:668 June 1, 1945.

150. McCay, C. M., and Will, Lois. Erosion of molar teeth by acid beverages. J. Nutrition 39:313 Nov. 1949.

151. Bridges, M. A., and Mattice, Marjorie R. Over 2000 estimations of pH of representative foods. Am. J. Digest. Dis. 6:440 Sept. 1939.

152. Haggard, H. W., and Greenberg, L. A. The acidity of saliva after ingestion of carbohydrate- and acid-containing substances. J. D. Res. 30:126 Feb. 1951.

153. Miller, C. D. Enamel erosion properties of fruits and various beverages. J. Am. Dent. Assn. 28:319 April 1952.

154. Miller, S. C., and Neuwirth, Isaac. Tooth decalcification due to hard candies. Dental Cosmos 77:453 May 1935.

155. West, E. S., and Judy, E. R. Destruction of tooth enamel by acidified candies. J. D. Res. 17:479 Dec. 1938.

Table 2 • The pH, percentage of acid, and percentage of sugar in prepared drinks and natural fruit juices

Product	Range in pH	Range in pH	Range in pH	pH of fluid	pH of saliva after 5 min.	Total carbohydrate %	Fiber %	Sugar %	Starch %	Acid % *
	McClure[32]	Bridges & Mattice[151]	Miller[153]	Haggard & Greenberg[152]		Chatfield and Adams[80]				
Cider (sweet)						12.5	—	10.5	—	0.52m
Coca Cola	2.6—2.7	2.40—2.65	(No. 1) 2.6	2.64	5.18	10.0†	—	10.0†	—	0.55†p
Cranberry juice (cocktail)	2.5—2.6									
Ginger ale	2.8—3.2	2.68—2.78	4.0—4.1							
Grape Juice			4.1	3.04	4.92	22.1		19.9		0.65m
Grapefruit juice	3.2—3.5					8.1	—	6.7	—	1.42c
Le monade		2.70—2.75	Comm. 3.1							
			Home 4.3							
Lemon juice (fresh)						8.3	—	2.3	—	5.96c
Limeade		2.56								
Lime juice (fresh)						8.3	—	1.4	—	6.90c
Orangeade		3.75	3.0—3.2							
Orange juice (fresh)		3.13—4.15	4.2	3.49	5.08	10.1	—	9.0	—	1.16c
Pineapple juice (canned)			3.8			12.8	—	11.8	—	1.02c
Prune juice (canned)		3.20—3.34				19.3	—	13.0	—	0.20c
Root Beer			3.0—3.2							
Tomato juice (canned)				4.22	4.84	4.3	0.2	3.4	—	0.40c
Vinegar (cider)		3.12								
Water	6.5—6.6									

*c = citric; m = malic; p = phosphoric. †McCay and Will[150]

to 3.7 as compared with 40 per cent solutions of sucrose in saliva which developed a pH of 3.2. They concluded that repeated use throughout the day leads to serious decalcification.

Bridges and Mattice[151] in 1939, reporting pH values of representative foods, commented that the least acid were bananas, figs, papayas, watermelons, cantaloupes and avocados with a pH of 5.0 to 6.0+; while those below pH 3.0 were some grapes, plums, some grapefruit juice, cranberry juice, limes and lemons. In a concluding statement, they reported:

> It is possible that the future will demonstrate that the pH of food is of little moment. Presumably the most important factor is the property of the individual food in stimulating the flow of gastric juice whether this be attributed to hydrogen-ion, to other constituents in the food, or to products of digestion. . . . None approximate normal gastric acidity at the height of digestion (pH 1.6-1.8).

Bridges and Mattice, of course, were not dentists.

Trask, Ziegler and Maloof[156] in 1940 reached five conclusions about the decalcification of fragments of freshly extracted teeth following quantitative studies of five-days' exposure to the dripping of a variety of solutions:

1. Starches and sugars per se do not decalcify teeth, but lactic acid formed from them may do so.
2. Acid foods having a pH of about 4 or below may prove to be the most important factors in dental decalcification.
3. Other acids than lactic acid in foods have been shown to cause extensive decalcification of teeth.
4. Many commonly used foods are acid and have a pH from 4 to 2.05.
5. The alkalis studied do not demineralize teeth.

McClure[32] in 1943 found hydrochloric acid, pH 1.5, more destructive of rats'

156. Trask, J. W.; Ziegler, E. E., and Maloof, E. C. The solvent action of various substances on teeth: a quantitative determination. J.A.D.A. 27:1013 July 1940.

molars than lactic acid, pH 2.4, but citric acid, pH 2.5, lactic acid, pH 3.0, and lactic acid, pH 4.0, also would erode rats' teeth. He experimented with young rats who were limited in their drinking of fluids (changed each 24 hours) to ginger ale, cola drink, grapefruit juice and cranberry juice cocktail. All rats on these acid beverages experienced decalcification of their molars. The control rats, drinking water, did not experience this decalcification.

McCay, Gortner and Restarski[157] and Restarski, Gortner and McCay[149] in 1945 reported:

In an initial experiment, some extracted human teeth were immersed in a common "cola" beverage. When first inspected after two days' immersion the enamel surfaces were found to be grossly decalcified.

Severe destruction of the enamel on the molars of white rats (200 rats) was produced by allowing the animals to drink the popular soft beverage for periods of five days or more. . . .

Preliminary observations on six puppies showed that consumption of soft drinks (500 c.c. per day) also produced gross and microscopic changes on their deciduous teeth.

Bieri and others[158] in 1946, proceeding on the assumption that limited amounts of acid beverage would produce erosion of the teeth of rats, dogs and monkeys, tested this assumption in an experimental situation. They reported:

1. Drinking of an acetic acid-sucrose solution of pH 2.6 for 1-2 weeks produced mild to moderate etching of rats' molars. Orange juice of pH 3.7 decalcified the teeth severely in two weeks. Solutions of pH 4.5 caused no etching when fed for two weeks.
2. The permanent teeth of dogs drinking a phosphoric acid-sucrose solution of pH 2.6 for 130 days showed etching of the enamel. . . .
3. Consumption of 16.5 ml. daily for one month of phosphoric and citric acid solutions of pH 2.6 produced noticeable erosion of the deciduous teeth of young monkeys.

Stafne and Lovestedt,[159] at the Mayo Clinic in 1947, reported their observations of the oral conditions of patients examined in this Clinic. Fifty patients who were using lemon juice as a therapeutic measure showed evidence of dissolution of tooth structure. They continued their report:

Restarski and co-workers stated that the results of their observations on animals (drinking a popular cola beverage) did not justify their making any definite conclusions as to what effects ingestion of acid beverages might have on the teeth of man. We have observed in some cases, however, destruction of enamel which we believe can be ascribed to the habitual use of acid beverages.

In 1948, Wynn and Haldi[160] summarized their observations of experiments with rats:

Erosion in the lower molar teeth of the albino rat resulted from the daily ingestion of each of the following canned fruit juices when they were served as the sole fluid intake over a period of 100 days: apple, grape, orange, tomato, sweetened grapefruit, pineapple, prune.

The greatest amount of erosion occurred in the teeth of the animals on apple, grape and grapefruit juice, and the least in those of the animals on tomato and prune juice.

Erosion of a milder degree also resulted from the ingestion of tomato, orange and sweetened grapefruit juice 3 times a day for 200 days.

Complicating the interpretation of studies of decalcification are the findings of Brudevold[161] in 1948. He found that there was a distinct difference in the solubility of powdered enamel and intact enamel, of different surfaces of the same tooth, of outer and inner levels of enamel from the same tooth, and of enamel from deciduous and young permanent teeth

157. McCay, G. M.; Gortner, R. A., and Restarski, J. S. The influence of fluoride on in vivo tooth decalcification in rats and dogs by acid beverages. Federation Proc. 4:158 March 1945.

158. Bieri, J. G., and others. Further studies on in vivo tooth decalcification by acid beverages. Arch. Biochem. 11:33 Sept. 1946.

159. Stafne, E. C., and Lovestedt, S. A. Dissolution of tooth substance by lemon juice, acid beverages and acids from some other sources. J.A.D.A. 34:586 May 1, 1947.

160. Wynn, Winfrey, and Haldi, John. The erosive action of various fruit juices on the lower molar teeth of the albino rat. J. Nutrition 35:489 April 1948.

161. Brudevold, Finn. A study of the phosphate solubility of the human enamel surface. J. D. Res. 27:320 June 1948.

when compared with older permanent teeth. The values obtained from different laboratories presumably will vary accordingly.

McCay and Will[150] in 1949 became interested in the dental effects of soft drinks which were claimed to have a retail value annually in excess of $700,000,000, and they tested the pH produced by a cola beverage in the salivas of 32 young officers at the Naval Medical Research Institute. Ten milliliters of the beverage were held in each mouth, agitated for 30 seconds and then expectorated. The range in pH of the saliva and solution recovered varied from 2.8 to 5.6.

Hicks[162] in 1950 and again in 1951[163] reported the effects of the daily ingestion of large amounts of citrus fruits and juices on a group of patients, selected because of periodontal irritation or "sensitive" teeth. One of the four patients with sensitive teeth ate daily large portions of sliced fruit salad and drank eight ounces of orange, tangerine or tomato juice. The decalcification of this patient's teeth was similar to that in the teeth of the patients described by Stafne and Lovestedt.[159]

Haggard and Greenberg[152] in 1951 concluded that the ingestion of carbohydrate-containing substances did not cause significant acidity of the saliva, although acidulated beverages such as common fruit juices or soft drinks did result in salivary acidity for short periods. They concluded, further, that decalcification of the exposed surfaces or sheltered areas of teeth by the action of fruit juices or soft drinks appeared unlikely in the amounts normally consumed. Their final conclusion may be quoted:

> It is true that there are, in fact, persistent areas of carbohydrate and corrosive acidity on sheltered surfaces of the teeth and under plaques which are protected from dilution and neutralization by the saliva: it would be expected that this protection would also operate against further acidification by the brief tides of acidity occurring in the saliva after ingestion of the acidulated beverages.

Inasmuch as the evidence submitted earlier in the portion of the present report dealing with bacterial plaques does indicate that a number of acids can diffuse into bacterial plaques, this latter assumption appears not to be borne out by scientific observations.

Elsbury[164] in 1952 tested the action of comparable solutions of hydrochloric, nitric, sulfuric, acetic and citric acids on exposed surfaces of extracted teeth. He concluded:

> The results suggest that dental erosion of this kind is mainly controlled by the pH of the solution, and is independent of the concentration of the solution by weight. The rate of attack varies, however, from acid to acid due to specific properties of the acids themselves. Noteworthy is citric acid which is more than twice as destructive as hydrochloric or nitric acid.

Gortner and Kenigsberg[165] in 1952 experimented with 28 white rats who were given daily 20 milliliters of grapefruit juice sweetened by 10 per cent of sucrose, and 25 rats given grapefruit sections, similarly sweetened and in daily amounts to provide 20 milliliters of juice. At the end of one week, the animals were sacrificed and the lower molars scored for decalcification on the basis of zero for "no effect" to six for "extensive destruction." The average tooth scores for the juice was 3.8 and for fruit sections 1.5, showing a higher rate of decalcification by the juice. To check the possibility that chewing stimulated a greater flow of saliva and hence more buffering, eight desalivated rats were tested similarly,

162. Hicks, Henry. Excessive citrus juice consumption. Clinical observations of its effect on superficial and deep tissues of the oral cavity. J.A.D.A. 41:38 July 1950.

163. Hicks, Henry. Excessive citrus juice consumption. II. Clinical observations of its effect on superficial and deep tissues of the oral cavity. Oral Surg., Oral Med. & Oral Path. 4:858 July 1951.

164. Elsbury, W. B. Hydrogen-ion concentration and acid erosion of the teeth. Brit. D. J. 93:177 Oct. 7, 1952.

165. Gortner, R. A., Jr., and Kenigsberg, R. K. Factors concerned with the different erosive effects of grapefruit and grapefruit juice on rats' molar teeth. J. Nutrition 46:133 Jan. 1952.

and it was found that the teeth still experienced the same amount of decalcification.

Soifer[166] in 1953 reported on a patient who had used excessive vinegar when she was between 15 to 25 years of age. This patient exhibited extensive erosion of the labial surfaces of her maxillary incisors.

At this point, it would seem, some legitimate conclusions about the decalcifying effect of sweetened beverages and acid fruit juices should be submitted. It would appear, from the evidence examined (1) that a number of soft drinks possess a high degree of acidity as is shown by their unusually low pH, (2) that a number of fruits and fruit juices provide a pH below the critical point for the decalcification of enamel, (3) that decalcification does occur when either are consumed in large amounts or at frequent intervals and (4) that such decalcification should not be significant unless abnormal amounts of such drinks are consumed.

Sweetened Beverages and Caries • The final task of this report is to examine the cariogenic properties of soft drinks. The essential role in the process of caries of fermentable carbohydrates already has been stated and documented. In addition, the necessity for the introduction into the oral cavity of a carbohydrate which can be degraded promptly to lactic acid by the bacterial enzymes produced in caries-active mouths has been pointed out as a part of this documentation. Furthermore, an adherent, selective bacterial plaque has been shown to be necessary in the formation and development of carious lesions in enamel, and the evidence of the ability of simple sugar solutions to penetrate into dental plaques promptly and to be utilized almost immediately after their ingestion has been summarized and documented. Finally, it should be noted that any cariogenic property to be ascribed to a sweetened beverage, such as the cola drink already discussed, must be based on the behavior of a 10 per cent or less solution of sucrose or other readily fermented sugar. A brief, chronological review of some pertinent conclusions of investigators of the damaging potential of sugar solutions now appears to be in order.

In 1937, Fosdick, Hansen and Epple[167] found that saliva stimulated by chewing gum (in order to provide sugar in solution) could be incubated with the powdered hard tissue of teeth and could produce decalcification of these tissues. They obtained similar results by adding 5 per cent glucose. This finding was incorporated into a test for caries activity.

In 1942 the Council on Foods and Nutrition of the American Medical Association[70] presented a report on wartime nutrition that appears appropriate to the present evaluation. Some of the final statement will be quoted:

> However, even with soldiers and sailors supplied with satisfactory rations, the tendency to say in effect "Let's give the boys what they want; it isn't going to hurt them," if allowed to go to the extent of permitting the indiscriminate use of soft drinks and candy, will undermine efficiency. Indiscriminate and uncontrolled supply of poor food for between-meal eating cannot be condoned with impunity anywhere.

This Council then submitted the final opinion:

> From the health point of view, it is desirable especially to have restriction of such use of sugar as is represented by the consumption of sweetened carbonated beverages and forms of candy which are of low nutritional value. The Council believes it would be in the interest of the public health for all practical means to be taken to limit the consumption of sugar in any form in which it fails to be combined with significant proportions of other foods of high nutritive value.

166. Soifer, M. E. Dental erosion. New York D. J. 19:111 March 1953.

167. Fosdick, L. S.; Hansen, H. L., and Epple Charlotte. Enamel decalcification by mouth organisms and dental caries: a suggested test for caries susceptibility. J.A.D.A. & D. Cosmos 24:1275 Aug. 1937.

Jay[59] in 1944 and again in 1947[87] stated bluntly from years of experience that no foods prepared with sugar are to be used during the first four weeks of control of an individual's caries by dietary means. The following statement encompasses his sixth specific suggestion for the planning of daily menus:

> Do not use the following: Confections of any kind; chewing gum; beverages prepared with sugar, similar to those served at fountains, and other soft drinks sold in bottles; chocolate milk and chocolate drinks. The use of sugar-coated pills and cough sirups should be restricted whenever possible. Avoid dentifrices and mouthwashes sweetened with sugar.

Jay, it must be noted, has succeeded in arresting the caries activity index consistently in over 80 per cent of his patients—83.4 per cent of the 809 patients listed as participating in the control diet for the first two weeks in his 1947 report.

Becks, Jensen and Millarr[168] in 1944 obtained similar results by restricting patients temporarily to a low-sugar diet in which they eliminated:

> Sugar in and on foods, all concentrated sweets, such as jams, jelly, sirup, honey, raisins, dates, figs, prunes, currants, and canned fruits and candy, chewing gum, confections, pastries and sweet beverages.

Of 1,228 patients experiencing rampant caries whose condition had been followed for one to four years, caries was halted in 1,004 (81.7 per cent). Of these patients, 499 were observed for one year, 111 for two years, 15 for three years, and six for four years, to establish that caries activity had ceased.

Kitchin and Permar[60] in 1948, with similar low-sugar diets prescribed for 216 caries-active patients (10,000+ lactobacillus counts), obtained a satisfactory reduction in the number of oral lactobacilli during the restricted initial two-week diet. Of these patients, 192 progressed to diet plan two (more starch permitted) for two weeks and 80 per cent maintained their low counts.

Anderson and others[169] in 1948 found more cavities in cotton rats who were fed dried milk than in those who were fed fluid milk, and concluded, in the cotton rat, that fluidity was an important factor in caries. However, both groups experienced considerably less caries than those animals fed on a regular dry cariogenic diet.

Volker and Pinkerton[94] in 1947 reported:

> Appreciable acid production has been observed to take place within fifteen minutes in mixtures of glucose, sucrose and concentrated salivary debris of carious persons. Various raw carbohydrates have been shown to be converted to acid at rates similar to those observed for refined sugar.

In this report, they also postulated that the salivary clearance time of glucose should be implicated in the process of caries as did Lundqvist[87] in 1952. Boyd and Speer[170] in 1950 determined the concentration of glucose in the salivas of laboratory workers after the slow ingestion of (1) sweetened beverage (one bottle), (2) 150 cc. of freshly squeezed orange juice, (3) four crackers, (4) one scoopful of vanilla ice cream and (5) three figs and sirup. Figs and crackers yielded the greatest amount of glucose and this glucose persisted longest in the salivas. The peak level was maintained for a short period only and returned to fasting levels in 10 to 20 minutes. Unfortunately, in none of these reports was the clearance time of sugars from the highly important bacterial plaque determined.

Neuwirth and Summerson[78] in 1951, as already has been reported, found that glucose in solutions, as dilute as 0.13 per cent, was transformed practically im-

168. Becks, Hermann; Jensen, A. L., and Millarr, C. B. Rampant dental caries: prevention and prognosis. J.A.D.A. 31:1189 Sept. 1, 1944.

169. Anderson, E. P., and others. Dental caries in the cotton rat. X. The effect of fluidity of the ration. J. Nutrition 35:371 March 1948.

170. Boyd, L. J., and Speer, F. D. The concentration of sugar in the saliva after the ingestion of carbohydrate containing substances. Bull. New York M. Coll. 13:104, 1950.

mediately by oral organisms to lactic acid. They concluded:

> If such food material were to be held within the oral cavity for more than a minute or two, in contact with saliva, our data indicate that a significant proportion of the glucose present might appear as lactic acid. . . .

Stephan, whose important contribution to the knowledge of the degradation of glucose under bacterial plaques will be reviewed again in a moment, found that a 10 per cent glucose solution could be retained in a plaque for 90 to 180 minutes after the mere rinsing of the mouth with the solution.

Miller,[153] of the University of Hawaii, in 1952 studied the five-day erosive action of fruits, fruit juices and soft drinks on rats' molars. He reported (1) that fruit juices had greater erosive effects (three to 17 times) than the fruits themselves, (2) that carbonated drinks, on the whole, produced less effect than noncarbonated prepared beverages, (3) that acid-flavored candy dissolved in water exerted the same decalcifying effect as two prepared noncarbonated beverages, (4) that citric acid, which had a marked erosive effect on enamel when used alone, lost that marked effect when it occurred naturally in fruit, and (5) that a cola drink exhibited a pH of 2.5 to 3.0 in 1948 (2.5 in Ithaca, N. Y.) and exhibited a pH of 3.0 in 1949. Miller concluded:

> Although no one has proved that enamel erosion is conducive to the carious process in human teeth, it seems reasonable to assume that overindulgence in acid beverages of various kinds might cause defects in the enamel of human teeth in situ. . . .

McCay[171] in 1952 fed white rats milk, sweetened water (10 per cent sucrose), coffee, and tap water to see if the fluid taken by rats interfered with their health or growth when the basal diet was kept adequate. On the sweetened water, the rats developed more than two decayed molars per rat; none developed in the rats drinking milk only.

Haldi, Wynn, Shaw and Sognnaes[172] in 1953 fed diets in different ways to three groups of albino rats (Wistar strain) for 90 days after weaning. Group I (eight rats surviving) received their entire ration by stomach tube and developed no cavities in their teeth; Group II (13 rats surviving) ingested granular sucrose orally and the balance of the ration by stomach tube and developed an average of 4.9 cavities per rat or 5.8 cavities per caries-active rat (11 rats), and Group III (13 rats surviving) ingested a 40 per cent solution of sucrose orally and the balance of the ration by stomach tube and developed an average of 0.8 cavities per rat or 1.7 cavities per caries-active rat (six rats).

Stephan[20] in 1940, Stephan and Miller[54] in 1943, and Stephan[146] in 1944 were among the first to test the behavior of a 10 per cent solution of sugar (used as a mouth rinse) in an adherent bacterial plaque. Such a test provides information about the degradation of a sugar solution as it actually has to take place in the human mouth in the process of producing a cavity. In 18 minutes after the rinse (within two minutes in the most caries-active mouths), all plaques were at a pH of 5.0 or less. The acidity reached its greatest intensity within the first 30 minutes and was overcome gradually until little acid effect remained in two to three hours. The renewal of the 10 per cent sugar solution by another rinse of the mouth repeated the same effect. It might logically be assumed that repeated exposure to a beverage containing 10 per cent sugar would produce the same effect.

It would appear from the evidence submitted regarding the behavior of dilute sugar solutions in dental bacterial plaques and the necessity of eliminating

171. McCay, G. M. Age changes in relation to the ingestion of milk, water, coffee and sugar solutions. J. Gerontology 7:161 April 1952.

172. Haldi, John, and others. The relative cariogenicity of sucrose when ingested in the solid form and in solution by the albino rat. J. Nutrition 49:295 Feb. 1953.

such solutions in the dietary control of caries, that the cariogenic property of sugar solutions has been established. Furthermore, it would appear that the dental profession and other interested agencies have a responsibility in warning the public of the cariogenic possibilities of sweetened beverages, at least until their precise relationship to human dental caries has been defined by acceptable controlled clinical studies.

SUMMARY

Because of the length of such a documented report, it may be helpful to provide a brief summary of its various areas in order to bring together the conclusions which reasonably may be achieved.

1. Five essential factors in the process of human caries have been reviewed and documented. They are (1) a caries-susceptible individual, (2) the presence of acid-producing and acid-tolerating microorganisms in the oral cavity, which are (3) capable of producing an optimum oral bacterial enzyme system for the prompt degradation of simple sugars to acid, (4) the availability of orally fermentable carbohydrates and (5) bacterial plaques adherent to the surfaces of the enamel of teeth.

2. Several additional factors which may contribute to the process of caries also have been listed without thorough documentation.

3. The behavior and essentiality of the dental bacterial plaque, through which sugar solutions must operate in caries, have been presented and documented in considerable detail.

4. The decalcifying effect of sweetened beverages and fruit juices has been examined in sufficient detail to warrant certain conclusions:

(a) Research has shown that some sweetened drinks and some natural fruit juices decalcify teeth because of their acidity.

(b) Decalcification has been observed when either are consumed in large amounts or at frequent intervals.

(c) Decalcification probably should not be significant unless such solutions are consumed in large amounts or at frequent intervals.

(d) Fruit juices provide some of the valuable food elements of human diets, while prepared soft drinks provide nothing but calories.

5. The examination of the scientific literature on the cariogenic properties of soft drinks and fruit juices also justifies a number of conclusions:

(a) Any sugar solutions, in order to stimulate caries, must be capable of diffusing into the adherent dental bacterial plaques on teeth.

(b) Available evidence indicates that starchy carbohydrates are of minor importance in the process of caries since complex carbohydrates cannot diffuse into bacterial plaques and cannot be degraded promptly to simple sugars in most mouths.

(c) Monosaccharides and disaccharides diffuse readily into bacterial plaques.

(d) The retention time of carbohydrates in the saliva appears of much less importance in the caries process than the retention time of simple sugars in bacterial plaques and the inability of the saliva to contact rapidly and buffer the acid produced under plaques.

(e) It has been demonstrated that solutions of simple sugars are degraded promptly to lactic acid in the bacterial plaques of caries-active individuals.

(f) This production of acid from retained solutions of sugar may continue in plaques for one-half to three hours following a brief rinse of the patient's mouth with a 10 per cent solution.

(g) The dental plaque appears to have the ability to store acid and to hinder the saliva from neutralizing it.

(h) The pH of an individual's saliva can be too high for decalcification to take place while, at the same time, the pH in

a bacterial plaque becomes so low that decalcification occurs rapidly.

(i) Solutions of some natural sugars, such as lactose, and sorbitol are fermented with difficulty in the human mouth.

(j) Data regarding the cariogenic potentialities of solutions of simple sugars that are derived from studies of laboratory rats must be appraised critically by dentists since great variations in susceptibility to caries exist for a number of strains of these animals and findings can be prejudiced merely by the selection of the strain of animal and the physical qualities of the diet which he is fed in an experimental situation.

CONCLUSIONS

1. The dental profession and other interested agencies have a responsibility to warn the public of the cariogenic property of sugars and their solutions and to point out that many of these products contain no highly important nutritional factors.

2. Research should be encouraged on the part of producers of sweetened beverages and confections to support the development of new or improved procedures or agents for the prevention and control of dental caries which may result from the use of their products.

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THE EFFECT OF CARBOHYDRATES ON EXPERIMENTAL CARIES IN THE RAT

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THE question of the role of sugar in human caries has been so frequently discussed that no attempt will be made to review the literature herewith.‡

In studies of the relationship of carbohydrates to dental caries in man, it has not been possible to recognize influences operating through oral as distinguished from those operating through systemic nutritional channels. Consequently, the interpretations of the effects of carbohydrates on caries in *man* have been controversial.

A feature of special interest which is gained in studies of the *albino rat* is that, at least to some degree, a separation is possible of the forces initiating caries from those forces advancing already established lesions. The albino rat is unique in that it does not have a significant incidence of dental caries when maintained for long periods of time on diets high in carbohydrates,[1-4] but the rat will show caries-like lesions in a relatively short time if maintained on rations containing coarse particles of maize or rice.[5] The nonoccurrence of caries in carbohydrate-fed albino rats indicates that carbohydrates are not potent initiating factors of caries in this species; the occurrence of carious lesions after diets containing identifiable initiating factors shows the albino rat to be susceptible to a tooth injury which our data show develops further like true dental caries.§

Shibata,[6] in 1928-1929, fed to rats diets containing rice and greens mixed (to the extent of 5 per cent) with sucrose, glucose, lactose, and maltose, respectively. He failed to recognize the importance of mechanical factors and ascribed the carious attack to the added carbohydrates. Subsequent to the demonstration of the role in rat caries of coarse corn particles by Hoppert, Webber, and Canniff[5] in 1931, Rosebury, Karshan, and Foley[7] were able to show that a similar causal role could be ascribed the coarse rice particles in the diets used by Shibata.

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‡The reader may find an introduction to the literature in the "General Analysis," p. 251 of Dental Caries, ed. 2, 1941, Lancaster Press, Inc., Lancaster, Pa.

§For the purpose of this discussion, it is suggested that two stages be clearly distinguished: (1) initiation of caries, and (2) promotion, development, acceleration, and cavitation. It is not necessary to assume that the factors involved in either are mutually exclusive or that these factors are ever present alone.

The recent findings by Keyes[9] that a high sugar diet increases the incidence of caries in *hamsters* has opened up the entire field of the relationship of sugar to experimental caries. There is some additional evidence available from studies on *albino rats*[10, 11, 12] and on *cotton rats*.[13, 14, 15]

It is peculiar in the light of the notable attention which has been directed to this subject that no studies have appeared heretofore of the effects on *rats'* teeth of adding excessive amounts* of sugar to the Hoppert-Webber-Caniff coarse corn particle diet.[5] Several such experiments are described in the following pages. However, the data in this report are not offered as conclusive evidence in a long debate, but only to point out a procedure which may be exploited profitably to gain additional knowledge.

The data reported herewith present, *first*, evidence that the maintenance diets containing no coarse corn, regardless of their starch and sugar content, produce caries only rarely when rats are maintained on them for as long as eleven months. *Second*, when rats are placed on a caries-producing diet for a short period and then subsequently maintained on diets containing various carbohydrates in excessive amounts, it was believed that the stage was set for testing the effect of carbohydrates on the incidence and continuation of rat caries. Three experiments have been performed. In the first one, the HWC† diet was just diluted by adding an equal weight of powdered sugar. In the second one, the HWC diet was followed by various diets containing no coarse corn but with high levels of selected carbohydrates. In the third experiment, each HWC ration was prepared using, as nearly as possible, only one carbohydrate, the same carbohydrate that was present at a high level in the diet during the continuation period when no coarse corn particles were present.

THE NONOCCURRENCE OF CARIES IN RATS ON DIETS WHICH DO NOT CONTAIN COARSE PARTICLES

Albino rats at 28 days of age were distributed into three groups and fed the rations shown in Table I. When the rats were 112 days old, they were mated. Some of the offspring were used in the experiments described in the following pages on the promotion of caries after initiation. One, two, and three litters of young, averaging 8.5 rats per litter, were born on these rations, and over two-thirds of the litters were weaned on each of the high carbohydrate diets. Many of the young were raised to maturity and third and fourth generations were obtained. Thus the rations appear to be reasonably complete for growth and reproduction.

The teeth of all rats which died during the course of experiments or which were killed for various reasons have been examined for caries. With one curious

*Rosebury and Karshan[5] added up to 28 per cent sugar to diets in which coarse rice or corn particles were present with inconclusive results.

†The coarse corn meal ration of Hoppert, Webber, and Canniff.[5]

exception,* no caries were found in any rats that had not been on diets containing corn meal. Two rats of the 76 studied showed small cavities. One rat on the glucose diet (Table I) had a small cavity in an upper molar; another rat on the same diet had several questionable cavities. In the other 74 rats that had not been on the corn meal diets, no caries was found. These rats ranged in

TABLE I

PERCENTAGE COMPOSITION OF THE DIETS USED IN THE STUDY OF THE COMPARATIVE EFFECTS OF DIFFERENT CARBOHYDRATES IN THE RATION OF RATS

	STARCH DIET	SUCROSE DIET	GLUCOSE DIET	LOW CARBOHYDRATE DIET*
Corn starch	65.5	–	–	12.0
Sucrose	–	66.0	–	–
Glucose hydrate	–	–	69.3	–
Hydrogenated fat†	5.0	5.0	4.5	26.0
Butter fat	5.0	5.0	4.5	26.0
Casein‡	20.4	20.0	18.1	30.0
Salt mixture§	4.1	4.0	3.6	6.0

*Note that the low carbohydrate diet is a high fat diet. All diets were designed to be isocaloric.

†Crisco.

‡Labco casein, chosen to minimize the presence of lactose. It has a minimum of fluorine.[11]

§Osborne-Mendel[15]

Daily supplement of 0.5 Gm. unirradiated dried brewer's yeast, 0.5 Gm. alfalfa leaf powder, and 0.1 drop of olive oil. About 1 Gm. of fresh beef liver was fed twice a week.

age from 3 months to 11 months, and they had been maintained after weaning on diets as listed in Table II.

TABLE II

DISTRIBUTION OF RATS IN THE VARIOUS DIETS

DIET	NUMBER OF RATS WITH NO CARIES
Starch	19
Sucrose	9
Glucose	6
Low carbohydrate	19
Starch, 2nd generation	4
Sucrose, 2nd generation	7
Low carbohydrate, 2nd generation	5
Sucrose, 3rd generation	4
Low carbohydrate, 3rd generation	1
Total	74

Of the total of 76 rats, 28 were on either the glucose or sucrose diet; these sugars have been shown to promote the development of caries induced by cracked corn meal. As only two rats on the glucose diet showed any trace of tooth decay, it can be concluded that sugars have little if any effect in starting rat caries. This conclusion is in agreement with the findings of Lilly,[1] that rats kept for a year on high sucrose diets do not develop caries.

*The rats of a single litter had a striking deformation of the jaw bones which consisted of an excavation of the bone surrounding the teeth. In some cases, the bone had disappeared or perhaps had failed to form to a level of about the tips of the roots of the teeth. The teeth of this litter showed many cavities but of a distinctly different appearance from that due to corn meal. Because of the probable connection between the deformity of the jaw bones and the decay of the teeth, and because of the evident familial relationship, this litter is not regarded as representative and is omitted from consideration.

ACCELERATION OF THE PROCESS OF CAVITATION BY CARBOHYDRATE

***Experiment No. 1.—Plan:** Cavities were initiated by maintaining the rats on the HWC diet for six weeks. The effect of sugar was tested by drastically increasing the dietary content of this substance, namely, half HWC diet plus half confectioner's sugar, and by giving access to this diet, to the amount desired, for about nine weeks.*

A group of 48 albino, Wistar-strain rats (29 male, nineteen female) were put on the Hoppert-Webber-Canniff diet[5] at weaning (about 4 weeks of age) and continued on this diet for six weeks. In the belief that at this time some of the teeth were suffering early damage from the coarse corn particles of the diet, a group of 13 rats (7 male, 6 female) were placed on a diet prepared by mixing equal weights of the HWC diet with powdered confectioner's sugar (grade marked "XXXX"). Since no extra vitamin or mineral supplements were supplied, it is not surprising that these rats grew somewhat less rapidly than did the control group. For example, the experimental male rats weighed on the average about 266 grams after 104 days on the sugar-rich diet, whereas the control male rats averaged about 336 grams.

At the time of sacrifice, the sugar-fed rats were in good coat and condition. Autopsies were performed and weights of each of a number of organs were obtained. The average organ weights of the control and experimental rats are given in Table III. The only abnormality observed in the experimental rats was the presence of striking quantities of nearly pure white fat in the omentum, mesentery, and perirenal regions. These milky fat deposits were not examined chemically. The organs of the peritoneal cavity, stripped of their excessively fatty surrounding tissues, appeared normal and were for the most part of normal average weights. The livers of the experimental rats, both male and female, were somewhat heavier than the livers of the control rats.

TABLE III
AVERAGE ORGAN WEIGHTS OF CONTROL AND EXPERIMENTAL RATS

	BRAIN (GM.)	LIVER (GM.)	SPLEEN (GM.)	KIDNEY (GM.)	LUNGS (GM.)	HEART (GM.)	STOMACH (GM.)
			Male				
Experimental group	1.80	9.89	0.70	1.47	1.60	1.01	0.99
Control group	1.66	9.43	0.78	1.78	1.69	1.15	1.09
			Female				
Experimental group	1.72	7.23	0.58	1.20	1.36	0.74	0.89
Control group	1.71	5.99	0.62	1.14	1.29	0.84	0.92

Blood samples taken from the descending aorta at the time of sacrifice were studied (a) for red blood cell counts and (b) for hematocrit values. The average red blood counts were as follows: control, 8.0 million; and sugar-fed, 8.3 million. Both of these averages are within normal limits for our rats. The average hematocrit readings were as follows: control, 45.6 per cent; and sugar-fed, 44.5 per cent. The latter value is somewhat low, but both values are probably within normal limits.

Fig. 1.—(×11). Lower jaw showing slight caries destruction. The size and place of the teeth may be judged from this picture.

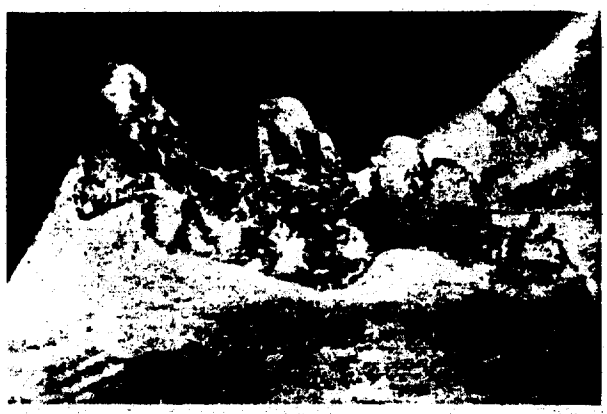

Fig. 2.—(×11). Lower jaw. The major portion of the first molar has been lost and considerably more than half of the second molar is missing.

Fig. 3.—(×11). Lower jaw. Advanced destruction. The third molar is intact, and a tiny fraction of the most anterior cusp of the third molar is missing.

Fig. 4.—(×11). Lower jaw. The third molar is practically intact, and the mesial cusp of the first is intact. The rest of the tooth tissue has been broken.

Fig. 5.—(×11). Lower jaw. Only the distal portion of the third molar is present above the gum line. Extremely advanced caries destruction.

Fig. 6.—(×11). Lower jaw. Practically complete loss of teeth from this jaw. This degree of caries loss was never seen prior to the observation of these rats on high sugar diets.

At death the jaws were freed and the teeth examined under a binocular microscope (15×) according to the technic previously described.[19] No attempt was made to differentiate between missing cusps and carious lesions because of the difficulty in making a clear distinction in the presence of advanced attrition. The data given in Table IV include the average number of teeth having at least one lesion, the average number of teeth totally destroyed, and the average number of cusps missing. In each case the values were calculated per rat.

TABLE IV

DATA ON EXPERIMENTAL CARIES IN RATS FED THE HWC DIET AND IN RATS FED A HWC + SUGAR DIET

DIET	NUMBER OF RATS	AVERAGE NUMBER OF CARIOUS TEETH	AVERAGE NUMBER OF TEETH TOTALLY DESTROYED	AVERAGE NUMBER OF CARIOUS CUSPS
HWC	35	4.9	0.85	12.8
HWC + sugar	13	5.5	1.80	15.0

Comparing the control and experimental groups, there was little difference between the values for the average number of carious teeth (4.9 vs. 5.5) and for the average number of carious cusps (12.8 vs. 15.0) per rat. In sharp contrast to these similarities, there was a 100 per cent difference in the average number of teeth totally destroyed (0.85 vs. 1.80) per rat; this difference was clearly significant by Fisher's "t" test.[20] This finding should be emphasized: twice as many teeth (per rat) were totally destroyed on the diet containing excessive amounts of sugar.

The simplest interpretation to be given these data is that excessive amounts of sugar added to the HWC diet do not initiate experimental caries in the rat; however, existing lesions tend to be extended rapidly and radically. In certain of the jaws of the rats receiving added sugar, not one molar tooth remained. Such a condition had never been observed previously in the scores of rats examined in various experiments in the Rochester laboratory (Figs. 1 to 6).

Summary for Experiment 1.—

1. Forty-eight rats were placed for six weeks on the Hoppert-Webber-Canniff diet, after which thirteen of the rats were given a diet made by adding an equal weight of powdered confectioner's sugar to the HWC diet. These diets were fed for the balance of the experiment of 104 days.

2. Although the experimental rats grew less rapidly than the control rats and laid down excessive amounts of peritoneal fat, there were no gross abnormalities in the organs of either group.

3. On the average, the experimental rats developed the same number of carious teeth and the same number of cusps missing per rat as did the control rats.

4. The experimental rats exhibited twice as many teeth totally destroyed as did the control rats. Apparently, excessive amounts of sugar do not initiate rat caries but existing cavities are rapidly extended.

Experiment No. 2.—Plan: Cavities were initiated by maintaining the rats for eight weeks on HWC diet. Then the colony was divided into groups with various diets as indicated after one week on a cleansing diet. The continuation diets for the next eighteen weeks had excessive amounts of selected carbohydrates.

Rats, at the age of 28 days, were given the HWC diet. Two such diets were used, one containing corn meal as obtained from the market; the other, containing corn meal ground fine, that is, fine enough to be passed through a sixty-mesh sieve. The screen analysis of the original corn meal was: none was retained on a twenty-mesh screen, 84 per cent retained on a forty-mesh screen, 13 per cent retained on a sixty-mesh screen, while 3 per cent passed a sixty-mesh screen. According to Hoppert, Webber, and Canniff,[5] no caries should be expected in the rats given the fine corn meal diet.

The teeth of the rats were prepared for examination by boiling the heads for one-half an hour with a soap solution. The boiled flesh was then easily removed from around the jaw bones. The jaws were removed and flesh brushed away with a tooth brush. The teeth were then cleaned by picking with a fine, sharpened dental broach under a binocular microscope with five power magnification.

After the teeth were dried, an attempt was made to obtain a numerical estimation of the extent of cavities by assigning values to a given size cavity. The values used were: 0 = no visible caries, 1 = a definite cavity, 2 = deep cavity with all cusps intact, 3 = some or all cusps gone, 4 = most of tooth above bone line gone, and 5 = all of crown of tooth gone. It was early found that cavities could not be sharply classified. Therefore intermediate values of ½ were used when desired.

It is obvious that such a numerical scale gives only a rough measure of the degree of caries. Also it is evident that the system does not progress by exact units as "2" is not exactly twice "1", and "4" does not guarantee that the factors have been exactly twice as intense as those which produced caries scored "2." Therefore, averages derived from such numbers must be accepted with caution.

At the end of eight weeks on the corn meal diets, it was found that sixteen of twenty rats on the coarse corn had caries with an average score of 4.8. Contrary to Hoppert, Webber, and Canniff, caries were found in ten of thirteen rats maintained on the fine corn meal diet with an average score of 2.6.

The entire group of survivors were transferred for one week to a low carbohydrate diet (see Table I). The purpose of this diet was to clean the teeth of the rats of corn meal debris. Two groups of rats were killed at this time and the extent of caries determined (Table V, Initial Phase). These included twenty rats maintained on the coarse ground corn diet and thirteen on the fine ground corn diet. If no cavities were evident, the rats were not considered in the average because continuation of caries was being studied.

The rats were next divided into groups as indicated and allowed free access to the respective diets according to the following schedule: 15 rats were returned to the HWC diet, 12 rats were returned to the fine corn HWC diet, 15 rats were placed on the starch diet (Table I), 16 rats were placed on the sucrose diet (Table I), and 16 rats were placed on the glucose diet (Table I).

The rats were killed after eights weeks on these diets and the teeth examined as described. The results are given in Table V. In the table are given the number of rats, the average caries score, the numbers of rats averaged, and the variability. The last column of Table V gives the summed squares of the deviations from the means, values needed for the interpretation of the means and necessary to show dispersion where the complete data are not given.

TABLE V

AVERAGE CARIES COUNTS OF RATS ON VARIOUS DIETS

DIET	NUMBER OF RATS	AVERAGE CARIES	NUMBER OF RATS AVERAGED	ΣD^2
Initial Phase				
Coarse corn (8 weeks)	20	4.8	19	236.7
Fine corn (8 weeks)	13	2.6	12	84.0
Continuation Diets				
Low carbohydrate	15	3.5	12	118.3
Fine corn	12	4.2	11	65.0
Coarse corn	15	7.5	14	224.3
Starch	15	5.0	13	85.5
Sucrose	16	4.8	9	68.6
Glucose	16	6.5	14	146.3

The interpretation of Table V is that diets high in carbohydrates probably promote rat caries more than does a low carbohydrate diet. Statistical analysis has shown that the chances are about 8 to 1 that glucose promotes rat caries more than does sucrose or starch. Since these odds are not sufficient for a conclusive statement that glucose is more provocative of a continuation of rat caries than is sucrose, it was decided to repeat the study with a more rigidly controlled set of conditions.

Summary for Experiment No. 2.—

1. One hundred twenty-two male and female rats were placed on HWC diets for a period of eight weeks. Twenty rats fed coarse corn and thirteen rats fed fine corn were sacrificed at that time; the average caries scores were 4.8 and 2.6, respectively.

2. The remaining rats were divided into groups of twelve to sixteen each, and they were fed diets containing (a) corn ground coarse or fine, and (b) low or high carbohydrate content, but without corn particles.

3. At the end of an additional eight-week period, the rats maintained on coarse corn had an average caries score of 7.5, whereas those on fine corn had an average score of 4.2. In both these groups, continuation on the diets produced increased caries scores.

4. The rats fed high carbohydrate diets in each instance had higher average caries scores than those maintained on the low carbohydrate diet.

5. When the caries scores of the rats fed continuation diets high in starch, sucrose or glucose, respectively, were compared, inconclusive difference were found.

Experiment No. 3.—Plan: Cavities were initiated in an eight-week period on an HWC diet modified to contain only one sugar. Then the rats were given access to a continuation diet (for eight weeks) high in the same sugar but lacking coarse corn particles.

Rats for this experiment were reared in the Pittsburgh laboratory. In order to provide rats as nearly as possible uniform from each breeding group, litters were reduced to eight after three days and to six after a week. The young were taken from their mothers at 21 days of age and placed on the HWC diet made up with a synthetic milk. The basic composition of this caries-producing diet was as follows: coarse, yellow corn meal, 66 per cent; basic mix, 13.75 per cent; butter fat, washed free of lactose, 8.85 per cent; and selected sugar or starch equivalent to 11.4 per cent sucrose. The basic mix of the above diet was casein, vitamin-free (lactose-free), 4.644 Gm.; Osborne and Mendel salt mixture, 1,257 Gm.; sodium chloride, 0.727 Gm.; alfalfa powder, 2,182 Gm.; and unirradiated brewer's yeast, 1.190 Gm.

The selected sugars were sucrose or glucose. Starch, in an equivalent percentage, was used in the diet of a control group. The casein was chosen to be free of lactose so that the HWC diet contained, as nearly as possible, only a single added carbohydrate. The advantages of this experiment over the preceding one were (1) the diet and life history of the parents were known, (2) the lactation conditions of the litters were practically uniform, and (3) a caries-producing diet was used with a selected carbohydrate component.

The rats were maintained on the caries-producing diets for eight weeks and on the caries-extension diets, the starch, sucrose, and glucose diets (Table I), containing no coarse corn particles, for eight weeks more. The rats were then killed and their teeth examined and scored.

Relative Incidence of Caries Among Males and Females.—In sixty-three cases, a male and a female rat from the same litter ate the same diet. In determining whether the male or female had a larger caries count, all pairs were considered except those in which both counts were zero. If only one had a zero count, that pair was included since this discussion is concerned with incidence and continuation of caries.

In the sixty-three pairs, thirty-nine males exceeded the females in the caries count; twenty-four females exceeded the males. The term exceeded here does not take into account the somewhat dubious averages which made the usual statistical methods applicable only with caution. The data, however, can be treated by the binomial distribution analysis. The mean expected of 63 pairs is 31.5. The distribution found was 24 and 9 or a deviation from the mean of 7.5. The standard deviation, σ, is $63 \times \frac{1}{2} \times \frac{1}{2} = 3.97$. The probability associated

with this deviation is 0.0588 corresponding to chance of 16 to 1. The data, therefore, show that the odds are only 16 to 1 that young male rats are more likely to have caries and more extensive decay than young female rats.

The Progress of Caries.—In order to determine the relative rates of progress of caries on the starch, sucrose, and glucose diets, respectively, the same method given above was used to consider the relative state of decay in pairs from the same litter. However, because of the chance that male rats may have a greater incidence of corn meal caries than females, no pairs consisting of male and female are used in this analysis. This treatment entails loss of data from only a few litters. As progress of caries is being determined, pairs in which either or both show a zero count are omitted from the analysis.

Of the pairs of rats of the same sex and from the same litter but on the starch and sucrose diets, respectively, eight pairs showed more decay on the starch diet and twenty-one on the sucrose diet. The probability associated with this deviation from the expected mean is 0.0155 or the chances are 63 to 1 that caries in young rats progresses faster on a diet high in sucrose than on one high in uncooked corn starch.

Thirty pairs of rats are available on the starch and glucose diets; of these ten show higher caries count on starch and twenty on glucose. The probability of this distribution, if due to chance alone, is 0.0688, or the odds that glucose promotes the caries to a greater extent than uncooked corn starch is 14 to 1.

If the preceding results are combined as starch versus soluble food carbohydrates, then eighteen pairs of rats show the starch-fed animals to have more tooth decay compared with forty-one pairs on the sucrose and glucose diets. The probability of this distribution is only 0.0027, or the chances are 370 to 1 that uncooked corn starch is less provocative of caries than are the soluble food carbohydrates.

Of the pairs of rats fed sucrose and glucose, respectively, twenty-one showed more decay on the sucrose diet and fourteen more on the glucose. The probability in this case is 0.238, or the chances are 3 to 1 that sucrose is more likely to cause continuation of caries than is glucose. Such a probability is, however, well within the limits of a chance distribution.

In Table VI is given the analysis of the decay on the three diets based upon the comparison of specific teeth. For example, in Line 1 of Table VI are given the results of a study of all the pairs of males eating the starch and sucrose diets, respectively. The same teeth in each pair were compared to determine which was more decayed. In a number of pairs the counts were the same for both teeth. In these cases a comparative re-examination of the teeth was made to determine if one was slightly more decayed than the other. Only cases in which decay occurred in both teeth were considered in this analysis.

Of the pairs of teeth in the male rats fed starch and sucrose, respectively, twenty-one showed more decay on starch and twenty-eight more on sucrose. The odds that sucrose in this case is not more provocative of caries are 2 to 1, showing the distribution to be mere chance. In contrast, the chances against sucrose in the case of the female pairs are 100,000 to 1 (Line 4, Table VI).

TABLE VI

ANALYSIS OF THE DATA OF COMPARATIVE DECAY OF INDIVIDUAL TEETH OF PAIRS OF RATS ON STARCH, SUCROSE, AND GLUCOSE

PAIRS	NUMBER OF PAIRS OF TEETH	DECAY GREATEST ON				PROBABILITY	CHANCES
		STARCH	SUCROSE	GLUCOSE	SUCROSE VS. GLUCOSE		
1. Starch vs. sucrose (males)	49	21	28			0.32	2:1
2. Starch vs. glucose (males)	55	27		28			1:1
3. Sucrose vs. glucose (males)	66		37	29		0.32	2:1
4. Starch vs. sucrose (females)	49	9	40			0.00001	100,000:1
5. Starch vs. glucose (females)	38	9		29		0.0014	700:1
6. Sucrose vs. glucose (females)	57		32	25		0.36	2:1
7. Starch vs. sucrose (males and females)	98	30	68			0.0001	10,000:1
8. Starch vs. glucose (males and females)	93	36		57		0.029	35:1
9. Sucrose vs. glucose (males and females)	123		69	54		0.18	5:1
10. Starch vs. sucrose vs. glucose (males and females)	191	66			125	0.00005	50,000:1

A third method of analysis of the data is to determine the average amount of decay per cavity. In all the rats fed the caries-producing and caries-continuation diets containing starch, 174 cavities were produced with a total count of 168. The average count per cavity is thus 0.97. For the rats fed sucrose-containing diets, the data are 206 cavities with a total count of 258 and average of 1.25. The glucose-fed rats had 233 cavities with a count of 250 and average of 1.07. These data on count per cavity are obtainable because of the nature of the values, that is, the caries counts, from which the averages are taken. However, the conclusions substantiate those drawn from the two previously described analyses and are included here as additional evidence.

The data therefore show that sucrose and glucose promote the development of corn meal caries in rats. This experiment does not show whether uncooked corn starch promotes rat caries; that is not essential to the solution of the problem of any caries promoting action of fermentable sugars.

Summary of Experiment 3.—

1. One hundred eighty rats were placed on a specially modified HWC diet in which a synthetic milk replaced the powdered milk usually included. The synthetic milk contained a single sugar, sucrose or glucose; in a control group, uncooked starch was added in an equivalent amount.

2. After eight weeks on these diets, the rats were given new diets in which no corn meal was present, but in which large amounts (about 65 per cent) of each diet was the same sugar or starch which had been in the initial diets.

3. After eight additional weeks on these continuation diets, on the basis of either whole mouth or tooth-by-tooth comparison, it was shown that rats fed uncooked corn starch had less progress of cavitation than rats fed fermentable sugars.

DISCUSSION

The question may be posed: why does sugar provoke the rapid and profound advance of caries in the rat molar? In answer, a few possible factors may be suggested: (a) the solvent action of sucrose for calcium compounds is well established;[21] (b) the sugars may provide a favorable medium for the growth or oral bacteria which are inimical;[22] and (c) sugar may be metabolized by organisms in the mouth to produce sufficient local acidity to decalcify tooth-hard tissues.[23] Experimental rat caries may well prove to be a testing ground for some of the hypotheses which attempt to describe the mechanisms of dental caries.

It seems reasonable to assume that diet may exert an influence on tooth health in ways which may be divided into three phases: (1) dietary factors in the development and calcification of teeth (pre-eruptive); (2) dietary factors in the erupted teeth but prior to the appearance of carious lesions (initiating factors); and (3) dietary factors in the progress of caries (cavitation).[10]

1. Pre-eruptive Factors. No attempt has been made in these experiments to control the pre-eruptive factors. In the third experiment, the maternal diets were considered to be part of the experiment. However, the only importance to the question as to the effects of starch versus fermantable sugars in the diets of the offspring is the clearly established fact that the young rats were susceptible to caries.

2. Initiating Factors. The initating factors were not studied in these experiments; all rats were placed on HWC diets in each of the three tests. A review of the literature indicates that the fermentable carbohydrates would not initiate caries except through a possible coarse particle effect. Thus, McClure[12] found a significant incidence of caries in rats fed granulated sugar as compared with rats fed finely ground sugar. In the experiments reported herewith, coarse corn particles were used as the initiating agents in all cases. In the third test, in order to control the influence of the carbohydrate as far as possible, the same sugar was added to the HWC diets as was used in the continuation diets. The effect of this procedure was only to add the specific promoting effect (if any) of the carbohydrate during the initiating period.

3. Extension Factors. Only one of the many possible factors involved in the destruction of tooth tissue has been studied, that is, the importance of the fermentable carbohydrates. The fermentable carbohydrates, sucrose and glucose, permit more rapid extension of existing cavities than does uncooked corn starch. Whether the initiating agents in experimental rat caries and in human caries are comparable, and whether the mechanisms for the enlargement of cavities in rat molars and in human molars are similar, are problems beyond the scope of this experiment. However, if an application of these findings to human caries prevention can be made, it would seem to stress the importance of insuring the perfection of tooth structure, because once the caries process is initiated, modern civilized diets would accelerate it.

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CARIOGENICITY OF VARIOUS SUGARS IN RATS*)

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Wistar strain albino rats were used for a comparative study of the cariogenicity of sucrose (powdered), glucose, fructose, lactose and potato starch. Recently FROSTELL, KEYES and LARSON (4) reported a lower cariogenicity of fructose, dextrose, maltose, hydrogenated starch and sorbitol than of confectionary sucrose in rats and hamsters. The present study was set up with a special interest into the effects of lactose. **)

Methods

Five groups of 7 male and 7 female weanling rats were given the experimental rations (table 1) ad libitum. The initial weights of the groups were the same. Growth and food consumption were recorded during the 4 weeks experimental period. Dental caries was evaluated microscopically in fuchsin stained sections of the right lower jaw quadrant (1,2). Wilcoxon's test (6) was used for the statistical evaluation of the data.

Table 1. Compositions of the experimental rations in percentages

Sucrose, glucose, fructose, lactose and starch resp. (calculated on water free basis)

	%		%
Sugar	40	Arachis oil	5
Casein	24	Liver concentrate	4
Wheat flour	23	Salt and vitamin mixture	4

Salt and vitamin mixture according to Sognnaes (5) (slightly modified)

NaCl	278.0	gram	choline	25158 mg
$CaHPO_4$	282.2	„	inositol	25158 „
K_2HPO_4	195.1	„	para amino benzoic acid	7547 „
$CaCO_3$	124.5	„	nicotinic acid	629 „
$MgSO_4$	41.5	„	pantothenic acid	503 „
KCl	47.7	„	vitamin B_1	88 „
Fe-citraat $3H_2O$	27.8	„	vitamin B_2	88 „
$MnSO_4$	3.74	„	vitamin B_6	88 „
$ZnSO_4$ $7H_2O$	3.50	„	Rovimix A/D 325/65	30 I.U. vitamin A + 6 I.U. vitamin D
$CuSO_4$ $5H_2O$	1.56	„		
$CoCl_2$ $6H_2O$	0.15	„		
KJ	0.66	„		

The salts and vitamins are mixed in the mentioned amounts and subsequently mixed with the other food ingredients.

Results and discussion

From table 2 is seen that for both male and female animals weight gain is highest in the sucrose and glucose groups, and somewhat lower in the fructose and starch

*) The study was sponsored by the Dutch Ministry of Social Affairs and Public Health.
**) Zie Voeding 17 (1956), 325; 18 (1957), 2.

Table 2. Growth and food consumption of the animals (averages and ranges)

Group	Ration with	Initial weight in grams	Ultimate weight in grams	P_2*) weight gain	Food consumption in grams	Food consumption in grams after the correction for differences in H_2O content of the sugars/starch	P_2*) food consumption	Food efficiency corrected values	P_2*) food efficiency
Males									
I	Sucrose	43.0 37–48	162.3 141–191	–	353.4 321.5–400.0	353.4 321.5–400.0	–	0.338 0.317–0.376	–
II	Glucose	43.4 41–46	164.1 136–196	n.s.	389.6 359.5–430.0	374.0 345.1–412.8	n.s.	0.323 0.275–0.363	n.s.
III	Fructose	43.0 38–47	153.7 136–164	n.s.	353.3 316.5–374.0	353.3 316.5–374.0	n.s.	0.313 0.293–0.331	0.026
IV	Lactose	43.0 38–46	112.8 103–132	<<0.001	296.9 253.0–324.0	290.7 247.7–317.2	<<0.001	0.240 0.202–0.292	<<0.001
V	Starch	43.3 40–46	147.4 139–153	0.04	419.6 376.5–467.5	379.7 340.7–423.1	n.s.	0.274 0.244–0.311	<<0.001
Females									
I	Sucrose	40.8 39–43	122.0 118–126	–	296.2 286.5–306.0	296.2 286.5–306.0	–	0.274 0.259–0.294	–
II	Glucose	40.3 36–45	123.1 114–136	n.s.	330.7 303.5–368.0	317.5 291.4–353.3	0.01	0.260 0.246–0.275	n.s.
III	Fructose	40.8 37–46	117.1 107–126	n.s.	303.3 280.0–324.5	303.3 280.0–324.5	n.s.	0.252 0.233–0.268	0.002
IV	Lactose	40.4 37–45	100.3 83–111	<<0.001	271.4 235.5–295.5	265.7 231.4–289.3	0.004	0.225 0.155–0.279	<<0.001
V	Starch	41.0 37–45	119.8 106–126	n.s.	376.8 332.0–412.0	341.0 300.5–372.9	<<0.001	0.231 0.212–0.256	<<0.001

*) [illegible] according to Wilcoxon's test for sucrose group against the other groups.

groups, whereas the animals in the lactose group gained significantly less weight than all the other groups. The difference between the weight gain of the male sucrose and fructose groups and that of the starch group is also statistically significant. The differences between the female groups are only small.

All animals remained in good health throughout the whole period; those on the lactose ration had slight diarrhoea, especially early in the experiment. Loss of calories can be expected to have occurred. The food consumption of this group is however also low and the same applies to the food efficiency. The relatively low food efficiency of the starch ration and its relatively high consumption in grams is as could be expected because of its rough fibre content. The difference between the sucrose and fructose groups point in the same direction as our earlier data on this point (3), e.g. a higher caloric cost to metabolize fructose than to metabolize glucose and sucrose.

The dental caries produced (table 3) is about the same in the sucrose, glucose and fructose groups but significantly less in the lactose groups as far as fissure lesions are concerned. The starch groups showed the lowest scores. Only in these groups there was also a tendency to lower scores for the approximal lesions. The latter are including the cervical lesions seen in the longitudinal sections of the jaw quadrants. Smooth surface caries was found in the animals of the various groups; its evaluation is however not possible in longitudinal sections.

Table 3. Dental caries scores of the animals (averages and ranges) and total number of cavities per group

Group *)	Ration with	Fissure caries **) scores	number of fissure cavities	Approximal caries **) scores	number of approximal cavities
I	sucrose	55.1 32 – 82	3	25.4 10 – 43	3
II	glucose	60.5 38 – 82	6	19.3 4 – 35	0
III	fructose	57.6 32 – 76	5	22.4 8 – 34	0
IV	lactose	46.9 32 – 67	1	24.4 16 – 46	1
V	starch	36.4 29 – 47	0	18.9 2 – 46	1

*) A sex difference in dental caries is not present.

**) The number of lesions was in all animals near the maximum with exception of those of the starch group. This maximum per jaw half means 6 fissure lesions and lesions of the proximal and distal surfaces of the three molars, also 6 in total. Most of these lesions showed pathological staining with fuchsin only and the number of cavities per group remained low.

The amounts of food consumed could have had some influence on the dental caries produced, but only if the period of contact of the food with the molars was influenced. Since the animals were fed ad libitum this could not be recorded. A sex

difference is not present in the average caries scores, although male animals eat appreciably more than females, but here also other factors could play a role. If the food consumption played a role, the lactose group scores could be considered a little too low, but the starch group scores would be then somewhat too high.
The high cariogenicity of glucose and fructose is in discordance with the observations of FROSTEL et al. (4). It is to be expected that differences in the caries producing bacterial strains in the oral flora — mainly streptococci, but also other bacteria can be involved — of the test animals play an important role in these discordant findings. A study of the dental plaque flora has been started in the meantime.

Conclusions

Comparing the five experimental groups, the animals on the lactose and starch rations showed the lowest weight gain, whereas the food consumption was low in the former but relatively high in the latter. Both had a low food efficiency in comparison with the animals on the sucrose, glucose and fructose rations. Dental caries scores were lower in the lactose and starch groups; starch being the least harmful to the molars. The other groups did not show distinct differences in fissure and approximal lesions scores.

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Dietary Control of Dental Caries

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Abstract

Dental caries is described as an ecological problem. Despite its multifactorial nature the disease can be reduced to manageable proportions in the majority of people by relatively simple means. The essential requirements of prevention are to reduce the intensity of the agent factors by reducing the frequency with which sweet sticky foods are allowed to remain in contact with the teeth and to increase the resistance of the teeth by ensuring an adequate intake of fluoride during tooth development. The major problem is not what to do to prevent caries but how to get the patient to do it. There is an urgent need to study the disease not as a pathological entity alone but also as a social problem and to encourage social scientists to study the attitudes of people to dental health and the behavioural factors involved.

If one accepts the idea of disease as an ecological problem then the health status of a community will be determined by a dynamic relationship between three factors: the *agent* of disease, the resistance of the *host* and the *environment* that can affect both. All mass diseases are subject to this general law. In the first two papers of this series Dr. Konig and Dr. Jenkins have shown that the diet can influence both the agent factors and the host or resistance factors in dental caries. The diet in turn is influenced by a large number of environmental factors such as socio-economic status, climate, urbanisation, war, taboos and fads, religious customs and methods of food processing and storage. Thus dental caries is a multifactorial disease. It is important, however, to recognize that in spite of the complexity of the process it is still possible to reduce the prevalence and incidence of the disease to within manageable proportions by relatively simple means.

According to Miller's Chemico-Parasitic Theory the pathological process which we call dental caries is initiated by demineralisation of enamel. The simplified model of the system is as follows:

Bacterial enzymes + Fermentable Carbohydrate → Acid

Acid + Enamel → Demineralisation.

Gnotobiotic experiments have established that bacteria play an essential part in the process. The role of fermentable carbohydrate is supported by four types of evidence — epidemiological studies, chemical studies, animal studies and controlled studies in humans.

This evidence is so well-known that it requires no elaboration. However, in the context of this paper it is worth reiterating the conclusions from two controlled studies in humans. The study of children living at a foundling home at Bowral in Australia (Lilienthal, Goldsworthy, Sullivan and Cameron, 1953) demonstrated that dental caries can be reduced to negligible proportions by excluding sugar and flour from the diet. However, the lactovegetarian diet given these children, while effective in reducing caries, is far too stringent for general application.

The Vipeholm Study at Lund in Sweden (Gustafson, Quensel, Lanke, Lundquist, Grahnen, Bonow and Krasse, 1954) disclosed that the incidence of caries is more closely related to the form and frequency with which sugar is consumed than to the quantity consumed. The following are the main conclusions which are of the utmost

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practical importance to the clinician:

(a) The consumption of sugar in a sticky form at meals increases the rate at which new lesions develop.

(b) The greatest increase in the number of new lesions results from the frequent consumption between meals of sugar in a sticky form.

(c) A high caries activity brought about by the consumption of sugar in a sticky form between meals is greatly reduced by withdrawing these foods from the diet.

(d) In a small number of patients new cavities may still appear even when the sugar and total dietary carbohydrates are reduced to a minimum.

Although the role of acid in demineralisation is often debated the fact remains that to date the only effective and practicable preventive measures are those which either reduce the frequency of acid formation on the surfaces of the teeth or increase the resistance of the enamel to the demineralising action of acid.

If the resistance of enamel is sufficient to withstand the attack of any demineralising agents that are formed, the teeth are in a state of equilibrium with their immediate external environment and thus remain intact or caries-free. But the quality and quantity of the demineralising agents and the frequency with which they are formed are subject to variation not only from day to day but also at different times of the same day.

While it is generally true to say that people who eat sweet sticky foods frequently will have more decayed teeth than people who eat such foods only occasionally, there are many exceptions. A few people who eat sweets frequently and regularly develop minimal caries whereas a few who do not eat sweets frequently develop large numbers of cavities.

In the first situation the frequent consumption of sweets is balanced by a high resistance of the teeth. In the second situation even a minimal consumption of sweets results in the production of demineralising agents (and indeed some will be produced from sources other than sweets) so that when the resistance of the enamel is low the teeth are attacked and caries results.

There are two main methods of reducing the production of demineralising agents — by reducing the frequency with which sweet sticky foods are eaten and by removing food debris from the teeth immediately after eating. Both methods can achieve a substantial reduction in the incidence of clinical caries but neither singly nor in combination will they prevent caries completely.

So far I have dealt with the effect of the diet on the local external environment of the teeth for dental caries occurs most frequently on tooth surfaces where food debris is retained. But the diet, of course, has a systemic as well as a local effect. After digestion the basic ingredients of the diet are absorbed and may then exert a nutritional effect on those teeth which are in the process of development.

It is generally conceded that nutritional factors can affect the resistance of teeth to caries but the manner in which they do so is debatable and it is doubtful if major nutrients play an important role. Yet there is much epidemiological evidence which is consistent with the hypothesis that, together with genetic factors, trace elements are most important factors in determining resistance of the teeth themselves to dental caries. And, of course, the most important trace element in this respect is fluoride. The inverse relationship between fluoride and dental caries has been consistently demonstrated throughout the world — in different ethic groups, in people with high and low standards of nutrition, in people with high and low economic status, and in people with high and low intakes of refined carbohydrate.

Let me now summarize the position briefly as follows:

1. Caries occurs when the state of balance between the agents which cause the disease overcome the resistance of the teeth to demineralisation.
2. The intensity of the attack by demineralising agents can be mini-

mised by reducing the frequency with which sweet sticky foods are eaten. This can best be achieved by:

a. avoidance of sugar in a sticky form. If the sweet sticky food is to be eaten, it is better to take it at meals instead of between meals.
b. by eliminating sugar between meals or, if this is not practicable, taking it in liquid form instead of solid form.
c. by ending meals with firm fibrous foods and immediate toothbrushing followed by vigorous rinsing of the mouth with warm water.

3. The resistance of the teeth can be maximised by ensuring an adequate intake of fluoride during the period of tooth development preferably in the form of fluoridated water or, if this is not possible, in tablet form to the extent of 0.5 mg. fluoride ion for children under three years of age and 1 mg. fluoride ion for children aged three years and over.

There is nothing new in this except to claim that if only this knowledge could be applied the prevalence of dental caries could be reduced to within manageable proportions in the majority of people. Let us not mince words. Dental caries *is* a preventable disease. This being so it is an unnecessary disease. I believe that much of our past efforts on prevention have been misdirected. Far too much of our educational material has been based on prohibitionist dogma with stress on "don't" and "avoid". Much time and money has been spent on presenting information to the public. But, as is patently obvious, education campaigns based on the dissemination of information have a negligible influence in preventing disease unless individuals are motivated to act on the advice they are given. Our knowledge of the attitudes of people to dental health is pitifully weak. We need to encourage social scientists to study the relation of human behaviour to health. For even if subsequent investigations lead to the discovery of methods which will enable caries to be prevented completely their success will depend upon the extent to which they are accepted and applied by the public.

In the meantime we have an obligation to do our best to encourage our patients to act on our advice. Since there are no definitive rules which determine how this can best be done it is necessary to adopt a flexible approach based on our understanding of each patient as a person.

The first objective is to gain the confidence of the patient. To do this it is necessary to give the new patient time to settle down on arrival at the surgery. As the Professor of Psychological Medicine, Professor Whitlock, says to our students — "have a talk before you get going.' Encourage the patient to tell you about his problems in his own words. With a bit of encouragement most adult patients will give you valuable clues to their attitude to their oral tissues and to their reaction to dental treatment. The mere fact that a patient is given an opportunity to ventilate his fear or hostility to dental treatment will often result in a complete change in his attitude if he gets the impression that he is talking to someone who is both understanding and sympathetic. This is in fact the first step in creating empathy or rapport with your patient.

The second objective is to make sure the patient understands the nature of his clinical problem, the relative importance of the causative factors and his role in its possible prevention. It is important to seek his active involvement from the outset. This can be promoted by requesting his cooperation in collecting a sample of saliva, and a diet record. The bacterial factor can be assessed by Snyder's test which, even though the results do not correlate particularly well with future caries, is nevertheless a useful health educational tool. The carbohydrate factor can be assessed by a simple analysis of a record of everything the patient eats and drinks over three successive days. This record can be analysed in terms of specific food groups to determine its general balance and adequacy. More specifically it can be analysed to determine the frequency of intake of sugar in solution, retentive sweets

and solid (non-retentive) sweets in the manner described by Nizel (1960). With the results of these assessments the dentist is now in a position to give positive advice and to relate his advice to the patient's attitude and emotional status (assessed during the consultation), dental status (assessed by clinical examination), bacterial status of his mouth (assessed by Snyder test) and dietary habits (assessed from the diet record). This advice can be given verbally or, and more effectively, summarized in the form of a letter supplemented by subsequent discussion with the patient. Such an approach gives the patient a sense of participation and the personalized nature of the advice makes it more meaningful to him than printed pamphlets or booklets.

Our experience with this type of preventive counselling is encouraging. Not only do most patients agree to cooperate but they are grateful for the time and sympathetic interest shown by the dentist in their problem. Once they realize that the major control lies in their hands they appear to be more willing to follow the advice that is given.

But this, of course, is an empirical approach necessitated by our lack of knowledge of the complex factors which influence the dentist-patient relationship. It is, I believe, no longer sufficient to study dental caries as a pathological entity; it must also be studied as a social problem. This is one of the great challenges that we shall have to face in the immediate future.

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Investigations into the relationship between saliva and dental caries.[1]

By

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Salivary influence on the caries process is most clearly demonstrated by the increased caries frequency in cases of either absent or substantially reduced saliva secretion, whether due to pathological conditions in man (PRINZ 1932, BORGMAN 1948) or to experimental removal of the glands in animals (summary: STELLING & GUSTAFSSON 1952). Which salivary factors play the main part in these cases and under normal conditions? Our theories on this question naturally depend on our general concept of the caries mechanism. An overwhelming array of evidence has now accumulated in favour of Miller's chemico-parasitical theory, even if it does not fully explain the initial phase of caries. According to this theory, as well as our present knowledge of salivary physiology and chemistry, the following factors may be held to be of the foremost importance in the formation of acid and dissolution of enamel. Hydrolysis by *amylase* is the first stage in the intraoral decomposition of starch into simpler sugars and acids. Acidification through these cleavage products may be substantially hampered by a high *pH value* and a strong *buffer effect*. The *calcium* and *phosphate* content should in theory counteract dissolution of the calcium phosphate, the chief constituent of the dental hard tissues. The *rate of secretion* should be of significance for diluting and rinsing away the substrate and products of the acid-forming bacteria. *Mucin* makes the saliva viscous, thereby

[1] This investigation forms part of co-ordinated work on dental caries, sponsored by the Swedish Medical Research Council.

retarding flow and diffusion; new investigations indicate, moreover, that mucin can serve both as a substrate for bacteria (ROGERS 1948) and as an inhibitor of salivary lysozyme (SIMMONS 1952).

The numerous experiments which have sought to correlate the incidence of caries with the above-named saliva factors may be appraised briefly as follows (the references cited cover more or less complete surveys). Amylase: varying and conflicting reports (ERICSSON 1951). pH value and buffer effect: negative correlation with incidence of caries, but seldom of statistical significance (ERICSSON 1949). Calcium, phosphate: also negative correlation, but even less significance (ERICSSON 1949). Mucin, viscosity: positive correlation, attained however by methods involving large sources of error (ERICSSON & STJERNSTRÖM 1951, RATHJE 1951).

The chief aim of this investigation has been to study the correlation of salivary glucose and lactate clearances with other saliva factors and with the caries incidence. The term oral glucose clearance was coined by VOLKER and PINKERTON (1947: 22, 23), who investigated the percentage of reducing sugar in saliva after ingestion of different carbohydrates. Earlier, TEUSCHER and FOSDICK had made investigations along the same line (1937: 20, 21). ERICSSON, working on salivary amylase, used a similar technique (1951), and LUNDQVIST pursued the same idea on a larger scale while engaged in the Vipeholm investigations (1952).

The effect of ingesting different carbohydrates on the lactic acid content of the saliva was first studied by NEUWIRTH and KLOSTERMAN (1940) and later more exhaustively by ERICSSON and HELLSTRÖM (1950, 1952).

A study of the course of both substrate clearance and lactic acid clearance in individual cases and larger groups, promised reward, since strong evidence had been presented in support of the theory that the dissolution of enamel by caries is a relatively acute and intermittent process, limited to short intervals after ingestion of carbohydrate when the changes in the sugar and lactic acid content of the saliva are most pronounced (STEPHAN 1940, ERICSSON 1949, LUNDQVIST 1952).

Theoretically, it seemed quite probable that a correlation could exist between glucose clearance and formation of lactic acid, and between each of these factors and some other saliva factors. The amylase activity might be assumed to affect first of all the salivary sugar content after ingestion of starch; further, the rate of secre-

tion and flow could well affect both glucose and lactate clearances. The secretion rate, viscosity and amylase activity seemed, therefore, to be the salivary factors worth closest study in connectio with glucose clearance and formation of lactic acid. Finally, it seemed advisable at the same time to study the possible correlation of all these saliva factors with incidence of caries.

Material.

The experiments were carried out on persons between the ages of 20 and 30 with extreme caries conditions. Most of the subjects chosen were students enrolled at the College of Dentistry and other colleges in Stockholm, and student nurses at the College of Dentistry. Subjects with less than 15 predilection surfaces that were either carious, filled or lost by extraction (DMF surfaces, not counting wisdom teeth) were designated as *caries-resistant.* During the last few years, each subject in this group had had an average of less than two new cavities per year. Persons belonging to the experimental *high-caries* group had more than 55 DMF surfaces; during the last few years, they had acquired an average of more than five new cavities per year.

The material comprises 31 subjects in the caries-resistant group and 32 in the high-caries group; the former numbered 8 women and 23 men, the latter 17 women and 15 men.

The mean of the caries index (according to the 100-surface system) is 0.08 (0.00—0.16) for the caries-resistant and 0.70 (0.58—0.86) for the high-caries group.

Certain minimum health requirements were demanded of the subjects in order to exclude factors which might have influenced the caries picture through impaired general health, gingival disorders on an endogenous basis, or pathologically influenced saliva. Thus, none of the participants were to have had diseases requiring long confinement in bed; no stomach or intestinal diseases, no diabetes, nephritis, anaemia or any other kind of blood disease; no medication with hydrochloric acid, iron salts, atropine, belladonna or other medicines having pronounced effects on the autonomic nervous system; and no cases of pregnancy.

A further requirement was that none of the subjects was to have indulged in extreme habits during the last five years with respect to frequency of carbohydrate ingestion, fruit consumption

and use of tobacco. Excessive brushing of the teeth was a disqualifying factor for the caries-resistant group, and the opposite for the high-caries group. Extreme exogenous influences were thus excluded as far as possible.

Methods.

Clinical examinations of all subjects were performed by one of the authors (L. S.), and covered status with respect to caries, gingiva and occlusion.

All subjects were required to give anamnestic accounts covering: places of birth and rearing, later places of residence and their dates, and condition of health from birth to the date of examination. Special importance was attached to any factors which could reasonably be expected to affect the secretion of saliva. Dietary anamnesis included times of main meals, times and composition of in-between meals, and other consumption of substances containing sugar and starch. In respect to oral hygiene note was made of the present and previous frequency of tooth-brushing, the subjects' own valuation of the effectiveness of their mouth hygiene, as well as the amount of dental care they had obtained. The dental treatment was specified in greater detail for the last few years, this making it possible to follow the recent development of caries in the subjects.

Saliva samples for the analyses were taken without stimulation and at a fixed time, 10 a. m. Subjects were not permitted to eat, smoke or brush their teeth for one hour prior to sample-taking. While in a relaxed state with their lips closed, the subjects let saliva accumulate in their mouths and then let it drip passively in a measuring glass. The time needed to collect 1.5 ml of saliva was noted in order to determine *rate of secretion;* this volume was then transferred to a Hess viscosimeter (ERICSSON & STJERNSTRÖM 1951). Actually, it is not the viscosity as such that is measured here, but its reciprocal, the *flow*. This was measured at room temperature every 10th minute for one hour, counting from the beginning of the secretion period. This method was adopted because wide variations in the secretion time were expected from the subjects: in view of the spontaneous reduction in viscosity, the comparison between individuals and groups had to take place at a standardized time after the start of secretion (ERICSSON & STJERNSTRÖM 1951). The figures given in the results correspond

to 25 cm of water, making the relative viscosity 25: (salivary flow reading).

When another 2.5 ml of saliva had accumulated in the measuring glass, 1 ml was pipetted and diluted with 2 ml distilled water for the estimation of *amylase*. This was done by analyzing the quantity of reducing sugar liberated by 1 ml of this diluted saliva from 100 ml of 1 % starch solution during a two-minute incubation period at a temperature of 37°. The starch solution was buffered at pH 6.8 by 0.005 M phosphate buffer, and contained 0.5 % NaCl.

1 ml of the saliva was used for determining the *fasting value of the reducing substance*, calculated as mg glucose/100 ml, in accordance with the colorimetric method of FOLIN and WU (1920). This volume was immediately pipetted into 2 ml HCl solution (0.30 ml 0.1-n HCl + 1.70 ml dist. water) in order to stop enzyme activity and to precipitate the protein of the saliva at its isoelectric point (ARNOLD & MCCLURE 1941).

0.2 ml of the saliva obtained was used to determine the *fasting value of lactate* in accordance with BARKER and SUMMERSON's colorimetric method (1941). Here the saliva was immediately pipetted into 0.5 ml of 20 % $CuSO_4 \cdot 5\,H_2O$ in order to check further enzyme activity.

After some minutes of rest, the subjects were permitted to eat white bread for two minutes. A few seconds before these two minutes were up, the subjects were made to swallow and then to remove bread crumbs from their teeth with a few simple movements of tongue and lips. The times for the following sample removals have been counted from the end of this operation.

The subjects were not allowed to rinse their mouths during the experiment.

During the next half-hour, samples of rest saliva were taken at intervals of 2, 5, 10, 20 and 30 minutes after completed ingestion of bread. The two-minute sample included the quantity of saliva accumulated between $1^1/_2$ and $2^1/_2$ minutes, the five-minute sample between $4^1/_2$—$5^1/_2$ minutes, etc.

0.2 ml of each sample was immediately pipetted into HCl solution for analysis of *reducing sugar* in accordance with FOLIN and WU. Dilutions:

2 and 5 min. samples:	0.2 ml saliva 0.5 „ 0.1-n HCl 9.3 „ dist. water	= dil. 1 : 50

Table 1.

Comparison of caries groups.

		Secr. rate			Flow						Amylase
		Both sexes	Male	Fem.	10 min.	20 min.	30 min.	40 min.	50 min.	60 min.	
Low caries group	n	32	24	8	18	30	32	32	32	32	32
	Max.	10.0	10.0	4.0	20.5	20.5	20.6	20.6	20.5	20.6	183.6
	Min.	1.0	1.0	1.5	1.1	1.8	2.1	2.3	2.7	2.9	3.9
	Med.	4.08	4.68	2.28	9.07	9.91	11.05	11.80	12.78	13.22	75.33
High caries group	n	31	14	17	12	27	29	30	30	30	31
	Max.	7.5	7.5	6.0	16.8	16.9	17.5	18.3	18.0	18.0	297.3
	Min.	0.4	1.5	0.4	0.8	1.0	1.2	1.2	1.3	0.1	18.0
	Med.	3.32	3.99	2.77	9.76	8.62	9.31	9.65	10.07	10.03	76.04
Comparison between groups	t	1.37	0.864	0.795			1.69				
	P	0.1—0.2	0.3—0.4	0.4—0.5			0.05—0.1				
	Sign.										

Table 1 (contd.).

Glucose							Lactate						
Fasting	2 min.	5 min.	10 min.	20 min.	30 min.	% red. 2—5 min.	Fasting	2 min.	5 min.	10 min.	20 min.	30 min.	% red. 5—20 min.
32	31	31	32	31	30	30	31	32	32	32	32	32	32
6.5	605.0	305.0	160.8	64.5	21.0	100.0	0.48	4.35	5.97	4.86	3.35	2.51	99.3
1.2	5.0	0	0	0	0	—3.3	0	0.15	0.29	0.21	0.01	0	—136.5
3.44	206.11	98.37	51.37	12.41	5.57	49.73	0.07	1.38	1.95	1.74	0.89	0.49	49.16
31	29	30	29	27	28	28	31	31	31	31	30	29	30
10.4	1,175.0	720.0	348.9	148.5	73.5	74.7	1.22	5.61	6.54	8.70	10.08	7.17	88.2
1.1	49.0	11.0	1.5	0.8	0	—28.6	0	0.42	0.46	0.41	0.22	0.10	—125.5
3.85	288.33	181.60	91.00	32.12	11.83	36.88	0.17	1.79	2.55	2.42	2.02	1.50	25.99
	1.60	2.51	2.02	2.39	2.07	1.90		1.44	1.62	1.67	2.81	2.67	2.01
	0.1—0.2	0.01—0.02 *	0.02—0.05 *	0.02—0.05 *	0.02—0.05 *	0.05—0.1		0.1—0.2	0.1—0.2	0.1	0.001—0.01 **	0.001—0.01 **	0.02—0.05 *

10, 20 and 30 minute samples	0.2 ml saliva 0.15 » 0.1—n HCl 2.65 » dist. water	= dil. 1 : 15

0.2 ml of each saliva sample was immediately pipetted into a copper sulphate solution for *lactate* analysis in accordance with the above.

Results.

The results of the saliva analyses are summarized in Table 1 together with a statistical evaluation, using Student's t-test, of the differences between the two groups. The 30-minute flow values were chosen for comparison, because sufficient volumes for determination were obtained at this time in all but two cases; extrapolated values for these two cases were, however, also included in this comparison. Calculation of viscosity in terms of reciprocal flow values seemed unprofitable, since the fluidity of the saliva as obtained from the Hess viscosimeter could just as well be combined directly with the other factors. For reducing sugar and lactate, the percentage reduction was calculated for the intervals of most pronounced decrease.

Since the sex distribution differed between the two caries groups, the analytical values of males and females were compared within each group. There appeared a difference in secretion rate which was almost significant in the low-caries group (Tab. 2). On account of this difference — to be commented on in the discussion — the secretion rates of sexes are also compared separately

Table 2.

Sex differences in secretion rate.

	Low caries group		High caries group	
	Male	Female	Male	Female
n	24	8	14	17
Max.	10.0	4.0	7.5	6.0
Min.	1.0	1.5	1.5	0.4
Med.	4.68	2.28	3.99	2.77
t	2.614		1.857	
P	0.01—0.02		0.05—0.1	
Signif.	*			

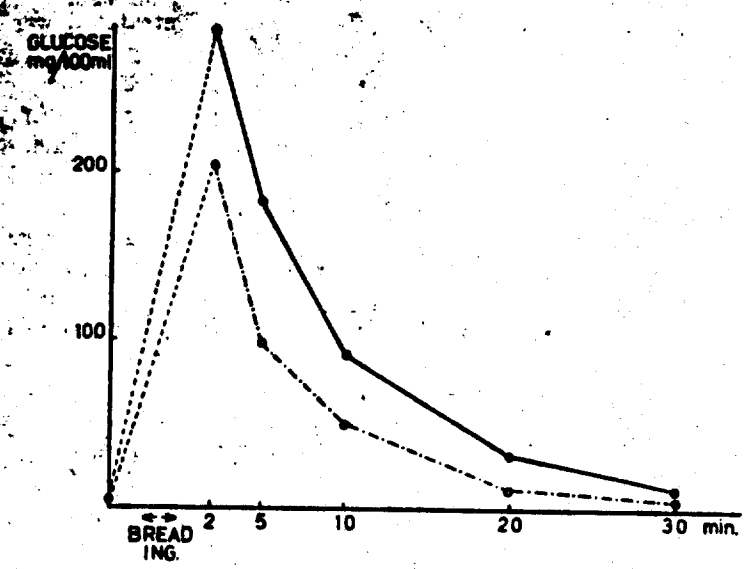

Fig. 1. Average salivary glucose values for susceptible group (———) and resistant group (—·—).

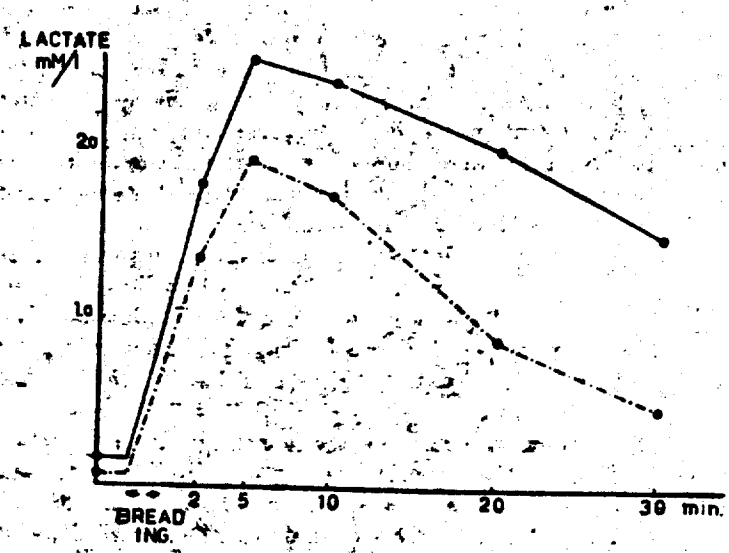

Fig. 2. Average salivary lactate values for susceptible group (———) and resistant group (—·—).

in Table 1. No significant sex difference or definite trend was found for the other analytical values.

It appears from Table 1 that the low caries group has throughout the theoretically more favourable values: higher secretion rate, lower viscosity, lower glucose and lactate values and more rapid reduction of these values. There is no difference between the group averages for the amylase activity.

The most significant differences are found for salivary sugar and lactate content. (** denotes "significant difference", $0.001 < P < 0.01$; * denotes "almost significant difference", $0.01 < P < 0.05$). Figs. 1 and 2 illustrate the averages of the two groups.

A graphic representation of flow, glucose and lactate values, which was made as a preliminary to the statistical treatment, revealed that of 13 cases with specially viscid saliva 11 belonged to the high-caries group.

Table 3 gives the correlations between some of the saliva factors. The analytical values to be compared were chosen on the following grounds. Secretion rate was combined with flow and amylase activity (columns a, d), since it has been previously reported that both these factors increase with stimulation of secretion in the individual (ERICSSON 1951, RATHJE 1951, ERICSSON & STJERNSTRÖM 1951). The 2-minute glucose values were combined with the amylase figures (g), since the amylase activity might be expected to influence the reducing sugar level, especially during the first minutes after the ingestion. The 20-minute and 30-minute lactate values were chosen because the greatest differences between groups and individuals occurred at these intervals; they were combined with the 10-minute glucose values (h, i) on account of the lag of the lactate maxima in relation to the sugar values. The reasons for the choice of the other values for comparison (b, c, e, f) have been given in the introduction. The significance of the correlation coefficients has been calculated according to Student's t-test. Asterisks in Table 3 have the same meaning as in Table 1.

Significant positive correlations are found for sugar and lactate concentrations. The correlations between secretion rate and glucose and lactate reductions are all positive but in no case significant. There are tendencies towards positive correlations between secretion rate and flow, and between flow and glucose and lactate reductions.

Table 8.

Correlations between salivary factors.

		a Secr. rate — Flow 30 min.	b Secr. rate — Glucose red. 2—5 min.	c Secr. rate — Lactate red. 5—20 min.	d Secr. rate — Amylase	e Flow 30 min. — Glucose red. 2—5 min.	f Flow 30 min. — Lactate red. 5—20 min.	g Gluocse 2 min. — Amylase	h Glucose 10 min. — Lactate 20 min.	i Glucose 10 min. — Lactate 30 min.
Low caries group	n	32	30	32	32	30	32	31	32	32
	r	0.015	0.28	0.139	0.172	0.154	— 0.030	— 0.167	0.828	0.90
	P	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	> 0.1	< 0.01	< 0.01
	Sign.								**	**
High caries group	n	29	28	30	31	28	29	29	28	27
	r	0.343	0.038	0.334	— 0.367	0.232	0.200	0.165	0.468	0.755
	P	0.05—0.1	> 0.1	0.05—0.1	0.02—0.05	> 0.1	> 0.1	> 0.1	0.01—0.02	< 0.01
	Sign.				*				*	**

Table 4.

Case history factors.

	Health	Occlusion, articulation	Diet	Oral hygiene
Low caries group	14 —	33 —	17 —	17 —, 5 +
High caries group ...	10 —	39 —	24 —	6 —, 12 +

The case histories were specially revised for the following factors which could be supposed to have contributed to the caries situation of the subjects: general health, occlusion and articulation, diet and mouth hygiene. The findings were estimated and tabulated in the following way:

health: 1—3 minus signs for factors which had impaired the general state of health for any appreciable time or led to hospitalization;

occlusion — articulation: 1—3 minus signs for disturbances which might have impaired the function and self-cleaning of the teeth;

diet: 1—3 minus signs for carbohydrate ingestions between meals;

mouth hygiene: 1—2 minus or plus signs for a standard below or above one daily tooth-brushing, respectively.

The sums of minus and plus signs for the two groups are given in Table 4. Of the results, which must of course be judged with great caution on account of their partly subjective background, the most striking is the difference in mouth hygiene in favour of the high-caries group. This group also has the more favourable record regarding general health, while articulation and dietary data are better for the low-caries group.

Discussion.

The results of this investigation should naturally be seen in the light of the previous knowledge of intraoral enzymatic acid formation and salivary influences on this process. The following may be said to be the most immediate theoretical expectation regarding the rôle of the factors analyzed in this investigation. A high amylase activity makes for a rapid saccharification of ingested starch and a high immediate salivary level of reducing sugar, but also for a rapid elimination of the starch from the oral cavity. A high level of reducing sugar in the saliva means a rapid dif-

fusion into the dental plaque and other oral bacterial deposits, resulting in rapid acid formation. This is reflected in a high lactate content in the saliva through diffusion from the deposits (ERICSSON & HELLSTRÖM 1952). On the other hand, a high salivary secretion rate makes for a rapid washing away of both sugar and lactate, as does a low viscosity of the saliva.

Assuming this course to be of crucial importance in the development of caries, all the differences found between the two groups in this investigation tend in the theoretically expected direction. The most important result is the strong connection between the salivary glucose and lactate levels, and between both factors and the caries picture. This confirms the impression that the intraoral sugar time (LUNDQVIST 1952) and acid production are the most important causal factors in dental caries. It also indicates that individual differences in the carbohydrate clearance may play an important part in the susceptibility to dental caries. It would, of course, be of great interest to study as well the effects of sucrose ingestion on salivary glucose and lactate clearances.

The small differences in secretion rate and flow between the caries extremes agree with the findings in numerous investigations on salivary pH, buffering, calcium and inorganic phosphate (ERICSSON 1949): the differences regularly go in the expected direction, but the statistical significance is in most cases rather weak. A possible explanation of this fact lies in the complexity of the caries process, where bacterial, dietary, gingival and enamel factors, to mention just the most obvious, also play their part. It is clearly difficult to prove the effect of one single factor in such a context.

The difference in secretion rate between the sexes was found to be rather great in this material. BECKS and WAINWRIGHT (1943), in a much larger material, found rather small and statistically non-significant differences between the sexes in all ages. The difference may simply depend on the smaller size of the female subjects — or their heads and salivary glands — and may have been especially large due to random factors in this particular investigation.

The revelation, in the survey of the case histories, of better average mouth hygiene in the high-caries group indicates that this factor is not particularly significant in the development of caries or lactic acid formation.

Summary.

The secretion rate, flow and amylase activity of the saliva, and its glucose clearance and lactate content after bread ingestion, were analyzed in 63 subjects between 20 and 30 years of age, about equally divided between one group with very high and one with very low caries frequency. The susceptible group was found to have higher average sugar and lactate levels in the saliva during the half-hour immediately following the ingestion. Positive correlations were found in both groups between glucose and lactate contents. These differences and relationships were statistically significant.

Non-significant trends were found towards higher values for secretion rate and flow for the resistant group, and also towards positive correlations between secretion rate and flow on the one hand, and glucose and lactate elimination on the other. The amylase activity showed no differences between the groups, and no consistent relationship to the other factors.

Zusammenfassung.

Sekretionsgeschwindigkeit, Fliessvermögen und Amylasewirkung des Speichels, gleichwie Glucoseclearance und Lactatgehalt nach Brotzufuhr, wurden analysiert bei 63 Versuchspersonen zwischen 20 and 30 Jahren, verteilt auf zwei etwa gleich grosse Gruppen mit sehr hoher bzw. sehr niedriger Kariesfrequenz. Es wurde gefunden, dass die kariesempfängliche Gruppe eine halbe Stunde unmittelbar nach dem Zufuhr durchschnittlich höhere Zucker- und Lactatwerte im Speichel hatte. Positive Korrelation zwischen Glucose- und Lactatgehalt wurde in beiden Gruppen gefunden. Diese Unterschiede und Korrelationen waren statistisch signifikant.

Nicht-signifikante Tendenz zu höheren Werten für Sekretionsgeschwindigkeit und Fliessvermögen wurde in der resistenten Versuchsgruppe gefunden, wie auch Tendenz zu positiven Korrelationen zwischen Sekretionsgeschwindigkeit und Fliessvermögen auf der einen Seite, Glucose- und Lactatelimination auf der anderen. Die Amylasewirkung zeigte keinen Unterschied zwischen den Gruppen und keinen konstanten Zusammenhang mit den übrigen Faktoren.

Résumé.

La vitesse de sécrétion et de coulage, l'action d'amylase et la clearance de glucose et d'acide lactique après ingestion de pain ont été étudiées chez 63 sujets ayant entre 20 et 30 ans, répartis à peu près également en un groupe à fréquence de carie très haute et un groupe à fréquence très basse. On a constaté que le groupe susceptible avait en moyenne des titres de glucose et de lactate plus hauts pendant la demi-heure suivant immédiatement l'ingestion que ne l'avait le groupe résistant. Des corrélations positives ont été trouvées dans les deux groupes entre les titres de glucose et de lactate. Ces différences et corrélations étaient statistiquement significatives.

Des tendances non-significatives ont été trouvées pour de plus hautes valeurs de vitesse de sécrétion et de coulage dans le groupe résistant, et aussi pour des corrélations positives entre la vitesse de sécrétion et de coulage d'un coté, l'élimination de glucose et de lactate de l'autre. L'action d'amylase n'a montré aucune différence entre les groupes ni aucun rapport constant avec les autres facteurs examinés.

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Effects of Sodium Chloride and Sucrose on Caries Activity in Rats

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Studies of caries activity in caries-susceptible white rats fed sucrose diets and drinking water that contained sodium chloride suggest that sodium chloride may alter the cariogenicity of sucrose.

Dental caries has been induced experimentally in appropriate strains of rats by feeding a corn and sugar diet[1,2] or diets that contained 70 to 80% whole wheat.[3] The basic diet determines the types of caries developed. The caries induced on a corn and sugar diet has been referred to mostly as sulcal caries, whereas on a whole wheat diet, smooth surface caries was induced.

In studies of the influence of phosphates on caries,[4] dietary supplements of Na_2HPO_4 and $Ca(H_2PO_4)_2 \cdot H_2O$ were highly cariostatic; precipitated basic calcium phosphate and $CaHPO_4$ were not cariostatic. The addition of sodium chloride to the diet was instrumental in rendering $CaHPO_4$ cariostatic and improved the cariostatic effect of $Ca(H_2PO_4)_2 \cdot H_2O$. Replacing $CaHPO_4$ with Na_2HPO_4 decreased the cariogenicity of the diet, and sodium chloride supplementation was also cariostatic in the presence of dietary $CaHPO_4$. Removal of $CaHPO_4$ from this diet did not alter its cariogenicity. Supplementation with sodium chloride was cariostatic in two diets formulated with and without $CaHPO_4$. The sodium content of the persistently cariogenic diets was approximately a fifth that recommended as needed for growth of weanling rats. Dodds and Howe[5] suggested that inadequate sodium is related to the cariogenicity of high cereal diets. Thus, of the two basic diets used in caries studies, sodium chloride has been shown to be cariostatic when added as a constituent to whole wheat diets. This report deals with the effect of sodium chloride when given in solution as drinking water to white rats on a basic cariogenic (corn and sugar) diet.

Materials and Methods

Two-hundred twenty male, weanling, caries-susceptible rats (Nelson), weighing 30 to 55 gm each, were paired and housed in stainless steel, screen-bottomed cages and separated into 11 equal groups. Standard laboratory procedures were followed.[6]

Sodium chloride solutions were prepared at 0.5, 1.0, and 1.5% and were available to the rats ad libitum in place of drinking water. The basic cariogenic diet consisted of granulated sucrose, 20%; yellow corn meal, 46.5% (99% retention on no. 20 screen); whole powdered milk, 28.5%; alfalfa meal, 4.8%; and irradiated yeast, 0.2%. The amount of sucrose was increased to two levels, 40 and 60%, at the expense of an equivalent amount of yellow corn meal. The diets were mixed every two weeks and kept refrigerated until used. Each basic diet was available daily in glazed ceramic containers 0.5, 1.0, or 1.5% sodium chloride solutions, respectively. The same procedure was repeated for the diets that contained 40 or 60% sucrose.

Control groups were given laboratory chow that contained 0.45% sodium but did not contain sucrose. The rats were weighed weekly and maintained on these conditions for 100 days. The rats were then killed with chloroform, the jaws were cleaned, and

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dental caries was evaluated by the method of Keyes.[7]

Results

A significant increase in enamel units destroyed was seen in groups 7 and 8 (Table), when compared with other groups in the experiment. Group 7 was given a 20% sucrose diet with 1.5% sodium chloride solution, and group 8 was given a 40% sucrose diet with 1.5% sodium chloride.

Generally, as the sodium chloride concentrations were increased, the mean enamel units destroyed were increased, with the exception of the groups on 60% sucrose. But whenever the different levels of sucrose are compared with the same concentration of sodium chloride, the mean enamel units destroyed values are considerably decreased with increased levels of dietary sucrose. For example, in the 20% sucrose diet with 0.5% sodium chloride, 7.8 mean enamel units were destroyed, whereas in the 60% sucrose diet with 0.5% sodium chloride, 4.0 mean enamel units were destroyed.

The average weight gain (in grams) for the groups for 92 days of the experiment was as follows: group 1, 192.0; group 4, 194.1; group 7, 175.6; group 2, 177.9; group 5, 163.4; group 8, 161.3; group 3, 145.6; group 6, 143.8; group 9, 133.6; group 10, 192.9; and group 11, 194.0.

Discussion

A review of the literature indicates that the specific effect of sodium or sodium chloride on caries is not clear. Most observations with sodium chloride have been coincidental with other studies. McClure and Muller[8] suggested that the cariostatic effect of $CaHPO_4$ probably is influenced by some other mineral, perhaps sodium chloride, or other factors in the diet. In a later study of diets composed largely of white wheat flour and cerelose,[4] they compared the influence of dietary supplements of Na_2HPO_4 and $CaHPO_4$, which were highly cariostatic, with $Ca_3(PO_4)_2$ and $Ca(H_2PO_4)_2 \cdot H_2O$, which were ineffective. The addition of sodium chloride to the diet was instrumental in rendering $CaHPO_4$ cariostatic and improved the cariostatic effect of $Ca(H_2PO_4)_2 \cdot H_2O$. When administered by intubation, Na_2HPO_4 was considerably less cariostatic than when ingested in the diet. Van Reen and Ostrom[9] have shown that, in a purified diet high in sucrose, Na_2HPO_4 and $CaHPO_4$ were cariostatic, but only Na_2HPO_4 behaved similarly in a whole wheat diet. However, their purified diet contained a 2% Hubbell, Mendel, Wakeman (HMW) salt mixture that provided at least 55 mg/100 gm of sodium in the diet.[10]

Navia, Lopez, and Harris[10] showed that feeding sodium trimetaphosphate during pregnancy had no influence in reducing the development of dental caries and a slight

TABLE
INFLUENCE OF SODIUM CHLORIDE AND SUCROSE ON DENTAL CARIES

Diet	Group	Sodium Chloride (%)	Mean Enamel Units Destroyed	Changes in Enamel Units Destroyed (%)	t Values
20% Sucrose	1	0.5	7.8 ± 0.8*	+ 7.7	0.46
	4	1.0	8.4 ± 1.0		
	7	1.5	13.0 ± 1.1	+ 55.0	3.09†
				− 62.7 (1 vs 7)	...
40% Sucrose	2	0.5	6.0 ± 1.5	− 16.6	0.55
	5	1.0	5.0 ± 1.0		
	8	1.5	11.3 ± 1.1	+ 126.0	4.23†
				+ 169.4 (1 vs 8)	2.84†
60% Sucrose	3	0.5	4.0 ± 0.9	− 45.0	1.82
	6	1.0	2.2 ± 0.4		
	9	1.5	2.7 ± 0.8	+ 22.7	0.56
				− 22.3 (3 vs 9)	...
Control	10	1.5	0	...	...
Controls without sucrose	11	(H O)	0	...	...

* Mean ± standard error.
† Significant at the 0.01 level.

effect when fed during the first 20 days postnatally. The supplement was highly effective when offered to rats immediately after eruption, from 20 to 40 days or from 40 to 80 days of age. They concluded that the cariostatic effect in rats appears to be because of the local action of the phosphate. It should be noted that the cariogenic diet contained 1.0% sodium chloride and the sodium trimetaphosphate supplement diet contained 0.5% sodium chloride. Dodds and Lowe[5] suggested that sodium ion is cariostatic, but that, because of the suboptimal sodium level of the whole wheat diet used, there is a metabolic impairment that may be responsible for its cariogenicity.

In the present study, when a 1% sodium chloride solution was given to the experimental rats, increases in dietary sucrose levels from 20 to 40% and 20 to 60% resulted in almost twofold and fourfold decreases, respectively, in total enamel units destroyed. The amount of enamel destruction appears dependent on both the sucrose level and the sodium chloride concentration. More work is needed to clarify the relationship of sodium chloride and sucrose to caries. The possibility exists that sodium chloride could have either a cariostatic or cariogenic effect, depending on the physicochemical conditions that exist in the oral cavity at the time of the experiment; such conditions might include osmotic pressure and active sodium transport and how these conditions influence plaque formation on the tooth surface. These effects may be exerted directly in the oral cavity or indirectly through the salivary glands and the saliva secreted in the oral cavity. The amount and time that these substances spend in the oral cavity in the process of eating and drinking would certainly seem to be of importance in inducing caries.

In considering particle size effects in the diet, these findings appear to suggest an inverse relationship between dental caries prevalence and dietary sucrose. Stewart, Hoppert, and Hunt[11] found granulated sugar less cariogenic than powdered confectioners' sugar. Konig[12] observed that diets that contain fine particles caused more initial lesions than the coarse particle diets; this difference was definite in the corn diets. Also, on the corn diets there were more suleal lesions under mastication than under nonfunction; the reverse was true for the sugar diets. It is possible that the results in the table indicate a dual effect on caries: the influence of sodium chloride and the resulting action of sucrose and corn in the diet.

Conclusions

Diets that contained 20, 40, and 60% sucrose and drinking water that contained 0.5, 1.0, and 1.5% sodium chloride were given to caries-susceptible white rats. Caries activity was compared in these rats by the amount of enamel destroyed. When sodium chloride levels were increased in diets that contained either 20 or 40% sucrose, significant increases of 62.7 and 109.4%, respectively, were noted in total enamel units destroyed. In the instance of rats given 60% sucrose, an overall decrease in total enamel units was noted when the salt content of their diets was increased. A decrease in total enamel units destroyed was also noted when comparing rats given increasing levels of sucrose but the same amount of sodium chloride.

Many studies have shown that increases in dental caries correspond to increases in dietary sucrose. The results of this study suggest that sodium chloride may alter the cariogenic effect of sucrose.

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DENTAL CARIES IN CHILDREN RELATED TO AVAILABILITY OF SWEETS AT SCHOOL CANTEENS

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HEALTH AUTHORITIES condemn the selling of sweets by school canteens because they claim that the practice is associated with an increase in dental decay (Lee and Lee, 1960; National Health and Medical Research Council of Australia, 1965; Stoll and Catherman, 1967). A further health hazard cited by the American Medical Association (1962) is that children, if given a choice, are quite likely to spend their lunch money on sweets instead of on more nutritious items which are necessary for growth.

The purpose of the present study was to determine whether the incidence of dental caries differed significantly amongst children according to the availability of sweets at school canteens.

MATERIAL AND METHOD

The material for the present study was derived from the results of the examination of 2,468 secondary schoolchildren in Adelaide, South Australia, from which investigation the effectiveness of fluoride toothpastes in reducing dental decay has been reported previously (Fanning *et alii*, 1967; Fanning *et alii*, 1968; Cellier *et alii*, 1968).

The original investigation compared the effects of two fluoride toothpastes and a non-fluoride control, and was designed so that the randomized blocks were formed by stratifying for sex, school, dental examiner, and initial condition of the teeth, as indicated by DMFS (decayed, missing, filled surface) scores. Equal numbers of children received the three dentifrices, and the children were divided approximately equally between the two examiners at each school; hence differences between schools were balanced in relation to dentifrices and examiners.

A total of 14 groups, each from a school with a canteen, were studied. There were seven all-male and seven all-female groups, 11 of which had access to sweets; sweets were not sold in the remaining three canteens.

Since there was no significant difference in the mean DMFS increment between the sexes in this study (Cellier *et alii*, 1968), pooled data were used to compare the DMFS increment at schools in which sweets were or were not sold.

RESULTS

Table 1 compares mean increments of decayed and missing teeth and filled tooth surfaces over a two-year period in children attending schools with and without the sale of sweets in the canteen.

TABLE 1

Mean Increments of Decayed, Missing and Filled Tooth Surfaces over a Two-Year Period

Sweets	Mean Increment	SE (Mean)	Number of Schools	Number of Children
Available	10·91	±0·26	11	981
Not available ..	9·34	±0·48	3	285

This table shows that children attending schools where sweets were not available had 1·57 fewer newly decayed tooth surfaces than those at schools where sweets were sold. The difference was significant ($P < 0·05$).

DISCUSSION

The findings of this study show a significant increase in the amount of tooth decay occurring over a two-year period in children attending schools which permit the sale of sweets in canteens.

The National Health and Medical Research Council of Australia has published "A Guide on School Tuck Shops" (1965), in which emphasis is placed on the health education role of the canteen. Reference is made in this book to the WHO/UNESCO report (1960) on "Teacher Preparation for Health Education", which states that teachers will not succeed in their health education efforts unless the school environment itself is conducive to good health practices. A canteen provides an educational experience because it is an activity which goes on at school premises as part of the school day. For this reason, items stocked in the canteen

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have the approval of the school in the child's mind, while implicit disapproval is given to undesirable foods which are not sold. Accordingly, the canteen has a considerable responsibility to encourage, by example, sound attitudes to nutritionally adequate foods. Attitudes learned as a child usually persist throughout life, so that properly taught children themselves become teachers of good dietary habits to their own children.

The findings of the present study related to increased dental decay from the sale of sweets, together with the educational advantages of a good canteen described by the National Health and Medical Research Council of Australia and the World Health Organization, should encourage school principals and parents' associations to give further consideration to the food items made available to children in school tuck shops.

The present investigation was restricted to secondary schools and was of short duration. Therefore, additional research should be undertaken to include primary schoolchildren and to extend over a longer period of time.

SUMMARY AND CONCLUSIONS

A total of 1,266 secondary schoolchildren were studied over a two-year period. Those attending schools where sweets were sold in the canteen had 1·57 more newly-decayed tooth surfaces than children at schools where sweets were not available.

In order to ensure the adoption of sound health practices by children, school authorities and parents' associations should be encouraged to consider carefully the type of food provided at school tuck shops.

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DENTAL PLAQUE pH IN RELATION TO INTAKE OF CARBOHYDRATE PRODUCTS

by

GÖRAN FROSTELL

INTRODUCTION

A number of authors have demonstrated a fall of the pH in the dental plaques following consumption of sugars and other carbohydrate products (*i.a. Stephan*, 1940, 1944; *Strålfors*, 1950; *Mörch*, 1961; *Frostell*, 1963). This fall is more pronounced in cariesactive persons than in cariesresistant, and it is more pronounced after consumption of food products considered caries promoting than after noncariogenic foods. It is generally considered that a foodstuff containing large amounts of fermentable carbohydrates is more cariogenic than a foodstuff containing only minute amounts of such substances. Consequently, it is believed that foods causing a considerable pH-decrease in the dental plaques for a long time after consumption are generally more cariogenic than a foodstuff giving no or only a slight pH-decrease, or a decrease for only a short period of time. Other factors, however, may also influence the cariogenicity of a foodstuff (*Jenkins*, 1965).

Kleinberg (1961) studied the effect of continuous addition of different concentrations of glucose on the pH of the dental plaques. He found that increasing the glucose concentration resulted in a corresponding decrease in the pH level until a maximum decrease and a steady state was obtained with a concentration of 5 per cent. With a glucose concentration of 50 per cent, the pH level of the steady state was raised.

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The aim of the present investigation was to study the effect of different physical and chemical factors on the pH-changes in the dental plaques after intake of sugar solutions and different types of candies and foods. It was the hope of the author that the knowledge so obtained could be of some value for the production of less cariogenic sweets and other foods.

MATERIAL AND METHODS

Patients having a tendency of accumulating dental plaque material were preferably chosen for these experiments. They were between 15 and 50 years of age, all having at least 20 teeth and average to high dental caries activity. All of them had fillings or other restorations.

The patients were told not to brush their teeth for two days before the experiment and to appear in the morning in the laboratory without having eaten or drunk anything with the possible exception of tap water. Dental plaque materials was collected with a blunt instrument from the buccal surfaces and from between the teeth at approximately 20 different spots, the material was pooled in a drop of distilled water and pH was determined immediately in a Beckman Zeromatic pH-meter. Only minute amounts of material were taken from each spot. The patient was then asked to rinse his mouth for 30 seconds with 10 ml of the solution to be tested or to eat and swallow a piece of the candy or food to be tested.

After 2, 5, 10, 20 and 30 minutes new samples were taken from about twenty spots near those tested before. The material was pooled and the pH was determined immediately. After each determination the pH-meter was tested against a buffer pH 6.00. At each experiment a test was also performed with a 5.00 standard buffer. Standard deviation at pH 6.00 calculated on 200 such determinations was $\pm$ 0.032 pH-units. Standard deviation of the meter, at pH 5.00 when calibrated at pH 6.00, was $\pm$ 0.025.

Since it was found that an appreciable pH-decrease of long duration generally occurred after a 30 seconds mouth rinse with a 50 per cent sucrose solution, which was to some extent characteristic of the patient, such a test was performed at least once with every patient.

Experiments were performed with either 18, 13 or 10 patients in each experimental group. All comparisons are made on experiments with groups containing the same patients.

The statistical evaluations showed that the differences between the original pH-value obtained before consumption and those obtained at a certain time point on the curves for a series of patients were distributed according to the normal curve and were more representative than the actual corresponding figures. Thus, the differences between pH at a certain time point and original

pH were used directly for statistical determinations. The pH-mean given for each time point is the arithmetical mean of the actual pH-values found, and the statistical deviations given are those within the limits of which the pH-values may be expected to fall in a large series of experiments. An Olivetti Programma Computer has been used for the calculations. Standard deviation of the method was determined at 0.13—0.26 (different for the H-different time points, Frostell, in preparation).

EXPERIMENTS AND RESULTS

1. *Effect of different sucrose concentrations*

Experiments were performed with a group consisting of 18 patients. Each patient rinsed his mouth with 10 ml of a sucrose solution. The concentrations used were:

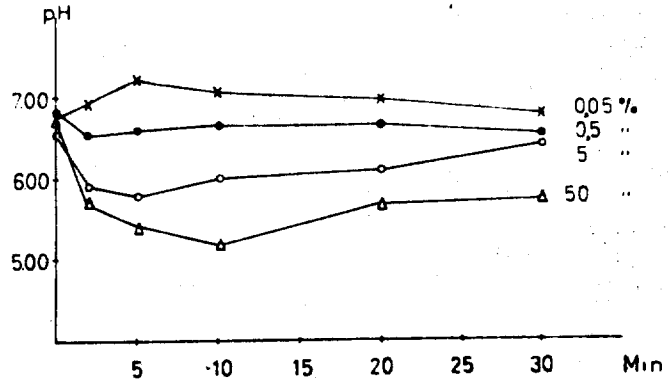

Fig. 1 a. Changes in the pH of the dental plaques of a person after a mouth rinse (30 seconds) with different concentrations of sucrose.

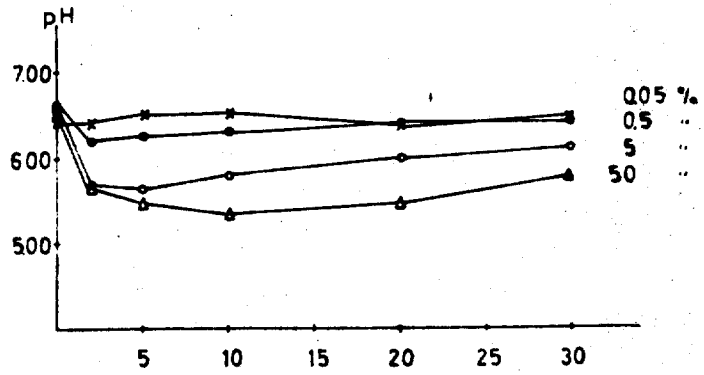

Fig. 1 b. Changes in the pH of the dental plaques after a mouth rinse (30 seconds) with different concentrations of sucrose. Mean of experiments with 18 persons.

Table 1.
Composition of the different products
Approximate figures as given by the manufacturers

a) *Gum Arabic — sorbitol tablets (US)*	
Gum Arabic	54 gram
Sorbitol	45 »
Essence, artificial colour, sweetener	1 »
	100 »
b) *Gum Arabic — sugar tablets (Tenor)*	
Gum Arabic	50 gram
Glucose	38 »
Sucrose	6.6 »
Extract glyz.	5 »
Essence, artificial colour	0.4 »
	100 »
c) *Hydrogenated potato starch candy (A-plus)*	
Product 6563	97 gram
Citric acid	2 »
Essence, artificial colour, sweetener	1 »
	100 »
d) *Conventional candy*	
Sucrose	55 gram
Dextrines	26 »
Dextrose, maltose	18 »
Essence, artificial colour	1 »
	100 »
e) *Sponge-cakes*	
Sucrose (or product 6563 + sodium cyclamate)	112 gram
Margarine	100 »
Oat meal	150 »
Baking powder	8 »
Vanilline	1 »
2 eggs	
f) *Ginger-cakes*	
Sucrose (or product 6563 + sodium cyclamate)	260 gram
Margarine	200 »
Oat meal	500 »
Sodium bicarbonate	1/2 teasponfull
Cinnamon	2 1/2 »
Ginger	1 »
Clove	1 »
Water	90 gram

g) *Marshmallows*

Sucrose	(or product 6563, 375 gram + sodium cyclamate)	300 gram
Glucose		75 »
Gelatin		35 »
Crystallized hens egg white		12 »
Water		175 »
Flavors		

h) *Chocolates*

Weight per cent (approximate) and additives in gram per kilogram

		Dark »sugarfree»	Dark with sugar	Light »sugarfree»	Light with sugar
Cocoa-pulp*)		100	45	16.25	10
Cocoa-butter		—	10	33.75	20
Milk powder**)		—	—	50 ***)	30
Sucrose		—	45	—	40
Lecithin	g/kg	3	3	3	3
Na-cyclamate	g/kg	5	—	5	—
Vanilline	g/kg	0.2	0.2	0.2	0.2

*) contains 54 % cocoa-butter
**) » 25 % butter fat and 38 % milk sugar
***) corresponds to 19 % milk sugar in the final product

0.05 per cent; below the lowest concentration that gives optimal acid production in suspensions of dental plaque material (*Frostell*, 1964):

0.5 per cent; a concentration which gives optimal acid production in suspensions of dental plaque material and which is often found in the saliva after consumption of fruits and other »natural» foods (*Lundquist*, 1952);

5 per cent; a concentration at which acid production in suspensions of dental plaque material is still optimal. Salivary concentration of sugar is very often around 5—10 per cent immediately after consumption of candy or sugar-rich foodstuffs;

50 per cent; at this concentration acid production in suspensions of dental plaque material is inhibited. Filled candies, marmalades and marshmallows coming in direct contact with the teeth often contain concentrations of sugar of this magnitude or more.

The results are given in Fig. 1 a and b. The 0.05 per cent sucrose solution often gave a moderate pH-increase in the plaques whereas the other concentrations gave pH-decreases of increasing strength and duration with increasing sugar concentration.

Table II.

Comparison between the results of the different experimental series

Tab. 5 and 6*)	2	5	10	20	30 min
50 % sucrose	0.82	1.07	1.14	0.99	0.71
50 % sucrose pH 7.8	0.35	0.74	0.78	0.68	0.49
Difference	0.47	0.33	0.37	0.31	0.22
t-value	5,17	3.04	3.76	4.37	3.33
Significance level	***	**	**	***	**
Tab. 5 and 7					
50 % sucrose	0.82	1.07	1.14	0.99	0.71
50 % sucrose pH 6.5	0.64	1.00	1.07	0.93	0.69
Difference	0.18	0.07	0.07	0.06	0.02
t-value	1.94	0.81	0.85	0.64	0.26
Significance level	—	—	—	—	—
Tab. 5 and 8					
50 % sucrose	0.82	1.07	1.14	0.99	0.71
50 % sucrose pH 4.9	0.65	0.89	0.91	0.75	0.55
Difference	0.17	0.18	0.23	0.24	0.16
t-value	2.42	2.65	3.53	2.61	2.09
Significance level	*	*	**	*	—
Tab. 5 and 9					
50 % sucrose	0.82	1.07	1.14	0.99	0.71
50 % sucrose pH 3.5	0.77	1.01	1.07	0.84	0.70
Difference	0.05	0.06	0.07	0.15	0.01
t-value	0.64	0.60	0.74	1.58	0.19
Significance level	—	—	—	—	—
Tab. 10 and 11					
50 % sucrose	0.79	1.10	1.11	0.91	0.66
3 % citric acid	1.30	1.15	0.86	0.63	0.35
Difference	+0.51	+0.05	0.25	0.28	0.31
t-value	3.59	—0.36	1.85	2.14	3.07
Significance level	**	—	—	*	**
Tab. 12 and 13					
50 % sucrose	0.76	0.97	1.07	1.00	0.72
50 % sucrose in fat	0.49	0.79	0.75	0.56	0.44
Difference	0.27	0.18	0.32	0.44	0.28
t-value	3.09	1.27	2.36	4.37	3.15
Significance level	**	—	*	***	**

*) These tables may be requested from the author.

Tab. 14 and 15					
5 % sucrose	0.76	0.89	0.70	0.51	0.40
5 % sucrose in fat	0.10	0.10	0.15	0.08	0.07
Difference	0.66	0.79	0.55	0.43	0.33
t-value	7.05	7.20	4.04	3.73	1.87
Significance level	***	***	***	**	—
Tab. 16 and 17					
50 % sucrose	0.85	1.09	1.19	1.06	0.77
Menthol + eucalypthol	0.57	0.87	0.91	0.78	0.54
Difference	0.28	0.22	0.28	0.28	0.23
t-value	3.71	2.03	2.68	2.99	3.05
Significance level	**	*	*	**	**
Tab. 18 and 19					
50 % sucrose	0.82	1.06	1.15	1.01	0.71
45 % sucrose + 5 % carbamide	+ 0.14	+ 0.02	0.10	0.27	0.20
Difference	0.96	1.08	1.05	0.74	0.51
t-value	11.11	12.81	19.18	9.52	7.13
Significance level	***	***	***	***	***
Tab. 21 and 22					
Sucrose tablets	0.26	0.55	0.57	0.48	0.26
Carbamide tablets	+0.13	+0.07	0.09	0.17	0.19
Difference	0.39	0.62	0.48	0.31	0.07
t-value	5.03	7.25	8.30	4.72	1.46
Significance level	***	***	***	***	—
Frostell 1965, Tab. 1 and 2					
Sucrose tablets	0.46	0.53	0.54	0.36	0.19
Sorbitol tablets	−0.36	−0.39	+0.29	−0.11	−0.04
Difference	0.82	0.92	0.83	0.47	0.23
t-value	10.66	9.97	7.15	5.21	5.07
Significance level	***	***	***	***	***
Tab. 25 and 26					
Marshmallow, sucrose	0.62	1.00	1.10	0.85	0.49
0563	0.27	0.30	0.22	0.19	0.10
Difference	0.35	0.70	0.88	0.66	0.39
t-value	4.71	9.07	10.75	9.75	5.01
Significance level	***	***	***	***	***

2. *Effect of buffer capacity and pH of a sugar solution*

Sucrose-phosphate buffer solutions containing 0.1 M phosphate buffer and 50 per cent (weight to volume) sucrose were used. The buffers were prepared according to directions given in »Handbook of Chemistry and Physics» (*Hodgman*, 1948).

The experimental group consisted of 18 people which on different occasions were given 10 ml of each sugar-buffer solution as a mouth rinse.

The results are given in Fig. 2 and Table II. There were only small differences between the buffers pH 3.5, 6.5 and the nonbuffered solution, whereas the pH 7.8 buffer gave significantly less pH-decrease in the plaques than the other three. The pH 4.9 buffer also gave less pH-decrease than the pure sucrose solution.

3. *Effect of citric acid*

Citric acid is often used in sour sweets and occurs in high concentration in citrus fruits. A solution containing 50 per cent sucrose and 3 per cent citric

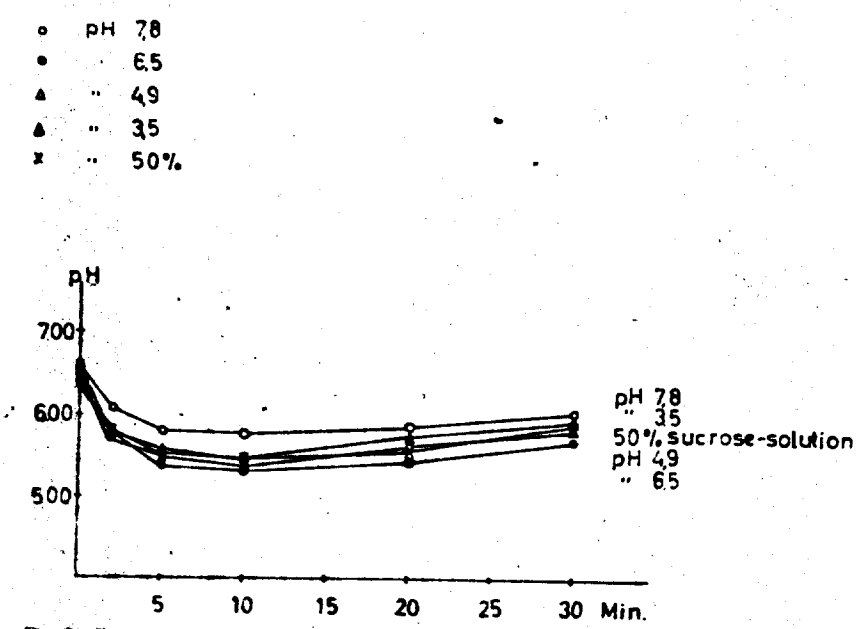

Fig. 2. Changes in the pH of the dental plaques after a mouth rinse (30 seconds) with a 50 per cent sucrose solution and different buffered phosphate-sucrose solutions. Only the results with the alkaline solution differs highly significant from the non-buffered solution. Each curve gives the mean of experiments with 18 persons.

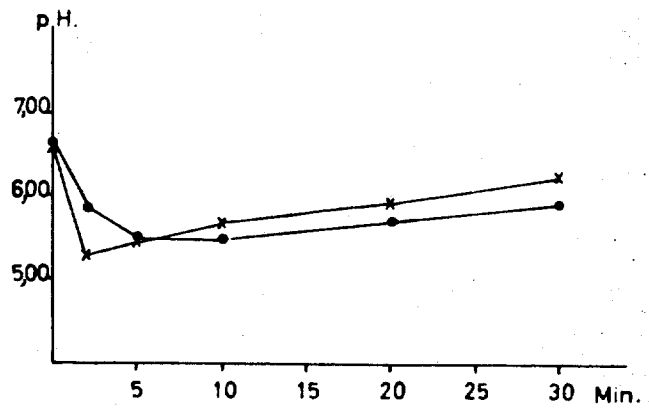

Fig. 3. Changes in the pH of the dental plaques after a mouth rinse (30 seconds) with a 50 per cent sucrose solution (•—•) and a 50 per cent sucrose solution containing 3 per cent citric acid. Each curve gives the mean of experiments with 18 persons.

acid, pH 3.7, was prepared. Experiments were performed with 18 persons and the results were compared with those obtained when the same persons rinsed their mouths with a solution containing 50 per cent sucrose only.

The results are given in Fig. 3 and Table II.

The presence of citric acid in the solution resulted in a significantly deeper pH-decrease in the plaques after 2 minutes but at 10, 20 and 30 minutes the pH was significantly higher.

4. *Effect of fat*

Sucrose-peanut butter mixtures (5 and 50 per cent, weight per volume) were prepared. The patients were asked to take a 10 ml quantity in the mouth, to chew it and to spit it out after the standard time. Experiments were performed with 10 patients and the results were compared with those of the same persons partaking in the experiments described under 1.

The results are given in Fig. 4 a and b and Table II.

The pH-decrease found after consumption of 5 or 50 per cent sucrose in fat was significantly less than those found after a rinse with the corresponding sucrose concentration in water.

5. *Effect of flavors (menthol and eucalypthol)*

Many sweets are flavored with menthol or eucalypthol or both. These are believed to be antibacterial and laymen often think that they would make

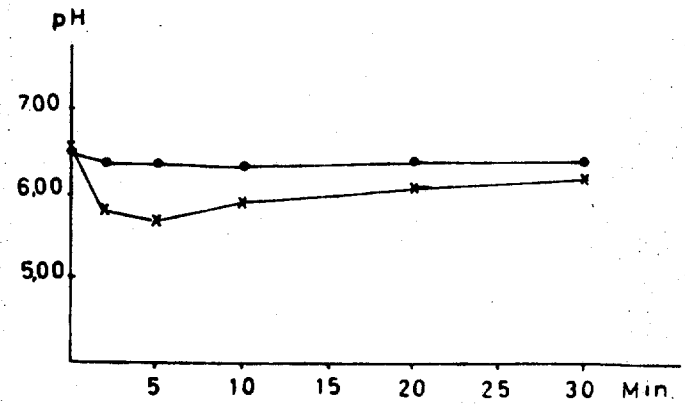

Fig. 4 a. 5 per cent sucrose in fat compared to 5 per cent sucrose in water. ● — ● sucrose in fat. Mean of experiments with 10 persons.

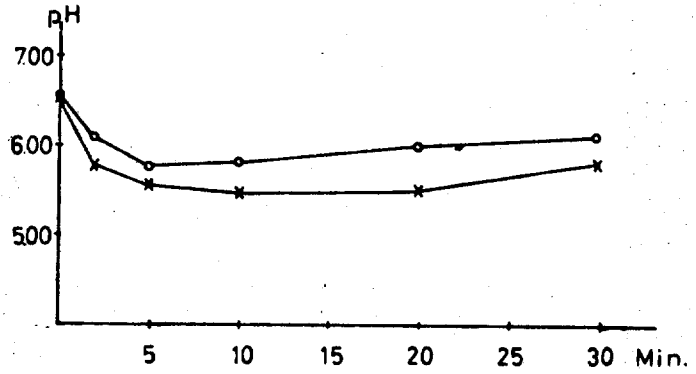

Fig. 4 b. 50 per cent sucrose in fat compared to 50 per cent sucrose in water. o—— o sucrose in fat. Mean of experiments with 10 persons.

sweets noncariogenic. Experiments were performed with a 50 per cent sucrose solution containing 2 per cent menthol and 2 per cent eucalypthol. These concentrations are approximately the maximum of those used in sweets and the taste was so strong that it was hard to tolerate. The patients were given a 10 ml mouth rinse with this solution and the results were compared with those obtained with a pure 50 per cent sucrose solution (see under 1). A group of 18 persons was tested.

The results are given in Fig. 5 and Table II.

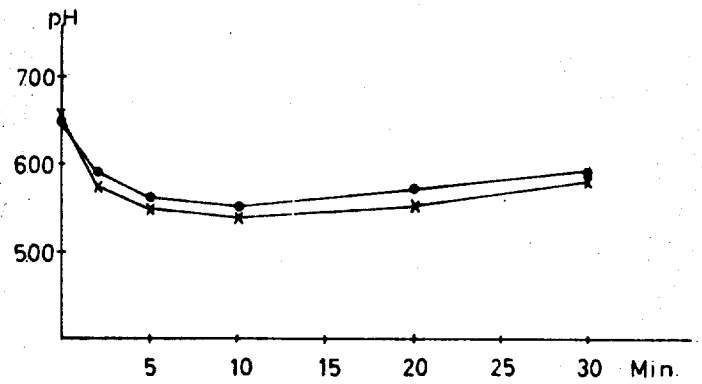

Fig. 5. 50 per cent sucrose plus menthol and eucalypthol compared to 50 per cent sucrose only. Mean of 18 experiments. ●— —● sucrose plus menthol and eucalypthol.

There was a significantly smaller pH-decrease at 2, 20 and 30 minutes when the flavors were used compared to the pure sucrose solution whereas the differences at 5 and 10 minutes were statistically probable.

6. *Repeated intake of sugar*

A study of the effect of repeated intake of sugar was performed with two patients using either a 5 per cent or a 50 per cent sucrose solution. These sugar solutions contained nothing but sucrose and water.

The experiment was started as usual with a mouth rinse and the pH of the pooled plaques was determined at the usual time intervals. Eight minutes after the start of the experiment the patient was given a second mouth rinse with a sugar solution of the same strength as the first one and the pH of the pooled plaque material was determined 2, 5, 10, 20 and 30 minutes after the start of this rinse.

A representative result of these experiments is given in Fig. 6.

There was no additional pH-decrease caused by the second sucrose rinse but the period in which the pH was below the initial value was probably prolonged.

7. *Effect of addition of carbamide*

Pilot experiments have shown (*Frostell* and *Erickson*, 1963) that addition of carbamide (urea) to concentrated sucrose solutions influence the pH-changes in the dental plaques after consumption of such solutions. It was found that

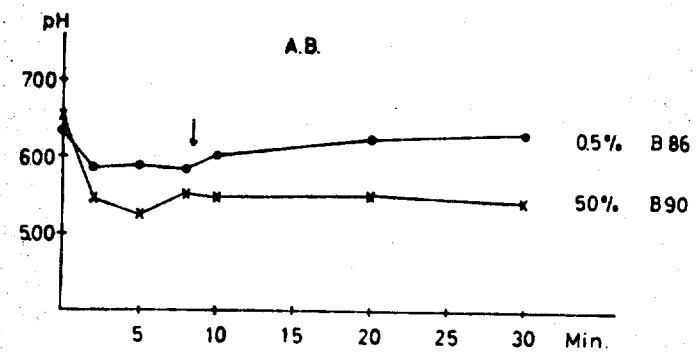

Fig. 6. Repeated rinsing with a sucrose solution, 0.5 per cent and 50 per cent. ● —— ● 0.5 per cent.

approximately 5 per cent carbamide was required in order to prevent the pH from dropping appreciably (Fig. 7). The authors suggested that carbamide might be added to sweets and other carbohydrate-rich foods in order to make them less cariogenic.

Experiments were performed with 18 persons using a 45 per cent sucrose solution containing 5 per cent carbamide (weight per volume). The results were compared with those obtained with the same group of patients given a 50 per cent sucrose rinse (see under 1).

The results are given in Fig. 8 and Table II.

The addition of carbamide (5 per cent) prevented to a great extent the pH from dropping in the dental plaques. The difference was statistically significant at all time points up to 30 minutes.

8. *Effect of addition of carbamide to tablets containing fermentable sugars*

In Sweden there is an appreciable consumption of hard or gummy sugar tablets, which is believed to be one of the many causes of the high prevalence of dental caries in the country. It has been proved that in a number of instances misuse of such candy has been associated with a very rapid tooth decay (*Ericsson*, 1954).

Experiments were performed with 18 persons who were given 2 sucrose tablets (ca 2 gram) at one experiment and 2 sucrose tablets containing 5 per cent carbamide at another experiment. The recipes for these tablets are given in Table I a and b.

The results are given in Fig. 9 and Table II.

The carbamide usually prevented the initial pH-decrease completely. After 10—20 minutes a slight pH-decrease was found.

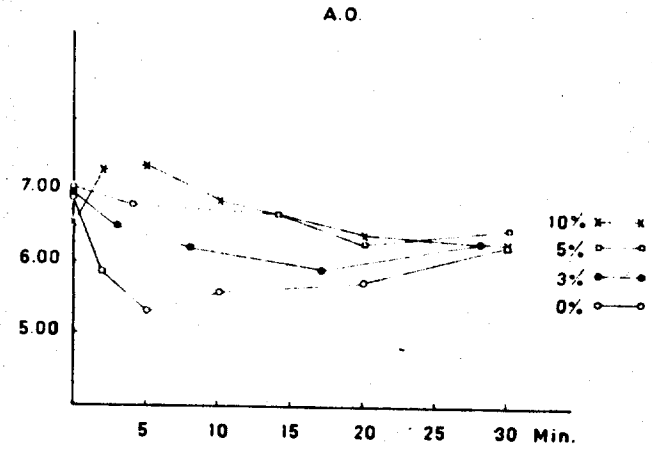

Fig. 7. Effect of addition of different concentrations of carbamide to a 50 per cent sucrose solution used for a mouth rinse. Experiments with one person.

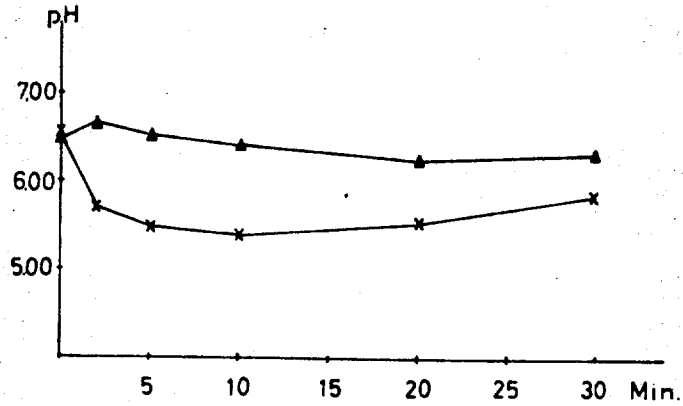

Fig. 8. Effect of addition of 5 per cent carbamide to a 45 per cent sucrose solution used for a mouth rinse. Means of 18 experiments.

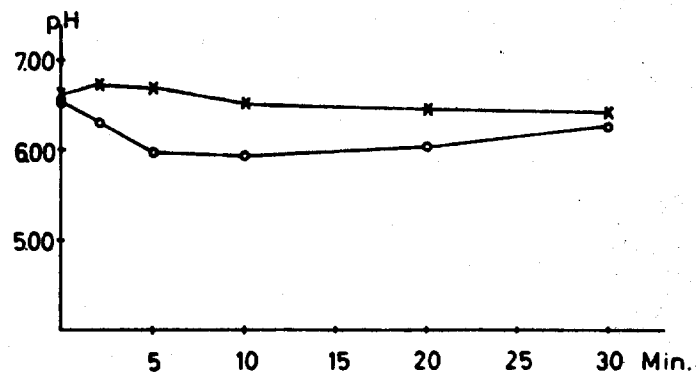

Fig. 9. Changes in the pH of the dental plaques after consumption of sucrose tablets containing 5 per cent carbamide. x — x sucrose-carbamide tablets. o — o sucrose tablets

9. *Substitution of fermentable sugars by sorbitol in tablets*

Sorbitol is widely recommended as a substitute for fermentable sugars in sweets in order to make them less cariogenic. A group of 18 persons was given 2 gummy tablets containing fermentable sugars at one experiment and 2 tablets containing sorbitol at another. Recipes are given in Table II.

The results, which have been published previously (Tables 1 and 2 *Frostell*, 1965), are given in Fig. 10.

The consumption of sorbitol tablets was usually followed by an increase in the pH of the plaques. The difference between the results with sorbitol tablets and those containing fermentable sugars was significant at all time points (Table II).

10. *Substitution of fermentable sugars by hydrogenated dextrines in lozenges*

In 1963 a new type of raw product for the candy and food industry — a hydrogenated starch hydrolysate — was developed (Product 6563 or Lycasin, Lyckeby Starch Refining Co, Lyckeby, Sweden), which was believed to be less cariogenic than ordinary sugars. This claim was to some extent supported by results of pilot experiments by *Frostell* (1963, 1965). Biochemical and nutritional studies on the product were carried out by *Dahlquist* and *Telenius* (1965). *Frostell*, *Keyes* and *Larson* (1967) showed that it was less cariogenic in experiments with hamsters and rats than sucrose, glucose, fructose and maltose.

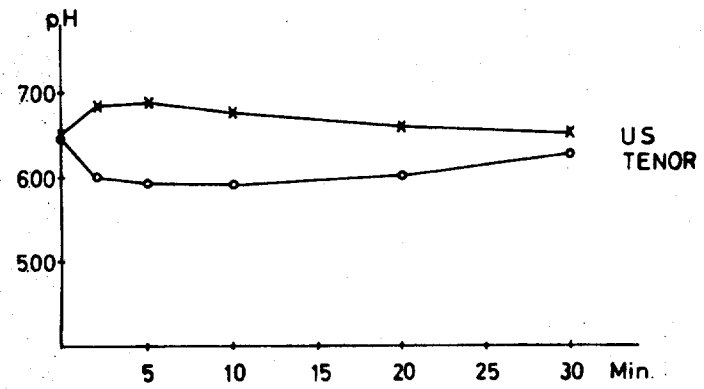

Fig. 10. Changes in the pH of the dental plaques after consumption of sorbitol tablets. Means of experiments with 18 persons.

x- - - x sorbitol tablets, 2 × 1 gram
o - - o conventional tablets containing sucrose

A group consisting of 18 persons were given a lozenge »conventional candy» (weight 4.5 gram, recipe given in Table I d) containing fermentable sugars in one experiment and a lozenge containing hydrogenated dextrines in another. (Table 1 c.

The results are given in Fig. 11 (and Tables 4 and 5, *Frostell* 1965).

No or only a very moderate pH-decrease occurred in the plaque material after consumption of the hydrogenated dextrine candy, whereas consumption of conventional candy was followed by an appreciable pH-decrease. The difference was statistically significant at all time points (Table 11). These results have been published in Swedish previously (*Frostell*, 1965).

11. *Substitution of fermentable sugars by hydrogenated dextrines in different types of candy and foods*

Experiments were performed with two or three persons in each group. They were given a conventional candy or food at one experiment and an experimental candy or food at the next in which the fermentable sugars had been substituted by hydrogenated dextrines (Product 6563).

a) *Orange marmalade*, semisolid substance containing strips of orange peel (Findus, Sweden) and an experimental marmalade containing approximately 60 per cent hydrogenated dextrines. The persons were given 10 grams of the marmalade.

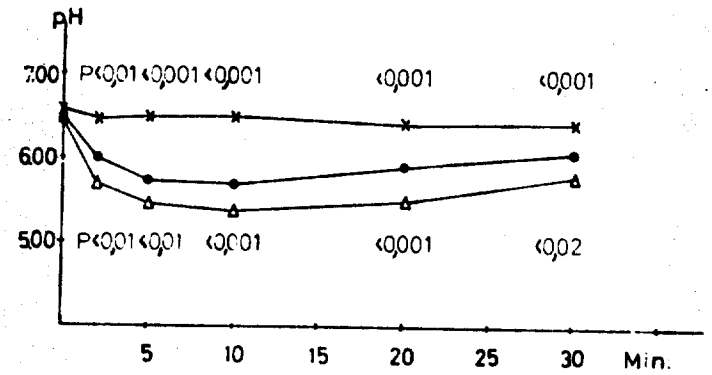

Fig. 11. Changes in the pH of the dental plaques after consumption of candy containing a hydrogenated starch hydrolyzate or sucrose. For comparison the results after rinsing with a 50 per cent sucrose solution are given.

×——× hydrogenated starch hydrolysate
●——● sucrose lozenges
△——△ sucrose solution, 30 seconds

The figure under and above the curves give the levels of significance of the difference between the top curve and the middle curve and the bottom curve and the middle curve, respectively.

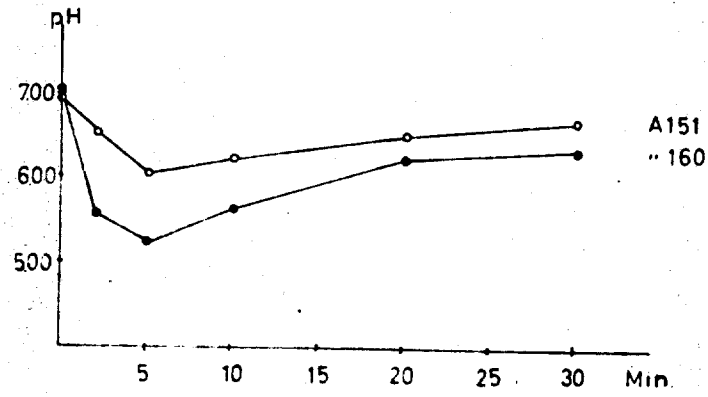

Fig. 12. Changes in the pH of the dental plaques of a person after consumption of marmalade.

o——o hydrogenated starch hydrolysate marmalade
●——● sucrose marmalade

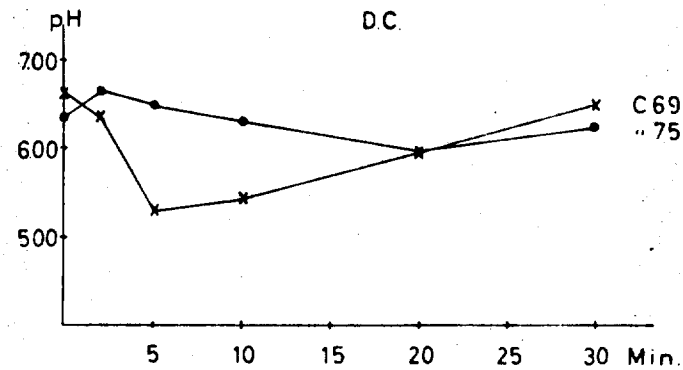

Fig. 13. Changes in the pH of the dental plaques after consumption of sponge-cake.

A representative result is given in Fig. 12.

Consumption of both marmalades was followed by a pH-decrease in the plaque material, the pH-decrease following consumption of the conventional product being more pronounced than that following the experimental product.

b) *Sponge-cake* containing fermentable sugars (recipes given in Table I e) was compared to a similar cake containing hydrogenated dextrines. Experiments were performed with two persons.

A representative result is given in Fig. 13.

The sponge-cake containing sucrose gave an appreciable pH-decrease in the plaque material whereas the substituted product gave no or only a very moderate pH-decrease.

c) *Ginger-cakes* (hard) containing sucrose were compared to similar cakes in which the sucrose was substituted by hydrogenated dextrines. Experiments were performed with 3 persons. Each person consumed 5 grammes. Recipes are given in Table I f.

A representative result is given in Fig. 14.

The changes in pH caused by consumption of ginger-cakes were rather slight. The experimental cake gave a less pronounced pH-decrease than the conventional product. The difference between the curves was, however, small, with exception for the results at 2 and 5 minutes.

d) *Marshmallows* containing fermentable sugars were compared to an experimental product containing hydrogenated dextrines. Experiments were performed with 13 persons. The conventional product is believed to give a high concentration of sugar on the plaque surfaces on the teeth. Recipe is given in Table I g.

The results are given in Fig. 15 and Table II.

The conventional product gave an appreciable pH-decrease in the plaque material whereas the substituted product gave only a slight pH-decrease.

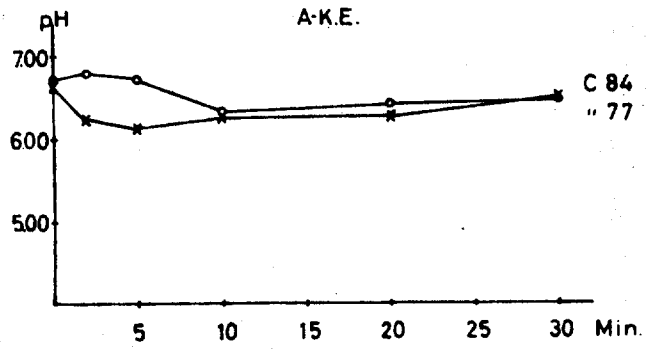

Fig. 14. Changes in the pH of the dental plaques of a person after consumption of ginger-cakes.

o——o hydrogenated starch hydrolysate cakes
x——x sucrose cakes

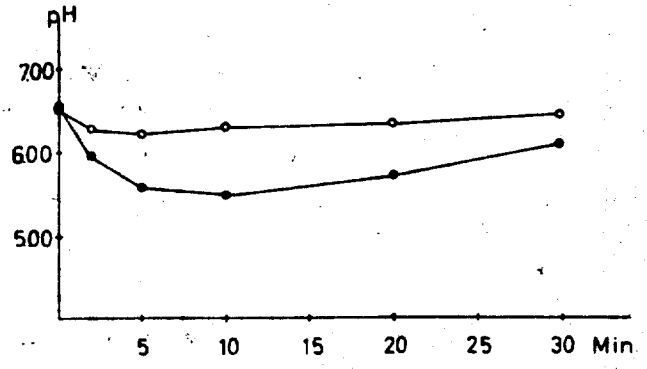

Fig. 15. Changes in the pH of the dental plaques after consumption of marshmallows.

o — o hydrogenated starch hydrolysate
• — sucrose marshmallows •

Mean of experiments with 13 persons.

12. *Experiments with different types of chocolate*

In the Vipeholm study it was found that milk chocolate containing 50 per cent fermentable sugars was cariogenic in persons under 30 years of age but not in older persons. On the other hand frequent consumption of toffees containing sugar increased caries activity much more. However, no direct comparison between chocolate and toffee may be made since the experimental conditions of the groups were not the same.

A number of authors have studied the presence of modifying or caries-inhibiting substances in chocolate and cocoa (*Frostell*, 1967). Such substances are believed to be fat, polyphenols, theobromin, tannic derivatives and perhaps others (*Strålfors*, 1967). It has been suggested that the comparatively low cariogenicity of cocoa and cocoa products would depend on the presence of substances which inhibit the acid production in the plaques. A more likely explanation is that the substances mentioned above influence the solubility of the enamel surface (*Strålfors*, 1967; *Frostell*, 1967). The high fat content may also be of significance.

Experiments were performed with 4 types of experimentally produced milk chocolates, the compositions of which are given in Table 1 h. For practical reasons the composition of the different chocolates differ from each other also in other respects than in sucrose concentration. Two types were intended to contain low concentrations of cocoa substances while the two

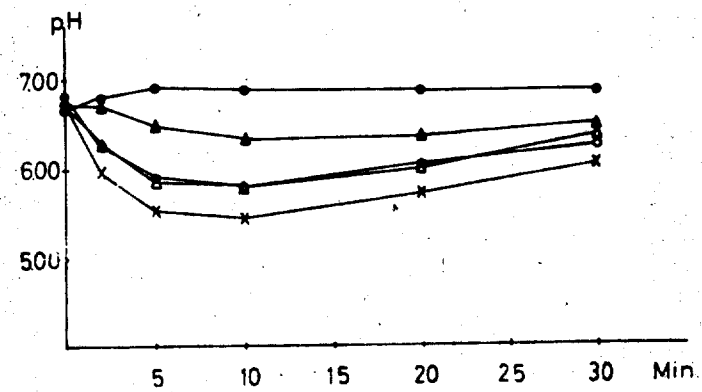

Fig. 16. Changes in the pH of the dental plaques after consumption of different types of chocolate. For comparison the results after rinsing with a 50 per cent sucrose solution are also given. Each curve gives the means of experiments with 18 persons.

● — ● dark sugar-free chocolate
▲ - - - ▲ light «sugar-free» chocolate (containing 18 per cent lactose)
○ — ○ dark chocolate containing sucrose
△ - - - △ light chocolate containing sucrose
× — × 50 per cent sucrose solution

others should be rich in such substances. Two types contained approximately 45 per cent of fermentable sugars whereas the two others contained only lactose, which is known to be less rapidly fermented by the oral microorganisms than sucrose, dextrose and fructose (*Frostell* 1964).

Experiments were performed with 18 persons, who were all given the 4 different chocolates at 4 different experimental occasions. Each piece of chocolate (4.5 grammes) was consumed as usual.

The result of this experimental series has been published elsewhere (*Frostell*, 1967) and is only briefly summarized here in Fig. 16. The tables may be requested from the author. The chocolate rich in cocoa and poor in fermentable sugars usually gave a pH-increase in the plaques whereas the cocoa-rich, sucrose-rich chocolate and the cocoa-poor, sucrose-rich chocolate gave curves which did not significantly differ from each other. The cocoa-poor, sucrose-poor chocolate (which contained 18 per cent of lactose) gave a less pronounced pH-decrease than the sucrose-rich chocolates.

DISCUSSION

The method used does not give the accurate pH of any special spot on the teeth or in the oral cavity. It will only give an indication in general of what is happening in the dental plaques. However, this method makes it possible to make comparisons between different kinds of carbohydrate foods provided the comparisons are made on a sufficient number of persons on which the foods to be compared are both tested. The results are valid for buccal and lingual plaques and superficial interproximal plaques but probably not to the same extent for plaques around the contact points or in the fissures.

Statistical evaluations based on several thousands of plaque pH-measurements have shown that a mean difference exceeding 0.3 pH-units (provided double experiments have been performed on 18 persons) is usually statistically significant at the one per cent level, whereas those exceeding 0.5 pH-units usually are significant at the 0.1 per cent level.

The experiments with different sucrose concentrations show — contrary to results of *in vitro* experiments on suspensions of oral microorganisms (*Frostell*, 1964) — that acid production in the plaques increased with increasing sucrose concentrations up to concentrations which are inhibitory in the *in vitro* experiments. The reason for this probably is the fact that in the *in vivo* experiments the substrate has to diffuse into the plaques. The results indicate that sugar concentration in the food is a factor of great importance for the initial acid attack in dental caries.

The experiments with different phosphate buffers must be evaluated with caution. The buffering action of the different buffers — although the total

phosphate concentration was the same in the different solutions — is widely different in the different pH-regions. Only in one pH-region — over pH 7 where the buffering action of the phosphate is appreciable — is there an extensive and highly significant effect of the buffer on the resulting plaque-pH. However, when experiments were performed with citric acid which buffers strongly at pH 3.7, a significant effect on plaque-pH reduction through a 50 per cent sucrose solution could be demonstrated after 2 minutes. After 10, 20 and 30 minutes the plaque-pH was significantly higher in the experiment with citric acid than with the pure sucrose solution. This is believed to be due to a stimulation of the production of alkaline, strongly buffering saliva caused by the citric acid. This belief is supported by the results of experiments with lemon juice and orange juice, which will be published later on.

Thus, it seems that in order to try to reduce the pH-drop in the plaques after consumption of foods containing fermentable sugars by use of buffers, the foods should contain a buffer with its maximum effect in the alkaline range. Acid buffers may, however, have a demonstrable effect on enamel surfaces not covered by plaque material. These views are in good agreement with clinical and experimental observations that acids taken with foods do not increase caries activity but result in erosion and abrasion (*Buonocore* and *Johansen*, 1958, *Dalderup*, 1960, and others).

The results of the experiments with sugar in fat are in good agreement with those of *Swenander-Lanke* (1965) and *Gustafson* et al. (1955). When sugar is mixed with fat it is not as easily released and cannot as easily diffuse into the plaque as when it is dissolved in water.

The results with flavors may be due to stimulatino of saliva or to an antibacterial effect in the plaques. Since there was still a significant difference after 30 minutes an antibacterial effect seems probable.

The results of experiments with repeated intake of sugar indicate that plaque-pH decrease is mainly a function of sugar concentration and not of consumption time. When the consumption time is prolonged, the period of pH-decrease in the plaque material is increased but not the pH-decrease itself.

The results with carbamide show that a significant reduction of pH-decrease in the plaques after consumption of foods containing fermentable sugars may be achieved. Experiments on hamsters and rats with sugar-rich diets containing carbamide have given some favourable results, which will be published in the future.

The results with sorbitol tablets are in good agreement with the results of *Fosdick* et al. (1957).

Sorbitol acts as a laxative when taken in large doses. It is very hydroscopic, a fact which reduces its use in candy industry. For these reasons a hydrogenated potato strach hydrolysate has been developed which is believed to be less cariogenic than sucrose and which is less hygroscopic and laxative than sorbitol. Since 1963, when it first appeared, this product has continuously been developed in order to reduce its content of sorbitol and to increase the content of low molecular dextrines and saccharides. A reduction of the sorbitol content reduces the laxative effect of the product and makes it less hygroscopic. Experiments on hamsters, rats and pigs indicate that the cariogenicity of the product is low compared to sucrose (*Frostell, Keyes* and *Larson* 1967, *Frostell*, unpublished results).

A number of pilot experiments have been carried out in order to study the possible usefulness of the product as a substitute for sugar in different kinds of sweets, foods, beverages and medicines. The present results indicate that it is worth while to continue the research in this field.

An important question is whether results obtained by measuring plaque-pH after consumption of various kinds of foods are relevant for judging the cariogenic properties of the foods. It is quite obvious that plaque measurements performed on sufficiently large groups of persons will give an information as to whether a food will provoke an intense initial acid attack or not. A number of other properties of a foodstuff, however, may be of importance for its cariogenicity such as its capacity to stimulate plaque formation and implantation of caries-inducing microorganisms, its tendency to accumulate on and between the teeth and its content of caries inhibiting or solubility reducing substances such as fibers, polyphenols, phytates, theophyllamin, theobromin and perhaps others. The present results with chocolate show that chocolates containing large amounts of fermentable sugars will provoke an appreciable pH-decrease in the dental plaques. In spite of this fact milk chocolate of the same type was not very cariogenic as shown in the Vipeholm study. Since acid production in the plaques was not inhibited even in experiments with the cocoa-rich black chocolate (provided it contained 50 per cent sugar), it is believed that its low cariogenicity is due to its content of substances causing a reduction of enamel surface solubility (*Stadlfors*, 1967) and not to an effect on the acid production in the dental plaques (*Frostell*, 1967).

SUMMARY

The changes in pH of the dental plaques after rinsing (30 seconds) with various sugar solutions or consumption of different sweets were determined according to a method elaborated by the author. The plaque pH was de-

termined 2, 5, 10, 20 and 30 minutes after the beginning of the test. The influence of the following factors was studied. sugar concentration (50, 5, 0.5 and 0.05 per cent), buffer capacity and pH of the sugar solution (0.1 M phosphate buffer, pH 3.5, 4.9, 6.5 and 7.8 in a 50 per cent sucrose solution), citric acid (3 per cent in a 50 per cent sucrose solution), fat (5 per cent or 50 per cent sucrose in peanut butter was compared to 5 per cent and 50 per cent sucrose in water), flavors (menthol and eucalypthol, 2 per cent of each in 50 per cent sucrose), carbamide (5 per cent carbamide in 45 per cent sucrose was compared to a 50 per cent sucrose solution), substitution of sucrose by sorbitol or hydrogenated starch hydrolysate (Product 6563, Lycasin) in tablets, lozenges and different types of candy and foods. Further, some experiments with four different types of chocolate were carried out.

Rinsing with 0.05 per cent sucrose for 30 seconds generally resulted in an increase in pH of the dental plaque whereas the more concentrated solutions induced a decrease in pH of increasing strength and duration with increasing concentration. Addition of 0.1 M phosphate solutions pH 3.5, 4.9 or 6.5 to a 50 per cent sucrose solution had little influence on plaque-pH compared to a nonbuffered solution whereas phosphate buffer pH 7.8 significantly reduced the influence of the sugar on plaque pH.

Addition of citric acid (3 per cent) caused an increased pH-fall in the plaques after 2 minutes but after 10, 20 and 30 minutes the pH was slightly higher than in the control experiments. Flavors (menthol and eucalypthol, 2 per cent of each) caused a significant reduction of the decrease in superficiel plaque-pH induced by a 50 per cent sucrose solution.

Five or 50 per cent sucrose in peanut butter fat gave significantly less changes in pH than the same concentrations of sucrose in water.

By addition of 5 per cent carbamide to a 45 per cent sucrose solution or to candies containing sucrose the pH of the dental plaques could be kept almost unchanged in the post-consumption period, i.e. decreases in superficiol plaque pH could be almost completely prevented.

Substitution of sucrose by sorbitol in tablets generally resulted in pH-increases in the dental plaque. Substitution of sucrose by hydrogenated starch hydrolysates generally resulted in no or only slight pH-changes in the dental plaques, i.e, the post-consumption decrease in plaque-pH could be practically completely prevented by these substitutions.

Experiments with four types of chocolate showed that the pH-changes in the plaques after consumption was dependent mainly on the sugar concentration whereas the concentration of cocoa did not significantly influence the results.

In the discussion it is pointed out that although the experiments (plaque-

pH measurements) give information as to the extent to which a foodstuff or a solution induces an initial acid attack on enamel surfaces, it does not give complete information concerning the cariogenicity of the product since this may be dependent on other factors also, for example influence on enamel surface solubility and influence on the oral flora.

RÉSUMÉ

RAPPORTS ENTRE LE pH DE LA PLAQUE BACTÉRIENNE SUR LES DENTS ET LA CONSOMMATION DE PRODUITS À BASE D'HYDRATES DE CARBONE

Les modifications du pH de la plaque bactérienne sur les dents après rinçages (30 secondes) avec différentes solutions sucrées ou après ingestion de diverses sucreries ont été déterminées suivant une méthode élaborée par l'auteur. Le pH de la plaque a été déterminé au bout de 2, de 5, de 10, de 20, et de 30 minutes après le début du test. L'influence des facteurs suivants a été étudiée: concentration du sucre (50 %, 5 %, 0,5 % et 0,05 %), pouvoir tampon et pH de la solution sucrée (tampon de phosphate de 0,1 M p. litre, pH 3,5, 4,9, 6,5 et 7,8, dans une solution de saccharose à 50 %), acide citrique (3 % dans une solution de saccharose à 50 %), matières grasses (comparaison entre 5 % et 50 % de saccharose dans du beurre de cacahuète d'une part et 5 % et 50 % de saccharose en solution aqueuse d'autre part), parfums (menthol et eucalyptol à raison de 2 % chacun dans 50 % de saccharose), urée (comparaison entre 5 % d'urée dans 45 % de saccharose d'une part et une solution de saccharose à 50 % d'autre part), remplacement du saccharose par le sorbitol ou par un hydrolysat d'amidon hydrogéné (produit 6563, Lycasin) dans des tablettes, des pastilles et dans différents types de bonbons et d'aliments. Enfin, quelques expériences ont été effectuées avec quatre sortes différentes de chocolat.

Les rin ages pendant 30 secondes avec le saccharose à 0,05 % déterminaient généralement une élévation du pH dans les plaques, tandis que les solutions plus concentrées causaient un abaissement du pH d'autant plus prononcé et d'autant plus prolongé que la concentration était plus forte. L'addition de solutions de phosphate de 0,1 M p. litre, de pH 3,5, 4,9, ou 6,5 à une solution de 50 % de saccharose influait peu sur le pH de la plaque par comparaison avec une solution non tampon, alors qu'un tampon au phosphate de pH 7,8 réduisait de manière significative l'influence du sucre sur le pH de la plaque.

L'addition d'acide citrique (3 %) causait une accentuation de l'abaissement du pH dans les plaques au bout de 2 minutes, mais au bout de 10, de 20 et de 30 minutes, le pH était légèrement plus élevé que dans les expériences témoins. Les parfums (menthol et eucalyptol, à 2 % chacun)

réduisaient de manière significative l'abaissement du pH produit dans la plaque par une solution de saccharose à 50 %.

Les concentrations de 5 % et de 50 % de saccharose dans le beurre de cacahuète causaient des changements du pH significativement moins importants que ces mêmes concentrations de saccharose en solutions aqueuses.

En ajoutant 5 % d'urée à une solution de 45 % de saccharose ou à des bonbons contenant du saccharose, le pH de la plaque bactérienne pouvait être gardé presque inchangé pendant la période suivant l'ingestion, c'est-à-dire que l'abaissement du pH dans la plaque pouvait être presque entièrement évité.

Le remplacement du saccharose par le sorbitol dans des tablettes déterminait en général une augmentation du pH dans la plaque bactérienne. Le remplacement du saccharose par des hydrolysats d'amidon hydrogénés ne déterminait en général que peu ou pas de changement du pH dans la plaque, c'est-à-dire que l'abaissement du pH dans la plaque pendant la période suivant l'ingestion pouvait en pratique être totalement évité par ces substitutions.

Les expériences effectuées avec quatre types de chocolat ont révélé que les changements du pH dans la plaque après l'ingestion dépendaient principalement de la concentration du sucre, tandis que la concentration du cacao n'avait pas d'influence significative sur les résultats.

Dans la discussion, l'auteur souligne que, bien que les expériences (mesures du pH des plaques) donnent des renseignements sur la mesure dans laquelle un aliment ou une solution cause une attaque acide initiale sur les surfaces d'émail, on ne peut y trouver de renseignements éclairant à fond le pouvoir cariogène du produit, puisque ce pouvoir peut dépendre aussi d'autres facteurs, tels que l'influence sur la solubilité de la surface de l'émail et l'influence sur la flore microbienne buccale.

ZUSAMMENFASSUNG

DENTALE PLAQUE-PH IN RELATION ZUR KOHLENHYDRATEZUFUHR

Die pH-Veränderungen in den dentalen Plaques nach Mundspühlungen (30 Sekunden) mit verschiedenen Zuckerlösungen oder nach Konsumtion von verschiedenen Süssigkeiten wurden nach einer Methode ausgearbeitet von dem Verfasser bestimmt. Das Plaque-pH wurde nach 2, 5, 10, 20 und 30 Minuten bestimmt. Man studierte den Effekt von den folgenden Faktoren: Zuckerkoncentration (50, 5, 0,5 und 0.05 Prozent), Pufferkapacität und pH der Zuckerlösung (0.1 M Phosphatpuffer, pH 3,5, 5,9, 6,5 und 7,8 in 50 Prozent Zucker), Zitronensäure (3 Prozent in 50 Prozent Zuckerlösung), Fett (5 oder 50 Prozent Zucker in Fett), Menthol und Eucalyptol

(2 Prozent in 50 Prozent Zuckerlösung), Karbamid (5 Prozent Karbamid in 45 Prozent Zuckerlösung), Substitution von Zucker durch Sorbitol oder einem hydrogenierten Stärkehydrolysat (Produkt 6563, Lycasin) in Tabletten und Bonbons. Ausserdem wurden einige Experimente mit vier verschiedenen Schokladesorten gemacht.

Mundspühlung mit 0.05 Prozent Zucker gab gewöhnlich eine pH-Steigerung während die mehr koncentrierte Lösungen eine pH-Senkung verursachten, die mit zunehmender Koncentration zunahm. Zusatz von 0.1 M Phosphatpuffer mit pH 3.5, 4.9 oder 6.5 zu einer 50-prozentigen Zuckerlösung beeinflusste wenig die resultierende pH-Veränderungen während ein pH 7.8 Puffer die pH-Senkungen signifikant reducierte.

Zusatz von 3 Prozent Zitronensäure verursachte eine vermehrte pH-Senkung nach 2 Minuten aber nach 10, 20 und 30 Minuten war das pH etwas höher als in den Kontrollexperimenten. Menthol und Eucalyptol verursachten eine signifikante Reduktion der pH-Senkung verursacht von der Zuckerlösung.

Fünf oder 50 Prozent Zucker in Fett verursachte signifikant wenige pH-Veränderungen als dieselben Koncentrationen von Zucker in Wasser.

Durch Zusatz von 5 Prozent Karbamid zu einer 50-prozentigen Zuckerlösung konnte man das pH beinahe unverändert behalten, also die pH-Veränderungen wurden beinahe vollständig verhindert.

Substitution von Zucker mit Sorbitol in Tabletten resultierte gewöhnlich in pH-Steigerungen in den Plaques. Substitution von Zucker mit einem hydrogenierten Stärkehydrolysat gab nur wenige oder gar keine pH-Veränderungen während der Postkonsumtionsperiode, also die pH-Veränderungen wurden beinahe vollständig gehebt.

Experimente mit vier verschiedenen Schokoladesorten zeigten dass die pH-Veränderungen nach der Konsumtion von Zuckerkoncentration abhängig waren aber nicht von der Kakaokoncentration.

In der Diskussion wird vorgehalten, dass obwohl die pH-Messungen eine Information der initialen Säureattacke, die von einem Nahrungsmittel verursacht wird, geben, geben sie jedoch keine kompletten Informationen über die Kariogenität dieses Nahrungsmittels. Die Kariogenität eines Nahrungsmittels ist auch von anderen Faktoren abhängig, z.B. von Einfluss auf die Lösligkeit der Emaljoberfläche und von Einwirkung auf die Mundflora.

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EFFECTS OF SUCROSE, STARCH AND A HYDROGENATED STARCH DERIVATIVE ON DENTAL CARIES IN THE RAT

by
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INTRODUCTION

Previous experiments with hamsters and rats (*Frostell, Keyes & Larson*, 1967; *Frostell*, in press), revealed a great difference in caries scores between groups of animals fed sucrose as compared to those fed a starch or a hydrogenated starch derivative, Product 6563, Lycasin*), diet. The latter product is used for production of candy, and is believed to be of low cariogenicity.

From previous experiments on calculus formation in rats (*Smith et al.*, 1963) it was known that animals given different starch diets often developed dental caries. Although starch is generally considered to be only moderately cariogenic, considerable dental destruction by caries was sometimes noted in these experiments.

Unmodified potato starch (raw starch) is known to cause insignificant changes in pH of the dental plaques (*Neff*, 1967; *Frostell*, in press). It is believed that the reason for this is that the large molecules of starch diffuse only slowly into the dental plaques. It is known that acid production in suspensions of dental plaque material in the presence of raw starch occurs rather fast, to an extent of about 80 per cent of the acid production in the presence of sucrose (*Frostell*, 1964). *Neff* found that cooked starch caused considerably greater pH-decreases in the dental plaques than raw starch, a

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finding confirmed by results of an investigation going on at present in this laboratory.

Pregelatinized starch is available as a white powdered product. The pregelatinization process is believed to break up the starch and to straighten out the twisted amylose molecule chains making them more available to amylase enzymes and more easily fermentable and digestible. Neither raw starch nor pregelatinized starch contain low-molecular saccharides as revealed by chromatographic studies in this and other laboratories.

In the present study a comparison was made between the caries promoting capacities of sucrose, unmodified potato starch, pregelatinized potato starch and a hydrogenated potato starch derivative (Product 6563, Lycasin*).

MATERIAL AND METHODS

Female weanling Sprague-Dawley rats were used in these experiments. The animals were 25 to 30 days of age when the experiment began. They were housed in wire bottomed cages, one or two per cage, and received distilled drinking water, *ad libitum*. They were weighed once a week. Seven groups of weanling female Sprague-Dawley rats were fed powdered diets containing 32 per cent skim milk, 2 per cent liver powder and 60 per cent test carbohydrate. The following carbohydrates were given to 4 groups for 30 days: sucrose, pregelatinized potato starch, a hydrogenated potato starch derivative supplemented with 0.5 per cent calcium carbonate. Three groups were given the following diets for 60 days: sucrose, unmodified potato starch and pregelatinized potato starch (Table I).

At the end of the experimental period the animals were weighed, killed by ether anesthesia and decapitated. The jaws were defleshed by the use of dermestid beetles (*Hall & Russel*, 1933). They were bleeched with 30 per cent H_2O_2 and dried with acetone. Caries was scored according to *Keyes* (1958).

RESULTS

Results are given in Table I. All animals kept on the diet containing the hydrogenated starch derivative developed diarrhoea.

Three animals out of 26, which were fed the hydrogenated potato starch derivative for 30 days, developed sulcal caries only.

Table I. *Number of carious enamel areas in the dietary groups*

Carbohydrate group	No. of animals	Days	CT	Bucco-lingual E	Morsal	Sulcal E	Proximal E	Total no of carious areas	Per cent vs control
Sucrose	20	30	5.2	8.6	0.2	3.2	2.6	14.6	100
	19	60	10.5	34.3	0.6	13.7	11.9	60.6	100
Pregelatinized potato starch	12	30	7.8	22.4	2.0	8.1	1.8	34.3	235
	23	60	9.5	13.2	3.8	13.7	2.2	32.9	54.3
Unmodified potato starch	22	60	6.4	6.6	0.2	9.6	1.2	17.6	29
Hydrogenated saccharides	26	30	0.2			0.3		0.3	2.1
Hydrogenated saccharides, $CaCO_3$	42	30	0.8			1.0		1.0	6.9

CT = carious teeth per animal
E = number of enamel areas

Six animals out of 42, which were given the hydrogenated potato starch diet with a 0.5 per cent calcium carbonate additive for 30 days, developed sulcal caries. The animals, however, were not in good physical condition, and the experiment was not extended beyond 30 days.

Seventeen animals out of 22 receiving the unmodified potato starch diet for 60 days developed caries. All animals had sulcal and bucco-lingual caries and seven animals also had proximal caries.

All animals, which were on the pregelatinized potato starch diet for 30 days (12), developed caries. All had sulcal and bucco-lingual caries. Nine animals also had proximal cavities.

All animals (23) that were on the pregelatinized potato starch diet for 60 days developed caries, which often penetrated into the dentine. All animals, except one, had sulcal and bucco-lingual caries in many teeth. That one animal developed bucco-lingual caries. Sixteen animals also had proximal caries.

All animals given the sucrose diet for either 30 or 60 days developed severe dental caries of all types. Deep lesions penetrating the dentine were found in great numbers in animals which had been on the diet for 60 days.

DISCUSSION

Previous results with sucrose, unmodified starch, and the hydrogenated starch derivative were confirmed in this study. In spite of the fact that the pregelatinized starch did not contain glucose, maltose or other low-molecular saccharides, it was considerably more caries-inducing than the unmodified starch diet. The results indicate that even a comparatively mild processing of the starch increases its caries-inducing properties.

Only sulcal lesions were found in the animals which were fed the diets containing the hydrogenated starch derivative. These lesions were confined to the enamel and no softening of the underlying dentine was observed. If the animals had been on the diets for periods of longer than 30 days, other lesions would probably have developed.

Since all the diets contained skim milk powder, which contains lactose fermentable by dental plaque material, it cannot be ruled out that this sugar did not to some extent contribute to the development of dental caries in these experiments. The hydrogenated starch derivative diet was not conducive to smooth surface caries but sulcal caries was found in 10 animals out of 58.

Accurate records were not kept of the animals eating patterns and food consumption. However, since some groups of animals gained weight somewhat more slowly than others, the results may to some extent depend on differences in eating habits and food consumption and not only on differences in the caries-inducing properties of the diets. However, the almost complete absence of soft deposits on the teeth and the excellent status of the alveolar bone gave the impression that the diets containing the hydrogenated starch derivative were not conductive to formation of dental plaque.

The addition of calcium carbonate was reported by *Gustafson, Stelling & Brunius* (1964) to reduce dental caries in animals or humans living on an otherwise calcium deficient diet. In these experiments calcium carbonate did not exert such an effect. Since all the diets used in these experiments contained 32 per cent skim milk, they were certainly not deficient in calcium, however.

In the previous rat experiments (*Frostell, Keyes & Larson*, 1967) the animals were infected with fecal flora taken from animals with rampant dental caries. In these experiments the animals were not infected in this way. In spite of this, dental caries developed in approximately the same way and to the same extent as in previous experiments. However, a group on pregelatinized starch for 30 days surprisingly developed much caries which may be due to the presence of an unusual bacterial flora.

SUMMARY

Seven groups of weanling female Sprague-Dawley rats were given powdered diets containing 32 per cent skim milk, 2 per cent liver powder and 66 per cent test carbohydrate. The following carbohydrates were given to 4 groups for 30 days: sucrose, pregelatinized potato starch, a hydrogenated potato starch derivative and the same derivative supplemented with 0.5 per cent calcium carbonate. Three groups were given the following diets for 60 days: sucrose, unmodified potato starch and pregelatinized potato starch.

At the end of the experimental period the animals were sacrificed and dental caries were scored.

The caries scores of the groups decreased in the following order: sucrose, pregelatinized potato starch, unmodified potato starch, hydrogenated potato starch derivative.

Animals on the sucrose diet developed rampant dental caries on smooth surfaces and in the sulci. Unmodified potato starch and pregelatinized potato starch also induced smooth surface caries and sulcal caries, which penetrated the enamel and softened the dentine, but considerably less than the sucrose diet. The hydrogenated potato starch derivative, which was given for 30 days only, was not conducive to smooth surface caries but sulcal caries confined to the enamel was found in 10 animals out of 58.

It was concluded that both unmodified and pregelatinized starch may be caries-inducing, that pregelatinization increased the cariogenicity of starch, and that hydrogenation of a starch hydrolysate considerably reduced its caries-inducing properties.

RÉSUMÉ

ACTION DU SACCHAROSE, DE LA FÉCULE ET D'UN PRODUIT DÉRIVÉ DE LA FÉCULE HYDROGÉNÉ

Des régimes en poudre administrés à 7 groupes de femelles de rats Sprague-Dawley venant d'être sevrées contenaient 32 % de lait écrémé, 2 % de poudre de foie et 66 % d'hydrate de carbone expérimental. Les hydrates de carbone suivants ont été donnés pendant 30 jours à 4 groupes: saccharose, fécule de pomme de terre prégélatinisée, un produit dérivé de la fécule de pomme de terre hydrogéné et le même produit additionné de 0,5 % de carbonate de calcium. Trois groupes ont reçu pendant 60 jours les régimes suivants: saccharose, fécule de pomme de terre non modifiée et fécule de pomme de terre prégélatinisée.

A la fin de la période expérimentale, les animaux ont été sacrifiés et l. caries dentaires ont été enregistrées.

Les taux de carie allaient en diminuant dans l'ordre suivant: saccharo. fécule prégélatinisée, fécule de pomme de terre non modifiée, produit d. rivé de la fécule hydrogéné.

Les animaux recevant le régime contenant du saccharose présentaient u polycarie évolutive sur les faces lisses et dans les sillons. La fécule de pomm de terre non modifiée et la fécule prégélatinisée provoquaient aussi l'appar tion de caries des faces lisses et des sillons, pénétrant l'émail et ramollissa la dentine, mais nettement moins que le régime au saccharose. Le produ dérivé de la fécule de pomme de terre hydrogéné, administré pendant 3 jours seulement, ne provoquait pas de caries des faces lisses, mais des cari des sillons limitées à l'émail ont été trouvées chez 10 des 58 animaux.

En conclusion, la fécule non modifiée et la fécule prégélatinisée peuve provoquer la carie; la prégélatinisation augmentait le pouvoir cariogè de la fécule, et l'hydrogénation d'un hydrolysat de fécule diminuait considérablement son pouvoir cariogène.

ZUSAMMENFASSUNG

EINWIRKUNG VON SACCHAROSE, STÄRKE UND EIN HYDROGENISIERTES STÄRKE DERIVAT AUF DER KARIESBEFALL IN DER RATTE

Sieben Gruppen, zusammengesetzt aus eben abgewöhnter weiblicher Sprague-Dawley Ratten, wurden verschiedene pulverisierte Diäte gegeben, die 32 Prozent Magermilch, 2 Prozent Leberpulver und 66 Prozent Testkohlenhydrat enthielten.

Während 30 Tagen erhielten 4 Gruppen folgende Kohlenhydrate: Saccharose, pregelatinisierte Kartoffelstärke, ein hydrogenisiertes Kartoffelstärkederivat und dasselbe Derivat mit 0,5 Prozent Kalziumkarbonat supplementiert. Drei Gruppen bekamen 60 Tage lang folgende Diäte: Saccharose, nichtmodifizierte Kartoffelstärke und pregelatinisierte Kartoffelstärke.

Nach abgeschlossener Experimentperiode wurden die Tiere getötet und der Kariesbefall wurde bestimmt.

Der Kariesbefall nahm in folgender Ordnung ab: Saccharose, pregelatinisierte Kartoffelstärke, nichtmodifizierte Kartoffelstärke, hydrogenisierte Kartoffelstärkederivat.

Tiere, die Saccharosediät erhielten, entwickelten hochaktive Karies an glatten Flächen und in den Sulci. Nichtmodifizierte Stärke und pregelatinisierte Stärke induzierten auch Glattflächenkaries und Karies in den Sulci.

aber sehr viel weniger als die Saccharose. Das hydrogenisierte Kartoffelstärkederivat, welches den Ratten nur während 30 Tagen gegeben wurde, war nicht glattflächenkariesinduzierend, erzeugte aber Emaillekaries bei 10 von insgesamt 58 Tieren in den Sulci.

Folgende Schlussfolgerung wurde gezogen: nichtmodifizierte Stärke und pregelatinisierte Stärke können Karies induzieren, Pregelatinisierung steigert die Kariogenität der Stärke und durch Hydrogenisierung eines Stärkehydrolysates wird die Kariogenität wesentlich reduziert.

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CARIES EXPERIENCE OF RATS FED VARIOUS STARCHES FOR STUDY OF EXPERIMENTAL CALCULUS FORMATION

by
GÖRAN FROSTELL
PAUL N. BAER

INTRODUCTION

Diets containing large quantities of starch have been found to enhance the formation of dental calculus when fed to weanling Sprague-Dawley rats (*Baer & White*, 1967). In some of the experimental groups rampant dental caries was observed. The purpose of the present investigation was fourfold:

1) to determine the ability of various starches to induce dental caries,

2) to determine whether pregelatinization of the starches influences their cariogenicity.

3) to determine whether amylopectin, the insoluble fraction of starch, or amylose, the soluble fraction, is the most effective in inducing dental caries.

4) to determine whether there is a correlation between calculus formation and dental caries experience.

I. EFFECT OF DIFFERENT STARCHES

MATERIALS AND METHODS

The experiments on calculus formation have been described in detail by *Baer & White* (1967). Eleven groups of weanling Sprague-Dawley rats of

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Table I
*Diets**)

Unmodified starch (66 %)	Pregelatinized starch (66 %)
Wheat (a)	Wheat (a)
Rice (b)	Potato (c)
Arrowroot (b)	Tapioca (d)
Tapioca (c)	Amioca (d)
Amioca (d)	
Potato (c)	
Cornstarch (e)	

*) In addition, all diets contained 32 % skim milk and 2 % liver powder

Sources: (a) Hercules Powder Co., New York; (b) Morningstar Paisley, Inc., Clifton, N. J.; (c) Stein Hall Co., New York; (d) National Starch & Chemical Co., New York; (e) Clinton Corn Processing Co., New York.

both sexes, containing 60 animals per group, were fed a powdered diet containing 32 per cent skim milk, 2 per cent liver powder and 66 per cent of one of the commercially available starches listed in Table I. Distilled drinking water was administered *ad libitum*.

Usually 24 animals in each group were sacrificed after a 30-day experimental period and an additional 24 per group after 60 days. The remainder were kept for study of calculus formation only and were not available for caries scoring. Upon termination of the experimental period the animals were decapitated, defleshed by the use of dermestid beetles and the molar teeth scored for calculus (*Baer, Stephan & White*, 1961) and for caries (*Keyes*, 1958). The total number of carious enamel areas was determined for each animal.

RESULTS

After a 30 day experimental period there were no statistically significant differences in the total number of carious enamel areas between animals on the regular corn starch and those on unmodified rice. Animals on unmodified amioca and unmodified potato starch had a tendency to have higher caries rates ($P < 0.05$). (Table II, Fig. 1.)

After 60 days (Table IIIa) there were no statistically significant differences between the animals fed the regular corn starch and the unmodified wheat, unmodified rice, unmodified tapioca and unmodified arrowroot. For the

Table II

Effect of various starches on calculus formation and dental caries. 30 day experimental period

Diet No	Diet Regimen (Starch)	Calculus scores N	$\bar{X}$	S.E.	Caries scores N	$\bar{X}$	S.E.	Vs Control t	P	Level of significance
S—1	Unmodified wheat	24	3.13	0.519	–					
Control	Cornstarch, regular	24	3.67	0.428	18	0.72	0.351			
S—2	Pregelatinized arrowroot		-	-	12	17.17	2.763			
S—3	Pregelatinized potato	24	1.63	0.300	12	34.67	5.855			
S—4	Unmodified rice	23	2.65	0.473	11	3.72	1.830	2.0211	0.10 > P > 0.05	-
S—5	Unmodified arrowroot	23	1.57	0.402						
S—6	Unmodified tapioca	23	2.70	0.442						
S—7	Pregelatinized amioca	22	0.77	0.130	10	25.00	3.952			
S—8	Pregelatinized tapioca	23	1.61	0.265	12	18.25	3.099			
S—9	Unmodified amioca	24	3.38	0.496	12	7.00	2.871	2.6564	0.05 > P > 0.01	*
S—10	Pregelatinized wheat	23	1.78	0.398	11	24.18	5.784			
S—11	Unmodified potato	15	0.47	0.369	9	3.67	1.607	2.4157	0.05 > P > 0.01	*

Table III a

Effect of various starches on calculus formation and dental caries, 60 day experimental period

Diet No	Diet Regimen (Starch)	Calculus scores N	$\bar{X}$	S.E.	Caries scores N	$\bar{X}$	S.E.	Vs Control t	P	Level of significance
S -1	Unmodified wheat	24	4.79	0.478	24	2.50	0.812	0.5343	0.6 P 0.5	
Control	Cornstarch, regular	23	4.57	0.555	12	1.83	0.694			
S 2	Pregelatinized arrowroot	--	–		12	24.58	3.253			
S 3	Pregelatinized potato	23	2.48	0.338	23	33.52	3.491			
S -4	Unmodified rice	24	4.67	0.476	24	0.50	0.458	1.6378	0.20 P 0.10	
S -5	Unmodified arrowroot	23	2.17	0.528	23	2.87	0.737	0.9119	0.40 P 0.30	
S -6	Unmodified tapioca	21	3.71	0.594	21	4.14	0.874	1.8126	0.10 P 0.05	
S 7	Pregelatinized amioca	22	1.77	0.436	22	18.05	3.708			
S 8	Pregelatinized tapioca	24	2.67	0.238	24	32.25	2.653			
S 9	Unmodified amioca	24	4.63	0.339	24	4.79	0.866	2.2339	0.05 P 0.01	
S -10	Pregelatinized wheat	24	2.83	0.280	24	6.42	1.268			
S 11	Unmodified potato	19	0.37	0.114	18	16.67	5.334	2.2452	0.05 P 0.01	*

*) ?

Table III b

Effect of pregelatinization of various starches on their caries-inducing properties. Values from Table II and IIIa.

Substance	Day	Unmodified N	$\bar{X}$	S.E.	Pregelatinized N	$\bar{X}$	S.E.	Vs Control t	P	Level of significance**)
Wheat	60	24	2.50	0.812	24	6.42	1.268	2.6023	0.05 P 0.01	*
Arrowroot	60	23	2.87	0.737	12	24.58	3.253	8.5624	P 0.001	***
Potato starch	30	9	3.67	1.607	12	34.67	5.855	4.4578	P 0.001	***
	60	18	16.67	5.334	23	33.52	3.491	2.7364	0.01 P 0.001	**
Tapioca	60	21	4.14	0.874	24	32.25	2.653	9.5096	P 0.001	***
Amioca	30	12	7.00	2.871	10	25.00	3.952	3.7620	0.01 P 0.001	**
	60	24	4.79	0.866	22	18.05	3.708	3.6213	P 0.001	***

*) If calculated on the number of lesions all the differences were significant on the 0.1 percent level

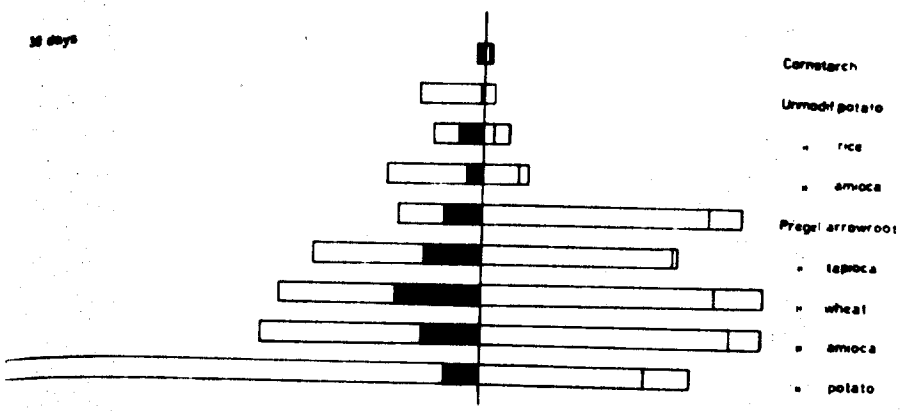
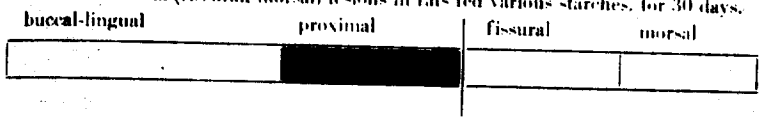


Fig. 1. Bar graph showing variations in the pattern of smooth-surface (buccal, lingual, proximal) and sulcal (fissural, morsal) lesions in rats fed various starches, for 30 days.



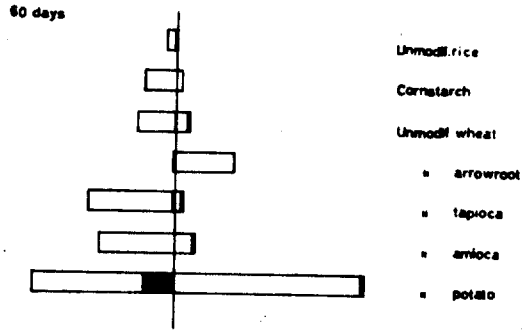


Fig. 2 a. Bar graph showing variations in the pattern of smooth-surface and sulcal caries in rats fed various unmodified starches for 60 days.

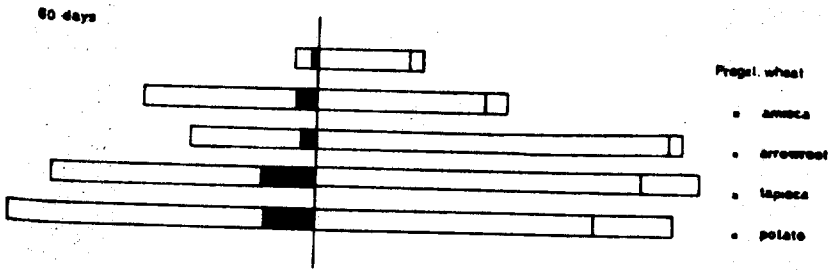


Fig. 2 b. Bar graph showing variations in the pattern of smooth-surface and sulcal caries in rats fed various pregelatinized starches for 60 days.

unmodified amioca and the unmodified potato starch diets there was a tendency towards a higher number of carious areas ($P < 0.05$).

Animals on all the pregelatinized starch diets (arrowroot, potato, amioca, tapioca and wheat) had several times more carious enamel areas than those fed the regular corn starch diet and those fed the unmodified starches. The differences were significant or highly significant. (Table IIIb), except for wheat for which there was a strong tendency (Figs. 2a and b.)

II. EFFECTS OF AMYLOSE AND AMYLOPECTIN

MATERIALS AND METHODS (A.)

Four groups of weanling Sprague-Dawley female rats, containing 20–25 animals per group, were fed for an experimental period of 30 days, one of the four diets shown in Table IV. Other experimental conditions were the same as in part I.

RESULTS

Animals on the corn amylose and the corn amylopectin diets (Table V) had more carious enamel areas than those animals on the high amylose corn starch. For amylopectin the difference was significant on the 1 per cent level.

Animals on the corn amylopectin diet had more carious enamel areas than animals on the corn amylose diet, but the difference was not statistically significant ($P > 0.10$). The calculus scores are given in Table VI. For additional information about the weight gain of the animals see *Baer & White* (1967).

Table IV
Diets)*

Diet*) (55 %)	Percent amylose or amylopectin contained in each starch fraction	
	Amylose	Amylopectin
a. Regular cornstarch (1)	28	72
b. High amylose cornstarch (2)	70	30
c. Corn amylose (3)	100	--
d. Corn amylopectin (3)	--	100

*) In addition, each diet contained 32 % skim milk and 2 % liver powder.

Sources: (1) Clinton Corn Processing Co., New York; (2) National Starch & Chemical Co., New York; (3) A. E. Staley Mfg. Co., Decatur, Ill.

Table X

Effect of various starches and starch constituents on the caries experience of rats

Substance	Day	Unmodified caries-scores N	$\bar{X}$	S.E.	Amylose caries-scores N	$\bar{X}$	S.E.	Amylopectin caries-scores N	$\bar{X}$	S.E.
Cornstarch, regular	30									
	60	20	3.10	0.767	20	7.60	1.297	20	10.85	2.071
	90	40	5.50	0.914						
Cornstarch, high amylose	30	17	2.71	1.325						
	60	16	3.38	1.221						
Potato starch	30	19	5.78	1.420						
	30	26	13.42	1.992	20	12.45	1.991	19	30.00	4.447
	60	28	16.43	2.954	19	34.00	4.438	17	47.29	5.535

	Day	t	P	Level of significance
Corn starch				
Regular – corn starch, high amylose	60	0.2018	P > 0.1	—
Amylose – corn starch, high amylose*)	30	2.6234	0.05 > P > 0.01	*
Amylopectin – corn starch, high amylose*)	30	3.1794	0.01 > P > 0.001	**
Amylopectin – amylose	30	1.3295	P > 0.1	
Potato starch				
Amylose – unmodified potato starch	30	2.7013	0.05 > P > 0.01	*
		0.3383	P > 0.1	
	60	3.4331	0.01 > P > 0.001	**
Amylopectin – unmodified potato starch	30	5.1830	P < 0.001	***
		3.7255	P < 0.001	***
	60	5.3788	P < 0.001	***
Amylopectin – amylose	30	3.6591	P < 0.001	***
	60	1.8885	0.10 > P > 0.05	

*) If calculated on the number of lesions the difference was highly significant.

Table VI

Effect of amylose vs. amylopectin on calculus scores

Diet	N	Calculus Scores $\bar{X}$	S.E.
Corn starch, regular	40	4.48	.41
High amylose cornstarch	40	1.45	.30
Corn amylose	40	0.65	.18
Corn amylopectin	37	2.89	.37

The difference between groups on the regular corn starch diet and those on the high amylose corn starch diet was not statistically significant ($P > 0.10$).

MATERIALS AND METHODS (B.)

Groups of weanling Sprague-Dawley female rats, containing approximately 20 animals per group, were fed for an experimental period of 30 and 60 days, unmodified potato starch, potato amylose and potato amylopectin diets, respectively. Other experimental conditions were the same as in previous experiment.

RESULTS

Animals on the potato amylopectin diet for 30 days had significantly more carious enamel areas than those on the potato amylose diet ($P < 0.001$). (Table V). After 60 days there was still a difference, but this difference was not statistically significant ($0.10 > P > 0.05$).

Animals fed the potato amylopectin diets had significantly more carious areas than animals fed the unmodified potato starch diets ($P < 0.001$).

There was no statistically significant difference in the number of carious enamel areas between animals on the potato amylose diet for 30 days and those on the unmodified potato starch diet, although there was a tendency for animals on the amylose diet to have more caries. After 60 days the difference was statistically significant ($0.01 > P > 0.001$).

III. CORRELATION BETWEEN CALCULUS FORMATION AND CARIES EXPERIENCE

MATERIAL AND METHODS

Since group means for both calculus formation and caries experience were available for some of the different dietary groups, a study was made of the

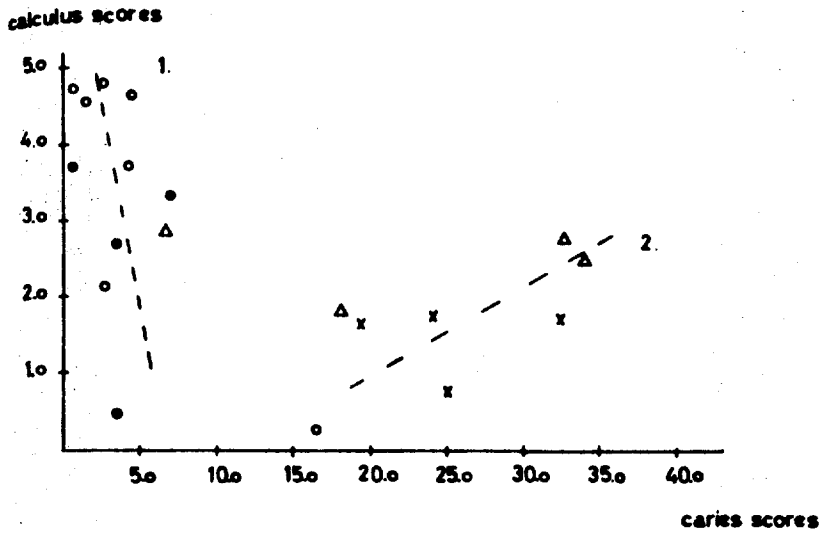


Fig. 3. Distribution of group means of calculus scores and caries scores.
1. Unmodified starches. 2. Pregelatinized starches.
O unmodified starches 30 days
● unmodified starches 60 days.
△ pregelatinized starches 30 days.
+ pregelatinized starches 60 days.

correlation between calculus formation and caries experience. At the time the scoring of dental caries was finished the scores for the individual values of calculus formation in the different animals were not available. Thus, it was not possible to carry through a detailed statistical study of the correlation between calculus formation and caries experience in the individual animal.

For those groups in which group means for calculus formation and caries experience were available, a correlation diagram was set up.

RESULTS

No clear correlation was found between calculus formation and caries experience (Fig. 3). In general, those animals fed the unmodified starches had very high calculus scores and very low caries scores. However, in experiments in which the animals were fed pregelatinized starches, there was a tendency for those groups with high caries scores also to have high calculus scores. Thus, those groups fed the pregelatinized starches usually had high caries scores and high calculus scores.

DISCUSSION

Since all the diets contained 32 per cent skim milk, which contains lactose, the lactose content of the diet may to some extent have contributed to the caries experience of the animals. Lactose, however, is considered to be only moderately cariogenic, a belief which is confirmed by the experiments with the unmodified starches.

Pregelatinization, however, caused a significant increase in cariogenicity of all the starches tested in all the experimental series. This finding is in accordance with the results of *Neff* (1967), who showed that cooked starch caused a considerable decrease in pH of dental plaques whereas raw starch did not. His results have been confirmed (*Frostell*, in press).

Amylopectin, the insoluble, branched constituent of starch, was generally more cariogenic than amylose, the soluble, linear constituent of starch. The cariogenicity of amylose was of the same order of magnitude as that of pregelatinized starch.

Thus, the results tend to show that the processing of starch increases its cariogenicity considerably and that this property is mainly ascribable to its amylopectin content and to a lesser extent to its amylose content, since regular starch contains much more amylopectin than amylose and since amylopectin was somewhat more cariogenic than amylose. Since in these experiments no polysaccharide production from sucrose was possible and still considerable plaque accumulation on smooth surfaces occurred, the question is raised whether amylopectin — which is not completely hydrolyzed by alfa-amylase and which chemical composition resembles that of the bacterial dextrans — may replace bacterial dextrans and favour plaque formation.

Acknowledgement. The authors wish to express their gratitude to Dr. R. Larson for valuable help and advice.

SUMMARY

Groups of weanling Sprague-Dawley rats were fed a diet containing 66 per cent starch, amylose or amylopectin. Twelve different types of unmodified or pregelatinized starches were studied. The diets were administered for 30 or 60 days in most experiments and caries and calculus were scored.

There was no statistically significant difference in the number of carious enamel areas between animals fed the regular corn starch control diet and those fed unmodified wheat, unmodified rice, unmodified arrowroot and unmodified tapioca, whereas animals fed the unmodified potato starch and

unmodified amioca had a tendency to have more carious areas. Animals on the pregelatinized starches (arrowroot, potato, amioca, tapioca and wheat) had several times more carious areas than those on the regular corn starch diet.

Animals fed corn amylose or corn amylopectin had significantly more carious areas than those on unmodified corn starch diets. Animals on amylopectin diets had more caries than animals on amylose. For potato amylose and amylopectin the difference was highly significant after 30 days on the diets, but not after 60 days.

No clear correlation was found between calculus formation and caries experience. In general, animals fed the unmodified starches had very high calculus scores and very low caries scores. However, in experiments in which the animals were fed pregelatinized starches there was a tendency for groups with high caries scores to have high calculus scores also.

RÉSUMÉ

CARIE DENTAIRE CHEZ DES RATS NOURRIS AVEC DIFFÉRENTES FÉCULES AU COURS D'UNE ÉTUDE SUR LA FORMATION EXPÉRIMENTALE DU TARTRE

Un régime contenant 66 % de fécule, d'amylose ou d'amylopectine a été administré à des groupes de rats Sprague-Dawley venant d'être sevrés. Douze types différents de fécule non modifiée ou prégélatinisée ont été étudiés. Les régimes ont été administrés pendant 30 ou 60 jours; la carie et le tartre ont été enregistrés.

Il n'y avait pas de différence statistiquement significative dans le taux de la carie entre les animaux ayant reçu le régime témoin à la fécule de maïs ordinaire et ceux qui étaient nourris à l'amidon de blé non modifié, la fécule de riz non modifiée, la fécule d'arrowroot non modifiée et la fécule de tapioca non modifiée, tandis que les animaux nourris à la fécule de pomme de terre non modifiée avaient un nombre significativement plus élevé de zones carieuses. Les animaux recevant les fécules prégélatinisées (arrowroot, pomme de terre, amioca, tapioca et blé) avaient plusieurs fois plus de zones carieuses que ceux qui recevaient le régime à la fécule de maïs ordinaire.

Les animaux nourris à l'amylose de maïs ou à l'amylopectine de maïs avaient un nombre significativement plus élevé de zones carieuses que ceux qui recevaient le régime à la fécule de maïs ordinaire. Les animaux recevant les régimes à l'amylopectine avaient plus de caries que les animaux recevant l'amylose. En ce qui concernait l'amylose et l'amylopectine de pomme de

terre, la différence était hautement significative après 30 jours au régime, mais pas après 60 jours.

ZUSAMMENFASSUNG

KARIESBEFALL VON RATTEN DENEN VERSCHIEDEN STÄRKESORTEN GEGEBEN WURDEN UM EXPERIMENTELLE ZAHNSTEINBILDUNG ZU STUDIEREN

Gruppen von Sprague-Dawley-Ratten, die eben abgewohnt waren, wurde eine Diät gegeben, die 66 Prozent Stärke, Amylose oder Amylopektin enthielt. Zwölf verschiedene Sorten von nichtmodifizierten oder pregelatinizierten Stärken wurden untersucht. Die Diäten wurden den Tieren während 30 oder 60 Tagen gegeben und Karies und Zahnsteinbildung wurden registriert.

Die Verschiedenheit im Kariesbefall zwischen Tieren, denen die nichtmodifizierte Kontrollstärke (Maisstärke) gegeben wurde und derjenigen die nichtmodifizierte Weissestärke, nichtmodifizierte Reisstärke, nichtmodifizierte Arrowrootstärke und nichtmodifizierte Tapiokastärke gegeben wurde war nicht statistisch signifikant, aber Tiere die nichtmodifizierte Kartoffelstärke und nichtmodifizierte Amiokastärke bekommen hatten einen Tendenz nach mehr Karies als die Kontrolltiere. Tiere, denen pregelatinizierten Stärkesorten gegeben wurde, hatten vielmals mehr kariierte Zahnflächen als Tiere der Kontrastdiätgruppe.

Tiere, denen Maisamylose oder Maisamylopektin gegeben wurde, hatten signifikant mehr Karies als Tiere der Kontrastdiätgruppe (High amylose corn starch). Tiere der Amylopektindiätgruppen hatten mehr Karies als Tiere der Amylosediätgruppen. Zwischen Kartoffelamylose und Kartoffelamylopektin war der Unterschied stark signifikant nach dreissig Tagen aber nicht nach sechzig.

Man konnte keine klare Korrelation zwischen Zahnsteinbildung und Kariesbefall finden. Tiere, denen nichtmodifizierte Stärkesorten gegeben wurde, wiesen starke Zahnsteinbildung und niedrigen Kariesbefall auf. Doch wenn Tieren pregelatinizierte Stärkesorten gegeben wurde, fand man bei Gruppen mit starkem Kariesbefall auch eine Tendenz starker Zahnsteinbildung.

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Effect of Various Sugars and Sugar Substitutes on Dental Caries in Hamsters and Rats

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ABSTRACT Two series of experiments, one in hamsters the other in rats, were conducted to compare the dental caries conduciveness of sucrose with that of several other sugars and carbohydrates. Hamsters and albino rats fed a diet containing 56% sucrose in the form of confectionery sugar developed highly active carious lesions in their molar teeth. The lesions were located on "smooth surfaces" as well as in crevicular areas. Fructose, dextrose, maltose, hydrogenated starch, sorbitol, and mixtures of starch were used as substitutes for the sucrose product. In hamsters, which are more prone to experience smooth surface caries, the substitutes used were followed by a reduction in plaque accumulations, less active progression of lesions, and little or no new lesion incipience. In the rats, which are susceptible to a wider variety of lesions, almost all the sucrose-free substitutes were associated with less active cavitation in crevices and on buccal, lingual, and proximal surfaces. However, only the hydrogenated starch product, which is fermented more slowly than the sucrose, was not conducive to circumferential lesions, but it was associated with a low grade form of activity in crevicular areas (sulci).

To account for the several types of carious lesions which are found in humans and which can now be induced in hamsters and rats, one needs to explore the supposition of Cox (1) that carious processes in one affected site may differ etiologically from carious processes in another site. Cavitation in crevices or sulci ("pit and fissure" cavities), on smooth surfaces (buccal, lingual, and proximal lesions), and on root surfaces does not always develop with equal severity. This phenomenon can be explained with the hypothesis that these lesions may differ with respect to the microorganisms involved and the type of carbohydrate consumed. In fact, there appears to be evidence available to support this postulation (2–4). New experimental procedures and deeper insight into processes associated with dental caries now permit further testing of the hypothesis by providing methods to study some of the interactions between various carbohydrates, bacterial flora, plaque formation, and variations in cavitation in the aforementioned areas.

Although several workers (5–7) have reported that the extent of cavitation in experimental animals may vary with the types of carbohydrate in the diet, generally speaking little distinction has been made between various lesions, apparently because only one kind has been found or studied. The present paper describes several exepriments with various dietary sugars and sugar substitutes which were undertaken to explore interactions between these ingredients and odontopathic bacteria and patterns of carious lesions in the molar teeth of hamsters and rats.

EXPERIMENTAL METHODS

A better appreciation of the interactions between dietary components, bacteria, and dental caries can be attained by using both hamsters and rats whenever possible. Hamsters are well-suited for studies of bacterial colonization on the teeth, smooth surface cavitation, and periodontal lesions (8–10), since these phenomena can be easily followed by examining the mouths of living unanesthetized animals. Rats are also satisfactory for studies of smooth-surface lesions, and their teeth are more suitable for studies on proximal and crevicular lesions (11). However, in unanesthetized rats it is not possible to examine the molar denti-

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tion and to evaluate lesions in recessed areas. Moreover, methods for assessing populations of caries-conducive bacteria in rats have been more difficult than in hamsters.

Diet formula no. 2000 (3) which is highly conducive to dental caries was used as a control ration in the series of assays to be described. It contains: (in percent) confectioner's sugar, 56; skim milk power, 28; whole wheat flour, 6; alfalfa powder, 3; brewer's yeast, 4; liver powder, 1; and sodium chloride, 2.[2] The effect of the various carbohydrates was compared by using the following substitutions for the sucrose fraction in diet no. 2000: fructose; dextrose; fructose (28%) and dextrose (28%); maltose; hydrogenated potato starch,[3] that is, product 6563 (12); hydrogenated potato starch with calcium carbonate, 0.5%; potato starch; potato starch (28%) and sucrose (28%). All of the sugars were powdered and analytically pure except sucrose and dextrose[4] which were commercial products.

Hamster experiments. Diet no. 2000 was fed ad libitum to control animals during the entire assay periods (exps. HA, HB, HC, and HD). In the experimental groups this diet was fed during a preassay induction period of 10 days, and then the sucrose was replaced by either fructose (exps. HA and HB), dextrose (exps. HB and HC), equal parts of dextrose and fructose (exps. HB and HC), maltose (exp. HC) and hydrogenated potato starch (exps. HB, HC, and HD).

Weanling golden or albino hamsters between 19 and 24 days of age weighed approximately 30 g when they were started on the various experiments. All animals were inoculated intraorally 3 times, at intervals of one week, with 0.1 ml of an 18-hour culture of caries-conducive streptococci and given about 5 ml of the culture in 200 ml of drinking water for 24-hour periods. The streptococcal strain used was a streptomycin-resistant mutant (E- 49) described previously (13, 14). After 1, 2, and 5 weeks, samples of plaques and saliva were taken by means of a cotton-tipped swab and were transferred to 1 ml of a 0.05% yeast extract water. The swabs were shaken in the yeast extract water and were then streaked on fuchsin-azide blood agar plates containing 2[illegible] μg/ml of streptomycin. The plates we[illegible] inoculated for 48 hours at 37° in 95[illegible] nitrogen plus 5% carbon dioxide. Th[illegible] number of microorganisms recovered [illegible] the plates was expressed as follows: ov[illegible] 1000 colonies per swab = 4+; 1000–500 [illegible] 3+; 500–250 = 2+; 250–50 = 1+; <[illegible] = ±; – means no recovery (13, 14). Th[illegible] development of bacterial plaques and co[illegible]onal cavitation was followed in the li[illegible] animals by periodic examination of th[illegible] teeth and gingivae with a dissecting m[illegible]croscope (magnification, × 15). Th[illegible] mouths of representative animals we[illegible] stained with eosin and then photograph[illegible] (15). At the end of the experimental i[illegible]terval, the extent of cavitation was eva[illegible]ated in living animals or in defleshed ja[illegible] (9, 16).

Osborne-Mendel rats experiments. Th[illegible] rats used in this series of studies were [illegible]tained from the NIH stock colony. This [illegible] a Cesarian-derived, relatively pathog[illegible] free colony maintained in a controlled [illegible]vironment, semi-barrier system.[5] Femal[illegible] were used throughout. At the start of a[illegible] experiment they were approximately [illegible] days of age and had an average weight [illegible] 45 g. They were housed in metal cages [illegible] groups of two or three, and provided w[illegible] their respective diets and water ad libitu[illegible] On the first day of an experiment ea[illegible] animal was infected with feces contain[illegible] bacteria conducive to both crevice a[illegible] smooth surface lesions. A few drops [illegible] softened mixed feces from caries-ac[illegible] rats were placed in the mouth of each a[illegible]mal, and a drinking solution of 15 f[illegible] fecal pellets per liter of distilled water [illegible] prepared daily and provided for the [illegible] 5 days of the experiment. Distilled w[illegible] was used thereafter. The animals w[illegible]

[2] The hamsters received a weekly supplement [illegible] puree of equal parts of apple, carrot, and kale. [illegible] animal received about 10 g of the blend. No s[illegible]ment was given to the rats.

[3] Hydrogenated potato starch (product 65[illegible]) manufactured by the Lyckeby Starch Refining [illegible]pany, Lyckeby, Sweden. It contains sorbitol a[illegible] drogenated dextrins with molecular weights [illegible]sponding to the equivalent of 2–10 glucose [illegible] with a mean of 4 glucose units. Each dextrin [illegible] sorbitol residue at one end of the chain.

[4] Cerelose, Corn Products Company, Argo, I[illegible]

[5] These rats are not specific pathogen-free a[illegible] However, they do not harbour a highly caries-co[illegible] microflora and develop very low levels of caries [illegible] unless they are infected with appropriate [illegible] organisms.

weighed again at termination of the 56-day experimental period.

Four experiments were set up at different times (exps. RA, RB, RC and RD). A control group which received diet 2000 was included in each experiment and each of the sucrose substitutes was assessed at least twice: fructose (exps. RA and RD), dextrose (exps. RA and RD), equal parts of fructose and dextrose (exps. RA and RD), maltose (exps. RA and RB), hydrogenated potato starch (exps. RA, RC, and RD), hydrogenated potato starch with calcium carbonate (0.5%) (exps. RA, RC, and RD), potato starch (exps. RB and RD), equal parts of potato starch and sucrose (exps. RA, RB, and RC), and sorbitol and starch (exps. RB, RC, and RD).

At the end of the experimental period the animals were killed and plaque accumulation was studied in animals selected at random in each of the dietary groups. The examinations were made at 25 × magnification immediately after death, after the teeth and gingivae were flooded with an alcoholic solution of green stain (FD&C green no. 3, fast green F.C.F.). Findings were noted as follows: negligible with traces in isolated areas in pits and fissures; continuous deposits along the gingival margin; and gross amounts on the surfaces.

The jaws and teeth were cleaned of soft tissues by dermestid beetles and odontal cavitation was evaluated by a method which included staining of lesions and the sectioning of molars so that sulcal and proximal areas could be examined as readily as the buccal and lingual surfaces (17).

RESULTS

Data obtained from studies of hamsters in experiments HA through HD are tabulated in table 1. Data derived from the examination of the teeth of rats in experiments RA through RD are tabulated in table 2. Figure 1 is a graphic presentation of average scores for rats in all experiments on each of the dietary variations studied. The average initial and final weights of the rats have been listed in table 3.

TABLE 1

Summary of observations in hamsters fed a diet containing sucrose for 10 days and then fed a sucrose substitute for the remainder of the experimental period

Principal carbohydrate	No. of animals	Preassay-assay period [1]	Streptococcal recovery	Coronal plaque	Avg caries score [2]	Final wt
			Experiment HA			g
Sucrose	6	10+40	++++	extensive	37	77
Fructose	6	10 / 40	++ to ±	sparse	7	68
			Experiment HB			
Sucrose	4	10+45	++++	extensive	83	80
Fructose	4	10 / 45	+++ to ++++	sparse	5	73
Dextrose	4	10 / 45	++ to ++++	sparse	2	85
Fructose-dextrose	5	10 / 45	++ to ++++	sparse	10	75
Hydrogenated starch (6563)	4	10 / 45	+	sparse	<1	84
			Experiment HC			
Sucrose	5	10+73	++++	extensive	169	92
Dextrose	6	10 / 73	+ to −	sparse	4	98
Fructose-dextrose	6	10 / 73	± to −	sparse	10	89
Maltose	6	10 / 73	± to −	moderate	22	97
			Experiment HD			
Sucrose	6	10+50	+ to ++++ [3]	extensive	139/40	86
Hydrogenated starch (6563)	5	10 / 50	± to +	sparse	56/0	63

[1] The number to the left of the virgule refers to the number of days the animals were fed sucrose (diet 2000); that to the right the days fed the test diet.
[2] The average values listed are for the entire dentition. In experiment HD the number to left of virgule is average score for dentition as a whole; that to the right is for third molars only; P. H. Keyes (9).
[3] No organisms were isolated from one animal.

TABLE 2

Comparison of various carbohydrates in caries test diets fed to Osborne-Mendel rats in experiments RA, RB, RC, and RD, showing the number of rats in each group and the number of areas of carious enamel on the bucco-lingual (BL), proximal (P), and sulcal (S) surfaces

Carbohydrate in diet	No. rats/group	Exp. RA			No. rats/group	Exp. RB			No. rats/group	Exp. RC			No. rats/group	Exp. RD		
		BL	P	S		BL	P	S		BL	P	S		BL	P	S
Sucrose	5	26.5	13.8	49.2	5	23.6	9.6	45.8	7	14.3	5.3	44.4	6	11.5	9.5	41.5
Dextrose	4	12.5	6.3	32.0									11	10.7	9.0	21.7
Fructose	4	6.0	7.5	28.3									4	9.8	11.5	22.1
Dextrose-fructose	2	8.5	5.0	20.0									12	8.6	8.0	24.6
Maltose	2	8.0	1.0	20.0	4	2.3	2.0	29.1								
Sucrose-potato starch	2	16.0	9.0	31.0	4	11.5	1.0	28.0	6	21.3	6.0	45.5				
Sorbitol starch					2	7.0	0.0	15.0	4	10.0	1.0	42.3	6	3.5	1.3	29.6
Potato starch					5	7.4	0.6	18.2					6	5.5	0.8	14.7
Hydrogenated starch (6563)	5	0.0	1.0	21.2[1]					6	0.2	0.0	16.8	6	0.0	0.0	11.0
Hydrogenated starch + $CaCO_3$	5	0.2	0.0	15.8[1]					6	0.0	0.0	23.6	6	0.0	0.0	6.0

[1] On experiment 63 days; all other groups assessed for 55 or 56 days.

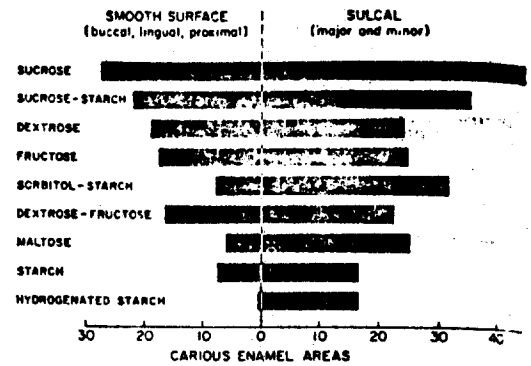

Fig. 1 Bar graph showing variations in the pattern of smooth-surface and sulcal (fissure) lesions which developed in rats fed various sugars and sugar substitutes.

Findings in animals fed 56% sucrose. In 21 hamsters which served as controls, dietary sucrose was consistently associated with extensive growth of bacterial plaque on the coronal surfaces of the teeth and in the gingival crevices. The caries-conducive streptococcus (E-49) was generally isolated from the teeth of all animals, except for 3 animals in experiment HD, in sufficient numbers to produce moderate-to-confluent growth on the culture plates. Cavitation of the smooth coronal surfaces was highly active and caused massive destruction, especially in the second and third molars (fig. 2). The severity of cavitation was greater in animals maintained for longer experimental periods. The terminal weights of animals was typical for this diet and the experimental conditions used.

In 23 rats the feeding of sucrose was also conducive to the formation of bacterial deposit along the buccal surfaces of the teeth and in the occlusal crevices. However, the deposits were not so extensive as those noted in hamsters. In all groups buccal-lingual (BL) and proximal (P) lesions were found repeatedly, and sulcal lesions were highly prevalent. Areas of carious enamel from individual animals ranged from 3 to 45 (average, 27) on the circumferential surfaces (buccal-lingual and proximal) and 21 to 49 (average, 45) in the sulci. The typical appearance of teeth from an animal fed sucrose is illustrated in figure 2. The growth of rats on this regimen was typical of

TABLE 3
Average initial and final weights and weight gain of rats fed various sugars and sucrose substitutes

Carbohydrate in diet	No. of animals	Avg initial wt	Avg final wt	Avg wt gain
		g	*g*	*g*
Sucrose	24	44.1	172.3	128.2
Dextrose	17	46.5	186.0	139.5
Fructose	10	45.8	188.4	142.6
Fructose-dextrose	18	46.1	174.7	128.6
Maltose	6	42.7	167.3	124.6
Sucrose-starch	12	39.0	161.3	122.3
Sorbitol-starch	12	47.4	115.9	68.5
Starch	11	47.2	160.4	113.2
Hydrogenated starch	18	42.2	157.7	115.5
Hydrogenated starch + $CaCO_3$	18	44.3	143.2	98.9

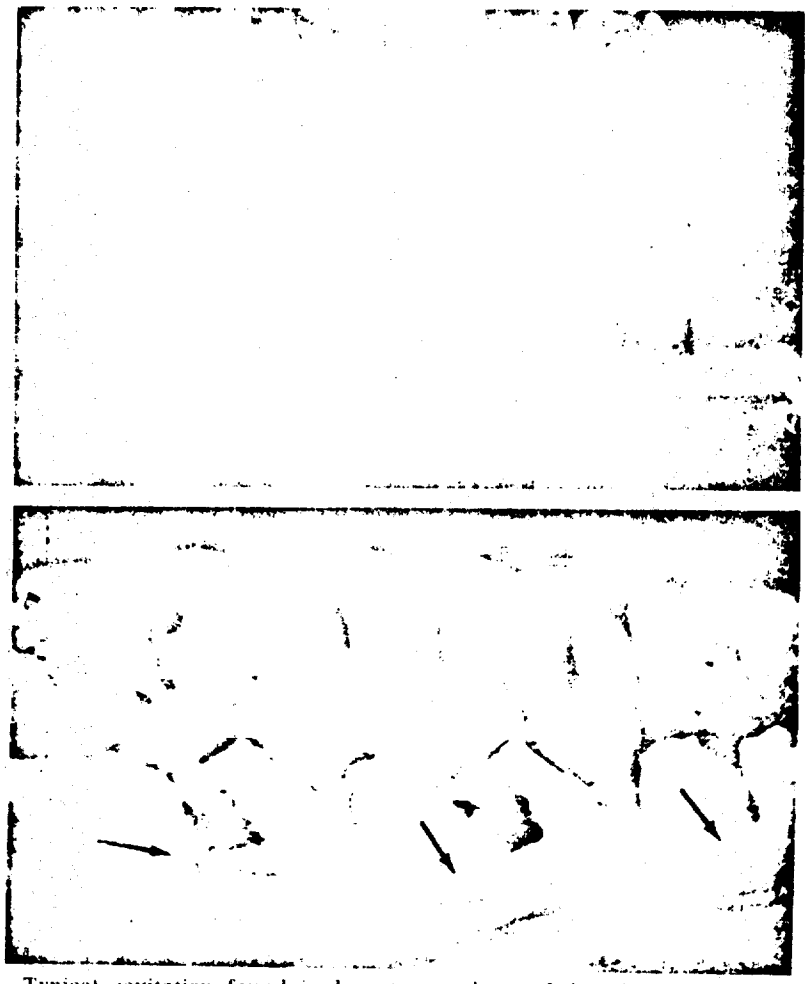

Fig. 2 Typical cavitation found in hamsters and rats fed a diet composed largely of sucrose and skim milk powder. (*A*) Photograph of maxillary molars of living hamsters shows extensive plaque and almost total destruction of second and third molars. (*B*) Sectioned mandibular molars of rat with lesions in all sulcal areas and smooth-surface lesions on the buccal surfaces of each tooth (arrows).

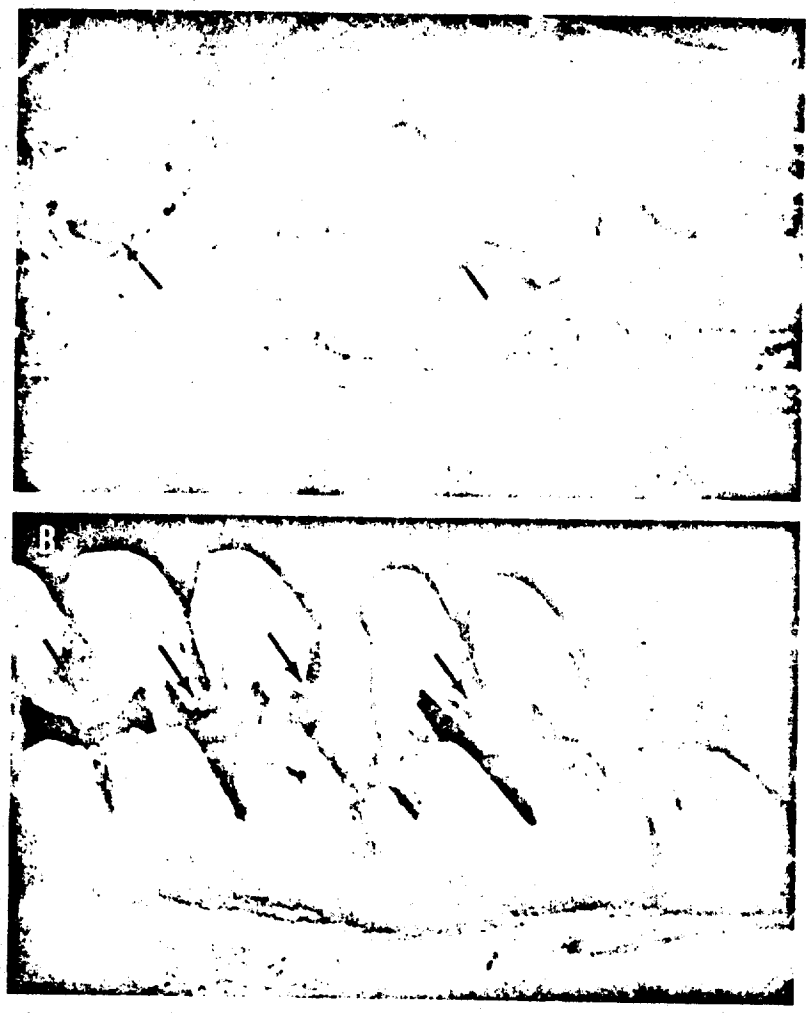

Fig. 3 Appearance of hamster and rat molars in animals fed sucrose substitutes. (*A*) The substitution of fructose, glucose, and hydrogenated starch was associated with a marked reduction in plaque deposits and less active cavitation shown in this photograph. Arrows point to relatively inactive lesions in maxillary first and second molars which started before the replacement of sucrose by fructose. (*B*) Teeth of rat fed hydrogenated starch (product 6563). No smooth decay is apparent, but small lesions are present in the occlusal sulci (fissures).

mals subjected to the conditions used (see table 3).

Findings in animals fed fructose as a replacement of sucrose. In hamsters (10 animals), plaque deposits which had commenced with the sucrose diet largely disappeared after fructose was substituted, and very little further accumulation occurred. When swabs of the oral cavities of these animals were plated out for recovery of streptomycin-resistant streptococci, plates in experiment HA tended to range between < 50 to 250–500 colonies plate. In experiment HB, however recoveries were higher and ranged tween 500–1000 colonies and confl growth per plate. Carious lesions w had started with sucrose remained sr and no smooth-surface decay was det on the third molars which erupted the change in diet. Figure 3 show representative photograph taken of maxillary teeth in living animals du the experimental period.

In rats (10 animals), the activity of lesions on the circumferential surfaces (buccal-proximal) was less than in sucrose-fed animals in experiments RA and RB, but it was not different in assay RD. The range in individual animals was from 3 to 31 (average, 17.4). Activity was consistently less in the occlusal crevices of animals fed fructose than in those continued with the sucrose diet, range 2 to 48 (average, 24.7).

Findings in animals fed dextrose as a sucrose substitute. Hamsters (10 animals) responded to dietary dextrose in much the same manner as those fed fructose. Again, plaque deposits which started to accumulate with sucrose became scanty, and only traces could be found on the third molars. Recoveries of the "labeled" streptococcus were variable. In experiment HB the microorganisms ranged between 250 colonies per plate and confluent growth, whereas in experiment HC the counts were lower, ranging between 50 to 500 colonies per plate. Carious lesions which had their inception with sucrose did not progress rapidly, and no smooth-surface activity was noted in third molars. The appearance of the mouths did not differ from that described previously for fructose and illustrated in figure 3. The growth of the animals and the terminal weights did not differ from those noted in animals fed sucrose.

In rats fed dextrose, the findings with respect to plaque deposits and cavitation appeared similar to those noted in animals fed fructose. Lesions occurred on all surfaces of the teeth, although on the basis of areas involved they had progressed somewhat less actively than those under the influence of sucrose.

Findings in animals fed equal parts of fructose and dextrose as a replacement for sucrose. In 11 hamsters (exps. HB and HC) fed the fructose-dextrose combination the results were similar to those when the simple sugars were fed alone. Neither plaque formation nor lesion formation (cavitation) was highly active. The streptomycin-resistant streptococcus was recovered in higher numbers from the mouths of animals assessed in experiment HB than from those tested in experiment HC. Terminal weights of animals did not appear importantly different from animals fed sucrose.

In 18 rats fed the combination of fructose and dextrose, plaque deposits and carious lesions did not differ in quantity and distribution from those found in animals fed fructose or dextrose alone.

Findings in animals fed maltose as a replacement of sucrose. In 6 hamsters (exp. HC) the consumption of maltose was associated with more noticeable plaque deposits although bacterial counts were low. Carious lesions progressed more actively than in animals fed either dextrose or the fructose-dextrose combination.

In 6 rats the consumption of maltose was associated with smooth-surface caries which were less active than those enhanced by sucrose. Activity on the smooth surfaces was somewhat lower for rats fed maltose than for those receiving dextrose, fructose, or the dextrose-fructose combination, but cavitation in the sulci was essentially the same for rats whether fed maltose, dextrose, fructose or dextrose-fructose (fig. 1).

Findings in animals fed hydrogenated potato starch (product 6563). The results were the same for the animals fed the hydrogenated potato starch with or without the addition of calcium carbonate. Animals of both species tended to develop diarrhea soon after they started to consume it, and the fecal deposits remained soft during the entire experimental period.

The growth and development of hamsters in experiment HB was satisfactory. However, all the animals in experiment HC developed diarrhea and died; none of the findings of this group are reported. Five of the six hamsters in experiment HD survived, although they did not grow well.

The teeth and gingival tissues of hamsters fed hydrogenated potato starch did not appear different from those in animals fed the other sucrose-free diets. Gross deposits of plaque could not be found in the teeth nor along the gingival crest. Very few odontopathic streptococci were recovered from the mouths of animals consuming this carbohydrate. In most instances fewer than 50 colonies per plate were noted. In experiment HB the pre-assay lesion did not progress rapidly, and new lesions did not form on the smooth surfaces of third

molars. As a result the overall level of activity was low and the teeth and gingivae resembled that depicted in figure 3. In experiment HD, cavitation in second molars progressed slowly but the final average score for the entire dentition was 56. However, smooth surface lesions did not form in third molars which had never been exposed to sucrose.

A total of 34 rats was fed one form or another of this product. Only negligible levels of plaque were noted along the buccal-lingual surfaces of the molars. However, deposits of debris and plaque were found in the occlusal sulci. The level of caries activity was lower on all surfaces for animals consuming this diet than for those fed any other carbohydrate studied. Smooth-surface activity was negligible, with the most active animal developing only 3 small lesions. A number of small lesions developed in the sulci with an average number of carious areas approximately one-third that of the sucrose fed animals (fig. 3).

Findings in rats fed potato starch alone or in combination with sucrose. The diet containing 56% potato starch was associated with more smooth-surface cavitation than the hydrogenated product, but it was not conducive to more active lesions in the crevicular areas (sulci). Rats fed equal parts of potato starch and sucrose experienced as much plaque and lesion formation as controls in experiment RC. In the other 2 trials, RA and RB, cavity formation was somewhat more active in the groups fed sucrose.

Findings in rats fed a sorbitol-starch combination. Rats (12 animals) fed a diet containing the sorbitol-starch combination developed essentially the same low level of smooth surface lesions as those with potato starch as the only source of carbohydrate. However, the level of sulcal activity with the sorbitol-starch diet was essentially the same as for those fed the sucrose-starch diet, and in experiment RC it was comparable to activity in rats fed 56% sucrose.

DISCUSSION

Dental caries usually has been considered a nonspecific bacterial disease associated with many types of acidogenic microorganisms, and usually little d tinction has been made between lesio originating in crevices, on smooth su faces, or on root surfaces. Some inves gators, assuming dental caries to microbiologically nonspecific, have post lated that experimental animals we resistant to either crevice or smooth-su face cavitation if lesions did not form while the animals were fed a certain ty of test diet (18, 19).

The possibility that etiologic facto (bacterial and carbohydrate) may diff for the various lesions has received m attention since the observation that ham ters and rats thought to be resistant various forms of dental caries beco susceptible when exposed to an appro ate bacterial-dietary challenge (20, 2 In order for lesions to form on smo surfaces, acidogenic microorganisms m adhere to the enamel surfaces in ten ous plaque deposits. In crevices t phenomenon does not appear to be ess tial, as bacteria and food residues may retained by wedging action and impacti It is now evident that investigations dental caries become more meanin when the different types of lesions considered separately, and when etiol factors are related to the potential of v ous microorganisms to colonize, to potential of various dietary compon to influence such colonization, and to by-products produced by the interac between bacteria and dietary substra Since more than one type of bact infection may be involved, this possi needs to be considered in the interp tion of results in animals and in extrapolation of results to humans.

In hamsters it was possible to s the bacterial plaques which had f in animals fed sucrose for 10 da appeared after the sucrose had be placed by fructose, dextrose, and genated starch. Since it was not p to assess plaque deposits in live ra jaws of the animal were examined the animal had been killed. Gross d were noted in animals fed sucrose, w as only small amounts or traces noted with other sugars, and ne amounts were present with hydrog starch. The marked difference in

accumulations seen in the mouths of living hamsters (figs. 2 vs. 3) was a more sensitive indication of a reduction in bacterial population on the teeth than the bacterial counts. The cultural methods used herein are useful to reveal the presence of caries-conducive streptococci but are apparently not suited to quantify amounts of plaque in a mouth. Large numbers of the E-49 type streptococcus were not recovered from one control hamster, although it developed extensive plaque and cavitation. This result may have been due to a technical error or to "competitive" exclusion (14).

The present experiments demonstrate that a diet which contains sucrose may have a caries-conducive potential which differs from one that contains other sugars and carbohydrates. Even the combination of fructose and glucose in amounts equal to those present in sucrose did not have the pathogenic potential of the disaccharide. Some of the explanation of these results may be found in the work of Gibbons and co-workers (22) who have isolated strains of streptococci from humans which are conducive to dental caries in rats and appear to be biochemically and serologically comparable to the hamster strains, for example, E-49 used in this experiment. These workers also reported that some strains form large quantities of extracellular non-dialyzable carbohydrate from sucrose and not from glucose. Wood and Critchley (23) observed that the extracellular polysaccharide synthesized primarily from sucrose is a dextran-like polymer and these investigators have postulated that extracellular polysaccharide synthesis by microorganisms contributes to the formation of dental plaque.

Fitzgerald and Keyes (13, 24) found that hamsters fed a diet containing 56% sucrose, as confectioner's sugar, developed abundant accumulations of coronal plaque and highly active cavitation only after they had been infected with a specific acidogenic streptococcus. Other equally acidogenic streptococci did not colonize on the teeth and form coronal plaques; nor were they associated with enamel demineralization and cavitation. The initial biochemical and fermentation tests did not disclose striking differences between the streptococcal strains which were associated with smooth surface caries and the types which were not. Quantitative determinations of the acid production rate of washed suspensions of caries-conducive and non-conducive streptococci did not reveal any significant differences, but the filament-forming bacterium *Odontomyces viscosus* (25), associated with plaque in the gingival crevice and root caries in hamsters (10), produced about one-fifth the acid released by the streptococci.[6]

Using a streptomycin-resistant mutant strain of caries-conducive streptococci, Krasse (7, 26) showed that sucrose in the diet favored implantation and cavitation in hamsters whereas dextrose did not; lactose also appeared to favor implantation of these microorganisms. Zinner et al. (27), Krasse (28), Gibbons and co-workers (22), and workers[7] at the National Institute of Dental Research (unpublished data) have found that human strains of streptococci which produce extracellular polysaccharides in the presence of sucrose may induce highly active caries in hamsters and gnotobiotic rats fed diets containing this sugar.

Jordan and Keyes (29) reported that microorganisms that are conducive to caries in hamsters and rats will colonize on surfaces of glass, plastic, steel wire, wax, and extracted teeth when they are periodically exposed to an environment containing sucrose and mucin. They did not colonize when sucrose was replaced with glucose and if mucin were not available. Microorganisms which have not been associated with plaque formation and smooth-surface lesions have not colonized in adherent masses under the conditions mentioned above.

Carlsson and Egelberg (30) have described the formation of large gelatinous plaques in human volunteers who did not brush their teeth, who consumed a basal diet of meat and eggs, and who sucked cubes of sucrose at regular intervals during the day. Neither fructose nor glucose consumed in the same manner was conducive to the formation of such deposits. Further

[6] Frostell, G., and O. Larje. Acid production from different carbohydrates in suspensions of rodent oral microorganisms associated with dental caries and periodontal disease (manuscript in preparation).

[7] Robert J. Fitzgerald, Harold V. Jordan and Paul H. Keyes.

work disclosed that the predominant organisms in the plaque deposits which formed on sucrose were streptococci (31).

Hydrogenated potato starch (product 6563) is made of potato starch by partial hydrolysis and hydrogenation at increased pressure and temperature and contains hydrogenated dextrins and disaccharides, and sorbitol. In Sweden this product is being used in the manufacture of low-caloric candies which are claimed to be less conducive to dental caries, or "non-cariogenic." Frostell (32, 33) has reported that the interaction between this product and bacterial plaques from human teeth does not produce as rapid a fall in pH as the interaction between sucrose and similar bacterial deposits.

Hydrogenated starch was not conducive to plaque formation nor to smooth-surface caries in hamsters of rats. However, small lesions had formed in the sulci of the rat molars (fig. 3). This product contains sorbitol which can be slowly fermented by the streptococci associated with dental caries (13). One should hesitate to conclude that this product, potato starch, or sorbitol and starch are "non-cariogenic" carbohydrates.

Since all of the diets used in these experiments contained 28% skim milk powder, they contained approximately 14% lactose. The investigations of Krasse (26) suggest that lactose favors the implantation of caries-associated streptococci. However, the caries-conducive potential of lactose has been difficult to assess, because animals do not remain reasonably healthy when fed diets containing large amounts of this sugar (6, 34). The finding that plaque disappeared from the smooth surfaces of hamster molars when sucrose was replaced and from rat molars when hydrogenated starch was fed suggest that lactose played no more than a supplemental role in smooth surface activity under these conditions. Lactose is not an essential factor for caries activity in either rats or hamsters, as infected animals have developed lesions when casein rather than milk powders has been used as source of protein (6, 35).

The results obtained in hamsters in this study do not appear to be in accord with the findings described by Gustafson et al. (6), who reported cavitation in hamst fed glucose, fructose, lactose, maltose, a sucrose. But in all of their assays the perimental periods ranged between 70 150 days. Moreover, sucrose and fruct were equally conducive to cavity for tion and favored more active lesions th the other sugars. There are obviously ma differences between the present study that of Gustafson and co-workers. diets were different and the animals bored plaque-forming streptococci. Un these conditions active smooth-surface sions and massive coalescence destro major portions of the crowns in about days. The relatively low "caries scores" ported by Gustafson et al. (6) sugg that the lesions observed may have b more of the crevicular type. If this w so, their findings would be in accord w the pathosis in albino rats described her

In an unusually well-managed stud rats Guggenheim and co-workers (36) e uated the effect of sucrose substitutes i diet which contained 25% skim milk p der, 43% wheat flour, and 7% ot supplements. The rats had been trea with erythromycin and maintained o low level of this antibiotic throughout experiment. At the beginning of the s they were infected with erythromycin sistant caries-conducive streptococci wh produce extracellular polysaccharide, a they were maintained on the experime regimen for 23 days. In general the f ings of Guggenheim et al. (36) coin with ours. Sucrose was conducive to plantation of the streptococci, to the mation of plaque-like aggregates ben which demineralization occurred, an highly active caries both on bucco-lin surfaces and in the occlusal sulci. Ani that consumed the sucrose substitutes bored fewer streptococci and devel less plaque, enamel decalcification, cavitation. The more striking reduc occurred in animals fed fructose, mal and starch. Guggenheim and co-wo (36) concluded that on smooth sur the aggregation of caries-inducing pl depends upon the establishment of n organism producing dextran-type pol charide; whereas in the deep narro sures the formation of plaque is not

tial for aggregation of microorganisms and the accumulation of acids.

Thus evidence from various sources strengthens the premise that the anatomical locus of carious lesions is related in part to the chemical structure of the carbohydrate retained in the mouth and to the specific type of bacteria present. Unquestionably, sucrose provides a substrate which is especially conducive to plaque formation and smooth surface decay if polysaccharide-producing microorganisms are present. Lesions originating in crevices (pits, fissures, sulci) apparently depend on the interaction between a wider variety of carbohydrates and a broader spectrum of acidogenic bacteria.

Until recent years few researchers seriously considered Cox's postulation that carious lesions may differ etiologically (1), partly because his suggestion has been difficult to assess experimentally. However, some workers have advocated the induction and evaluation of all types of carious lesions in experimental animals since assay procedures which affect one type of cavity may not affect another (2, 21, 37, 38). It would be unusual to have oral conditions which would favor smooth-surface cavitation and negligible crevice decay, although such findings have been reported in rats (39). However, the teeth of the animals described were not sectioned to reveal sulcal lesions, and in at least one re-examination of such teeth after sectioning, both crevice and smooth surface lesions were equally prevalent (40).

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Dental Caries in Seventh-Day Adventist Children

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THE tenets of the Seventh-Day Adventist religion include the belief that man's diet and his physical and spiritual health are related. Adventist dietary counsels advise (in addition to abstinence from certain animal protein) limitations on the use of sugar, sticky desserts, highly refined starches and between meal eating.[1] These counsels, written during the nineteenth century, were not originally intended for the specific prevention of dental caries. However, these principles today are generally advocated as part of a dietary program to control dental caries. The recommendation to limit refined carbohydrate consumption may constitute a significant dietary variable in Adventist children, since there is religious motivation for compliance. This provides a basis for evaluating the effect of such dietary recommendations upon caries prevalence, in what might be considered comparable to a dietary program designed to control caries.

Several investigators have reported caries prevalence to be lower among Adventist than non-Adventist children.[2, 3] The purpose of the present study was to extend this comparison to Seventh-Day Adventist (SDA) children residing in an area of naturally occurring, water-borne fluoride in Southern California.

Method

The study population consisted of white school children in grades 4-6. SDA children were available for study in a SDA parochial school in Loma Linda, California. The comparison group of non-SDA children consisted of public school children from the same grades in the nearby city of Redlands. The drinking water of each community contained on the average, 0.7 ppm fluoride. As far as could be determined, both groups were of similar socio-economic status.

Dental examinations were made by the authors with mirror and explorer and adequate illumination. Each examiner participated in the examinations of each group. Diagnostic criteria had been discussed and agreed upon in an earlier clinical study.[4] A permanent tooth was considered erupted when any part projected through the gingiva. Caries was diagnosed on the basis of loss or change in consistency of tooth structure and resistance to the removal of a fine explorer point, not on the basis of explorer catches alone.

Caries prevalence has been evaluated in terms of decayed and/or filled permanent tooth surfaces, since there were so few teeth missing due to extraction (5 extracted teeth in each group).

All statistical analyses have been performed by analysis of variance.

Results

A total of 290 children 9-11 years of age were examined, 132 in the SDA and 158 in the non-SDA group. Prior to pooling the findings of the two examiners, between-examiner differences were tested for statistical significance. No significant differences were observed in age, D/F and total erupted teeth. However, sig-

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nificant between-group differences were found in the tooth population at risk in children of the same age. SDA children consistently demonstrated delayed permanent tooth eruption in separate analyses of males and females alike.

In order to adjust for this different tooth population at risk of becoming carious, caries prevalence was compared by formulating a rate for each child by dividing the number of D/F surfaces by the total number of tooth surfaces erupted. Analyses of these average rates (× 100) are shown in Table 1. Prevalence rates for SDA males, females and combined sexes are consistently lower than those of the non-SDA groups. However, the difference is statistically significant ($p < 0.05$) only when the sexes have been combined.

Discussion

Donnelly[3] has reported that SDA children have lower average DMF teeth than non-SDA children in Maryland. Water supplies in this section of Maryland had been fluoridated for about eight years. Average values reported for DMF teeth were lower and the differences between SDA and non-SDA groups greater than observed in the present study. This may be partially the result of different examiner criteria. The Maryland SDA group had more erupted permanent teeth than the non-SDA group at most ages. However, tooth eruption has been recognized to vary according to diet and other environmental factors.[5] Differences in DMF were small below age 10, those age groups which had received the greatest benefits from fluoridation. The decreased DMF observed in SDA children 10-13 years of age, approximated that expected from fluoridation, in a comparison with pre-fluoridation baseline data from non-SDA children.

The data from the present study in California, an area of naturally occurring fluoride, indicate that any caries inhibiting effect of SDA living may be superimposed upon the benefits of fluoridation. While the observed decrease in SDA caries prevalence has been small, it suggests that dietary education may be a worthwhile adjunct to fluoridation.

TABLE 1

COMPARISON OF 290 SDA AND NON-SDA CHILDREN

Caries prevalence in permanent teeth expressed as a rate: D/F surfaces divided by number of tooth surfaces at risk (× 100)

	SDA		*Non-SDA*		
	MEAN	S.D.	MEAN	S.D.	*F*
Males	4.79	4.19	5.79	4.49	2.98
Females	5.21	3.91	6.14	4.20	1.37
Total	4.97	4.07	5.97	4.35	4.72*
n	132		158		

* $p < 0.05$.

Conclusions

Significantly ($p < 0.05$) lower caries prevalence has been observed in a group of SDA school children, in comparison with a non-SDA group in an area of water-borne fluoride. This difference may be associated with SDA recommendations to limit the intake of highly refined carbohydrates.

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The effect of uncooked and roll-dried wheat starch, alone and mixed in equal quantity with sucrose, on dental caries in the albino rat

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1. Eighty weanling albino rats, five from each of sixteen litters were distributed between five dietary groups in sixteen randomized blocks. Each block was formed from within a litter and each animal in the block received a different diet.

2. The main features of the diets were: group 1, 72% sucrose; group 2, 72% uncooked wheat starch; group 3, 72% roll-dried wheat starch; group 4, 36% sucrose and 36% uncooked starch; group 5, 36% sucrose and 36% roll-dried starch.

3. The rats were killed after 20 days on the diets and assessed for dental caries. The rats consuming diets containing sucrose (groups 1, 4 and 5) had significantly more caries than animals receiving diets in which starch was the sole carbohydrate. The diet containing roll-dried wheat starch produced significantly more caries than uncooked starch. The mixture of uncooked starch and sucrose was more cariogenic than the mixture of roll-dried starch and sucrose.

It has been shown (Green & Hartles, 1967) that rats fed on a diet containing sucrose as the sole source of carbohydrate had a greater caries incidence than those fed on diets containing a similar quantity of either uncooked or roll-dried maize starch. Sucrose diets were also more cariogenic than diets containing equal amounts of sucrose and either of the two forms of maize starch. The roll-dried maize starch produced significantly more caries than the uncooked starch. Somewhat surprisingly it was found that a mixture of sucrose and uncooked maize starch produced significantly more caries than a mixture of sucrose and roll-dried maize starch.

Grenby (1965) reported that he could not demonstrate any significant difference in caries incidence in Wistar rats receiving diets containing either uncooked or cooked wheat starch. The total consumption of maize starch in Britain is very small compared with that of wheat starch. In view of this and the findings of Grenby (1965) it was decided to repeat our earlier studies in which maize starch had been used but in this case to substitute wheat starch for maize.

EXPERIMENTAL

Eighty weanling albino rats of both sexes, five from each of sixteen litters, were assigned, one from each litter, to the five dietary groups. The animals were housed and managed as described by Green & Hartles (1966*a*, *b*). The rats were weighed at the beginning, and at the end of the experiment. They were maintained on the diets for 20 days and then killed by diethyl ether inhalation.

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Diets

Diet RC 14 (group 1) contained 72% sucrose in the form of icing sugar (Green & Hartles, 1966*b*).

Diet RC 27 (group 2) was as RC 14 except that sucrose was replaced by uncooked wheat starch (Energen Limited).

Diet RC 28 (group 3) was as RC 14 except that sucrose was replaced by roll-dried wheat starch (Energen Limited).

Diet RC 29 (group 4) was as RC 14 except that half the sucrose was replaced by uncooked wheat starch.

Diet RC 30 (group 5) was as RC 14 except that half the sucrose was replaced by roll-dried wheat starch.

The diets and deionized water were provided in unrestricted amounts.

Dental caries was assessed as described by Green & Hartles (1966*a*).

RESULTS

Growth of animals. The animals remained healthy throughout the experiment and grew normally.

Caries incidence. The results are given in Table 1; dental caries is expressed as the number of lesions per rat in the first and second mandibular molars. Cumulative T includes all lesions (maximum value is 12), and cumulative B includes the more severe lesions (see Green & Hartles (1966*a*) for description of classification).

Table 1. *Caries experience of five groups each of sixteen rats maintained on different high-carbohydrate diets for 20 days*

Group	Diet	Cumulative T (all lesions). Mean	Cumulative B (more severe lesions). Mean
1	RC 14, sucrose	7·2	2·1
2	RC 27, uncooked wheat starch	0·9	0
3	RC 28, roll-dried wheat starch	3·2	0·1
4	RC 29, sucrose and uncooked starch	8·1	2·2
5	RC 30, sucrose and roll-dried starch	6·5	1·1
	Pooled standard error of group means	±0·40	±0·31

The total number of lesions present was subjected to an analysis of variance in which the total variation of the experiment was divided into that due to treatments, that due to blocks, and the residual or error variation. The total treatment variation was then further divided into a series of orthogonal comparisons so that each comparison could be given a valid level of significance, using Snedecor's *F*-test. The

within-litter comparisons which have been made together with their levels of significance are shown in Table 2. The comparisons permit the following conclusions.

(1) Diets containing sucrose were significantly more cariogenic than those containing starch alone.

(2) Roll-dried wheat starch produced significantly more caries than uncooked starch.

(3) The mixture of uncooked wheat starch and sucrose was significantly more cariogenic than the mixture of roll-dried starch and sucrose.

Table 2. *Within-litter comparisons of the total number of carious lesions arising as a result of different dietary carbohydrates, with the significance level for each comparison*

(The number of rats compared is shown in parentheses)

Dietary treatments compared			Level of significance
Pure starches. Groups 2 and 3 (32)	against	diets containing sucrose. Groups 1, 4 and 5 (48)	$P < 0.001$
Uncooked starch. Group 2 (16)	against	roll-dried starch. Group 3 (16)	$P < 0.001$
Sucrose. Group 1 (16)	against	sucrose/starch mixtures. Groups 4 and 5 (32)	NS
Sucrose and uncooked starch. Group 4 (16)	against	sucrose and roll-dried starch. Group 5 (16)	$P < 0.025$

NS, not significant.

DISCUSSION

In the production of dental caries wheat starch behaves in a similar manner to maize starch when incorporated in the diet of the caries-susceptible rat.

Diets containing uncooked wheat starch resulted in a low incidence of dental caries, and the roll-drying of the starch significantly increased its cariogenicity. However, the really intriguing finding was that a diet containing equal quantities of sucrose and uncooked starch was more cariogenic than one containing the same amounts of sucrose and roll-dried starch. This result is similar to that obtained with maize starch (Green & Hartles, 1967) and poses the interesting question as to why the consumption of a mixture of highly cariogenic sucrose with a virtually non-cariogenic starch should lead to more dental caries than when sucrose is mixed with moderately cariogenic roll-dried starch. We suggested earlier (Green & Hartles, 1967) that in the case of roll-dried maize starch there may be present other metabolizable substrates which might lessen the utilization of sucrose by the flora of the dental plaque, but so far we have not obtained any evidence to support this view. We are now establishing techniques to determine the frequency of eating of rats on different diets, for this may prove to be a factor in the relative cariogenicity of diets.

Our finding that roll-drying of wheat starch increases its cariogenicity is apparently in conflict with results obtained by Grenby (1965). He prepared samples of precooked starch in his own laboratory and the resulting material was probably different

from that produced by commercial roll-drying. These results focus attention on the possible effects of methods of processing on the caries-producing potential of starches.

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THE EFFECT OF DIETS CONTAINING DIFFERENT MONO- AND DISACCHARIDES ON THE INCIDENCE OF DENTAL CARIES IN THE ALBINO RAT

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Summary—In two of three separate experiments diets containing sucrose were more cariogenic than similar diets in which sucrose was replaced by glucose. The caries produced by diets containing fructose, galactose, lactose or maltose was not significantly different from that produced by diets containing equivalent amounts of glucose. It should be stressed that whilst sucrose does appear able to promote significantly more occlusal caries than other sugars, all the simple sugars tested were highly cariogenic.

EVIDENCE has accumulated from animal studies suggesting that sucrose is a more potent cariogenic agent than other common sugars. SHAFER (1949), GRENBY (1963) and KRASSE (1965a, b) have all reported that, in rodents, diets containing glucose are less cariogenic than otherwise similar diets containing sucrose. SHAFER (1954) and GUSTAFSON *et al.* (1955) found that diets were more cariogenic when they contained sucrose than when the sugar present was maltose. SHAFER (1954) recorded a similar finding when fructose replaced sucrose in the diet but GUSTAFSON *et al.* (1955) reported that these two sugars were equally cariogenic.

More recently the work of GUGGENHEIM *et al.* (1966), of SHAW, KRUMINS and GIBBONS (1967) and of FROSTELL, KEYES and LARSON (1967) has in general confirmed these findings.

LEACH (1965) has demonstrated that the extracellular polysaccharide found in the dental plaque is of bacterial origin. WOOD and CRITCHLEY (1966) reported that cariogenic streptococci produced extracellular polysaccharide from sucrose, and GIBBONS *et al.* (1966) showed that potentially cariogenic streptococci could produce more such extracellular polysaccharide from sucrose than from glucose. CRITCHLEY *et al.* (1967) demonstrated that such extracellular polysaccharide is composed of dextran and levan. The inference to be drawn from these observations is that sucrose may favour plaque formation to a greater degree than other sugars, and that this difference may be not only quantitative but qualitative. It is further inferred that the apparently greater cariogenicity of sucrose may be related to its influence as a substrate for extracellular anabolism within the dental plaque.

The present study was undertaken because previous animal work on the relative cariogenicity of sucrose and other sugars was inconclusive and in some cases ignored such important factors as inter-litter variation.

Our animals have in the past developed occlusal lesions exclusively. Many of the studies which have shown sucrose to be more cariogenic than other sugars have been concerned with smooth surface lesions. *A priori* any interaction between micro-organisms and substrate which resulted in increased polysaccharide synthesis on the tooth surface could be of greater cariogenic significance in relation to smooth surface caries than in the case of occlusal lesions since accumulation of organisms and substrate in the fissures is unlikely to be so dependent on the synthesis of adhesive polysaccharides.

It was of interest therefore to study the relative cariogenicity of sucrose and other sugars in rats that normally developed occlusal caries only.

EXPERIMENTAL

Animals

Two hundred and fourteen weanling albino rats were used in the five experiments. They were born and reared by dams fed Spillers autoclaved small-animal diet, which produces no microscopically observable carious lesions even after many months. The animals were housed and managed as described by GREEN and HARTLES (1966a).

Experiments 1 and 3 were each constructed from 14 within-litter blocks, each block containing three and two animals respectively. Experiments 2 and 4 each consisted of 14 within-litter blocks of four rats, and experiment 5 consisted of 16 blocks of two animals.

The duration of the experiments was 20 days except in experiment 4 where owing to the necessity for premature sacrifice several experimental periods were used, but the animals within each individual block were always killed at the same time. The animals were weighed at the beginning and end of, and at least once during, the experiment.

Diets

Diet RC 14, a cariogenic diet containing 72 per cent sucrose (GREEN and HARTLES, 1966a). All the other diets differed only in their carbohydrate content.

Diet RC 25, contained 72 per cent glucose.
Diet RC 26, contained 36 per cent of both sucrose and glucose.
Diet RC 31, contained 72 per cent fructose.
Diet RC 32, contained 36 per cent of both glucose and fructose.
Diet RC 33, contained 72 per cent lactose.
Diet RC 34, contained 72 per cent galactose.
Diet RC 35, contained 36 per cent of both glucose and galactose.
Diet RC 36, contained 72 per cent maltose.

Diet and demineralized water were available to the animals at all times.

Assessment of dental caries

The jaws were processed and the caries assessed by the techniques described by GREEN and HARTLES (1966b, 1967). In addition before the jaws were sectioned the

teeth were examined under a dissecting microscope for signs of buccal or lingual smooth surface lesions.

Experiment 1

Three groups of fourteen animals on diets containing sucrose (RC 14), glucose (RC 25) and a mixture of sucrose and glucose (RC 26).

Experiment 2

Four groups of fourteen animals on diets containing sucrose (RC 14), glucose (RC 25), fructose (RC 31) and a mixture of glucose and fructose (RC 32).

Experiment 3

Two groups of fourteen animals on diets containing maltose (RC 36) and glucose (RC 25) respectively.

Experiment 4

Four groups of fourteen animals on diets containing lactose (RC 33), galactose (RC 34), glucose (RC 25) and a mixture of equal parts of glucose and galactose (RC 35). The animals receiving diets RC 33 and RC 34, excreted large volumes of urine, produced abnormally soft faeces and did not gain weight. Whenever an animal appeared distressed it was killed together with the other three rats in the block. Only one block of animals survived until the twentieth day, the others were sacrificed between the sixth and sixteenth days. In this way, although the experimental periods of the blocks differed, valid comparisons between the treatments could be made. Food intake was not measured but subjectively it appeared that animals on diets RC 33 and RC 34 did not utilize noticeably less food than animals receiving diets RC 25 and RC 35.

Experiment 5

Two groups of sixteen animals on diets containing sucrose (RC 14) and glucose (RC 25) respectively.

RESULTS

Growth of animals. With the exception of the rats receiving diets containing lactose (RC 33) and galactose (RC 34) the animals remained active and grew normally. The extent of the weight gains is shown in Table 1.

Caries incidence. The results shown in Table 1 have been subjected to analyses of variance in which the variation due to treatments, that due to blocks and the residual or error variation have been calculated. Where appropriate the variations attributed to the treatments have been sub-divided by a series of orthogonal comparisons. The test of significance used was Snedecor's F-test. The only significant effect which could be demonstrated was that in two experiments out of three, diets containing sucrose were

TABLE 1. THE MEAN CARIES EXPERIENCE ARISING FROM THE CONSUMPTION OF DIETS CONTAINING DIFFERENT SUGARS, TOGETHER WITH THE AVERAGE DAILY GAIN IN WEIGHT

		Total lesions (cumulative-T)	Advanced lesions (cumulative-B)	Wt. gain/ rat/day
Experiment 1	Sugar in diet			
RC 14	Sucrose	6·7±0·9†	1·7	3·5
RC 25	Glucose	4·5±0·8	1·6	3·3
RC 26	Sucrose + glucose	5·9±0·6	0·9	3·4
Experiment 2				
RC 14	Sucrose	6·6±0·6	1·6	3·5
RC 25	Glucose	6·8±0·6	2·7	3·5
RC 31	Fructose	6·3±0·5	3·3	3·1
RC 32	Glucose + fructose	6·4±0·6	2·4	3·2
Experiment 3				
RC 25	Glucose	5·1±0·7	1·6	3·3
RC 36	Maltose	4·6±0·2	0·6	2·8
Experiment 4				
RC 25	Glucose	6·9±0·5	1·6	3·4*
RC 33	Lactose	7·7±0·7	1·7	−0·2*
RC 34	Galactose	7·8±0·8	3·6	−0·3*
RC 35	Glucose + galactose	7·0±0·6	1·4	2·9*
Experiment 5				
RC 14	Sucrose	9·4±0·5	3·7	3·2
RC 25	Glucose	7·7±0·5	4·1	3·4

* Average exposure to diet 10 days.
† S.E.M.

Experiment 1. RC 15 and RC 26 were significantly more cariogenic than RC 25 ($P<0·05$) but not significantly different from each other.
Experiment 5. RC 15 and RC 25 were significantly different ($P<0·025$).

more cariogenic than those containing purely glucose. All other sugars and sugar mixtures were indistinguishably cariogenic in the conditions of these experiments.

In all the animals examined only one lesion was observed which could be categorized as a smooth surface lesion. This occurred in a rat maintained on the lactose containing diet (RC 33).

DISCUSSION

Evidence has accumulated from many sources to show that there are significant differences between the cariogenicity of different carbohydrates when they are included in equivalent amounts in the diets of rodents (HARTLES, 1967). In man the two major sources of dietary carbohydrate are starches of one kind or another which have been subjected to varying degrees of processing, and sucrose. In our strain of rat un-

cooked starch prepared from maize or wheat is relatively non-cariogenic, heat treatment of these starches by roll-drying increases their cariogenicity (GREEN and HARTLES, 1967b, c). The degree of caries resulting is however much less than that arising from the consumption of a diet containing an equivalent amount of sucrose.

Currently there is much debate concerning the role of sucrose in the production of dental caries. In certain circumstances sucrose is more cariogenic, in the rat, than lactose, maltose or glucose (SHAW, KRUMINS and GIBBONS, 1967). KRASSE (1965a, b) has shown that, following the implantation of caries-inducing streptococci in the mouths of hamsters, more caries is produced when the diet contains sucrose in place of glucose.

In the present study a group receiving the glucose diet was common to all experiments. Sucrose diets were included in three of the experiments, and in two of these

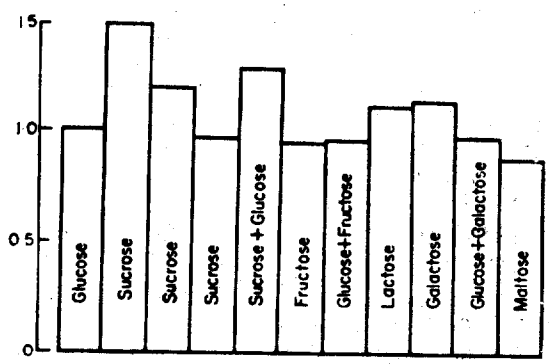

FIG. 1. Relative cariogenicity of various sugars deduced from the results of the five experiments. Glucose is given an arbitrary value of unity.

sucrose was significantly more cariogenic than glucose. None of the other sugars was significantly different from glucose in its cariogenicity.

In the production of experimental dental caries in the rat there are three major factors involved; (1) strain of animal, (2) bacterial and chemical composition of the plaque, and (3) diet. At present, only the diet has been deliberately altered, but changes in diet may influence the composition of the plaque by selectively encouraging particular micro-organisms. Our results indicate that sucrose is probably more effective in potentiating the initiation of occlusal caries than is any other sugar. However it is necessary to emphasize that this is a matter of degree only since all the sugars tested were cariogenic. Since a glucose diet was included in each experiment it is possible to compare the relative caries incidence with glucose and other sugars. This comparison, with the effect of glucose having an arbitrary value of unity, is shown in Fig. 1.

A recent report by FROSTELL, KEYES and LARSON (1967) is worthy of comment. *Inter alia* they studied the effect of various sugars on the incidence of caries in the rat. Their animals developed both sulcal (occlusal) and smooth surface lesions. These rats were from a "relatively pathogen-free colony maintained in a controlled environment

semi-barrier system . . . and develop very low levels of caries activity unless they are infected with appropriate micro-organisms". They were rendered "caries-active" by placing faeces from caries-active animals in their mouths and their drinking water. Four experiments were carried out using variable and relatively small numbers of rats in each treatment group. No mention is made of any precautions taken to minimize the effect of between-litter variation and the results must therefore be viewed with caution. The general conclusion reached that sucrose is more conducive to caries than any other sugar is probably correct, but the design of the experiment is such that figures purporting to show variation in caries experience arising from the consumption of other sugars must be treated with reserve. In all studies on experimental caries in the rat a wide range of caries activity is observed, especially between litters; unless precautions are taken to minimize the effect of these variations by distributing treatments within litters, the results may be of uncertain value.

The evaluation of the caries-promoting effects of diets containing sucrose must be approached with caution. The known facts are that certain streptococci convert sucrose into dextrans and levans, thus helping to increase plaque formation. When these micro-organisms are implanted in the mouths of animals receiving diets containing sucrose, caries is produced. When the diet contains glucose in place of sucrose the bulk of the plaque formed and the incidence of caries are both reduced (KRASSE, 1965a, b).

GUGGENHEIM *et al.* (1966) produced caries in the rat by implanting a strain of streptococcus which utilized sucrose preferentially, indeed it did not thrive in its absence. It would therefore be surprising if this organism did not produce more caries on diets containing sucrose than on diets containing other sugars. Thus in these rather atypical circumstances differences between the effects of sucrose and other sugars may be exaggerated.

It is likely that circumstances leading to an increased accumulation of plaque would be of greater significance in caries of the smooth surfaces than in fissure caries (LEACH *et al.*, 1969). Our animals experienced occlusal fissure lesions only, and the findings are in general similar to those of SHAW *et al.* (1967). From our experiments we conclude that in the rat all the sugars studied were highly cariogenic, but that the presence of sucrose in the diet exacerbated the disease.

Sucrose is the major sugar consumed by man, and is a potent caries-producing agent. It is less certain that the substitution of equivalent quantities of other sugars for sucrose would have any appreciable effect on the pattern of human dental caries. Sucrose is not an essential factor in the aetiology of caries although it is probably the most cariogenic of all common dietary carbohydrates.

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Résumé—Au cours de deux sur trois expériences séparées, des régimes contenant du saccharose sont plus cariogènes que des régimes identiques où la saccharose est remplacé par le glucose. Les caries provoquées par des régimes, contenant du fructose, galactose, lactose ou maltose, ne sont pas différentes de celles produites par des régimes contenant des quantités équivalentes de glucose. Alors que le saccharose est capable de produire plus de caries occlusales que les autres sucres, tous les sucres étudiés sont hautement cariogènes.

Zusammenfassung—In 2 von 3 getrennten Versuchen erwies sich Rohrzucker enthaltend Futter kariogener als ähnliche Kostformen, bei denen Rohrzucker durch Glukose ersetzt war. Die durch Futter mit Zusatz von Fruktose, Laktose, Galaktose oder Maltose verursachte Karies unterschied sich nicht signifikant von den aequivalenten Mengen Glukose enthaltenden Futterformen. Ees muß hervorgehoben werden, daß alle untersuchten einfachen Zucker in hohem Grade kariogen wirkten, während Rohrzucker dazu in der Lage zu sein scheint, signifikant mehr Okklusalkaries als andere Zucker zu entwickeln.

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Research Association of British Flour-Millers, Cereals Research Station, St. Albans

Investigations in Experimental Animals on the Cariogenicity of Diets Containing Sucrose and / or Starch

T. H. Grenby

Reasons for the use of animals in experimental caries research are too well known to require reiteration. Although not comparable with man in all dental respects, laboratory animals may provide a valuable pointer to control of the disease in man, especially in respect of the cariogenicity of different foods. From many points of view the monkey is the most suitable animal for caries research, but in studying the effect of diet on dental caries, it suffers from the disadvantages that a long experimental period is required, and feeding a diet of closely controlled composition is difficult. Most animal experiments on the connexion between diet and caries have therefore used caries-susceptible strains of rodents: a survey of the literature between 1948 and 1960 reveals 733 references to the use of the white rat, 217 to the hamster, 60 to the cotton rat, and 5 to the monkey (Bislin and Cox, 1964).

Certain strains of experimental rodents appear to be more susceptible to caries than others, and these caries-prone strains are generally used for testing the cariogenicity of different diets. Although little information is available on this point, 'stock' diets, such as the Medical Research Council diet 41B used in Britain for normal breeding and maintenance purposes, do not appear to give rise to a heavy incidence of caries. More severe caries occurs when specially-devised diets, usually high in carbohydrate, are fed to the rodents for periods ranging from 2 or 3 weeks to several months, the length of the experimental period depending largely on the method of assessing caries.

Studies of the effect of diet on rodent caries were first carried out in the early 1920's. Some of the diets used in the 1920's and 30's contained hard, relatively coarse cereal particles, which caused mechanical damage to the

teeth. To avoid this, it became general practice to grind all constituents of the diet to a fine powder, and diets of this type were used in most of the work reviewed in this paper. Carious lesions from these diets are said to be histopathologically analogous to those in man.

EARLY WORK ON HIGH CARBOHYDRATE DIETS

In most of the studies of experimental rat caries before 1939, the bulk of the carbohydrate in the diet was supplied by cereal foods, and was therefore in the form of starches rather than sugars. Corn (or maize) was the cereal most commonly used, but sometimes it was replaced by rice or wheat. (See, for example, Agnew, Agnew and Tisdall, 1932; Rosebury, Karshan and Foley, 1932; Keyes, 1946; see also Cox, 1952, for a review of this early work). Research workers of that time inclined to the view that starchy foods were responsible for rodent caries, and some noted the importance of coarse cereal particles in initiating caries (Hoppert, Webber and Cannipe, 1932; Rosebury, 1938). Little was said about the effect of sugar intake on caries in rodents, and in fact some experiments were reported which failed to establish a connexion between the two (Agnew, Agnew and Tisdall, 1932; Johnston, Kaake and Agnew, 1933).

During and shortly after the 1939–45 war it was confirmed that coarse cereal particles cause fracturing in rodent teeth (Hunt and Hoppert, 1948), and reports began to appear of caries from high-sugar diets of soft consistency (see, for example, Arnold, 1942; Shaw *et al.*, 1944; McClure, 1945; Cox *et al.*, 1948; Sognnaes, 1948). It was previously believed that sugar only promoted caries once lesions had been initiated by fracture, and these results with finely powdered diets in which the sugar content exceeded 66 per cent, provided the first evidence that sugar could initiate carious lesions.

In addition to this discovery, strains of rats reasonably uniform in their susceptibility to caries had been bred through many generations (see for example Hunt, Hoppert and Erwin, 1944). More sensitive methods of assessing rodent caries were also developed. The way was now open for fuller investigations of the effect of dietary carbohydrates on rodent caries.

RESULTS WITH FINELY-POWDERED DIETS

Although a vast number of experiments have been carried out in the last 20 years, relatively few were designed specifically to compare sucrose with

starch in the diet. However, nearly all the diets contained some type of sugar and/or starch, although the starch was often not pure, but present as a constituent of cereal products. A brief survey of these results nevertheless sheds some light on the comparative cariogenicity of sugar and starch.

In some of the earliest experiments SHAW and his co-workers (1944) observed that the teeth of cotton rats fed on stock rations of 'crude' materials were highly resistant to caries, but extensive fissure caries occurred when the rats received synthetic rations containing 67 or 73 per cent of sucrose for 10–14 weeks. Replacement of all the sucrose by dextrin, a partially degraded form of starch, prevented caries almost completely. Similar results were obtained by SOGNNAES, (1948). Replacement of only half the sucrose by dextrin did not reduce caries, but replacement of three-quarters of the sugar gave some protection (SCHWEIGERT, SHAW, PHILLIPS and ELVEHJEM, 1945). Glucose, dextri-maltose, fructose, maltose and a lactose-sucrose mixture were all cariogenic. Incorporation of additional casein or lard in the diet at the expense of sugar reduced caries, which suggested that the cariogenic agent in the diets was the 56 to 73 per cent of sugar. (SCHWEIGERT, SHAW, ZEPPLIN and ELVEHJEM, 1946; RESNICK, WHITE and SHAW, 1958). The other ingredients of the diets were casein 18 to 24 per cent, 'celluflour' 12–15 per cent, corn oil 4–5 per cent, dried liver 4 per cent, and vitamin and mineral supplements (Table I). Limiting the intake of the diet reduced caries (SHAW, 1950). SHAW (1965) observed that caries was more closely related to the length of time food was available than to the amount of food ingested. Raw sugar and refined sugar were equally cariogenic (SHAW, 1949). Replacement of some of the sugar by sorbitol or mannitol, hexoses in which the group on

Table I. Approximate composition of cariogenic diets

	%
SHAW et al.	
Sucrose or other carbohydrate	56–73
Casein	18–24
'Celluflour'	12–15
Corn oil	4– 5
Dried liver	4
Vitamin and mineral supplements	
McCLURE	
Commercial glucose	18
Cereal products	80
Small amounts of vitamin, mineral and liver additions	

the 1-C atom has been reduced to $-CH_2OH$, consistently diminished caries (SHAW and GRIFFITHS, 1960). This may have important applications, because these derivatives of the sugars are sweet-tasting, and could conceivably be used in confectionery instead of sugars.

In other experiments attention was focussed on cereal foods, which are rich in starch but contain little free sugar. Still using the cotton rat, CONSTANT, PHILLIPS and ELVEHJEM (1952) found that whole-grain cereals were less cariogenic than their processed products, corn flakes, wheat flakes and oatmeal. A mixture of foods found in the USA human diet containing 17 per cent sucrose, produced as severe caries as a 67 per cent sucrose synthetic ration (ZEPPLIN *et al.*, 1950).

Thus the results of SHAW and his co-workers suggested that sugar was the agent which caused caries in the cotton rat, but that other dietary factors could promote or diminish its cariogenic action. This has been confirmed by many other workers. MCCLURE (1945) found that a high-sugar diet gave rise to caries in rats, but later was able to produce caries from diets containing 18 per cent commercial glucose as the only sugar, together with about 80 per cent of cereal products and small amounts of vitamin, mineral and liver additions (Table I; MCCLURE, 1952; MCCLURE, 1958; MCCLURE, 1960; MCCLURE and MULLER, 1959). These diets produced carious lesions on the lingual, buccal and proximal surfaces of the molars, as well as in the occlusal fissures, whereas high-sugar diets seem to give rise to very little smooth-surface caries. As the cereal part of the diet, MCCLURE used flour, bread or mixtures of various powdered cereal products. Caries occurred in all cases, but these diets were less cariogenic than those of higher sugar content (see ERSHOFF, BRANN and BAVETTA, 1962). Another type of cariogenic diet used by MCCLURE (1955) contained glucose 18 per cent, corn starch 45 per cent, and milk powder 35 per cent (see also EDMONDS, MADSEN and SMITH, 1963): its cariogenicity probably depended on the presence of the glucose and the processing the milk powder had undergone, rather than on the starch.

Other workers have continued to use high-sucrose diets to induce severe experimental rodent caries. Two diets containing 64 per cent sucrose have been studied at Emory University (HALDI *et al.*, 1958; WYNN *et al.*, 1953; WYNN *et al.*, 1959) Caries has been produced in hamsters at the US National Institute of Dental Research by diets containing 56–59 per cent confectionery sugar and 27–28 per cent skim milk powder as the major constituents (see for example KEYES, 1959; ENGLANDER and KEYES, 1964; ENGLANDER, KEYES and FITZGERALD, 1965). This type of diet is said to cause more proximal and fissure caries than the diet of MCCLURE (1955), which

contained glucose and cornstarch instead of sucrose (LARSON, ZIPKIN and FITZGERALD, 1963). A diet originated by STEPHAN and HARRIS (1955), containing 66 or 67 per cent sucrose and 32 per cent skim milk powder, has also been widely used to give rise to caries rapidly (see for example MÜHLEMANN, MARTHALER, KÖNIG and SCHMID, 1956; KÖNIG, LARSON, FITZGERALD and MÜHLEMANN, 1965; KÖNIG and MÜHLEMANN, 1964; MARTHALER, KÖNIG and MÜHLEMANN, 1964). To produce caries the sugar should be fed in solid form, because there is some evidence that sugar solutions are less cariogenic (VOLKER, 1959; BIBBY, 1955).

The evidence from these and many other trials indicts sucrose and glucose as the main cariogenic agents, but gives less information on the role of starch, apart from one small study by SHAFER (1949), who directly compared sucrose, glucose and corn-starch at a level or 61 per cent in the diet of hamsters, and found that sucrose was highly cariogenic, corn-starch was non-cariogenic, and glucose was intermediate. Some experiments by LARSON (1963) also showed that raising the corn-starch content of a diet at the expense of the sugar content reduced caries, although the diets were isocaloric. The low cariogenicity of corn-starch compared with sucrose was also suggested by the results of MCCLURE (1945) and COX and his co-workers (1948). In contrast to this, ARNOLD (1942) observed cervical and fissure carious lesions in hamsters fed on a diet containing 66 per cent corn-starch but no glucose or sucrose.

There is some evidence that finely ground cereal products containing starch can affect the incidence of caries from diets containing about 79 per cent cereal and 18 per cent glucose. Wheat was more cariogenic than corn, rice and oats, and raw wheat produced more smooth-surface caries than processed wheat (DODDS, 1960; DODDS, 1964; DODDS and LAWE, 1964). In general, cereal products seem to be less cariogenic than sugar. MILLER (1958) observed that a diet of 'natural foods' containing several cereal products gave rise to little caries in rats, but the inclusion of 17 per cent of sugar at the expense of whole wheat flour increased the caries scores. Similarly, replacing 12 per cent of the rice flour in a rice diet by sucrose increased its cariogenicity (MILLER, 1963).

Reviewing early work on the subject, STEPHAN (1948) found that starchy foods give very low experimental caries scores in rats compared with sugars. VOLKER (1959) considered that poly-, di- and monosaccharides all have similar capacities to produce tooth decay in experimental animals, but most recent results do not confirm this. BIBBY (1961) noted the importance of sugar in promoting experimental caries, but suggested that under certain conditions complex carbohydrates are as cariogenic as simple sugars.

BRAMSTEDT (1964) concluded that starch and glycogen are far less cariogenic than glucose, although both have glucose molecules as their basic unit of structure. This is the view most commonly held to-day.

TESTS COMPARING WHEAT STARCH WITH SUGAR

The work described so far provided ample evidence of the detrimental effect of sugar on rodent teeth, but shed little light on the behaviour of starch as opposed to cereal products containing only a proportion of starch. Furthermore, in the small number of trials with pure starch, corn- or maize-starch was used in preference to wheat starch, whereas the consumption of wheat products in the human diet far exceeds that of corn. Wheat starch was tested, however, in one series of experiments. GUSTAFSON, STELLING and BRUNIUS (1952) found that diets containing 47 to 67 per cent wheat starch in the absence of sugar caused caries in only 3 per cent of a large group of hamsters, whereas 94 per cent developed caries when the starch was replaced by sucrose.

Among the foods sometimes suspected of promoting caries in man are white bread, cakes and biscuits, all containing wheat flour. Thus we at St. Albans set up some trials to compare wheat starch and other wheat products with sugars in the diet of caries-susceptible albino rats. The usual experimental periods were 5, 8, 11 or 14 weeks from weaning, and the teeth of balanced groups of rats were then examined and scored for caries by the method of SHAW and his co-workers (1944), which has recently been compared with that of KÖNIG, MARTHALER and MÜHLEMANN (1958), and found to give similar results (see KÖNIG and GRENBY, 1965, or Nutrition Reviews, 1965). All the diets were fed ad libitum, and contained 32 per cent skim milk powder and 2 per cent liver powder. The remaining 66 per cent was carbohydrate or cereal foods.

The first experiments (GRENBY, 1963) compared sucrose, glucose and raw wheat starch, and confirmed that sucrose was more cariogenic than glucose. The wheat starch diet produced little or no caries, and was indistinguishable from the non-cariogenic stock diet in this respect. The results of two experiments are presented in Table II, and clearly show that the differences in cariogenicity throughout the experiments were highly significant.

In these early experiments raw wheat starch was tested. For human consumption, however, it is generally cooked or processed in some way, and because its peculiar structure is easily altered by processing, cooked

Table II. Caries scores in groups of rats fed on diets containing 66 per cent carbohydrate, 32 per cent skim milk powder and 2 per cent liver powder. (Figures in brackets are numbers of rats)

Weeks on diet	*Experiment 1* *sucrose*	*Raw wheat starch*	*Experiment 2* *sucrose*	*Glucose*
5	10 (8)	2 (8)	21 (8)	6 (8)
8	23 (8)	2 (7)	28 (8)	9 (8)
11	29 (10)	1 (9)	36 (10)	16 (9)
14	36 (8)	3 (6)		

starch should also be tested in animal experiments. Wheat starch consits of granules of up to 0.05 mm in diameter. In making bread or flour confectionery, the cooking process causes the granules to swell and perhaps rupture, distorting their structure and rendering them more liable to attack by bacterial and salivary enzymes (Figures 1 and 2). However, in several

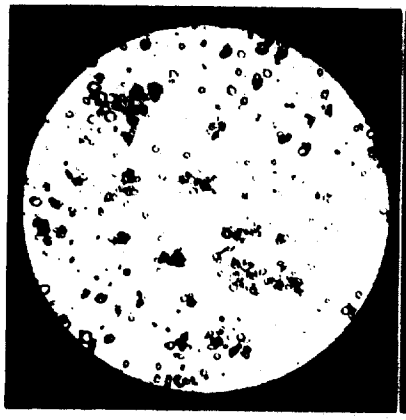

Fig. 1. Raw wheat starch, stained with Congo red (x 120).

Fig. 2. Cooked wheat starch, stained with Congo red (x 120).

Table III. Caries in rats fed for 8 weeks on diets containing 66 per cent wheat starch, 32 per cent skim milk powder and 2 per cent liver powder

	Experiment 1				*Experiment 2*			
	Raw starch		Cooked starch		Raw starch		Cooked starch	
	Males	Females	Males	Females	Males	Females	Males	Females
Mean caries scores	1.2	1.3	2.5	3.5	2.9	3.0	1.9	2.4
Numbers of rats	(13)	(11)	(13)	(10)	(10)	(10)	(8)	(8)

tests in which finely powdered cooked and raw starch were compared at a level of 66 per cent in the diet for 8 weeks, all the caries scores were very low, and there was no consistent difference in cariogenicity between the two (Table III). The rats meanwhile retained their susceptibility to caries on a 66 per cent sucrose diet.

Thus wheat starch appears to be non-cariogenic. Wheat flour, which contains about 60 to 75 per cent starch, and bread were tested next. In the same type of experiment as before, diets containing either white or wholemeal flour were of low cariogenicity, unless they also contained sucrose (GRENBY, 1966; KÖNIG and GRENBY, 1965). This is shown in Table IV: the inclusion of 10 per cent sucrose in the diet at the expense of flour caused a significant increase in caries. Bread which had been dried and powdered behaved in the same way as flour, and gave similar caries scores. Again no difference could be established between white and wholemeal bread. It was possible that drying and pulverising the bread had altered its physical characteristics by making it less sticky. In one final experiment, therefore, fresh bread was fed to large groups of rats for 11 weeks. As shown in table V there was very little caries, indicating that under the conditions of our experiments, the gelatinised wheat starch in bread was not cariogenic (GRENBY, 1966).

Table IV. Comparison of white flour and sucrose in the diet of caries-active rats. There were 8 rats in each group. All diets contained 32 per cent skim milk powder and 2 per cent liver powder

	Remainder of diet:		
	66% flour	*56% flour 10% sucrose*	*66% sucrose*
Caries scores after 5 weeks	6	14	17
Caries scores after 8 weeks	8	19	30

Table V. Caries in rats fed for 11 weeks from weaning on white and wholemeal bread alone. Rats from 9 litters were randomised between the two groups, and each group contained approximately equal numbers of males and females

	White bread	*Wholemeal bread*
Number of rats	33	38
Mean caries score	1.9	1.4
S.E. of means (69 D.F.)	± 0.26	± 0.24

Difference between means: *Not significant* (p 10%)

The cariogenicity of a food depends not only on its suitability as a substrate for certain oral micro-organisms, but also on its physical nature, especially the tenacity with which it adheres to the teeth and may bring fermentable carbohydrates into contact with the enamel surface. Although there was no evidence from our experiments so far that starch, flour or bread were harmful in this respect, further trials were set up to compare the two main constituents of bread, starch and gluten. The starch content of white bread is usually between 55 and 65 per cent and the gluten (protein) content 7–10 per cent. The gluten gives bread its cohesive texture, and under certain circumstances can form a sticky, pasty mass with water. When it was incorporated in rat diets, however, it was non-cariogenic, even in the presence of 10 per cent sucrose. By comparison, cooked wheat starch was significantly more cariogenic than gluten, but remained far less cariogenic than sucrose. Dry, powdered bread was intermediate between gluten and starch, and significantly different from both of them.

Thus there is ample evidence from this work that sucrose produces very much more caries than starch, although in some of the more recent trials starch diets gave rise to a detectable level of caries.

DISCUSSION AND CONCLUSIONS

Carbohydrates, including both sugars and starches, form the major cariogenic fraction of the diet. Under favourable conditions *in vitro*, a wide variety of carbohydrates can be attacked by a combination of oral micro-organisms and salivary enzymes, producing acid which might participate in the caries process. Not all of these fermentable carbohydrates give rise to caries, however. It appears from the results of animal experiments that sucrose is the most highly cariogenic of all the sugars tested, while others such as glucose and lactose are also cariogenic but not to the same extent as sucrose.

Conclusions from animal experiments with the polysaccharides are less positive, because in much of the published work the product used has not been fully defined. Wide variation can occur in the number of monose units constituting the polysaccharide molecule, the way in which they are linked together, and the degree of branching of the chains of monose units. Further, the polysaccharide molecules are arranged in a specific way inside starch granules, depending on the type of plant from which they are obtained. When fed to animals, the starch granules may be intact, or disrupted by extraction or cooking processes which render the starch more

liable to enzymic attack and degradation. Taking into account not only experiments in which the diets contained starch, but also those with flour and other cereal products rich in starch, it is clear that starch is far less cariogenic than sucrose. This is probably because it cannot be attacked by oral bacteria rapidly enough and close enough to the tooth surface to produce caries. There is a possibility that certain forms of starch may promote caries by adhering to the teeth and bringing sugar into close contact with the enamel surface, but no evidence for the occurence of this in *vivo has* been obtained yet.

SUMMARY

Some general observations are made on the use of laboratory rodents to study the connexion between carbohydrate in the diet and dental caries. The early work on experimental rodent caries is briefly described, including the use of coarse corn diets to induce caries, and the subsequent discovery that finely powdered diets were also cariogenic provided that they contained a high proportion of sugar.

The large number of soft, cariogenic diets which have been fed to rodents are divided into groups according to their composition, and the experimental findings relating to the cariogenicity of the sucrose and starch they contain are surveyed. High-sucrose diets produced severe fissure caries, while diets containing cereal products rich in starch and 18% glucose induced both fissure and smooth-surface caries. A limited amount of evidence about corn-starch suggested that it was non-cariogenic.

Experiments at St. Albans on the cariogenicity of carbohydrate foods are described. Sucrose was highly cariogenic, glucose less so, and wheat starch far less still. No difference was detected between cooked and raw starch in this respect. Diets containing white and wholemeal flour and bread produced little caries, unless they also contained sucrose. In the most recent work the order of cariogenicity was starch > bread > gluten, but sucrose produced much more caries than any of these cereal products. Reasons for the difference between sugars and starch are discussed briefly.

ZUSAMMENFASSUNG

Zur Verwendung von Nagern als Versuchstiere zum Studium der Beziehungen zwischen Kohlenhydraten der Nahrung und Zahnkaries werden einige allgemeine Bemerkungen gemacht. Frühere Arbeiten auf dem Gebiet der experimentellen Nagetierkaries werden kurz beschrieben, darunter die Verwendung von grobkörnigen Diäten zur Karieserzeugung und die spätere Entdeckung, dass auch fein pulverisierte Diäten kariogen sind, wenn ihr Zuckergehalt hoch ist.

Die vielen weichen kariogenen Diäten für Nagetiere werden entsprechend ihrer Zusammensetzung klassifiziert, und eine Übersicht über die experimentellen Befunde in Beziehung zum Saccharose- und Stärkegehalt gegeben. Diäten mit hohem Saccharosegehalt führten zu schwerer Fissurenkaries, während Diäten mit stärkereichen Getreideprodukten und 18% Glukose sowohl Fissuren- wie Glattflächenkaries induzierten. Die wenigen Befunde mit Getreidestärke deuteten darauf hin, dass diese nicht kariogen ist.

Es werden Versuche über die Kariogenizität kohlenhydrathaltiger Nahrungsmittel beschrieben, die in St. Albans durchgeführt wurden. Saccharose war stark kariogen, Glucose weniger, und Weizenstärke noch sehr viel weniger. Zwischen gekochter und roher Stärke wurde in dieser Beziehung kein Unterschied gefunden. Diäten mit Weissmehl, Vollkornmehl und Brot

verursachten wenig Karies, wenn sie nicht zugleich Saccharose enthielten. In den letzten Versuchen war die Rangfolge der Kariogenizität Stärke > Brot > Gluten, wobei Saccharose sehr viel mehr Karies induzierte als alle diese Getreideprodukte. Gründe für diese Unterschiede zwischen Zucker und Stärke werden kurz diskutiert.

RÉSUMÉ

Quelques remarques générales sont faites quant à l'utilisation de rongeurs pour l'étude des relations entre les hydrates de carbone alimentaires et la carie dentaire. Nous décrivons quelques travaux plus anciens sur la carie expérimentale chez les rongeurs, entre autre sur l'utilisation d'aliments concassés grossièrement pour induire la carie et sur la découverte plus tardive montrant que les aliments réduits en poudre fine pouvaient être cariogènes s'ils étaient riches en sucre.

Nous classifions les nombreux régimes cariogènes mous pour rongeurs d'après leur composition et nous présentons une revue des constatations expérimentales en relation avec le taux du saccharose et d'éléments énergétiques. Les aliments riches en saccharose conduisent à de graves caries de fissure, alors que les aliments énergétiques à base de graines et contenant 10% de glucose, induisent des caries de fissures et de surfaces lisses. Les rares observations portant sur l'alimentation énergétique à base de graines montrent que ce régime n'est pas cariogène.

Nous décrivons les expériences que nous avons faites à St. Albans sur l'activité cariogène d'aliments contenant des hydrates de carbone. Le saccharose était très cariogène, le glucose moins et l'amidon de graines bien moins encore. Nous n'avons pas trouvé de différence entre l'amidon cuit et cru. Une alimentation à base de farine blanche, de farine complète ou de pain ne produisait que peu de carie, si elle n'était pas supplémentée de saccharose.

Au cours des dernières expériences nous avons pu classer en ordre d'activité cariogène décroissante l'amidon, le pain et le gluten et nous avons constaté que le saccharose était plus cariogène que tous ces produits végétaux. Nous discutons les causes des différences observées entre les actions des sucres et de l'amidon.

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THE EFFECTS OF DIETS CONTAINING SUCROSE, GLUCOSE OR FRUCTOSE ON EXPERIMENTAL DENTAL CARIES IN TWO STRAINS OF RATS

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Summary—Fructose, glucose and sucrose were compared at a level of 33 per cent in the diet of two strains of rats. In three of the four sets of results sucrose produced more caries than glucose or fructose. There was no consistent difference in cariogenicity between glucose and fructose.

The two strains of rats differed in their susceptibility to caries on high-sugar diets, and caging rats from both strains together in one experiment did not appear to alter their relative susceptibility to caries.

MANY trials with laboratory animals have shown that diets containing certain sugars cause dental caries. The sugars which have been most frequently tested are sucrose and glucose (GRENBY, 1967b). Both are known to be highly cariogenic, but there is some evidence that under comparable conditions sucrose produces more caries than glucose (SHAFER, 1949; GRENBY, 1963; SHAW, KRUMINS and GIBBONS, 1967). As the disaccharide sucrose molecule yields one glucose and one fructose unit on hydrolysis, it seems desirable to compare the cariogenicity of fructose [D (—) laevulose] with that of sucrose and glucose. Relatively little information on this is available, but GUSTAFSON *et al.* (1955) observed that in hamsters, sucrose and fructose produced more caries than glucose, while FROSTELL, KEYES and LARSON (1967) found that in small numbers of hamsters and rats, sucrose generally gave rise to more caries than glucose or fructose. We have accordingly carried out two trials with rats at different times in these laboratories to compare fructose with both sucrose and glucose.

Within each trial two distinct caries-susceptible strains of albino rats were used, one originally supplied by the Nederlands Instituut voor Volksvoeding (Wistar strain), and the other bred from animals of the Osborne–Mendel strain kept at the Zahnärztliches Institut der Universität, Zürich. As these strains differed in several respects (GRENBY, 1967a), their relative susceptibility to caries was assessed in a preliminary trial with a single cariogenic diet containing sucrose. In a second trial comparing caries susceptibility, a smaller number of rats of both strains were kept in one large cage throughout the experimental period, to see in addition whether susceptibility to caries was transmissible under these conditions.

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MATERIALS AND METHODS

The experiments were started when the rats were weaned at about 4 weeks. They were kept in large cages which could hold up to 12 rats of either sex on one or other of the diets, and were weighed weekly. The rats were killed 5 or 8 weeks after the start of the experiments, and the heads were preserved in methylated spirit until the mandibles were dissected out and examined for caries under a magnification of × 25 by a modification of the method of SHAW *et al.* (1944). Whereas these authors graded lesions in the six main molar fissures from 1 to 5, in the present work scores of 1 or 2 were assigned to lesions on smooth surfaces, in approximal areas, and in any of the smaller fissures other than the six main ones, because of the heavy incidence of caries in some of the groups. The caries scores thus represent the extent of caries in the molar teeth of both the left and right mandibles of each animal.

The basic cariogenic diet (STEPHAN and HARRIS, 1955) used in the two preliminary trials contained 66 per cent sucrose in the form of caster sugar (fine crystals); 32 per cent skim milk powder; and 2 per cent liver powder. The nutritional value was improved in the third and fourth experiments (i.e. the main trials) by the replacement of half of the sugar by white Manitoba flour of about 70 per cent extraction. The diets thus contained 33 per cent of sucrose, glucose or fructose. They were always fed in powder form *ad libitum*, and tap water was always available.

In experiment 3, some 60 rats were drawn from each strain, providing 3 litter-matched groups to be fed on the 3 diets, with two distinct but roughly equally sized sets per strain. This permitted the examination of about half of the animals of each strain after 5 weeks on the experimental diets, while the remainder were allowed to continue for another 3 weeks.

In experiment 4 all the rats were kept for 8 weeks on the same diets as in experiment 3. Two groups of rats, 60 from 7 Osborne–Mendel litters and 48 from 8 Wistar litters, were subdivided to provide intercomparisons of the 3 diets, not only within each strain as in experiment 3, but also within each sex, and between the sexes.

RESULTS

The results of the first preliminary trial (Table 1) show two points of difference between the two strains of rats when fed on the diet which contained 66 per cent sucrose. First, the Osborne–Mendel strain gained more weight than the Wistar ($P < 5$ per cent); second, the caries scores were also significantly higher in the Osborne–Mendel strain than in the Wistar ($P < 5$ per cent). The lesions were greater in both number and extent in the Osborne–Mendel strain, and in some cases two or more lesions in a tooth had progressed so far that they had fused, with the complete destruction of a cusp.

In view of this difference in caries attack, six male rats of each strain were kept together in a single large cage, and the 66 per cent sucrose diet was fed for 8 weeks, as before. The results (Table 2) show that the Osborne–Mendel strain again grew significantly faster than the Wistar, and the disparity in the caries scores between the strains was at least as pronounced as before ($P < 5$ per cent), showing that the susceptibility of the Osborne–Mendel strain or the resistance of the Wistar strain had not been seriously affected by the presence of the other strain.

TABLE 1. COMPARISON OF THE SUSCEPTIBILITY TO DENTAL CARIES OF TWO STRAINS OF RATS FED ON A DIET CONTAINING 66 PER CENT SUCROSE, 32 PER CENT SKIM MILK POWDER, AND 2 PER CENT LIVER POWDER

	Wistar strain		Osborne–Mendel strain	
	Males	Females	Males	Females
No. of litters represented	7	7	9	9
No. of rats	7	7	9	9
Mean weight gain (g)	133	82	201	135
S.E. of means	±7·3	±3·0	±10·8	±4·4
Difference in mean weight gain between the two strains: significant for both sexes ($P<5\%$).				
Mean caries scores	9·4	19·1	40·2	32·4
S.E. of means	±3·3	±2·2	±1·5	±2·1
Difference in mean caries scores between the two strains: significant for both sexes ($P<5\%$).				

TABLE 2. CARIES SCORES IN MALE RATS OF TWO STRAINS, CAGED TOGETHER FOR 8 WEEKS, AND FED ON THE SAME DIET AS IN THE PREVIOUS EXPERIMENT

	Strain	
	Wistar	Osborne–Mendel
No. of rats (each from a different litter)	6	6
Mean gain in weight (g)	72	157
S.E. of means (5 D.F.)	±16	±16
Difference in mean weight gain: significant ($P<5\%$)		
Mean caries scores	6·5	43·8
S.E. of means (5 D.F.)	±1·9	±2·8
Difference in mean caries scores: significant ($P<5\%$)		

In experiment 3, as in the earlier experiments, the Osborne–Mendel strain gained more weight than the Wistar, and was the more caries-prone of the two. Most of the lesions in both strains occurred in the fissures, but some smooth-surface lesions were observed in the Osborne–Mendel animals, generally on buccal surfaces.

A cursory inspection of the mean caries scores (Table 3) suggests that the order of cariogenicity of the three diets was fructose < glucose < sucrose, but statistical examination of the mean scores for each strain after experimental periods of 5 or 8 weeks showed that in no case were the differences in caries scores between the diets significant at the 5 per cent level. If the 8-weeks scores of the fructose and glucose groups were combined, however, the mean was significantly lower than that of the sucrose group ($P<5$ per cent) in both strains of rats.

In experiment 4 there were no significant differences in the weight gains of the animals fed on the three different diets over the 8-week experimental period. As before, the Osborne–Mendel rats grew faster than the Wistar and in general developed more dental caries. The six mean caries scores for the Wistars given in Table 4 (each of the 3 sugar diets in both sexes) were statistically indistinguishable from each other, except

TABLE 3. THE EFFECTS OF FRUCTOSE, GLUCOSE AND SUCROSE ON CARIES TESTED IN TWO STRAINS OF RATS. THE DIETS CONTAINED 33 PER CENT OF A SUGAR, 33 PER CENT WHITE FLOUR, 32 PER CENT SKIM MILK POWDER, AND 2 PER CENT LIVER POWDER

	Diets					
	Fructose		Glucose		Sucrose	
	Males	Females	Males	Females	Males	Females
(A) Results after 5 weeks						
Wistar strain						
Number of rats	6	5	6	4	4	5
Mean weight gain (g)	113	90	126	87	119	80
Mean caries scores (Males + Females)	6·6		10·8		18·6	
S.E. of mean caries scores (27 D.F.)	±3·5		±3·7		±3·8	
Difference between the two extreme means: N.S. (10% > P > 5%).						
Osborne–Mendel strain						
Number of rats	5	7	5	7	4	7
Mean weight gain (g)	149	115	155	111	172	107
Mean caries scores (Males + Females)	16·8		26·0		25·2	
S.E. of mean caries scores (26 D.F.)	±3·7		±3·7		±3·8	
Difference between means: N.S. (20% > P > 10%)						
(B) Results after 8 weeks						
Wistar strain						
Number of rats	6	3	5	4	4	5
Mean weight gain (g)	144	118	171	119	164	112
Mean caries scores (Males + Females)	12·4		13·7		20·9	
S.E. of mean caries scores (17 D.F.)	±2·9		±2·9		±2·9	
Difference between the means: N.S. (P = 10%)						
Difference between means of sucrose group and (frucrose + glucose groups): significant (P < 5%)						
Osborne–Mendel strain						
Number of rats	5	6	5	5	4	7
Mean weight gain (g)	230	158	251	158	257	158
Mean caries scores (Males + Females)	33·8		41·1		50·0	
S.E. of mean caries scores (11 D.F.)	±3·4		±3·5		±3·4	
Difference between means: N.S. (10% > P > 5%)						
Difference between means of sucrose group and (fructose + glucose groups): significant (P < 5%)						

for the group of females fed on the fructose diet, which was higher than any of the other 5 groups (P < 5 per cent). It is doubtful whether any importance should be attached to this one result, however, because there was no confirmation for it either in experiment 3 or in the Osborne–Mendel strain.

TABLE 4. THE EFFECTS OF DIETS CONTAINING 33 PER CENT FRUCTOSE, GLUCOSE AND SUCROSE ON CARIES IN TWO STRAINS OF RATS

	Males			Females		
	Fructose	Glucose	Sucrose	Fructose	Glucose	Sucrose
Wistar strain						
Number of rats	8	8	8	8	8	8
Mean weight gain (g)	166	165	172	100	101	100
S.E. of means of 8 rats		±3·9 (14 D.F.)			±3·2 (14 D.F.)	
Difference between any two groups of the same sex			N.S.			
Mean caries scores	12·0	12·0	10·6	28·2	10·4	16·1
S.E. of means of 8 rats			±2·8 (35 D.F.)			
Comparisons of diets:		*Males*			*Females*	
fructose (F) vs. glucose (G)		N.S.			F>G, $P<5\%$	
fructose (F) vs. sucrose (S)		N.S.			F>S, $P<5\%$	
glucose (G) vs. sucrose (S)		N.S.			N.S.	
Correlation coefficient between caries scores and weight gains			−0·22 (34 D.F.) N.S. ($P>10\%$)			
Osborne–Mendel strain						
Number of rats	10	10	10	10	10	10
Mean weight gain (g)	226	218	229	158	157	160
S.E. of means of 10 rats		±5·3 (12 D.F.)			±2·9 (12 D.F.)	
Difference between any two groups of the same sex			N.S.			
Mean caries scores	15·7	12·4	34·3	25·3	15·4	36·6
S.E. of means of 10 rats			±2·3 (50 D.F.)			
Comparisons of diets:		*Males*			*Females*	
fructose (F) vs. glucose (G)		N.S.			F>G, $P<5\%$	
fructose (F) vs. sucrose (S)		F<S, $P<5\%$			F<S, $P<5\%$	
glucose (G) vs. sucrose (S)		G<S, $P<5\%$			G<S, $P<5\%$	
Correlation coefficient between caries scores and weight gains			+ 0·46 (29 D.F.) ($P = 1\%$)			

In this strain (Osborne–Mendel) the results for males and females on each of the 3 diets were again treated separately (Table 4). It was shown by Hartley's Sequential Test that in this part of the experiment the sucrose diet had produced significantly more caries than either fructose or glucose in both sexes ($P < 5$ per cent). The difference between fructose and glucose was not significant in the males, but in the females the fructose group had significantly higher scores than the glucose group ($P < 5$ per cent). In addition, there was a low but significant correlation between caries scores and weight gains for the Osborne–Mendel strain ($r = +0{\cdot}46$; 29 D.F.; $P = 1$ per cent), whereas the correlation for the Wistar rats was not significant ($r = -0{\cdot}22$; 34 D.F.; $P > 10$ per cent), possibly because of their much slower rate of growth.

DISCUSSION

The results with the Osborne–Mendel strain of rats (Tables 3 and 4) indicate that a diet containing 33 per cent sucrose tended to be more cariogenic than similar diets

containing the same level of either glucose or fructose. The Wistar strain was less sensitive, and had significantly higher caries scores on sucrose than the monosaccharides only in experiment 3. Thus three out of the four sets of results from the two strains in two trials indicate that sucrose was somewhat more cariogenic than the other two sugars, although the differences were not large. The general level of caries on these sugar + flour diets indicates, however, that all are appreciably more cariogenic than the sugar-free bread or flour diets of earlier trials (GRENBY, 1966).

It was not possible to distinguish between glucose and fructose in their effects on the incidence of caries. Although in experiment 4 the female rats of both strains appeared to be more susceptible to caries from fructose than from glucose, this difference was not observed either in the males in experiment 4 or in 3. This is in contrast to the work of GUSTAFSON *et al.* (1955), which demonstrated that fructose was more cariogenic than glucose in the hamster. The overall results of the present work do not establish any difference in cariogenicity between fructose and glucose, both being somewhat less cariogenic than sucrose.

The results do not show whether the hydrolysis of sucrose to its less cariogenic constituent monosaccharide units occurs in the production of caries on diets containing sucrose, and no tests were carried out on the mixture of glucose and fructose (invert sugar) produced by the hydrolysis of sucrose. However, sucrose may be a better substrate than the monosaccharides for the specific oral micro-organisms which cause caries. KRASSE (1965) found that implanted caries-inducing streptococci were present in large numbers in the mouths of hamsters on a high-sucrose diet, but only a few could be recovered from animals fed on a diet in which all the sucrose was replaced by glucose (see also GUGGENHEIM *et al.*, 1966).

Recent evidence suggests that sucrose is more cariogenic than the two monosaccharides because it may be a better starting-material for the synthesis of bacterial polysaccharide. The action of the bacterial enzymes, dextransucrase or levansucrase (see HEHRE, 1955; WOOD and CRITCHLEY, 1966), on sucrose gives rise to extra-cellular dextrans and/or levans, probably contributing to the formation of a heavy dental plaque. In support of this, CARLSSON and EGELBERG (1965) have found that in man a sucrose diet produced a heavier dental plaque than either glucose or fructose. This might promote caries by holding acidogenic bacteria and fermentable carbohydrates close to the tooth surface.

The other interesting point emerging from these results (Tables 3 and 4) is the difference in caries susceptibility between the two strains of albino rats (see also GRENBY, 1967a; KÖNIG and GRENBY, 1965). The caries scores of the Osborne–Mendel strain were consistently higher than those of the Wistar strain, and in experiment 3 the increases in caries scores between 5 and 8 weeks were greater in the Osborne–Mendel strain than the Wistar. Further, when rats of both strains were caged together for the 8-week experimental period (Table 2), the susceptibility or resistance to caries of one strain did not appear to influence the level of caries in the other. Differences between strains of rats in their caries response to certain sugars in the diet have also been observed recently by SHAW, KRUMINS and GIBBONS (1967). In the present work, the Osborne–Mendel strain in particular showed an increased incidence of caries from

sucrose, compared with glucose or fructose, but no consistent difference in cariogenicity between glucose and fructose was observed in either strain.

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Résumé—Du fructose, glucose et saccharose ont été ajoutés dans une proportion de 33 pour cent à l'alimentation de souches de rats. Dans trois sur quatre essais, le saccharose a produit plus de caries que le glucose et le fructose. Le glucose et le fructose ne semble pas posséder un pouvoir cariogène différent.

Les deux souches de rats ne réagissent cependant pas de même, lorsqu'on les soumet à des régimes riches en sucre. Le fait de les maintenir dans les mêmes cages ne semble pas modifier cette différence de susceptibilité à la carie.

Zusammenfassung—Fruktose, Glukose und Rohrzucker wurden auf der 33 Prozent-Basis im Futter von zwei Rattenstämmen miteinander verglichen. Bei 3 von den 4 Ergebnissen verursachte Rohrzucker mehr Karies als Glukose oder Fruktose. Zwischen Glukose und Fruktose gab es keinen einheitlichen Unterschied hinsichtlich der Kariogenizität.

Die beiden Rattenstämme unterschieden sich hinsichtlich ihrer Kariesanfälligkeit bei zuckerreichem Futter; wurden Ratten beider Stämme in einem Versuch gemeinsam in Käfigen gehalten, so änderte sich ihre relative Kariesanfälligkeit nicht.

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Dental Caries
– reduction rate
– evaluation of caries
– – tooth missing
– dietary habits
– cariogenic diet
– xylitol
– mannitol
– sorbitol
– oral microorganisms
– rat

Xylitol and Dental Caries

Efficacy of Xylitol in Reducing Dental Caries in Rats

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Summary: Approximately the same degree of dental caries was induced in rats fed a cariogenic diet alone or supplemented with either 10% glucose or sucrose. When the cariogenic diet was supplemented with a 10% level of the sugar alcohols, mannitol or sorbitol, there was slightly less caries induction of the first and third molars. The incidence of caries in the second molars was reduced about one-third, while the extent of the carious lesions was reduced about one-half. In rats fed the diet supplemented with 10% xylitol, no carious lesions developed in the first and third molars and only minimal involvement of the second molars was observed. These data suggest that, under the conditions described, xylitol is appreciably more effective in reducing the incidence of dental caries than is either mannitol or sorbitol.

Introduction

It is well recognized that inclusion of carbohydrates in the diet is an influential factor in determining the incidence of dental caries in humans [6, 11, 12]. While the evidence is circumstantial, elimination of carbo-

hydrates, particularly sucrose, from our diet would in all probability drastically reduce the incidence of carious lesions on the crowns of human teeth [12]. Unfortunately, the dietary habits of most individuals are such that reduction, let alone elimination, of carbohydrates would be extremely difficult. Experimentally it has been shown that sugar alcohols added to a cariogenic diet will reduce the incidence of caries in rats compared to that seen when the same diets are supplemented with free sugars [5, 9, 13] and thus, might be useful as sugar substitutes. The present investigation was therefore undertaken to determine which of three sugar alcohols (mannitol, sorbitol or xylitol) would be most effective in reducing the incidence of caries formation in rats compared to a monosaccharide (glucose) or a disaccharide (sucrose). The results of these studies are reported herein.

Materials and Methods

For induction of caries, male Swiss albino rats (strain Wistar) weighing approximately 100 g at the start of the experiment were used. The animals were divided into 7 groups of 20 rats each and fed either a normal laboratory diet (Charles River), a cariogenic diet consisting of 66 % coarsely ground polished rice, 30 % whole milk powder, 3 % alfalfa leaf meal and 1 % sodium chloride, [1] or the cariogenic diet supplemented with glucose, sucrose, mannitol, sorbitol or xylitol. When the cariogenic diet was supplemented with the sugars or sugar alcohols the concentrations of carbohydrates were added at 5 % of the total cariogenic diet for the first 2 weeks of the experiment, 7.5 % for the next 2 weeks and finally 10 % from the fifth week to the termination of the experiment at 21 weeks. The gradual increase in the addition of the carbohydrates was made to eliminate or reduce any resulting diarrhea in the rats [13]. To compensate for the addition of the carbohydrates, the milk powder per 1000 g of total diet was appropriately reduced 50 g during the first 2 weeks, 75 g during the second 2 weeks and 100 g from the fifth week to the termination of the experiment. The sugars and sugar alcohols were reagent grade and were powdered or granular in form.

The rats were weighed at the beginning of the experiment and at the termination of the experiment at 21 weeks. The rats were provided with their respective diets and water ad libitum. The lower molars of the rats were examined at 3 week intervals for the gross appearance of carious lesions.

Rats surviving 21 weeks were sacrificed, tissue was removed from the skull and the first, second and third mandibular molars were examined for the presence of caries. Each tooth was evaluated for carious lesions on the following basis:

0 = intact tooth. 1 = up to one fourth of tooth missing. 2 = up to one-half fo tooth missing. 3 = up to three-fourths of tooth missing. 4 = entire tooth missing.

Results

The effects of the cariogenic diet alone or supplemented with the various carbohydrates on growth and survival are shown in Table 1. Rats fed the normal diet, the cariogenic diet alone or supplemented with 10 %

Tab. 1: Growth and survival of rats fed normal or cariogenic diets supplemented with sugars or sugar alcohols

Group	Type of Diet	Av. weight (g)		No. Surviving/ Total
		Beginning of Exp.	Termination of Exp.	
1	Normal	103	556	17/20
2	Cariogenic (C)	102	521	13/20
3	C + 10 % Glucose	101	500	11/20
4	C + 10 % Sucrose	101	497	8/20
5	C + 10 % Mannitol	102	450	13/20
6	C + 10 % Sorbitol	100	462	8/20
7	C + 10 % Xylitol	100	430	7/20

glucose or sucrose (groups 1–4) increased in weight approximately 5-fold over the 21-week experimental period. Those rats fed the cariogenic diet supplemented with the sugar alcohols (groups 5–7) exhibited approximately a 4-fold increase in weight. Survival was greatest in the group fed the normal diet and poorest in those groups fed the cariogenic diet supplemented with sucrose, sorbitol or xylitol. Animals which died during the experimental period were not included in the evaluation of caries since the deaths occurred before carious lesions were observed [13].

Shown in Table 2 are the effects of the different diets on the incidence of dental caries. The percentage of surviving rats showing carious lesions for each of the groups is recorded for the first, second and third left mandibular molars only. The incidence of caries in the first, second and third right mandibular molars was virtually identical to that shown for the left molars. No data is given for the maxillary molars since caries was not observed in these teeth. From the table, it can be seen that caries did not occur in any of the mandibular molars of rats fed the normal diet (group 1). When the cariogenic diet (group 2) or the cariogenic diet sup-

Tab. 2: Incidence of caries in rats fed normal or cariogenic diets supplemented with sugars or sugar alcohols

Group	Type of Diet	Percentage of Rats Showing Caries Left Lower Molars		
		1st	2nd	3rd
1	Normal	0	0	0
2	Cariogenic (C)	46	92	69
3	C + 10 % Glucose	73	82	64
4	C + 10 % Sucrose	50	63	38
5	C + 10 % Mannitol	23	54	15
6	C + 10 % Sorbitol	38	63	38
7	C + 10 % Xylitol	0	14	0

plemented with 10 % glucose (group 3) was fed to rats, 82–92 % of the animals exhibited caries in the second molars, while a smaller percentage of rats (46–73 %) exhibited caries in the first and third molars. The cariogenic diet supplemented with 10 % sucrose, mannitol or sorbitol (groups 4, 5, 6) induced caries in a smaller percentage of rats. The greatest incidence of caries in rats fed the sucrose-, mannitol- or sorbitolsupplemented diets was seen in the second molars (54–63 %), while a smaller percentage of rats exhibited caries in the first and third molars (15–50 %). Only one rat of seven fed the cariogenic diet supplemented with 10 % xylitol (group 7) displayed caries in the second molars (14 % incidence), while caries was entirely absent in the first and third molars.

When the lesion scores for the left molars (e. g. 0 to 4) were averaged for each group, rats fed the cariogenic diet alone or the diet supplemented with 10 % glucose or sucrose showed approximately the same degree of caries involvement (Table 3). The extent of the carious lesions was greatest in the second molars (i. e. average lesion score 2.5–2.6), with less involvement noted in the first and third molars (i. e. average lesion score 0.8–1.5). In the groups of rats fed the cariogenic diet supplemented with 10 % mannitol or sorbitol, the average lesion score for the second molars was about half that noted for rats fed the cariogenic diet (i. e. average lesion score 1.1–1.2 versus 2.6). Slightly less caries involvement was also observed in the first and third molars of the latter two groups of rats as compared to rats fed the cariogenic diet (i. e. average lesion score 0.4–0.8 versus 0.8–1.2). Minimal lesion involvement was observed in the second

Tab. 3: Lesion scores for molars of rats fed normal or cariogenic diets supplemented with sugars or sugar alcohols

Group	Type of Diet	Average Lesion Score * Left Lower Molars		
		1st	2nd	3rd
1	Normal	0	0	0
2	Cariogenic (C)	0.8	2.6	1.2
3	C + 10 % Glucose	1.5	2.5	1.4
4	C + 10 % Sucrose	1.5	2.5	0.9
5	C + 10 % Mannitol	0.6	1.2	0.4
6	C + 10 % Sorbitol	0.8	1.1	0.5
7	C + 10 % Xylitol	0	0.3	0

* The average lesion score was obtained by adding the value of 0 to 4 scored for each rat's tooth in each group of rats and then dividing by the total number of rats in that group.

molars (i. e. average lesion score 0.3) of rats fed the cariogenic diet supplemented with 10 % xylitol, while the first and third molars of this group of rats did not show any carious lesions. The average lesion scores for the right mandibular molars were virtually identical to those observed in the left molars.

Discussion

Because of their frequent isolation from carious teeth and their ability to induce caries in experimental animals, the lactobacilli and streptococci are the most frequently implicated microorganisms in dental caries. A number of investigations dating back to 1930 have shown that these microorganisms ferment dextrose and sucrose rapidly with the production of high yields of lactic acid. On the other hand, sorbitol and mannitol have been shown to be fermented much more slowly by these microorganisms in vitro [3, 7, 8, 15], in carious lesions themselves [14] or in mouth rinse studies [4]. Based on these observations, it has been suggested that sorbitol or mannitol might be useful in the prevention or control of dental caries. SHAW and GRIFFITHS [13] concluded from their studies in rats that neither sorbitol nor mannitol supported the initiation and progression of carious lesions at a rate comparable to sucrose or dextrin. Since mannitol does not possess sweetening properties while sorbitol does, these investigators sug-

gested that sorbitol be employed in confections as a substitute for free sugars to reduce caries.

MUEHLEMANN *et al.* [10] showed that xylitol was less cariogenic than sorbitol when administered to rats at levels of 10%, 20% and 30% in the diets. More recently, KARLE and BÜTTNER [9] reported that xylitol and sorbitol were significantly less cariogenic than sucrose when administered in the diet fed to rats. The results of the present study confirm the earlier observations that sugar alcohols are more effective than free sugars in reducing the incidence and extent of caries in experimental animals. The data presented also clearly demonstrate that xylitol is more effective than either sorbitol or mannitol in reducing caries formation. As far as we are aware, information in the literature regarding fermentation of xylitol by oral microorganisms is not available. However, it has been shown that caries-producing strains of streptococci, isolated from hamsters and one human source, were unable to ferment xylitol in vitro [2].

Moreover, these strains could not be adapted to grow in a medium containing xylitol as the only carbohydrate source. These observations might well explain the superiority of xylitol (compared to sorbitol or mannitol) in reducing the incidence of caries. Since xylitol possesses satisfactory sweetening properties, it should be considered as a substitute for sucrose to aid in the reduction or prevention of dental caries.

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The Cariogenicity of Different Dietary Carbohydrates Tested on Rats in Relative Gnotobiosis with a Streptococcus Producing Extracellular Polysaccharide

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Abstract.—Low-fat test diets containing 25 per cent sucrose, glucose, fructose, lactose, maltose or uncooked wheat starch were found to be consumed in amounts not differing significantly, and continuous recordings of intake of food and water revealed similar feeding patterns. Caries activity was tested on groups of 9 Osborne-Mendel rats the indigenous flora of which had been depressed prior to inoculation of a cariogenic streptococcus (OMZ-61) known to induce surface caries. The test organism failed to thrive on the diets which did not contain sucrose; caries incidence was highest on the sucrose diet, and extremely low on the diets containing fructose, maltose, or starch.

In experiments on hamsters it has been shown that caries activity is related to the sucrose concentration of the caries test diet (GUSTAFSON *et al.* 1953 [1]), and that other saccharides are less cariogenic than sucrose (GUSTAFSON *et al.* 1955 [2]). KRASSE (1965 a [3], 1965 b [4]) observed, in originally caries-inactive humans, that cariogenic microorganisms established themselves more easily on a high sucrose than on a high-glucose diet. Correspondingly, caries activity was high on the sucrose diet and low on the glucose diet. CARLSSON and EGELBERG (1965 [5]) have found formation of voluminous colonies of plaque when sucrose was given to the test subjects every hour during the day, whereas thin films of plaque were formed after glucose administration, and even thinner ones after fructose administration. Colonies of microorganisms isolated from human plaque and incubated with glucose have been found to synthesize iodophilic intracellular polysaccharide (GIBBONS and SOCRANSKY 1962 [6]; GIBBONS 1964 [7]). From sucrose not only intracellular, but also extracellular polysaccharide has been described to be produced by microorganisms in salivary sediment (DAIN and MANLY 1961 [8]), by *Streptococcus salivarius* (NIVEN *et al.* 1941 [9]);

SNYDER *et al.* 1955 [10]; WOOD 1964 [11]), and by "specific" streptococci which were cariogenic in animals (DONOHUE, KESTENBAUM and KING 1966 [12]; KRASSE 1966 [13]). Extracellular plaque polysaccharides (dextran and levan, ratio 10:1) have been shown to be the predominant constituent of plaque matrix, and have been suggested not only to serve as a carbon source for plaque bacteria when other sources are depleted, but to contribute to rendering the plaque an effective diffusion barrier (CRITCHLEY *et al.* 1966 [14]).

In view of these findings, differences in cariogenicity between sucrose, glucose and other saccharides should be revealed best in investigations on smooth-surface caries activity. It was the object of the present experiment to study establishment and caries promoting action of streptococcus OMZ-61 in rats fed diets containing different saccharides. In addition the synthesis of extracellular polysaccharide by strain OMZ-61 was to be investigated. This organism is a streptococcus which is serologically identical with the HS-strains isolated by FITZGERALD and KEYES at the National Institutes of Health (DE CROUSAZ and GUGGENHEIM 1966 [15]) and is known to induce surface caries (GUGGENHEIM *et al.* 1965 [16]; KÖNIG *et al.* 1965 [17]).

When different carbohydrates are tested, a methodical difficulty consists in the occurrence of unspecific effects which may be caused by dietary factors altering the taste and herewith the pattern of intake of the diet to be tested (KÖNIG *et al.* 1964 [18]). As the time of local availability of fermentable substrate influences the caries rate in human (LUNDQVIST 1952 [19]) and rat teeth (KÖNIG 1965 [20]), continuous recording of intake of food and water was to be tested in a pilot study. Its results were to provide the basis for comparability of the diets investigated in the main experiment.

MATERIALS AND METHODS

Animals. Weanling Osborne-Mendel rats raised on a low-fluoride stock diet were used in the pilot study and the main experiment. Large litters were reduced to 10 animals a few days after delivery and after weaning were distributed at random among the treatments irrespective of sex. Each litter, regarded as a homogeneous block of experimental units, constituted one complete replication of all treatments. The animals were weighed at the beginning and at the end of the 23-day experimental period. The rats were housed in stainless-steel cages with screen bottom and front. 2 animals belonging to the same treatment group were housed together, except the rats on the pilot study during the time of continuous recording.

Recording of eating pattern. Recording was done in a pilot study on 6 animals of 5 different dietary treatments for 4 successive days. The recording apparatus has been described in detail previously (KÖNIG *et al.* 1964 [18]). Each unit, for one animal, consisted of a cage with separate access to food cup and water vessel, each placed on an electromechanical balance connected with a 12-channel multipoint compensating recorder. The chart speed was 60 mm per hour. From the chart the frequency of eating and drinking, total eating time per day and amounts consumed were derived. The data were tabulated and subjected to detailed analyses of variance. The main object of analysing the data in detail was to eliminate the "time and litter" variation caused by the necessity to take recordings on the rats of the first litter at the beginning, and the last litter towards the end of the study when the animals were nearly 30 days older than the first ones. The treatment mean square was tested for significance against the interaction "animals × treatments".

Introduction of relative gnotobiosis. For the main experiment 9 litters with 10 animals each were available. They had all been delivered on the same day. When 13 days old they were transferred with the dams from the breeding cages to stainless-steel cages without bedding. At the same time and for the same reason the stock diet was removed and the animals were fed a non-cariogenic finely powdered diet in order to avoid obliteration of the fissures of the erupting molars. This diet was fed until the 22nd day. The animals were weaned when 19 days old and the litters randomly distributed among 10 treatment groups. From day 19 to 22, drinking water was administered containing 1000 mg erythromycin◊ per liter in order

◊ Glucoheptonate; Ilotycin® Gluceptate Lilly.

Table I Description of 10 treatments for testing caries activity of rats on differing diets in relative gnotobiosis with streptococcus OMZ-61.—Ingredients of diets given in per cent

Treatment	Depression erythromycin	Inoculation str. OMZ-61	Diet No.	Ingredients of test diets	
1	+	—	590	25 sucrose	(regarding other ingredients, refer to description of treatments 5–10)
2	—	—	590	25 sucrose	
3	+	+	2000a	56 sucrose, 7 wheat flour (70% extraction), 28 skim milk powder (spray process), 2 Gevral Protein® 5 powdered brewer's yeast, purified (Vitamin- und Hefe AG, Rheinfelden), 2 NaCl reagent grade Merck	
4	+	+	580s	66 sucrose, 32 skim milk powder, 2 Gevral Protein®	
5	+	+	590	25 sucrose (Pharmacopoeia Helvetica V, Siegfried	+ 25 skim milk powder, 43 wheat flour, 2 Gevral Protein®, 5 brewer's yeast
6	+	+	591	25 D (+) glucose (Ph.Helv.V, purum, Siegfried)	
7	+	+	592	25 D (—) fructose (Ph.Helv.V, S.II purissimum, Sandoz)	
8	+	+	593	25 D (+) lactose (Ph.Helv.V, purum, Siegfried)	
9	+	+	594	25 D (+) maltose purum, Siegfried	
10	+	+	595	25 wheat starch (amylum tritici, Ph.Helv.V)	

to depress the indigenous Gram-positive flora in groups 1 and 3–10. On day 22 the concentration of erythromycin was reduced to 100 mg/l which was then administered continuously through the whole experiment. Also on day 22, feeding on the experimental diets was started. On days 23, 24, 25, 26 and 27, two inoculations daily with suspensions of erythromycin-resistant streptococcus OMZ-61 in saline were done in rats on groups 3–10. This test organism was known to get quantitatively established under these conditions which had been tested previously (GUGGENHEIM *et al.* 1965 [16]; KÖNIG *et al.* 1965 [17]), and it was known to induce a typical decay pattern characterized by smooth surface lesions and carious attack on distal cusps and shallow distal fissures of first and second mandibular molars. Bacterial checks of establishment of the inoculated test organism were taken 3 hours before the rats were sacrified at the age of 46 days, at the end of the 23-day caries test period. Smears of the swabs taken from 4 rats per group were coded and screened after staining with fluorescent antibodies. The procedures and the test organism have been described previously (GUGGENHEIM *et al.* 1965 [16]).

Diets. The 10 treatments tested are shown in Table I. The first 4 of them were controls. The test treatments were intended to evaluate, in the state of relative gnotobiosis, the relative cariogenicity of the six diets fed to groups 5–10. These diets contained the respective test carbohydrate (25 per cent), 25 per cent skim milk powder, 43 per cent wheat flour (70 per cent extraction rate), 2 per cent of a commercial vitamin-mineral-protein supplement (Gevral Protein® Lederle) and 5 per cent powdered brewer's yeast. The 6 saccharides tested at a 25-per-cent concentration in diets 590–595 were sucrose, glucose, fructose, lactose, maltose and uncooked wheat starch. As baseline control group 1, the 25 per cent sucrose diet 590 was fed to depressed, non-inoculated rats. Control group 2, on the same diet, was the non-depressed non-inoculated control which, in contrast to rats on treatments 3 and 4 harbored not an inoculated, but the indigenous cariogenic microorganisms. On treatments 3 and 4, standard caries-test diets were adminis-

tered to rats in relative gnotobiosis. 2000a was a modification of diet 2000 (KEYES and JORDAN 1964 [21]), 580s a modification of diet 580 (STEPHAN and HARRIS 1955 [22]). All ingredients of the rest diets were finely powdered and mixed thoroughly. The diets and tap water, renewed daily, were available *ad libitum*. Intake was measured and continuously recorded in the pilot study on treatments 5, 6, 7, 9 and 10; day-to-day measurements only of the total daily intake were done on three cages of each treatment in the main experiment.

Scoring of carious lesions. At the end of the experimental period, the animals were sacrificed, the jaws removed and fixed in 10-percent buffered formalin. After fixation the soft tissues were scraped off the mandibles and while drying the surfaces of the lower molars were inspected for smooth surface lesions under a dissection microscope at 16 times magnification. The extension of smooth surface lesions was recorded on a scoring chart and scores attributed using KEYES (1958 [23]) area units E. Afterwards serial mesio-distal sections (7–8 per half jaw, about 150 microns thick each) were prepared from the non-decalcified mandibles, with an internal-rim rotary cutting machine (JANSEN 1950 [24]). The sections were stained, serially mounted on slides and covered with Scotch tape. Then the slides were coded and put in random order. The total of 12 fissures in the first and second lower molars were examined microscopically at 16 times magnification for carious dentinal lesions according to methods described previously (KÖNIG 1966 [25]; GREEN and HARTLES 1966 [26]). The numbers of dentinal fissure lesions encountered were subjected to a detailed two-way analysis of variance.

Scoring of surface deposits. Deposits on tooth surfaces were scored on the intact maxillary molars, differentiating between thin films on the mesial surface of the first molars (±), thick layers at the same site (–), and thick layers also on other surfaces (++).

Bacteriological checks. Gram-stained smears were examined as described before (GUGGENHEIM *et al.* 1965 [16]). In addition to this procedure, smears were stained with highly specific fluorescent antibodies which were prepared from homologous rabbit anti-sera according to methods described by WOOD, THOMPSON and GOLDSTEIN (1965 [27]). The preparations were evaluated semiquantitatively under a Leitz fluorescence microscope equipped with a HBO 200 lamp.

Production of extracellular polysaccharide. Cariogenic streptococci were grown in 500 ml trypton broth (NIVEN, KIZIUTA and WHITE 1946 [28]) containing 5% sucrose and fortified with 3% beef extract. This batch was inoculated with 5 ml from a 10 hour culture in the same medium and incubated at 37 °C for 18 hours. Two polysaccharide fractions were prepared and analysed following in principle the methods described by HOBSON and MACPHERSON (1953 [29]). Fraction I was a soluble polysaccharide in the metabolic liquid and fraction II an amorphous polysaccharide surrounding and embedding the bacterial cells. The only modification in the preparation of fraction I was the final dialysis against distilled water for 48 h during which water was changed several times. A seamless regenerated cellulose tubing type Visking 36 D.C.◊ with an average pore radius of 24 Å was used. HOBSON and MACPHERSON (1953 [29]) successfully separated fraction II (the extracellular polysaccharide which in their case was capsular material from bacterial cells) by heating for 25 min (100 °C) in acid solution

Table III Average daily consumption of food and water per cage (housing 2 rats) on the test diets of the main experiment. Measurements on 3 cages per treatment for 3 successive days

Treatment, diet				Food	Stand. deviations	Water	Stand. deviations
1	590	25	sucrose	26.6	2.4	38.0	5.1
2	590	25	sucrose	25.9	2.7	43.1	4.3
3	2000a	56	sucrose	26.5	3.5	42.3	5.6
4	580s	66	sucrose	20.2	2.1	29.7	4.1
5	590	25	sucrose	25.0	1.7	41.6	3.2
6	591	25	glucose	25.0	1.7	47.2	10.3
7	592	25	fructose	24.6	1.5	40.3	7.2
8	593	25	lactose	23.6	3.7	44.6	11.7
9	594	25	maltose	26.6	2.1	48.5	4.2
10	595	25	starch	27.1	2.5	38.9	4.5

◊ Visking Company, Division of Union Carbide Corporation 6753 West, 65th Street, Chicago 38, Illinois, USA.

Table III Results of pilot test investigating the spontaneous eating activity of Osborne-Mendel rats on differing diets. Average weight gain during a 34-day experimental period; average daily intake of diet per animal (g), average total eating time per day, in minutes, eating frequency, and intake of water recorded on 6 animals out of each dietary group for 4 successive days

25% carbohydrate component	Weight gain (g)	Intake of food (g)	Eating time	Eating frequency	Intake of water (ml)
5 sucrose	108	11.0	195	11.9	20.8
6 glucose	126	12.2	166	12.2	23.6
7 fructose	124	11.0	158	10.7	22.6
9 maltose	132	12.3	208	10.9	21.9
10 starch	123	12.0	205	11.8	26.4
Standard deviations					
5	29	2.5	69	2.2	5.6
6	40	2.6	30	2.0	9.3
7	31	2.2	40	2.7	6.6
9	32	2.2	51	3.3	4.7
10	33	3.1	37	2.6	5.1

(pH 2). By this method when applied to cariogenic streptococci not enough material was removed from the cells. Ultrasonic treatment for 5 min with a Branson Sonyfier S-250 at 3.5 D.C. amperes removed more material, though this procedure was far from providing maximum yield as could be revealed by microscopic examination in wet indian ink preparations (MACKIE and MCCARTNEY 1960 [30]). Prior to final precipitation with ethanol this fraction was also dialysed as described above.

Diffusion-in-gel analyses were made using purified polysaccharides of both fractions as antigens and the corresponding homologous antisera. Preparation of antisera has been described by DE CROUSAZ and GUGGENHEIM (1966 [15]).

The dried polysaccharides were hydrolysed in 2 N-H_2SO_4 in stoppered glass tubes for 3 h at 97 °C. The acid hydrolysates were neutralized with $BaCO_3$. Suitable amounts of the neutralized hydrolysates were subjected to thin-layer chromatography. Silica gel G/boric acid layers according to PASTUSKA (1961 [31]) were used. For our purpose N-butanol/acetic acid/water (60 + 30 + 10) proved to be the most effective solvent system. Chromatograms were developed in a sandwich-chamber, applying

† Camag, Muttenz, Switzerland.

Table IV Presence (+++, ++, +) or absence (–) in smears of streptococcus OMZ-61 staining with fluorescent antibodies, in 4 rats per group

Diet	Animal	Streptococcus OMZ-61
1 590	1a K	—
25% sucrose†	7a K	—
	13a K	—
	19a K	—
2 590	6a K	—
25% sucrose††	12a K	—
	18a K	—
	24a K	—
3 2000a	1a H	++
56% sucrose	7a H	++
	13a H	++
	19a H	+++
4 580s	2a H	+++
66% sucrose	8a H	+++
	14a H	+++
	20a H	+++
5 590	1a G	++
25% sucrose	7a G	++
	13a G	++
	19a G	++
6 591	2a G	±
25% glucose	8a G	±
	14a G	±
	20a G	±
7 592	3a G	—
25% fructose	9a G	±
	15a G	±
	21a G	—
8 593	5a G	—
25% lactose	11a G	+
	17a G	±
	23a G	±
9 594	4a G	—
25% maltose	10a G	—
	16a G	—
	22a G	—
10 595	6a G	+
25% starch	12a G	+
	18a G	+
	24a G	+

† Depression-non-inoculation control.
†† Non-depression, non-inoculation control.

ascending chromatography. Visualization was done with naphtoresorcinol-H_3PO_4 spray (GEE 1962 [32]).

RESULTS

Food intake and weight gain. The average figures resulting from the pilot test are shown in Table II. The individual data were subjected

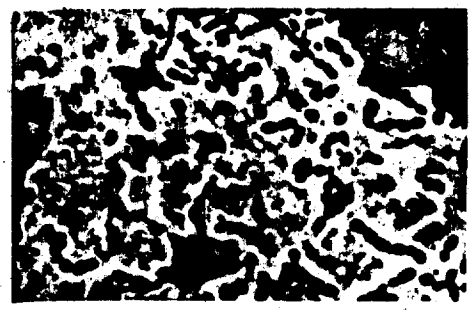

Fig. 1 Wet-ink preparation (MACKIE and MCCARTNEY 1960) of OMZ-61 from 5% sucrose broth photographed in phase-contrast. Cells embedded in amorphous polysaccharide. Magnification 1000 ×

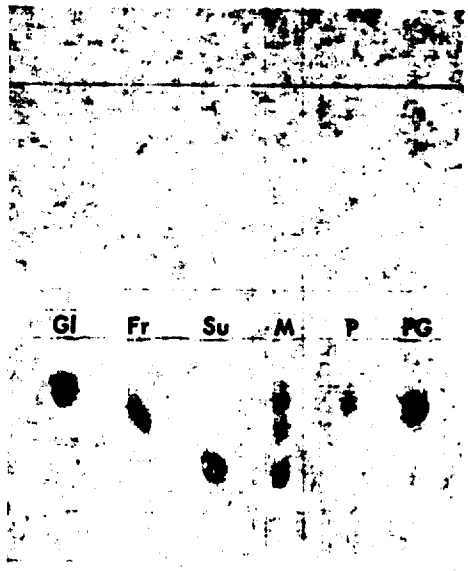

Fig. 2 Thin-layer chromatogram of the hydrolysed fraction II of extracellular polysaccharide synthesized by OMZ-61 grown in broth with 5% sucrose, and of reference compounds. Gl glucose, Fr fructose, Su sucrose, M mixture of glucose, fructose and sucrose, P hydrolyzed polysaccharide fraction II, PG hydrolyzed polysaccharide fraction II with added glucose

to analyses of variance. No differences were found between the diets with respect to daily intake of diet, total eating time per day, eating frequency and intake of water. Weight gains were slightly lower on the sucrose diet. In the main experiment, this was only true for the animals on the high-sucrose diets 2000a (56 per cent sucrose) and 580s (66 per cent sucrose; Table V). Intake of food and water in the main experiment (Table III) were homogeneous ($P_F > 0.1$) with exception of diet 580s on which significantly less food was consumed than on the others ($P_F < 0.001$). Moreover, the intake of water was considerably lower in the group of rats on diet 580s.

Bacteriological checks. The result of examination of the smears stained with fluorescent antibodies is shown in Table IV. The absence of streptococcus OMZ-61 in all the animals on depression-non-inoculation control treatment 1, and on non-depression non-inoculation control treatment 2 indicated that there was no unintended contamination with the test organism. Of the inoculated groups 3–10, the test organism got quantitatively established only in the animals on sucrose diets 2000a, 580s and 590. Only few organisms of the test strain were detected on the glucose, fructose, lactose and starch diets, and none at all on the maltose diet.

Extracellular polysaccharide formation. Preliminary investigations had shown that production of excessive amounts of polysaccharide by cariogenic streptococci (HS- and FA-strains) was only achieved in substrates containing sucrose or raffinose. From raffinose the extracellular polysaccharide was only produced by strains wich fermented melibiose. In broth supplemented with 5% sucrose these streptococci produced a voluminous, flocculent sediment adhering to the glass walls of the culture tube. The remainder was a cellfree, fairly opaque and slightly viscous supernatant. A photograph of such an aggregate from the sediment is shown in Figure 1.

Thin-layer chromatograms of the hydrolysed OMZ-61-polysaccharides contained in fractions I and II showed glucose to be the only constituent of these macromolecules. The following R_f-values were calculated from 10 chromatograms: glucose 0.497 ± 0.003 (average ± standard deviation), fructose 0.442 ± 0.005 and sucrose 0.377 ± 0.002. Upon visu-

Table V Results of 23-day caries test in relative gnotobiosis of 10 groups ($N = 9$) of Osborne-Mendel rats fed on diets containing different carbohydrates. Average number per rat of dentinal fissures lesions and of teeth in which the shallow distal fissure was more severely decayed than the corresponding deep central one, in first and second lower molars; average area score E of smooth surface lesions; average weight gain during 23-day test period

Treatment, diet number	Cariogenic component, per cent	Depression with erythromycin	Inoculation cariogenic str. OMZ-61	Dentinal fissure lesions	Distal-lesion predominance	Buccal surface lesions	Lingual surface lesions	Weight gain (grams)
1 590	25 sucrose	+	—	2.7	0.0	0.7	0.0	105
2 590	25 sucrose	—	—	7.9	0.4	1.8	0.0	94
3 2000a	56 sucrose	+	+	11.7	4.0	19.2	12.9	66
4 580s	66 sucrose	+	+	10.0	3.8	17.3	7.0	67
5 590	25 sucrose	+	+	7.6	2.8	6.4	4.9	95
6 591	25 glucose	+	+	2.4	0.0	0.1	0.0	88
7 592	25 fructose	+	+	0.7	0.0	1.2	0.0	91
8 593	25 lactose	+	+	2.9	0.0	1.8	0.0	82
9 594	25 maltose	+	+	0.7	0.0	0.0	0.0	94
10 595	25 starch	+	+	1.1	0.0	0.7	0.0	103
Standard deviations								
1				2.1		–	–	14
2				3.3		3.9	–	12
3				0.7		7.2	5.4	8
4				3.5		7.9	5.7	12
5				4.7		5.8	5.5	16
6				2.9		–	–	20
7				1.4		3.3	–	17
8				2.1		2.5	–	9
9				1.0		–	–	20
10				1.1		0.9	–	19

alization with naphtoresorcinol reagent, glucose was easily detected by its steel-blue colour, whereas the two other sugars stained red to red-black. Figure 2 shows a typical TCL-plate where the hydrolysed fraction II prepared from strain OMZ-61 was chromatographed together with reference compounds. The polysaccharides of both fractions did not stain with Lugol's solution. According to the physical properties these polyglucans were most probably dextrans. This conclusion will have to be verified by structural analyses.

In agar-gel diffusion the polysaccharides of both fractions showed no precipitation with homologous antisera from rabbits immunized with streptococci cultured in broth containing glucose as carbohydrate source.

Caries incidence. The average incidence of carious lesions of fissures and smooth surfaces is shown in Table V. The analysis of variance of fissure lesions, Table VI, indicated highly significant differences between treatments.

Considering fissure caries incidence in the control groups on sucrose diets (1–4), the first of the detailed orthogonal comparisons—lower part of Table VI—differentiates between baseline control group 1 (depression, non-inoculation) and the groups of animals harboring a caries-active flora (2–4). The difference was highly significant. Comparison 2 revealed a significant difference between group 2, animals with an indigenous oral flora, and groups 3 and 4, animals inoculated with the cariogenic test strain after depression of the original cariogenic flora. However, an additional difference has to be taken into account when these groups are compared: besides the bacteriological difference, the effect of 1 low sucrose diet. 590 was tested against 2 high-sucrose diets, 580s and 2000a. The difference of the fissure caries rate in animals on the 2 high-sucrose diets 2000a and 580s both tested in relative gnotobiosis with streptococcus OMZ-61 was not significant (comparison 3; $0.2 > P > 0.1$).

Table VI Analysis of variance of frequencies of dentinal fissure lesions in 10 groups ($N = 9$; 9 litters of 10 rats each used as blocks) of rats on diets with different carbohydrates, tested for cariogenicity in relative gnotobiosis. Lower part of table, orthogonal subdivision of treatment sum of squares into 9 comparisons (C_1–C_9) with one degree of freedom each. Abbreviations: s sucrose, g glucose, f fructose, l lactose, m maltose, st starch

Source of variation	Degrees of freedom	Sum of squares	Mean square	Probability of error
Treatments	9	1 375.51	152.83	$P_F < 0.001$
Litters	8	174.22	21.78	$P_F < 0.001$
Remainder (error)	72	354.89	*4.929*	
Total	89	1 904.62	–	

Treatment	1	2	3	4	5	6	7	8	9	10		
Depression	+	—	+	+	+	+	+	+	+	+		
Inoculation	—	—	+	+	+	+	+	+	+	+		
Diet	590	590	2000a	580s	590	591	592	593	594	595		
Carbohydrate, %	25 s	25 s	56 s	66 s	25 s	25 g	25 f	25 l	25 m	25 st		
Average number of dentinal fissure lesions	2.7	7.9	11.7	10.0	7.6	2.4	0.7	2.9	0.7	1.1		
Comparison 1	−3	+1	+1	+1							348.48	$P_F < 0.001$
2	0	−2	+1	+1							52.02	$P_F < 0.01$
3	0	0	−1	+1							12.50	$0.2 > P_F > 0.1$
4					−2	+1	+1				216.00	$P_F < 0.001$
5					0	−1	+1				14.22	$0.1 > P_F > 0.05$
6								−2	+1	+1	24.00	$P_F < 0.05$
7								0	−1	+1	0.89	
8					−1	−1	−1	+1	+1	+1	54.00	$P_F < 0.01$
9	+3	+3	+3	+3	−2	−2	−2	−2	−2	−2	653.40	$P_F < 0.001$
Total of treatment sum of squares											1 375.51	

The analysis of fissure lesions on the 25%-carbohydrate diets 5–10 was subdivided in two groups of comparisons. The first included the sucrose, glucose and fructose diets (comparisons 4 and 5), and the second dealt with the lactose, maltose and starch groups (comparisons 6 and 7). Comparison 4, sucrose *v.* glucose and fructose, confirmed the higher incidence of fissure caries on the sucrose diet 590 to be significant at the 0.1-per-cent level of probability of error. Regarding the significance of the difference between glucose and fructose diets (averages of 2.4 and 0.7 lesions respectively), the probability of error would be only slightly higher than 5 per cent if one rejected the null hypothesis (comparison 5).

The higher caries rate on lactose (2.9 lesions) in comparison with maltose (0.7) and starch (1.1) was significant ($P_F < 0.05$; comparison 6), while there was no real difference between the cariogenicity of maltose and starch (comparison 7). Comparisons 8 and 9 match the groupings, making up for orthogonality. Their meaning is self-explaining from the matrix shown in the table.

Regarding the average total number of fissure lesions as shown in Table V, there was no difference between diet 590 fed to rats with their indigenous flora (7.9 lesions on non-depression, non-inoculation control treatment 2), and diet 590 fed to rats in relative gnotobiosis with strain OMZ-61 (7.6 lesions on treatment 5). There was, however, a marked difference with respect to distribution of lesions. Table V and Figure 3 show that the indigenous flora induced lesions mainly in the central fissures of first and second mandibular molars, whereas the test organism OMZ-61 induced lesions predominantly in the area of the shallow distal fissures in the same teeth. Such a "distal-lesion predominance" was found in rats on treatments 3, 4 and 5, all three groups having been both inoculated with OMZ-61 and fed sucrose-containing diets. In addition these three groups of rats are the only ones which developed smooth surface caries

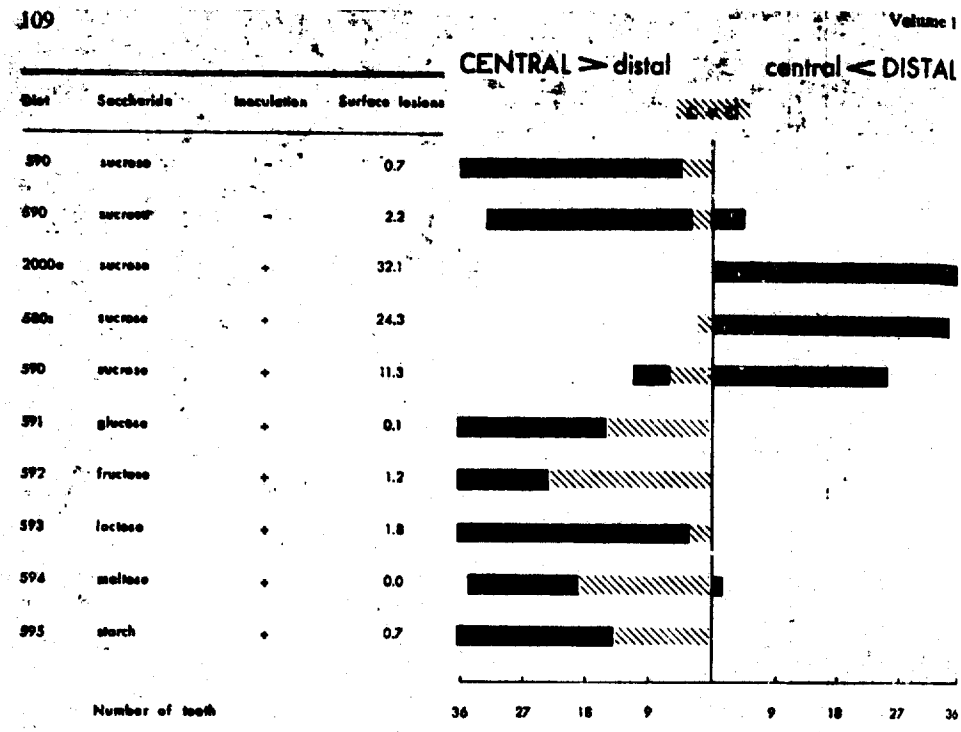

Fig. 3 Numbers of first and second mandibular molars (total 4 × 9 = 36 teeth per group) with carious lesions more severe in central than in distal fissures, or equally severe (left of vertical line); and with lesions more severe in distal than in central fissures: "distal-lesions predominance" (right of vertical line). Average numbers of buccal + lingual smooth surface lesions for comparison

to an appreciable extent (refer to Table V and Figure 3). On all other treatments the score of carious smooth-surface areas was extremely low.

Surface deposits. In general only slight or very slight accumulation of deposits was found. On the sucrose diets on treatments 2, 3, 4 and 5 some animals showed thick and rough soft aggregates which seemed to have formed by coalescence of single colonies. Most of the thick plaque-like aggregates were associated with demineralization underneath. From this finding it was concluded that the deposits were not calcified. On all other treatments only thin smooth films had formed on the mesial surface of the first upper molars. They had a yellow-brownish color and remained transparent after drying.

Discussion

The findings confirm previous results indicating that starch is nearly non-cariogenic (Gustafson *et al.* 1955 [2]; Grenby 1966 [33]) and that glucose is less cariogenic than sucrose (Gustafson *et al.* 1955 [2]). One of the results of the present experiment is in striking discordance with the findings of Gustafson *et al.* (1955 [2]) who found fructose (carious area 44.4) as cariogenic as sucrose (43.5). Looking at the caries rate of male and female hamsters separately, males had carious areas of 55.2 on sucrose and 50.6 on fructose, whereas females showed 27.7 on sucrose and 40.7 on fructose. In connection with the considerable variation and the inconsistency of findings in males and females, growth was inhibited and the mortality rate was high in the group of hamsters on this

65%-fructose diet. It may therefore be assumed, that to some extent unspecific effects were interfering in this hamster experiment. Its outcome was the main reason for recording intake in the present experiment, and for using diets with a lower percentage of the test carbohydrate, with the wheat flour and starch diet 595 as the baseline the low cariogenicity of which was known.

Another explanation for the low caries activity on the fructose diet in the present experiment may be the specificity of the cariogenic flora, induced by depression of the indigenous Gram-positive microorganisms and subsequent inoculation with streptococcus OMZ-61 which mainly causes surface caries. Moreover the experiment was done on rats with "empty fissures"; under these conditions caries activity is more dependent on formation of plaques than in animals with impacted fissures harboring microorganisms and retaining considerable amounts of substrate (refer to the last paragraph of the discussion).

The similarity of the patterns of spontaneous intake of food and water, and of amounts of food consumed in the pilot study as well as in the main experiment strongly indicates that the different effects of the diets compared in groups 5–10 were related to a different and specific caries-conduciveness of the 6 carbohydrates tested. As it was shown in previous experiments on Osborne-Mendel rats fed the same sucrose diet, differences in total daily eating time smaller than 30 minutes are not likely to exert a significant influence on the caries rate (König *et al.* 1964 [18], König 1965 [20]). Another unspecific substrate effect might have consisted in slow oral elimination of a highly cariogenic carbohydrate, and rapid elimination of a less cariogenic one (Bibby, Goldberg and Chen 1951 [34]). The results of an oral sugar clearance study on Osborne-Mendel rats, however, have shown that it takes more time until the nearly non-cariogenic maltose is eliminated than in the case of sucrose, glucose, fructose and soluble starch, no significant differences being found between the latter 4 carbohydrates (Herzog *et al.* 1966 [35]). With equal time of presence of substrate for bacterial metabolism, acid formation and growth on treatments 5–10 the chances for the test strain to get established quantitatively and form enough acid for development of lesions would have been the same, provided that the caries-conduciveness of the test carbohydrates was similar. However, since the effects on the caries rate differed significantly, the diet-microbiota relationship (Keyes 1962 [36]) which was most favorable on the sucrose diet, must have been disturbed when other carbohydrates were administered. Several possibilities might offer an explanation: (1) the carbohydrate in question as a substrate did not sufficiently support oral establishment of the cariogenic test organism and/or did not give rise to large amounts of acid in deep plaque layers; (2) there was no thick plaque because the carbohydrate could not be utilized for synthesis of extracellular polysaccharides providing a plaque matrix acting as diffusion barrier.

Regarding possibility (1), it has been shown that the test strain *in vitro* fermented sucrose, glucose, fructose, lactose and maltose, but no starch (Guggenheim *et al.* 1965 [16]). This would explain non-establishment on the starch diet, but not non-establishment on the maltose and the other diets. The low caries activity on the starch diet in spite of the presence of amylase *in vivo* might be due to the slow diffusion of the large starch molecules into the plaque. The rate of formation of acid in deep plaque layers would be low because of the slow rate of release of maltose (Frostell 1965 [37]; Neff 1966 [38]). Lack of a suitable substrate for acid formation cannot explain the low caries activity on e.g. the glucose diet; the classical experiments of Stephan and Miller in 1943 [39] and other workers have clearly demonstrated that ample amounts of acid are produced when applied to tooth surfaces on which plaque had already accumulated.

In view of the findings on the role of extracellular polysaccharides as an important constituent of the plaque matrix, cited in the introduction of the present paper, possibility (2) offers the best explanation of the results encountered in this experiment. In particular, the observation of Carlsson and Egelberg (1965 [5]) that plaque accumulations were volumi-

nous after administration of sucrose but thin when glucose and even thinner when fructose were given, is in accordance with the ranking of fissure caries activity on the sucrose, glucose and fructose diets (7.6, 2.4 and 0.7 lesions respectively). Extensive smooth surface lesions of rat molars which occur under plaque accumulations were found only on treatments 3, 4 and 5 on which both the test organism was present and sucrose was administered in the diet. The rough soft deposits observed in animals on treatments 2, 3, 4 and 5 were similar morphologically to those found by CARLSSON and EGELBERG (1965) after administration of sucrose. Extracellular polysaccharide synthesis by streptococci isolated from the human oral cavity was known for a long time (OERSKOV and POULSEN 1931 [40]). In a careful study SHERMAN *et al.* (1943 [41]) confirmed this finding and elaborated the criteria which allowed to distinguish between the levan-producing *Streptococcus salivarius* and the heterogenous group named *Streptococcus mitis*. SNYDER *et al.* (1955 [10]) isolated *Streptococcus salivarius* from saliva in large numbers ($5 \cdot 10^8$). Nevertheless he believed that the water-soluble nature of levan made its participation in the formation of dental plaques highly improbable. In fact MCDOUGALL (1964 [42]) and CRITCHLEY *et al.* (1966 [14]) demonstrated that dental plaques contained only 1–2 per cent levan. The latter group stated that the amount of dextran (8–9%) was greatly in excess of the amount of levan.

So far there are only two recognized species of streptococci known to produce dextran from sucrose, viz. *Streptococcus bovis* (DAIN, NEAL and SEELEY 1956 [43]) and *Streptococcus sanguis* (NIVEN, KIZIUTA and WHITE 1946 [28] and WHITE and NIVEN 1946 [44]). CARLSSON (1965 [45]) found that bacteria which closely resembled *Streptococcus sanguis* constitute about half of the streptococcal flora in plaque and suggested that plaque is the usual habitat of this organism. However, cariogenic streptococci (HS- and FA-strains) are not identical with these species being essentially different in other metabolic properties.

The relatively late observation of extracellular polysaccharide synthesis by cariogenic streptococci may be explained by the fact that these strains, in contrast to *Streptococcus salivarius*, form small and unpretentious colonies on 5% sucrose agar. However, extracellular polysaccharide formation cannot be overlooked in broth supplemented with 5% sucrose. GIBBONS *et al.* (1966 [46]) called this extracellular polysaccharide "extracellular capsular material". Figure 1 demonstrates that this term is not adequate. Obviously it is not capsular material, in fact large amounts of amorphous extracellular polysaccharide spreading between and embedding the cells are formed in broth containing sucrose.

Apparently oral establishment of streptococcus OMZ-61 in large quantities was dependent on feeding of sucrose. Regarding localization, carious lesions in the rat molars induced by strain OMZ-61 were not only found on smooth surfaces; occurrence of extensive lesions in the area of the shallow distal fissures of the first and second lower molars were also found to be a characteristic sign of establishment of this organism (KÖNIG *et al.* 1965 [17]). These lesions often are observed to start at the morsal plateau of the minor distal cusps of the first and second molars and progress along the dentin-enamel junction towards the depth of these fissures. It was therefore assumed that these distal-fissure lesions also are a surface-type of caries.

In the present material, even rats with a depressed indigenous flora on diets not favoring establishment of the test organism showed some minor degree of caries activity. In most animals not having dentinal lesions, slight decalcifications of the enamel which are always completely missing in germfree rats, had occurred. This finding indicates that a microflora or an organism which is not identical with the test streptococcus OMZ-61, and which was not effectively depressed by erythromycin, may have been involved in the caries process under the conditions studied. It may preferably be located in the deep narrow central fissures in which some impaction of sponge-like material frequently takes place inspite of administration of a soft diet, and in which formation of plaque is not *conditio sine qua non* for formation of aggregates of microorganisms and accumula-

tion of cariogenic acids. On smooth surfaces accessable to functional cleansing, aggregation of caries-inducing plaque and its sequels seems to be dependent on the establishment of microorganisms producing polysaccharide of the dextran type.

Zusammenfassung. In der Aufnahme fettarmer Versuchsdiäten, die 25% Saccharose, Glukose, Fruktose, Laktose, Maltose oder ungekochte Weizenstärke enthielten, wurden keine signifikanten Unterschiede gefunden; kontinuierliche Registrierung der Futter- und Wasseraufnahme liessen sehr ähnliche Gewohnheiten erkennen. Die Kariesaktivität unter dem Einfluss dieser Diäten wurde an Gruppen von je 9 Osborne-Mendel-Ratten getestet, deren ursprüngliche Mundflora unterdrückt und durch einen Testorganismus grösstenteils ersetzt wurde (Streptococcus OMZ-61). Der Testorganismus, der Oberflächenkaries induziert, etablierte sich quantitativ nur mit der Saccharosediät bei welcher der Kariesbefall am stärksten war; extrem niedrig war der Befall auf den Fruktose-, Maltose- und Stärkediäten.

Résumé. L'enregistrement continu de l'incorporation de régimes expérimentaux pauvres en matière grasse contenant 25% de saccharose, glucose, fructose, lactose, maltose ou d'amidon de froment non cuit, a donné des résultats similaires pour ces 6 régimes. L'ingestion d'eau, enregistrée de même façon, ne montrait pas de différences significatives entre ces 6 groupes. La cariogénicité de ces régimes différemment sucrés a été déterminée sur des groupes de 9 rats de la souche Osborne-Mendel, dont la flore indigène avait été supprimée avant l'inoculation d'un streptocoque cariogène (OMZ-61) induisant des lésions de surface. Cet organisme a prospéré seulement au régime contenant du saccharose et l'attaque carieuse a été la plus marquée dans ce groupe. Elle était extrêmement faible dans les groupes alimentés avec des régimes contenant fructose, maltose ou amidon.

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THE VIPEHOLM DENTAL CARIES STUDY

The Effect of Different Levels of Carbohydrate Intake on Caries Activity in 436 Individuals Observed for Five Years [1]

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It is still too early to assume a definite attitude to the many theories about the etiology and pathogenesis of dental caries. Cumulative evidence strongly suggests that dental caries is related mainly to carbohydrate intake, especially to the amount of sugar consumed.

Indirect evidence produced in favour of this assumption appears overwhelming. Observations made in epidemiological, biochemical and bacteriological investigations and animal experiments argue strongly for the consumption of carbohydrate being a necessary requirement for the development of caries.

A question that then presents itself is whether the evidence produced by the investigations reported in the literature is not sufficient to decide this point one way or the other. Epidemiological studies have shown that caries activity decreases in the entire population of a country in times of war when sugar is rationed. The incidence of caries increases in primitive races as soon as they adopt diets resembling those of civilised peoples. These observations have sometimes been accepted as evidence that sugar favours the development of dental decay. A serious objection that

[1] This paper is a revised edition of a publication in Swedish in Svensk Tandläkare-Tidskrift vol. 45 Suppl. 1952. This revision was made necessary by later analysis of further data concerning the relationship between the consumption of chocolate and dental caries, which in turn necessitated modification of the Discussion. In addition, some sections, which will be the subjects of separate papers, have been abridged.

may be raised against this line of thought is that reduction in the consumption of sugar is not the only change in the mode of living during times of war, and increased sugar consumption is not the only change following in the wake of civilisation of natives races.

It is doubtful whether conclusions based on observations made in animals hold true for human beings. As to other studies such as lactobacilli counts of the saliva and acid production in the plaque, it is not always possible to say with certainty whether the point under discussion really is related to the pathogenesis of dental caries.

Long-term studies on human beings seem to be the only method capable of answering the question of causal relationship between carbohydrate intake and dental caries. The action or effect of the experimental variable on dental caries activity must therefore be assessed by continuous direct determination of the number of new carious lesions of the teeth per unit of time. This is the only satisfactory method available for measuring caries activity in a scientific investigation until an indirect method has been devised and found satisfactory, i. e. capable of reflecting clinical dental caries activity in detail.

In the evaluation of the relationship between dietary carbohydrates and dental caries certain types of sugar, are of greatest importance. The high consumption of refined sugar in certain countries in which the frequency of dental caries is also high again suggests the possible importance of sugar as a causal factor. The importance of bread and other high starch foodstuffs has not received any special attention in long-term studies on human beings.

The total consumption of sugar in Sweden with its population of 7,000,000 is about 300,000 tons per year i. e. round 45 kg. per person per year. The household consumption is about 60—70 per cent of this quantity. The rest was used mainly by foodstuff manufacturers.

Earlier clinical studies of dental caries have been surveyed and analysed by GUSTAFSSON (1954), who suggested that investigations that might be able to provide a definite information on any relationship between the amount of refined sugar in the diet and dental caries are:

1) Studies of dental caries activity in man associated with assessed and adjusted carbohydrate consumption.

2) Tentative therapy of caries by restricted carbohydrate consumption.

Only few investigations of this type are on record. In most of them the experimental period was short and the results are sometimes difficult to judge.

Thus, an overwhelming amount of experimental evidence is available in favour of sugar promoting dental caries, but clinical experimental studies hitherto performed give conflicting results. Clinical studies must therefore still be directed to fundamental questions.

THE PURPOSE OF THE PRESENT INVESTIGATION

A very important question in the study of carbohydrate intake and dental caries is: *Does an increase in carbohydrate (sugar) intake cause an increase in dental caries? If so, what factors related to consumption are of importance?*

Critical analysis of the earlier studies and results suggests that factors other than the *quantity* of carbohydrates (sugar) ingested are involved, e. g. type of sweetmeat or foodstuff in which sugar is incorporated, ingestion at or between meals and the type of basic diet. These factors may be many and their effect is hard to evaluate. Therefore, in investigations of this type it is necessary to have adequate control groups.

Another question of paramount importance is: *Does a decrease in carbohydrate (sugar) intake produce a decrease in dental caries activity?*

An important subordinate question in this connection is whether the activity falls towards nil if carbohydrates are restricted as much as possible. The answer to this question would indicate whether we must suppose the existence of caries, which is related to factors other than the dietary carbohydrates.

The purpose of the present investigation of the relationship between caries activity and carbohydrate intake was therefore to find answers to the following questions.

A. Whether and, if so, how caries activity, as studied under controlled conditions, is influenced

a) by the ingestion, *at meals*, of refined sugar with only a *slight tendency to be retained* in the mouth (non-sticky form),
b) by the ingestion, *at meals*, of sugar with a *strong tendency to be retained* in the mouth (sticky form, sugar-rich bread),
c) by the ingestion, *between meals*, of sugar with a *strong tendency to be retained* in the mouth (sticky form, sweets etc.).

B. Whether and, if so, how dental caries activity is influenced by the omission of a variable proved to be capable of increasing caries activity.

C. Will any new carious defects arise if the consumption of sugar is reduced as far as practically possible?

GENERAL CONSIDERATIONS ON THE PLANNING OF THE INVESTIGATION

In the clinical approach to the problems mentioned in the preceding section it was decided to choose an institution for mentally deficient, where the patients — on account of their mental disorders — might be expected to remain for a long time.

After a preliminary registration of the dental status, attention was directed to the question whether such persons could be regarded as suitable from a medical and an odontological point of view for an investigation of this type. The subjects were then examined dentally at regular intervals and at least once a year. On the basis of these examinations, numbered here as first to ninth, dental caries activity was assessed.

For a certain period all patients of the entire institution received one and the same basic diet. As judged by accepted standards, this basic diet was adequate in all respects except regarding calories. To the basic diet of every experimental group was added some supplement, which varied from one group to another. These supplements constituted the experimental variables. Every group consisted of patients from one or more independent departments.

The investigation, which is still in progress, began in 1945. The first year was a preliminary and adjustment period (Preparatory Period). For 18 months (1946—1947) various vitamins

and mineral substances were studied for their effect on caries in different groups. This part of the investigation will be referred to as the *Vitamin Study Period*. These studies have been reported earlier (1948). The caries activity in the series studied was found to be *low* and without any significant differences between the groups.

In the light of this observation it was assumed that the patients were highly resistant to dental caries, and the investigation of the relationship between carbohydrate intake and caries was planned accordingly. It was therefore considered that the investigation should include an initial stage during which the experimental conditions should represent extremes as far as the carbohydrate consumption is concerned. This period covered the years 1947 to 1949 and is referred to here as *Carbohydrate Study I*.

During Carbohydrate Study I the amount of refined sugar used in the preparation of the basic diet was kept to a minimum. However, in view of the natural sugar content of the foodstuffs, the diet was not "sugar-free". In some groups the subjects received nothing to eat between meals, in others they received a certain amount of sweets, which they were allowed to eat when they liked. The sweet ration varied from one group to another. In other groups the subjects received a supplement of refined sugar at meals, which brought the total sugar ration up to about twice as much as the daily consumption of sugar per head of the Swedish population. Some groups also received sugar-rich bread, while others received only the basic diet plus fat to bring up the caloric level.

When working out the experimental conditions for the 1949—1951 period attempts were made to set up a menu resembling that of an ordinary Swedish household as far as circumstances would permit. This part of the investigation is referred to below as *Carbohydrate Study II* and the conditions used may be briefly summarised as follows. The basic diet was roughly that of an ordinary Swedish family. The amount of sugar used per day in the preparation of the food corresponds roughly to the average daily household consumption per head of the Swedish population. It was forbidden to eat between meals except in those groups, where sweets were offered to the patients between meals in such amounts that the total consumption of refined sugar did not ex

ceed the average Swedish consumption. One group received sugar dissolved in the beverages they drank at meals, while another group received only the basic diet plus fat to make up for the calories. Finally some patients received sugar-rich bread instead of the ordinary bread of the basic diet.

The experimental conditions chosen were thus such that both during Carbohydrate Study I and II four essentially different types of groups were represented, so that the relationship between carbohydrate intake and caries could be studied from the following points of view:

1) Basic diet without additional carbohydrates but with supplementary fat to bring up the caloric level. Observations made in this group elucidate the relationship between the basic diet and dental caries activity. (*Control Group.*)

2) Basic diet with additional sugar in solution (not sticky form at meals). The amount of sucrose is somewhat larger than in any of the other groups. Observations made in this group elucidate the relationship between the total sugar intake and dental caries activity. (*Sucrose Group.*)

3) Basic diet plus addition of sugar in bread (sticky form) consumed at meals. Observations made in this group elucidate the relationship between dental caries activity and the retention of sugar consumed in low concentration at meals. (*Bread Group.*)

4) Basic diet plus addition of sugar in the form of sweets (sticky form) consumed between meals. Observations made in this group elucidate the relationship between caries activity and sugar retention, between meals, from preparations containing sugar in high concentration. (*Chocolate, Caramel, 8-toffee, 24-toffee Groups.*)

MATERIAL

The Vipeholm Hospital is an institution for mentally deficient. The institution receives patients from all parts of Sweden, so that the selection is geographically representative of the entire country.

The hospital is situated in a large park just outside of Lund. For purposes of administration the hospital is divided into 12 departments. These departments are accommodated in 6 buildings with one ward in each of the two floors. Every department is

closed, so that the inmates are not allowed outside except under supervision. During the daytime the patients are under continuous observation of well trained, experienced personnel. Therefore it was not difficult to check how the dietary regime was followed. The personnel consists of 300 persons.

The fluorine content of the water

Drinking water is supplied from the water-works of the city of Lund. It consists of a mixture of surface- and well water. The drinking water was analysed on several occasions during the experimental period. As measured by 8 determinations made since 1945, the fluorine content is 0.4 p. p. m. (ranging from 0.7 to 0.2 p. p. m.). On 2 occasions the hardness of the water was found to be 63 and 116 mg.Ca/litre.

THE PATIENTS

At the end of Carbohydrate Study II, in June 1951, there were 964 patients at the institution. Of these, the dental status of 663 (506 males and 157 females) was noted. To be accepted for the investigation the patient had to fill the following requirements:

1. The patient should not object to dental examination.
2. The patient should show no signs of active tuberculosis or other disease requiring dietary measures.
3. The patient should have a minimum of 10 teeth.

Most of the patients spend the whole of their lives at the hospital. They have a feeling of security there and do not want to leave it. In view hereof one might expect the series to be practically constant and that most of the 633 patients examined in 1946 would still be at the same ward 5 years later. However, only two thirds, i.e. 436 patients, were in the same ward and thereby in one and the same dietary group throughout the Vitamin Study, and the Carbohydrate Studies I and II. This is due to the transfer of patients from one department to another and to deaths as well as exceptionally to tentative releases.

For reasons given later, is was thought justified to include in the present account only these 436 patients — the constant mat-

TABLE 1

Age distribution of the main material 1946—1951

Group		Number of pat.	Born				Average age	
			1910 or before	1911—1920	1921—1930	1931 or later	1946	1951
Control Group		60	24	25	9	2	34.9	39.9
Sucrose Group		57	26	19	8	4	34.7	39.7
Bread Group	male	41	12	15	14	—	30.4	35.4
	female	42	8	17	15	2	28.0	33.0
Chocolate Group		47	12	18	12	5	29.1	34.1
Caramel Group		62	32	19	11	—	35.6	40.6
8-toffee Group		40	9	10	14	7	26.3	31.3
24-toffee Group	male	48	16	15	15	2	31.0	36.0
	female	39	12	10	15	2	31.1	36.1
Total		436	151	148	113	24	31.9	36.9
	male	355	131	121	83	20	32.2	37.2
	female	81	20	27	30	4	30.5	35.5

erial. Thus patients entering a group after August 1946 or leaving it before June 1951 were not included in the analysis.[1] Therefore, unless otherwise stated, all data given below refer to these patients, who have been termed the *main material* 1946—1951.

The average age of these patients was 31.9 years (males 32.2: females 30.5, Table 1). The commonest types of mental disease in the material were idiocy and imbecility. In many cases a hereditary factor was obvious. There were also a number of cases of epilepsy, some in the dement stage and some in combination with oligophrenia.

In the evaluation of the degree of oligophrenia it was often not possible to use ordinary methods for estimating the degree of underdevelopment.

FRÖDERBERG (1948) devised a method for evaluating the intelligence of a patient according to reactions to certain types of

[1] In the Chocolate Group the total number of patients was included in an extended analysis.

stimulation. With the use of this method six different degrees of oligophrenia could be recognised.

Group 0, which consists of the lowest degree of development, comprises patients who in some respects differ widely from normals. They react poorly to extrinsic stimuli and must as a rule be helped at meals. Only rarely were such patients accepted in the present series.

In groups 1—3 the patients show a tendency to imitation and try to speak.

The groups in which the patients most resemble normals are 4—6. In these groups the patients could do light, simple work and could give correct answers to simple questions.

Oligophrenic deficiency of the severity seen in the present series is often associated with certain peculiar habits which may influence the state of the teeth. (Table 2.)

An observation of interest was that in only 40 per cent of the subjects in the present series was paraffin stimulated saliva available.

The ability of the patients to perform their toilet by themselves, to sit at table and eat without help varied widely from one patient to another, and the personnel often had to help them. Only some of the least defective cleaned their teeth. Of the entire series, only 82 subjects brushed their teeth regularly. The better oral hygiene in the female bread group is explained by the fact that the nurses in that department brushed the patients' teeth every morning.

Difficulties have always been encountered in the conservative dental treatment of these patients. Some of them have, however, received such treatment by dentists outside the hospital. In view of the mental condition etc. of the patients, dental treatment had formerly usually consisted of extraction, while most of the cavities had been left untreated. Thus in the beginning of the investigation only 625 (5.6 per cent) of the 11,238 cavities had been filled. At the end of the Carbohydrate Study the number of untreated cavities had increased to 13,363. Since 1950 the patients have received more systematic dental treatment which thus consists mainly of filling cavities that had existed before the commancement of the investigation.

TABLE 2

Oral hygiene etc. in the main material

		Number of patients[1]							
Group	Nr. of patients	Brush teeth regularly	Stim. saliva possible	Chew tobacco	Need help at table	Regurgitate	Grind teeth	Swallow food without chewing	Put stones etc. in mouth
Control Group	60	9	20	4	6	0	2	5	0
Sucrose Group	57	2	21	11	4	2	3	3	4
Bread Group male	41	2	21	4	1	4	9	3	6
female	42	38	19	0	7	0	2	0	2
Chocolate Group	47	1	13	0	1	1	0	0	0
Caramel Group	62	10	20	4	0	1	0	1	2
8-toffee Group	40	0	20	2	9	5	2	3	3
24-toffee Group male	48	1	20	2	1	0	1	3	1
female	39	19	18	0	7	0	2	2	6
Total	436	82	172	27	36	13	21	20	24

[1] Some patients appear in more than one column.

METHODS

Records were made of the number of decayed teeth, the surfaces involved, the number of teeth extracted, not erupted or retained. Plaster models of the bite were also made for recording malocclusions of the teeth. The condition of the periodontium was also noted at the beginning of the study.

1. Recording of Caries

DIAGNOSIS AND CLASSIFICATION OF CARIOUS LESIONS

In the present investigation the following types of dental lesions were accepted as carious:

A. PRIMARY CARIES

1. *Such macroscopic* defects of the enamel, dentine and cementum as did not show characteristics of hypoplasia or erosion. The properties of the bottom of the cavity or its colour and contents were not considered in deciding whether or not the lesion should be classed as dental caries. If the floor of the cavity was hard, a note of this was, however, made in the record.

2. "*Caries without defect*" (Cwd). Visible, decalcified spots of enamel that had lost their normal translucence and assumed a chalk-like consistence (precaries).

3. *Pit and fissure caries* was also said to be present when the probe was "caught".

4. *Roentgen caries* (Rtg-caries). Well defined decalcified areas of the proximal surfaces in the x-ray film, which could not be verified as cavities on inspection with mirror and probe.

B. SECONDARY CARIES

Caries adjacent fillings was, as usual, classed as secondary caries. This type of caries was not included in the statistical analysis in the present investigation.

The Moulage System

The lesions thus observed were classed according to the Scandinavian Moulage System, (Fig. 1 a—d) devised in accordance with suggestions made by WESTIN (1940), DAHLBERG (1940) and

LINDSTRÖM (1940). The system has been described in detail by WESTIN & WOLD (1943), RÖNNHOLM, MARKÉN & ARVILL (1951), and also used by SELLMAN (1945) and BÖÖK & GRAHNÉN (1952).

The way in which this method was applied in the present investigation was described earlier by LUNDQVIST, BONOW & GRAHNÉN (1948).

According to this method, a cavity is compared with respect to position, depth and extent with models of teeth with cavities, each of which has been made on the basis of a large number of observations of all types of caries lesions. Every type of caries is represented by a series of models containing cavities of varying size and depth. Every lesion has its own size and type number. For example a cavity in the distal surface of a premolar or molar included in the Moulage System is designated 11. If this cavity increases in size, it is classed as 12, 13, 14 etc. In the following the Moulage number is given with an M as index, *e.g.* M 1, M 31 etc. With this system, then, it is possible to describe

1) the position, type and size of any cavity at a given time,
2) the number of cavities occurring between two consecutive examinations,
3) any increase in the size of the cavities recorded earlier.

The method is superior to simply recording whether or not a tooth or dental surface is decayed. This is especially the case in investigations like the present one, in which the changes were to be followed for a number of years. In such studies the state of the teeth on one occasion should be described as accurately as possible, so that any progress might be detected at a later examination. It will later be apparent how useful the method proved for overcoming difficulties encountered in the evaluation and recording of caries activity in the present investigation.

Photographic Recording

The most objective methods available for recording dental caries are, of course, those using photography. It was, however, not possible to apply such a method in the present investigation of such a large material. A specially devised method of intra-oral

Fig. 1 a. Moulage System Series 1.

photography (GUSTAFSSON & LUNDQVIST, 1953) was, however, employed for the recording of certain types of new lesions. Figs. 2 and 3 thus show changes of the type Cwd and M 1 photographed intra-orally.

DENTAL EXAMINATION

Before the patient was sent from the ward to the dental department the teeth were brushed by the personnel and then again by the examiner when the patient was in the chair. The mouth was after-

Fig. 1 b. Moulage System Series 2.

wards rinsed with a water spray and dried with the air syringe. The teeth were then examined, tooth by tooth and surface by surface, with the mirror and probe, generous use being made of the compressed air to remove the remains of any loose debris and saliva. Curved, sharp probes of the type Maillefer Inox 13 and 14 were used. The source of illumination consisted of a Dentatus spot light, type VLA. At the examination the dentist had at his disposal a bitewing-status of 5 films taken a few days beforehand. All the

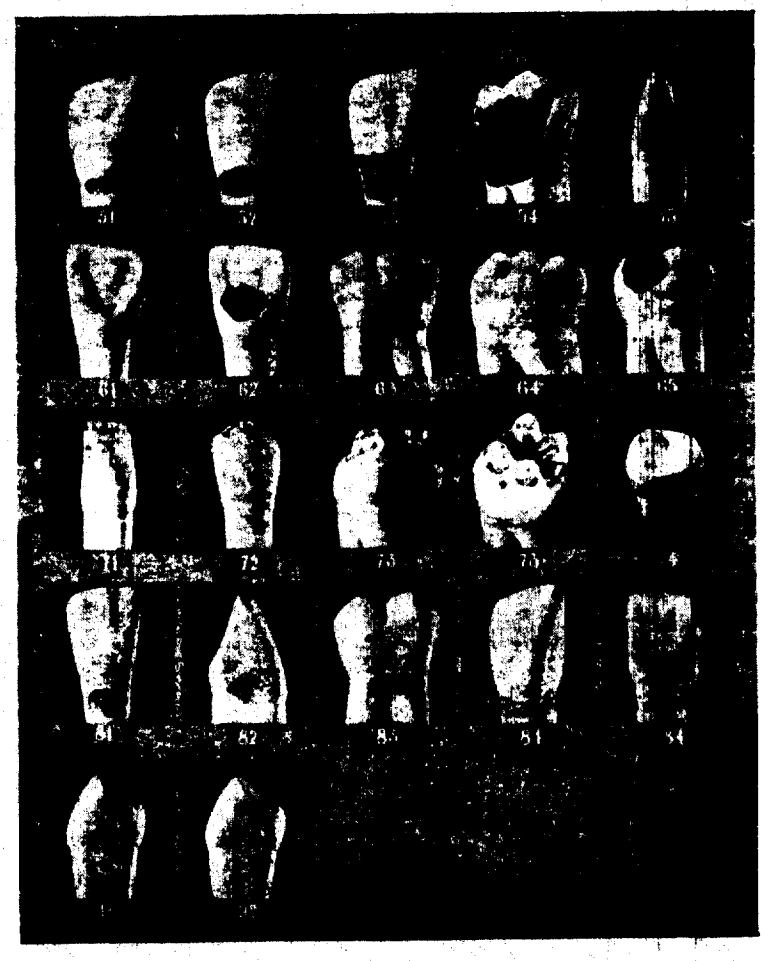

Fig. 1 c. Moulage System Series 3.

findings were dictated to a secretary who recorded them in the dental cards of a loose-leaf register.

If the examiner did not observe a cavity that had been recorded at a preceding examination, the secretary immediately reported the fact to the examiner, who then re-inspected the surface under consideration and the lesion was duly noted. However, if the examiner was unable to find a defect in a surface reported earlier as decayed, the surface was still recorded as carious and was marked

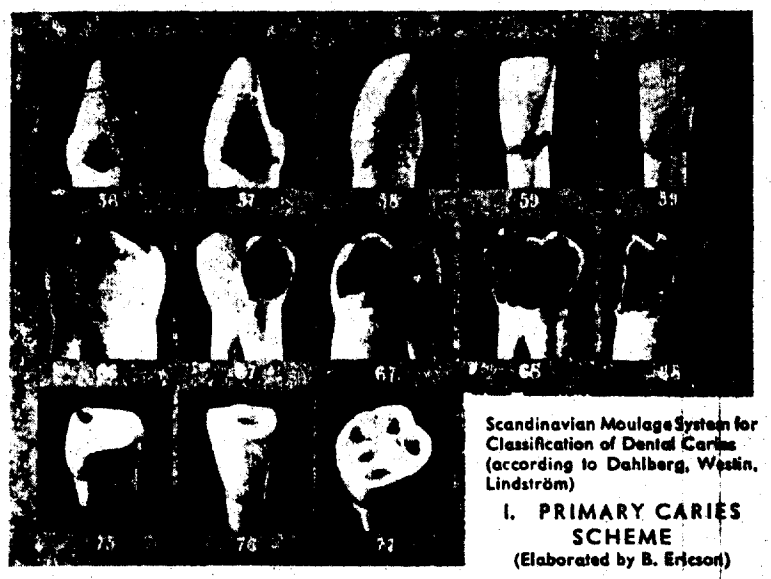

Fig. 1 d. Moulage System Series 4.

with a red K in the register. It was necessary to make this correction in order to be sure that cavities that were registered for a certain period as new really were new and did not represent doubtful registrations re-appearing after an interval of varying length during which they were not regarded as cavities. This problem is discussed in detail in a later section (page 255).

Dental Records

In the beginning the data about the extent of the caries defects were entered in a record at every examination. Therefore in the analysis of the material 2 sheets or more had to be compared. Owing to the size of the record sheets it was both difficult and time-consuming to follow the changes in the state of the individual surfaces from one examination to another without making mistakes.

Therefore a new system was tried during Carbohydrate Study II. This consisted of a loose-leaf register: the dental record of each patient consisted of 32 tooth cards, the leaves (tooth cards and summarising cards) inserted between covers so that the foot of the

Fig. 2 a and b. Caries without defect (Cwd) intra-orally photographed. In Fig. 2 b the change has partly developed into a cavity of the type M 51.

Fig. 3. Initial fissure caries (M 1) intra-orally photographed in mirror.

card with the tooth number was always visible. The tooth card contained vertical columns for each examination, which was given a number, and horizontal columns for each tooth surface (Fig. 4). For the mesial, distal and facial surface there are two lines. In the lower one those cavities are entered in which the cementum is engaged. The carious lesions were entered here according to the Moulage numbers of the surfaces involved or, if caries without defect or roentgen caries was demonstrable the designations Cwd or Rtg.

Any increase in the number of carious surfaces from one examination to another was noted in the row in the visible part of the tooth card as was also the number of intact and decayed surfaces. Here a note was also made if the tooth was extracted or retained. If the tooth was intact, the visible part of the card was blank.

In order to obtain necessary data for assessing caries activity and frequency, 4 summarising cards were allotted to each patient. The first of these included the patient's name and date of birth,

hospital record number and department. As a rule it was possible to read the desired data in the visible part of the card.

TREATMENT OF THE PRIMARY MATERIAL FOR STATISTICAL PURPOSES

In the investigation of a factor of possible importance in the causation of a given disease the role played by such a factor should be evaluated as morbidity frequency, i. e. how many healthy individuals develop the disease on introduction of the factor under consideration. Owing to the commonness of dental caries, this procedure has not often been possible in the study of dental caries in man. It is necessary to note the extent of caries in the bite and study it for any subsequent variation. In caries investigations 3 possibilities are available for recording the dental status on a given occasion.

1. Number of cavities (untreated or filled).
2. Number of damaged surfaces[1] (decayed or filled as well as surfaces of missing teeth).
3. Number of damaged teeth (decayed or filled and missing teeth).

In recording of the *state of the teeth or caries frequency* the absolute figure or any form of index based on the above-mentioned units or measures may be used for individual persons and for groups. A difficulty inherent in method 2 is the evaluation of extracted teeth or teeth with crowns. In most investigations an extracted tooth is said to represent 3 decayed surfaces. WESTIN & WOLD (1943) introduced the so-called 3—2 method, i. e. an extracted molar or premolar was said to represent 3 decayed surfaces and an incisor and cuspid 2.

BODECKER (1939) gives the number of decayed surfaces of the 180 surfaces he distinguished by his own classification or of the 148 anatomical surfaces (Bodecker's index).

WESTIN based his classification on the assumption that in the correct evaluation of caries frequency consideration should be given to the difference between the individual surfaces regarding their susceptibility to decay. Because of the difference between the lingual surfaces and others with respect to retention, and as the

[1] Mesial, occlusal, distal, facial (buccal) and lingual surfaces.

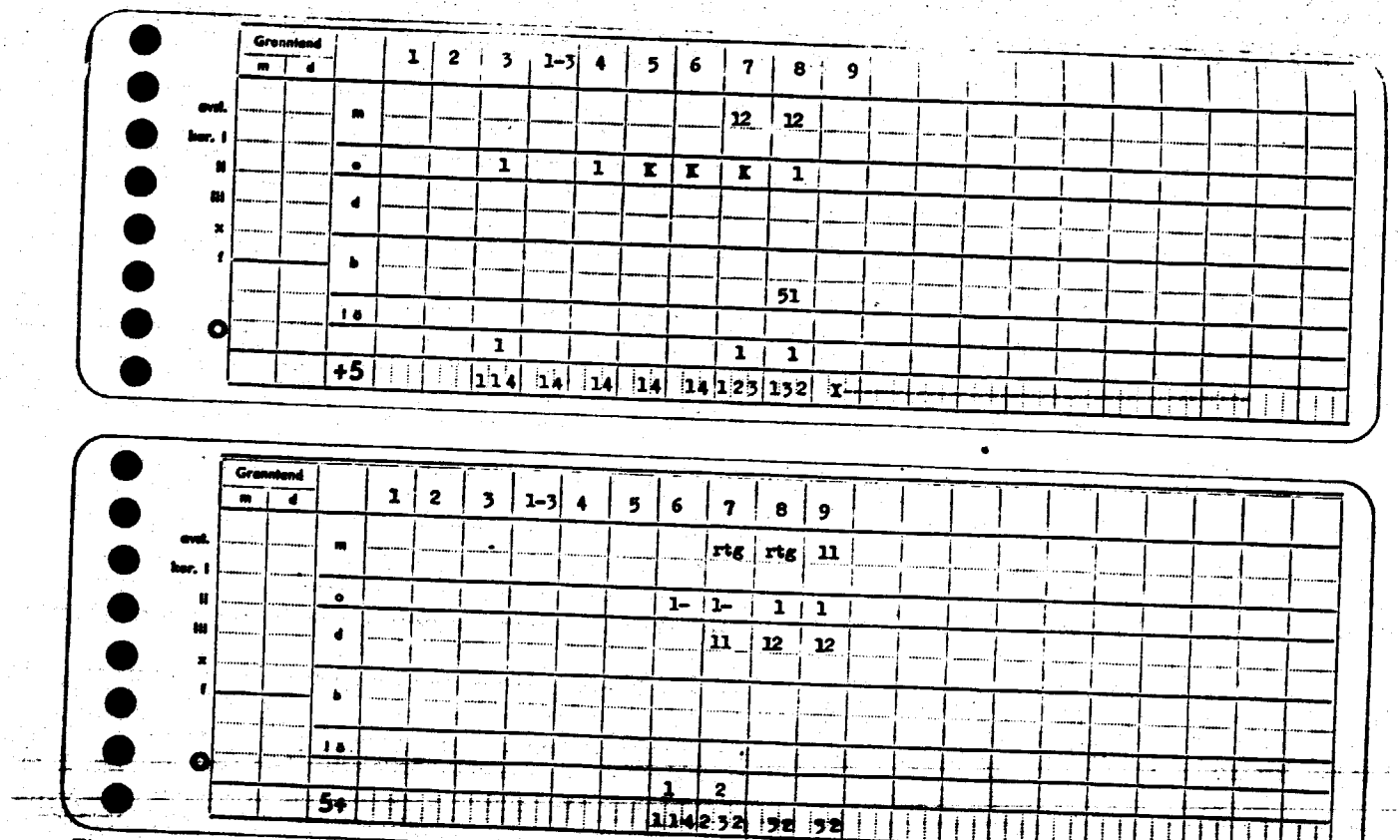

Fig. 4. Example of dental cards in the loose-leaf system. Examinations 1—9registered according to the Moulage System

third molars differ from the rest in susceptibility to caries, e. g. on account of irregular eruption, WESTIN excluded these surfaces from his classification and calculation. This reduced the number of surfaces of relevant interest to 100 (148 surfaces minus 4×5 surfaces per third molar and 28 lingual surfaces). In the present investigation certain calculations were made of the lesions occurring in these 100 surfaces, which are hereinafter referred to as *Westin's index surfaces*.

Broadly speaking, *caries activity* may be conceived as the rate at which the teeth are destroyed by caries. The term thus includes all new primary and secondary carious lesions as well as intact surfaces interfered with in the treatment of dental caries and by the enlargement of existent cavities.

In the evaluation of caries activity in investigations of the etiology and pathogenesis of caries, primary defects are of special importance.

The most suitable way of assessing caries activity is therefore to use the number of new cavities appearing per unit of time. A difficulty attending this type of recording is the registration of two cavities in one and the same surface. Sometimes it is impossible to decide with certainty whether a new cavity in an earlier carious fissure system really is a new cavity or simply an extension of a lesion already recorded. This also applies to cavities in the facial surfaces. These difficulties can be more or less circumvented by measuring the caries activity by the number of new decayed surfaces per person per unit of time.

According to the DMF method, caries activity is measured by determining the difference between the DMF-values at the end and at the beginning of the study period and dividing the value found by the time. A disadvantage of this method is, however, that surfaces and teeth can be damaged by factors other than caries. This is particularly the case with elderly people in whom diseases other than caries are capable of causing larger losses of surfaces than is caries.

It is therefore of paramount importance to determine which surfaces are caries-free and which are decayed at a given examination, and at the following examination, which of these caries-free surfaces have in the meantime become decayed.

In this way caries activity is expressed as the mortality rate of the surfaces. This surface mortality may be expressed as the numerical value per person per year and as the mean value of a group of persons. In the calculation of the mean value for a group, however, it must be supposed that the number of intact surfaces in the individuals of the groups compared are roughly equal. The influence of such individual variation can however, be avoided by giving the surface mortality during the study period per 100 originally caries-free surfaces. By taking into account only changes in the number of previously intact surfaces it is possible to avoid the source of error accompanying the assessment of the statistical value of missing teeth, which has also been suggested by WESTIN & WOLD (1943) and SELLMAN (1945).

It is, however clear that an objective analysis of caries activity requires classification of the individual surfaces according to their susceptibility to caries, it being known that some teeth vary widely from one another in this respect and surfaces still more so (LEIGH, 1923). Extreme variants are the occlusal surfaces of the first molars of the lower jaw and the lingual surfaces of the incisors and canines of the lower jaw. The risk of certain surfaces developing caries may, however, increase with advancing decay of neighbouring teeth, the presence or occurrence of other dental diseases, conservative treatment, crowns and bridges etc. Thus, proximal surfaces usually show a high caries-risk, which decreases considerably if the neighbouring teeth are extracted.

The evaluation of caries activity by this method requires that the entire series be examined, tooth by tooth and surface by surface, and any changes found be systematically recorded. These requirements were filled in the present investigation in which a card was assigned to every tooth and punched according to a special code and the data thus registered were analysed by the Hollerith method.

Data obtained from the Primary Material

The dental caries activity found in the various groups and described below is expressed as the number of new carious surfaces per unit of time observed in

1. Westin's 100 index surfaces, or
2. the 148 surfaces of the 32 teeth, or
3. the 148 surfaces of the 32 teeth with the exception of lesions difficult to judge (Cwd, Rtg and the earliest stages of caries in the fissure system, M 1).

Statistical analysis of caries activity is based on Westin's index surfaces, because the values refer to surfaces less different with regard to susceptibility to caries.

It is, however, obvious that it is important in biochemical and bacteriological studies to know whether a person or group is caries active or not. In such investigations the activity must be calculated on the basis of all types of lesions in all surfaces.

The exclusions listed under point 3) were made because of the difference of opinion between the examiners in the classification of a surface as carious or non-carious when the lesions were Cwd, M 1 and Rtg. The calculations were based on "major cavities", and the cavities were only looked upon as new if they were of the type M 2, M 11, M 51, M 41—43, regardless of the fact whether lesions of the type M 1, Cwd or Rtg-caries had been observed at the same site at the preceding examination.

No account will be given of the caries activity of the individual teeth, but, in order to elucidate certain relevant problems, the following calculations were also made:

a) activity in incisors and cuspids as compared with that in premolars and molars,[1]
b) activity in occlusal, proximal, facial and lingual surfaces of previously intact teeth,
c) the frequency of cementum caries.

In these special calculations of the distribution and site of new cavities the third molars were not considered.

No analysis was made of secondary caries or of enlargements of cavities except for the enlargement of certain types of lesions (Cwd, M 1, Rtg) in those groups in which the caries activity was highest.

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[1] In the following the terms incisors (inc.) and molars (mol.) denote incisors+cuspids respectively premolars+molars.

The caries frequency in the different groups was calculated as the number of DMF teeth per person. With the aid of the DMF curves it was also possible to compare the present series with those of earlier investigations regarding the state of the teeth at the beginning and at the end of the study period. Here, too, the third molars were excluded.

In order to calculate the activity of caries in these different ways it was necessary to collect extensive data for every patient.

After due codification the data were analysed, usually by the Hollerith punch card system.

Discrepant Recordings

When checking the data for statistical analysis it was observed (QUENSEL, 1948) that cavities recorded in the first or second examination were sometimes missed at the second or third. These registrations had been performed without the examiner knowing what findings had been made on the previous occasion. Therefore, at the fourth examination the examiner dictated the number and site of cavities to the secretary who had the records before her and who checked the findings with the entries made at previous registrations. She reported any cavities that had been previously noted but now missed. In this way all entries made at previous examinations were checked and corrected (QUENSEL, 1948).

However, it was found at still later examinations that cavities sometimes appeared at the same site and with the same Moulage number as before on some surfaces for which correction had been made at the fourth examination. The dental records of the entire series were again checked in 1949 for discrepant recordings. It was found that cavities found in the earlier part of the investigation had fairly often been missed later. The seriousness of this source of error must receive attention.

By repeated examinations of the same patient some inconsistent recordings must probably be expected (Table 3). All variants from 1 to 5 will be referred to here as discrepancies. In certain cases such as 1 and 2 the variation is surely due to recording errors, but it is questionable whether this assumption will hold good for types 3—5.

This variation may be a serious source of error in studies based on differences in the number of newly carious surfaces recorded at conse-

TABLE 3

Examples of theoretically possible discrepant recordings

Variant	Examination 1	2	3	4	5	6	7	8	9
1	Cav.	—	—	—	—	—	—	—	—
2	Cav.	Cav.	Cav.	—	Cav.	Cav.	Cav.	Cav.	Cav.
3	Cav.	Cav.	Cav.	—	—	Cav.	Cav.	Cav.	Cav.
4	—	Cav.	—	—	—	Cav.	Cav.	Cav.	Cav.
5	—	—	—	—	Cav.	—	—	—	—

cutive examinations, when e. g. cavities detected at the second but missed at the third, fourth and fifth are recorded as new cavities at the sixth examination. In view of what was said on page 252, only those cavities appearing in surfaces found intact at all the previous examinations should be classed as new.

Correction for Discrepancies

The method used will be best understood from a few examples (Table 4).

In patient C 7 five cavities had been registered at two of the first, second or third examinations, but the surfaces had been considered non-carious at the fourth examination. In patient C 10 one cavity had been excluded (mes.). One cavity (dist.) had been recorded as new at the third examination and was again recorded at the fourth. It was then missed at the fifth and sixth and recorded again at the seventh, at which it was classed as *new*. In a case like this, it may with certainty be assumed that the cavity recorded at the seventh examination was *not* new. This also applies to the cavities excluded in patients C 7 and C 10 at the fourth examination but which later "re-appeared" at the same site and with the same Moulage number.

At the fifth, sixth and seventh examinations of these patients, then, there were 6, perhaps 7, "new" cavities which were in reality old cavities that had been missed at some of the previous examinations. In view of the findings made at later examinations, one is tempted to believe that too many cavities had been excluded by the correction made during the fourth examination. The cavities were observed at two independent examinations and each time they were given the same Moulage number, which strongly suggests that the cavities really existed. Every dental surface recorded at any single examination as decayed was therefore also afterwards considered as carious (Table 4). This infers that the number of surfaces recorded as intact at the fourth and subsequent examinations was fictitiously low. This in turn also means that the number of new cavities recorded was, if anything, too low. Thus the caries activity

figures deduced from the records will be slightly too low throughout, but this is no disadvantage, as it will only strengthen the reliability of any differences found.

As mentioned, the correction was entered as a capital red K in the tooth records. In patients C 7 and C 10, for example, this means that the number of new cavities recorded as such at the sixth and seventh examination was smaller than it would have been if the correction made at the fourth examination had been allowed to stand (Table 4).

In an investigation of this type it is of interest to know not only whether a cavity has developed but also *when*. As far as proximal cavities are concerned, such information is usually obtainable from the X-ray films. In some cases later knowledge of the presence of a definite cavity facilitates a more reliable interpretation of small differences in the density of the enamel shadow which would not by themselves be sufficient to class a surface as carious. Therefore the X-ray films of all the proximal cavities were reviewed. If earlier changes could be demonstrated at the site of the cavities, the entries in the tooth records were corrected in the manner exemplified for patients C 20 and C 24 in Table 4.

Frequency of Discrepancies

At inspection of the tooth records for discrepant recordings in 1949, altogether 1,125 surfaces had been recorded as decayed in at least one of the first 7 examinations in the Caramel Group (which included a slightly larger number of patients than that of the Caramel Group of the main material 1946—1951). Of these cavities, 797 had never been missed after discovery. The remaining 328 surfaces had at one examination or other been missed. Of these, 140 were found to be caries-free and 188 decayed at the seventh examination.

In 106 of these 328 cases the cavity had been missed at one examination only. When a recorded cavity had afterwards been missed more than once, the cavity was classed in 71 cases as "new" in subsequent recordings.

At the seventh examination and before correction, 153 of the surfaces in the Caramel Group had been classed as newly carious. Of these, 19 cavities (12.4 per cent) were of the type: cavity, missed cavity, "new" cavity. The true number of newly involved surfaces after the correction was thus 134.

The frequencies given above are based on the total number of discrepancies including those observed at the seventh examination. It was thought of interest to know how the number of these surfaces marked with a red K, varied from one examination to another. Table 5 shows that most of these discrepancies are referable to the first three examinations. The number of surfaces for which the recordings required correction was low (2—3 per cent) in comparison with the total number examined.

TABLE 4

Examples of the effect of correction of discrepancies on the number of new carious surfaces noted

Pat. Nr.	Tooth	Surface	Moulage number recorded																	
			According to correction at fourth examination									According to successive correction								
			1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
C 7	8 —	o	—	1	1	—	—	—	**1**	1	1	—	**1**	1	K	K	K	1	1	1
	4 +	o	—	1	1	—	**1**	1	1	1	1	—	**1**	1	K	1	1	1	1	1
	+ 4	o	—	1	1	—	**1**	1	1	1	1	—	**1**	1	K	1	1	1	1	1
	6 —	b	—	**63**	*63*	63	63	—	—	—	—	—	**63**	K	63	63	K	K	K	K
		d	91	91	—	—	—	**16**	16	16	16	91	91	K	K	K	16	16	16	16
	— 4	o	1	1	—	—	—	—	**1**	1	1	1	1	K	K	K	K	1	1	1
C 10	1 —	m	—	—	55	—	—	—	**56**	56	56	—	—	**55**	K	K	K	56	56	56
		d	—	—	**56**	56	—	—	**56**	56	56	—	—	**56**	56	K	K	56	56	56
C 20	1 +	m	—	—	—	—	—	**31**	33	33	35	—	**Rtg**	*Rtg*	*Rtg*	*Rtg*	31	33	33	35
C 24	+ 5	o	1	1	1	1	1	1	—	—	X	1	1	K	1	1	1	K	K	X
		d	—	—	—	—	**13**	**13**	**16**	**16**	X	—	—	**Rtg**	*Rtg*	13	13	16	16	X

1, 2 etc. — recordings rejected at fourth examination
Italics — corrections inserted at fourth examination
Rtg — recordings inserted at re-examination of the radiographs
Bold print indicates new cavities

K designates a surface in which a cavity has been demonstrated at an earlier examination but overlooked at later examinations or found doubtful

TABLE 5

Relative frequency of corrections (K) for discrepancies in recording. The Caramel Group of the main material

	Examination Nr.							
	2	3	4	5	6	7	8	9
Number of new K	91	166	36	16	17	7	9	26
Number of K noted earlier		55	136	146	139	118	85	74
Total K	91	221	172	162	156	125	94	100
Number of intact surfaces			5411	5330	5230	5020	4698	4567
K in per cent of intact surfaces			3.2	3.0	3.0	2.5	2.0	2.2

It was interesting to note that the discrepancies were commonest for cavities of type M 91 and M 92 ("arrested caries") neither of which, however, were classed as new lesions in the present study of caries activity. Then came M 1 with 20.5 per cent of the total number of discrepancies, roentgen caries with 13.7 per cent and caries without defect with 5.0 per cent. However, these frequencies tell us only little, because the types of cavities under consideration are the most common. Although an account is given of only one patient group, in which caries activity was high, the distribution of discrepancies was found to be roughly the same in the others.

Discrepant recordings of the kind described above have probably often been observed in long-term studies of caries (BOYD, 1950), although it is seldom mentioned in the literature.

In a series of the present type, i.e. in which it is difficult to examine the patient, the frequency of discrepant recordings must be fairly high. The effect of this source of error has, however, been essentially eliminated by the correction performed. This correction, which was done in part long after the examination, would not have been possible if the recording had not been made by the Moulage System.

It should, however, be stressed that these discrepancies need not

be ascribable entirely to missed cavities. They may be an expression of a true variation in the course of the disease shown by morphologic changes of the carious lesions, which though slight, may be of considerable biologic importance. This directs one's thoughts to the "healing" and to the arrest of caries.

ACCURACY OF RECORDING METHOD

This point received special attention by QUENSEL *et al.* (1954) here only the size of the error of the method will be accounted for.

In a series of persons with low caries activity (at most 3 new carious surfaces per year) the error of a single observation is of the magnitude of 1 carious surface. The standard error of the mean caries activity, as judged by observations made in 40—50 persons will be in the range of 0.2 new carious surface per person per year.

If the activity is still higher (4—6 new carious surfaces per person per year) the error of a single observation will be 2 new carious surfaces. The standard error of the mean of 40—50 persons will be about 0.5 new carious surface per person per year.

2. The Periodontium and Tooth Position

The state of the periodontium was also recorded at the beginning of the study. In the analysis of these data, which has not yet been performed, attention must be directed to the accuracy of the method. The frequency of gingivitis and periodontitis was fairly high. This might be partly ascribable to the poor oral hygiene. As the average age of the series was fairly high, the changes were sometimes advanced and led to the loss of teeth. Of the surfaces lost in this way during the 1946—1951 period, 7.9 per cent were due to periodontitis.

In order to record malocclusions impressions were taken and models were made in plaster of Paris. These models were then studied in order to get an idea of frequency and types of anomalies. This study showed that the groups did not differ essentially from one another regarding the anomalies under consideration.

3. Plan of the Investigation

The Vipeholm dental caries investigation proper consists to date of 4 consecutive studies (the Preparatory Period, the Vitamin Study and the Carbohydrate Studies I and II). During each of these study periods the subjects in one group were compared with those in another. This type of comparison will be referred to as *inter-group comparison*. But the subjects belonging to each of these groups and living under conditions varying from one experimental period to another were also compared. The advantage of this procedure is that the comparisons are made throughout in the same patients and the effect of individual variation on the statistical analysis diminished. This type of comparison will be referred to as *intra-group comparison*.

During each of these four study periods the subjects were repeatedly examined for carious lesions, which were then recorded for the calculation of caries activity. It was originally intended to carry out the individual trial for equal periods, i.e. 1 year or for multiples of such a period; this would facilitate the appraisal of caries activity, which is best expressed as the number of new cavities per person per year. As the interval between any two consecutive study periods was never more than about a month, the lesions recorded at the end of one study period were taken as the starting values of the text. (The interval of 1 month between the study periods was necessary for carrying out preliminary analyses of the observations made and for planning final details of the procedure to be followed during the next study period.)

The studies covered the following periods (see also Fig. 5):

Preparatory Period	March 1945—Jan. 1946
Vitamin Study Period	Jan. 1946—July 1947
Carbohydrate Study Period I	July 1947—July 1949
Carbohydrate Study Period II	July 1949—July 1951

DENTAL EXAMINATIONS

The entire material was first dentally examined in as short a time as possible (main registration). In addition, some groups were examined at shorter intervals for special purposes (supplementary registration). (See Fig. 5.)

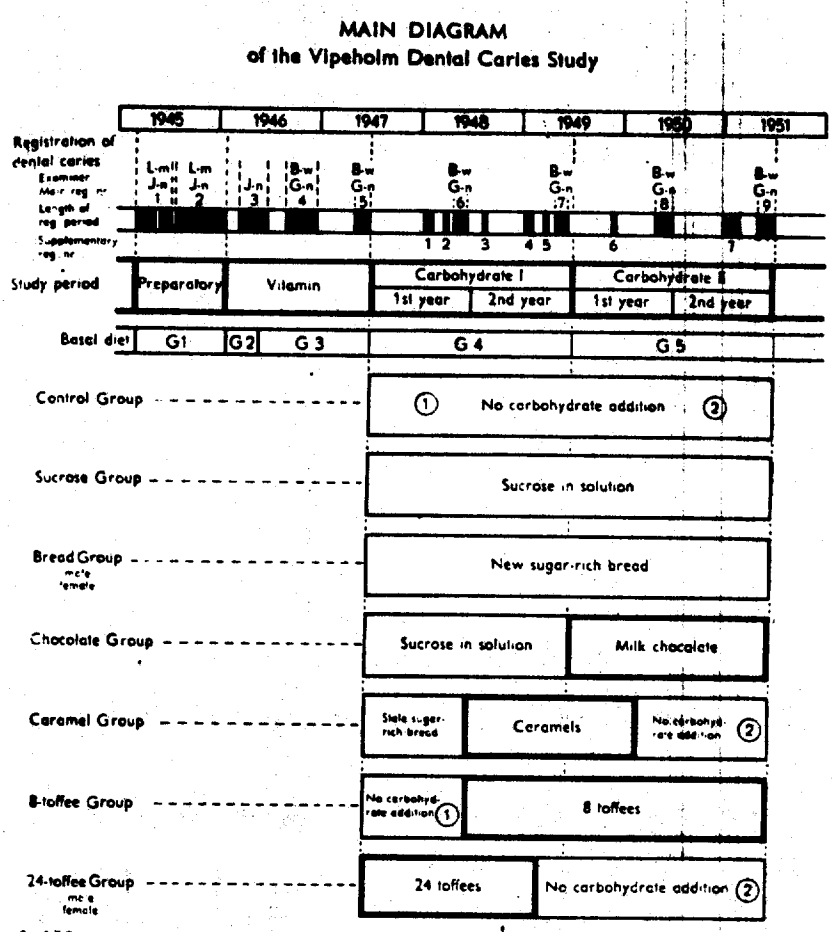
At the first three examinations the examiners had no reference to the patients' dental cards. In order to diminish the risk of missing any earlier defects, from the fourth examination on the examiners had before them the dental cards containing records of earlier findings.

All the main dental recordings were performed by two dentists (Bonow and Grahnén) except the first three, which were carried out by two others (Lindholm and Jacobsson). In supplementary recordings three dentists participated (Bonow, Grahnén and Krasse).

As the first three examinations were performed in a different way (without knowledge of earlier cavities) from those carried out later, when the examiner had the subject's dental card before him, and as all of the main examinations from the fourth on were done by the same examiners, *only observations made at the fourth and subsequent examinations will be considered here.* As the fourth examination was carried out in the middle of the Vitamin Study Period, only those observations made during the latter half of that period i. e. 9 months instead of 18 will be accounted for. Therefore, in order to calculate the number of new carious lesions per year, the figures found should, by rights, have been multiplied by 1.3. However, in view of the magnitude of the error of the method, which is independent of the length of the study period, it was decided to refrain from such correction, because it might cause undue changes in the distribution of the patients with respect to caries activity.

DESCRIPTION OF THE GROUPS

Every department had its own initials and numbers before the time of the present investigation. In this presentation, however, the departments will not be referred to by these designations but by descriptive names (see Fig. 5 and Table 6). Two departments, for example, received a dietary addition of a special type of bread throughout the investigation and were therefore termed the Bread Groups. In certain cases, the subjects were first taken as an inactive control group and afterwards received an experimental variable (chocolate) that caused an increase in caries activity. As the main point of interest in this group was the reaction ob-



1 150 g. margarine as caloric compensation.
2 40 „ „ „ „ „

Fig. 5.

served during the latter period, it was named the Chocolate Group, despite the fact that it was originally (during Carbohydrate Study Period I) a kind of control group. Another reason why this type of nomenclature was chosen was that the relationship between caries activity and the experimental variable is based mainly on intra-group comparison. The hospital designations and the descriptive names of the various groups are summarised in Table 6.

BASIC DIETS AND DIETARY SUPPLEMENTS

The Preparatory Period

March 1945—January 1946. That year the diet (G 1) consisted of the ordinary hospital diet.

The Vitamin Study

The basic diet G 2 for the period 15.1.1946 to 1.6.1946 contained 2800 calories per person per day. This diet was replaced on 1.6.1946 by diet G 3, which was a slight modification of diet G 2 and which was continued until the end of the Vitamin Study Period in 1947. The nutritional values of the various diets are summarised in Table 7.

The main purpose of the vitamin trial was to find out whether inbalance of the amounts of vitamins ingested was capable of producing any changes in caries activity. The basic diet therefore contained an adequate amount of all nutritional constituents and the supplements were usually given as an extra daily dose. Thus one group received an excess amount of vitamin A, another of vitamin C, etc. Some groups received supplements of all the vitamins to compensate the imbalance of the amounts of vitamins. Supplementary calcium and fluorine were also studied for any caries inhibiting effect. One group received calcium as the lactate, one group received a daily dose of 1 mg. fluorine in the form of a sodium fluoride tablet. Another group received 1 mg. fluorine in the form of bone meal that was spread on the bread. The control groups consisted of two wards in which the subjects received the basic diet only. The various groups and the dietary supplements are summarised in Table 6.

CARBOHYDRATE INTAKE DURING THE VITAMIN STUDY PERIOD

Carbohydrates were supplied mainly in the form of potatoes (500 g. per day) and white, soft bread (200 g. per day). The daily amount of refined sugar used in the preparation of the basic diet was 60 g. per person. The daily carbohydrate intake per person was thus 350 g., the total sugar content of which was 90 g. (Table 8). The subjects received nothing between the meals.

TABLE 6. *General survey of the study*

Unless otherwise stated all dietary supplements were served at meals

Group	Number of patients	Department	Vitamin Study Basic diet G_3: 3000 kcal [1]) 1946—1947	Carbohydrate Study I (CH I) Basic diet G4: 1800 kcal [2]) 1st year (CH I: 1) 1947—1948	Carbohydrate Study I (CH I) 2nd year (CH I: 2) 1948—1949	Carbohydrate Study II (CH II) Basic diet G5: 2700 kcal 1st year (CH II: 1) 1949—1950	Carbohydrate Study II (CH II) 2nd year (CH II: 2) 1950—1951	Sugar consumption during the Carbohydrate Study	
Control Group	60	A_{3-4} D_{1-2}	Ca A+C+D; Ca	No supplementary carbohydrates Calorie compensation 150 g margarine + [3])		40 g margarine		Sugar in less sticky form	Sugar at meals
Sucrose Group	57	C_{3-4}	1 mg F (tablet, swallowed whole)	Sucrose in solution 300 g + [3])		75 g			
Bread Group male	41	D_{3-4}	A	New high-sugar bread 345 g at one meal		substitutes basic diet bread		Sugar in sticky form	
Bread Group female	42	K_{3-4}	—						
Chocolate Group	47	B_{1-2}	D	Sucrose in solution 300 g + [3])		Milk chocolate 65 g in 4 portions between meals			Sugar also between meals
Caramel Group	62	B_{3-4}	C	Stale, high-sugar bread 345 g at one meal	22 caramels 155 g between meals + 200 g sugar in sol. + [3])	155 g in 4 portions between meals (10 % red. of basic diet)	No suppl. carbohydr. Calorie compensation 40 g margarine		
8-toffee Group	40	A_{1-2}	—	No suppl. carbohydr. Calorie compensation 150 g margarine + [3])	8 toffees 60 g between meals + 250 g sucrose in sol. + [3])	60 g in 4 portions between meals + 25 g sucrose in sol.			
24-toffee Group male	48	C_{1-2}	1 mg F (bone meal)	24 toffees 180 g between meals + 150 g sucrose in sol. + [3])		No suppl. carbohydr. Calorie compensation 40 g margarine			
24-toffee Group female	39	K_{1-2}	A+C+D; Ca						

[1] The supplements during the Vitamin Study have been given in ordinary symbols. Vit. A=8000 I.U.; C=100 mg; D=2000 I.U.; 0.8 g Ca as calcium lactate. [2] One tablet daily containing: 1 mg vit. B_1; 0.6 mg vit. B_2; 8 mg niacine and 8 mg Fe. [3] Daily supplement of rose hip powder corresponding to 20 mg vit. C.

TABLE 8. *Carbohydrate ration in the different types of diet (g per person per day)*

Conditions		Starch + dextrin		Sugar								Total carbohydrates		
				Mono- and disaccharides in foodstuffs		Added sucrose			Total sugar					
Basic diet	Supplements	At meals	Btw. meals	At meals	Btw. meals	At meals	Btw. meals	Total	At meals	Btw. meals	Total	At meals	Btw. meals	Total
G 3	No suppl. carbohydrates	260	—	30	—	60	—	60	90	—	90	350	—	350
G 4	No suppl. carbohydrates (150 g margarine)	100	—	30	—	—	—	—	30	—	30	130	—	130
	300 g sucrose in sol.	100	—	30	—	300	—	300	330	—	330	430	—	430
	345 g high-sugar bread at one meal	250	—	30	—	50[1]	—	50	80[1]	—	80	330	—	330
	22 caramels + 200 g sucrose in sol.	100	30	30	40	200	30	230	230	70	300	330	100	430
	8 toffees + 250 g sucrose in sol.	100	10	30	10	250	30	280	280	40	320	380	50	430
	24 toffees + 150 g sucrose in sol.	100	40	30	40	150	80	230	180	120	300	280	160	440
G 5	No suppl. carbohydrates (40 g margarine)	260	—	50	—	70	—	70	110	—	110	370	—	370
	75 g sucrose in sol.	260	—	50	—	140	—	140	190	—	190	440	—	440
	345 g high-sugar bread (subst. basic diet bread)	270	—	50	—	110[1]	—	110	160[1]	—	160	430	—	430
	65 g milk chocolate	260	—	50	10	70	20	90	110	30	140	370	30	400
	22 caramels	230	30	40	40	60	30	90	100	70	170	330	100	430
	8 toffees + 25 g sucrose in sol.	260	10	50	10	90	30	120	140	40	180	390	50	440

[1] 50 g sucrose in sticky form.

food was reduced as far as possible (1 g. per person per day), inter alia by employing saccharine instead of sugar in the preparation of the food.

The basic diet was on the whole poor in carbohydrates owing to the low sugar- and starch content of the constituents. The most important sources of carbohydrates were the bread ration (80 g. white bread per day) and the potato ration (250 g. per day). In order to get a constant level of vitamins A and D, milk powder was used instead of milk in the preparation of the food. The beverages drunk at meals consisted of a weak malt drink, coffee or cocoa. This diet differed from an ordinary Swedish menu in that part of the mineral and vitamin requirements were filled either by a tablet given once a day in all groups except the bread group or by a ration of dry powdered hips that was sprinkled on the food (Table 7).

The basic diet G 5 during Carbohydrate Study II was roughly representative of Swedish standards.

The composition of the basic diet and the amount of some of its constituents are given in Table 7.

The daily caloric value of the basic diet was 2700 and the daily carbohydrate intake, 370 g., roughly the same as that given during the Vitamin Study.

The total consumption of sugar was about the same as during the Vitamin Study, or 110 g. per day. The other main sources of carbohydrates were bread (245 g.) and potatoes (370 g.). Basic diet G 5 contained much larger quantities of these foodstuffs than diet G 4.

During this period milk was used (not milk powder) and no supplementary vitamins were given.

TYPES OF DIETARY REGIMES

Experimental variables were introduced by supplementary foodstuffs (see below) with a caloric value of 1200 during Carbohydrate Study I and 300 during Study II. In this manner each of the groups was placed on a dietary regime with at total caloric value of 3,000, which included different components to be studied for their effect on caries activity (see Fig. 5, Tables 6 and 8).

A. *Diet with only slight retention tendency of the sugar*

Basic Diet. No supplementary carbohydrates

A caloric intake of 3,000 kcal per day was secured by the addition of 150 g. non-vitaminised margarine during Carbohydrate Study I and 40 g. margarine during Carbohydrate Study II. The margarine was spread on the bread, but part of it had also to be added to the food during Carbohydrate Study I.

Basic Diet + sugar in solution

During Carbohydrate Study I a daily ration of 300 g. sugar was given partly as an addition to the beverages drunk at meals and partly to the food after it had been prepared. It was intended to add the entire ration to the drinks only, but this proved hardly possible.

During Carbohydrate Study II a 50 per cent solution of sucrose was prepared in the central kitchen. Such quantities of this solution were then added to the beverages consumed at meals that every subject received 75 g. supplementary sugar per day. This guaranteed that the supplementary sugar was really given in solution during this period.

B. *Diet with a pronounced retention tendency of the sugar*

a. *Sugar in sticky form at meals*

Basic Diet + new bread with high sugar content

Each subject received 345 g. of bread containing 50 g. of refined sugar. The composition is given in Tables 9 and 10. Bread of this type is widely consumed in Sweden in the afternoon with coffee. During Carbohydrate Study I the subjects received this bread once a day with coffee in the afternoon. Most of the patients ate the bread first and drank their coffee afterwards. In order to secure a high degree of "stickiness" the bread was served very fresh.

During Carbohydrate Study II all bread in the basic diet was replaced by the sweet type, which was thus served at *all* meals.

TABLE 9

Composition of additional sweets and bread during carbohydrate studies

Calculated on the basis of the ingredients

	Protein %	Fat %	Carbohydrate %	Ca %	P %
Toffee	—	2.2	87.6	—	—
Caramel	8.4	13.3	67.2	0.23	0.19
Milk chocolate	9.1	35.7	50.0	0.27	0.21
High-sugar bread	5.9	12.7	57.4	0.01	0.05

Average weight of toffee 7.18 g.
" " " caramel 7.11 g.

Basic Diet + stale bread with high sugar content

During Carbohydrate Study I bread of the sweet type was stored for at least 24 hours, after which it was served once a day to the afternoon coffee. No stale bread was used during Carbohydrate Study II.

b. *Sugar in sticky form between meals*

Basic Diet + pieces of chocolate

Chocolate was given only during Carbohydrate Study II and ordinary commercial milk chocolate was used. The composition

TABLE 10

Carbohydrates of additional sweets and bread

	Calculated on the basis of the ingredients						Analysis (average)
	Starch + Dextrine %	Sucrose %	Lactose %	Maltose %	Monosaccharides %	Total sugar (as monosaccharides) %	Total sugar (as monosaccharides)
Toffee	21.9	44.4	—	12.2	9.1	68.6	70
Caramel	19.9	19.0	9.3	10.9	8.1	49.3	51
Milk chocolate	3.5	35.7	10.8	—	—	48.8	48
High-sugar bread	42.6	14.5	—	—	—	15.2	13

of the chocolate is given in Tables 9 and 10. The chocolate ration (65 g.) corresponds to 30 g. sugar.

Basic Diet+caramels (max. 22)

These caramels were of the same type as those sold in shops. The quantities placed at our disposal by the manufacturers had been taken directly from ordinary stock. The composition of these caramels is given in Tables 9 and 10. The amount given (about 160 g.) corresponds to 100 g. carbohydrate per person per day, of which 70 g. consists of different sugars. Furthermore, in order that the total carbohydrate supplement would be the same as that in the Sucrose Group (Table 8) every person received 200 g. sugar in solution per day.

During Carbohydrate Study II the amount of sugar in the caramels corresponds to the 75 g. given in the Sucrose Group. Therefore no additional sucrose in solution was given during this period. An increase in body weight was soon noted for the patients, for which reason the daily ration of the basic diet was decreased by 10 per cent.

Basic Diet+toffees (max. 8)

As it was desired to study the effect of sticky *sugar* in as pure form as possible, the binders and other ingredients used in the manufacture of ordinary toffee and sweets were omitted. The toffees were prepared especially for the investigation, and their composition is given in Tables 9 and 10. These toffees therefore differed somewhat from the ordinary types of Swedish sweets, but they resembled English toffees.

The 8 toffees (about 60 g.) correspond to 50 g. carbohydrates, of which 40 g. were various sugars. In order to bring up the total carbohydrate supplement to the same level as in the Sucrose Group 250 g. of sugar in solution were given during Carbohydrate Study I. During Carbohydrate Study II 25 g. sucrose in solution were given for similar reasons.

Basic Diet+toffees (max. 24)

This group represents, like the Caramel Group, a practically unrestricted consumption of sweets. The toffees were of the same type as those used for the 8-toffee Group.

The amount of sweets given (about 180 g. per day) corresponded to about 160 g. carbohydrate per person per day, of which 120 g. consisted of sugars. An additional ration of 150 g. sucrose in solution was given for the same reasons as described above.

This regime with practically unrestricted consumption of toffees was used only during Carbohydrate Study I.

DAILY ROUTINE

During Carbohydrate Study I the subjects received the sweets after meals, one half was served after breakfast, the other half after lunch. During Carbohydrate Study II the sweets were given in 4 portions. Many of the patients ate their entire ration as soon as they got it. Many of them ate two or three sweets at a time, some even swallowed them without chewing and some ate no sweets at all. The patients themselves decided how much of their ration they wanted to eat, and therefore the actual consumption varied widely. This point will be reverted to in connection with the presentation of the results. (Cf. the survey-tables for the different groups.)

The daily routine during Carbohydrate Study II was the same as that in the earlier periods except regarding the distribution of sweets:

Awakened	6.30 a. m.
Breakfast	7.30
Distribution of 1/4 of daily ration of sweets	9.30
Distribution of 1/4 of daily ration of sweets	10.30
Dinner	11.30
Distribution of 1/4 of daily ration of sweets	1.30 p. m.
Distribution of 1/4 of daily ration of sweets	2.30
Coffee	3.15
Supper	5.30
Bed-time	6.00

CONTROL OF EXPERIMENTAL CONDITIONS

None of the patients in the Vipeholm investigation were requested or encouraged to eat more of the sweet additions than they desired. It was therefore necessary to check how much of the

supplements really was consumed. This was done in the following ways:

1. Any changes in the general condition of the patient and changes in appetite or amounts of supplements consumed were noted in special forms issued to every department and returned to the Station at the end of the month.

2. Dietary supplements given in the wards were supplied by the central store and kitchen, where an account was kept of all supplies issued.

3. All waste and food returned from the departments was weighed from time to time and studied in order to find out whether the calorie supply was too high or whether the dietary supplement appealed to the taste of the patients.

4. Individuals selected at random were occasionally observed regarding the amount of the ration they consumed.

5. At the main dental examinations the personnel in charge was also questioned about how much of the dietary supplement the patient generally ate.

4. Control of the General Condition

The patients were under the daily observation of departmental physicians, who co-operated with the Station. During the Vitamin Studies and Carbohydrate Study I, a special physician was employed for the purpose of controlling the general condition of the patients. The methods used have been described by FRÖDERBERG (1952). In addition, a large number of laboratory tests were performed (SWENANDER LANKE, 1952; survey: HÖJER & MAUNSBACH, 1954). The purpose of these laboratory tests was to check any extra-oral effects of the experimental conditions.

RESULTS AND DISCUSSION

Survey

The results are summarised in the main tables recording the caries activity on Westin's 100 index surfaces (Table 11), caries activity for all surfaces (Table 12) and caries development calculated on the basis of the number of DMF teeth (Table 13 and Fig. 6, page 284).

As the risk of caries is more uniform for Westin's index surfaces, all statistical analyses were based on the values noted for caries activity observed in these surfaces. Tables 11 and 12 show that the activity figures calculated on Westin's index surfaces and on all 148 surfaces run parallel, though with the natural difference that the former are slightly lower than the latter. Tables 11 and 12 also show that in the present material a large number of intact surfaces per unit of time were lost for reasons other than primary caries, e. g. involvement of neighbouring surfaces by existing cavities, fractures, extractions and periodontitis. It would therefore have been misleading if caries activity had been estimated only by calculating it from the difference between two determinations of the number of surfaces lost. The total surface losses distributed among the causes are accounted for in Table 12.

As a background to the following statistical analysis of every group of patients, it might be convenient to give a general survey of the results.

General Condition

Reports of the general condition of the patients during the investigations described here have been presented by FRÖDERBERG (1952) and SWENANDER LANKE (1952). It was shown, *inter alia*, that the morbidity of tuberculosis markedly decreased during the investigation. The mortality also showed a tendency to decrease. Bodyweight showed a slow but definite increase in the entire material (Table 14), and was most marked during Carbohydrate Study II.

The Vitamin Study

At the beginning of the Vitamin Study Period accounted for here, the patients had on the average 15 DMF teeth (Table 13). The number of intact surfaces (Westin's index surfaces) ranged from 53 to 68 per person in the various departments. These values for the frequency of caries showed that the state of the teeth at the beginning of the investigation was better in this material than in the population as a whole. According to WESTIN & WOLD (1943), military conscripts aged 20 had 18.44 carious teeth per individual and a Westin's caries index of 0.37.

During the Vitamin Study, when the patients received a diet relatively low in sugar and no additional sugar between meals, the average caries activity was 0.34 new carious surfaces per patient per year, calculated on Westin's index surfaces (Table 15). This average is, however, somewhat too low (see page 262), because the period was shorter than one year.

A statistical analysis of caries activity gave the following results. Comparisons of the occurrence of new caries in the various departments showed an χ^2-value of 7.13 (8 degrees of freedom), which corresponds to a probability of $P > 50$ per cent for the differences between the departments being ascribable to chance. The results obtained by the analysis of variance are given in Tables 15 and 16. Even by this analysis, the differences between the departments appear to be due to chance.

The analysis thus showed no difference in caries activity between the different groups of patients during the Vitamin Study. Tables 11 and 12, however, show that the average number of new cavities per group of patients per period varied considerably. The average for all departments was 0.34 new carious surfaces per person per year. The means for the different departments varied between 0.15 and 0.50.

Even occasional cases of relatively many new carious surfaces per person per year are sufficient to increase the mean, often by 0.1 unit. An extreme case was found, for example, in the Caramel Group, where one individual with 13 new carious surfaces increased the mean for that group from 0.28 units to 0.48 units.

Summing up, then, the variation of 0.30 in the averages of new carious surfaces per person per year was not remarkable. This is

TABLE 11. *Number of new carious surfaces per Westin's*

Group	Period	Conditions: Basic diet	Conditions: Additions	Nr. of pers.
Control Group	Vit.	G 3	Vit. A, C, D; Ca	60
	CH I: 1	G 4	150 g margarine	
	: 2	G 4	150 » »	
	CH II: 1	G 5	40 » »	
	: 2	G 5	40 » »	
Sucrose Group	Vit.	G 3	1 mg F in tablet	57
	CH I: 1	G 4	300 g sucrose in sol.	
	: 2	G 4	300 » » » »	
	CH II: 1	G 5	75 » » » »	
	: 2	G 5	75 » » » »	
Bread Group male	Vit.	G 3	Vit. A	41
	CH I: 1	G 4	345 g new high-sugar bread at one meal	
	: 2	G 4	345 » » » » » » »	
	CH II: 1	G 5	345 » » » » » all meals	
	: 2	G 5	345 » » » » » » »	
female	Vit.	G 3		42
	CH I: 1	G 4	345 g new high-sugar bread at one meal	
	: 2	G 4	345 » » » » » » »	
	CH II: 1	G 5	345 » » » » » all meals	
	: 2	G 5	345 » » » » » » »	
Chocolate Group	Vit.	G 3	Vit. D	47
	CH I: 1	G 4	300 g sucrose in sol.	
	: 2	G 4	300 » » » »	
	CH II: 1	G 5	65 » milk chocolate	
	: 2	G 5	65 » » »	
Caramel Group	Vit.	G 3	Vit. C	62
	CH I: 1	G 4	345 g stale high-sugar bread at one meal	
	: 2	G 4	22 caramels + 200 g sucrose in sol.	
	CH II: 1	G 5	22 caramels	
	: 2	G 5	40 g margarine	
8-toffee Group	Vit.	G 3		40
	CH I: 1	G 4	150 g margarine	
	: 2	G 4	8 toffees + 250 g sucrose in sol.	
	CH II: 1	G 5	8 » + 25 » » » »	
	: 2	G 5	8 » + 25 » » » »	
24-toffee Group male	Vit.	G 3	1 mg F (boné meal)	48
	CH I: 1	G 4	24 toffees + 150 g sucrose in sol.	
	: 2	G 4	24 » + 150 » » » »	
	CH II: 1	G 5	40 g margarine	
	: 2	G 5	46 » »	
female	Vit.	G 3	Vit. A, C, D; Ca	39
	CH I: 1	G 4	24 toffees + 150 g sucrose in sol.	
	: 2	G 4	24 » + 150 » » » »	
	CH II: 1	G 5	40 g margarine	
	: 2	G 5	40 » »	

index-surfaces in the main material 1946—1951

Initially intact surfaces	New carious surfaces per period			Distribution of persons according to new carious surfaces per period						
per person	total	per person	per 100 initially intact surfaces	0	1	2	3	4—6	7—9	10 or more
59.5	9	0.15	0.25	52	7	1				
59.1	7	0.12	0.20	52	7					
58.6	4	0.07	0.11	57	2	1				
58.0	34	0.57	0.98	44	10	2	2	1	1	
56.4	26	0.43	0.77	48	7	1	2	1	1	
57.3	21	0.37	0.64	42	11	2	2			
56.8	25	0.44	0.77	39	12	5	1			
56.0	44	0.77	1.38	42	9	2		2	1	1
54.8	40	0.70	1.28	42	7	5	1	1		1
53.4	44	0.77	1.45	46	3	4	2	1		1
56.9	15	0.37	0.64	30	9	1		1		
56.2	7	0.17	0.30	36	4		1			
55.6	18	0.44	0.79	31	8		1		1	
54.8	25	0.61	1.11	32	3	4		1	1	
53.3	82	2.00	3.75	20	9	4	1	2	2	3
61.0	10	0.24	0.39	32	10					
60.4	20	0.48	0.79	31	5	3	3			
59.9	13	0.31	0.52	31	9	2				
59.5	22	0.52	0.88	32	5	3	1		1	
58.7	41	0.98	1.66	29	6	2	3		1	1
53.1	19	0.40	0.76	35	6	5	1			
52.7	9	0.19	0.36	39	7	1				
52.2	11	0.23	0.45	39	5	3				
51.3	52	1.11	2.16	26	11	4	2	2	2	
49.7	58	1.23	2.48	21	12	8	1	3	2	
59.9	30	0.48	0.81	49	7	5				1
58.9	21	0.34	0.58	49	7	4	2			
57.8	119	1.92	3.32	22	15	8	8	5	1	3
55.0	187	3.02	5.48	19	10	7	3	12	9	2
50.9	41	0.66	1.30	39	12	8		3		
68.1	20	0.50	0.73	28	8	2	1	1		
68.4	32	0.80	1.17	24	10	2	3		1	
67.7	121	3.03	4.47	12	11	3	2	8	2	2
64.7	111	2.78	4.29	14	7	4	5	3	5	2
60.3	143	3.58	5.93	9	7	6	3	9	1	5
61.2	16	0.33	0.54	39	4	3	2			
60.3	187	3.90	6.47	18	9	2	3	8		8
55.7	199	4.15	7.44	18	8	1	4	4	4	9
50.5	10	0.21	0.41	40	6	2				
49.4	19	0.40	0.80	33	11	4				
62.7	9	0.23	0.37	33	4	1	1			
62.2	239	6.13	9.85	8	8	2	1	6	5	9
55.5	252	6.46	11.64	1	4	4	5	8	5	12
47.9	11	0.28	0.59	32	5		2			
46.6	15	0.38	0.83	30	4	4	1			

TABLE 7

Nutritional value of basic diets G3, G4 and G5
(per person per day)

		Vitamin Study	Carbohydrate Study		Nutritional standard in Sweden 1940 [2]
			I	II	
		G3	G4 [1]	G5	
Calories	kcal	3000	1800	2700	3791
Fat	g	125	98	88	148
Carbohydrates	g	349	130	366	483 [3]
Proteins	g	93	85	87	90
Calcium Ca	g	0.9	0.9	0.9	1.1
Phosphorus P	g	1.6	1.4	1.7	1.6
Iron Fe	mg	12.6	9.9	14.5	15
Vitamin A	I.U.	2700	3200	2900	3300
Vitamin B_1	mg	1.2	0.9	1.6	1.5
Vitamin C	mg	43	45	63	73

[1] During CH I one tablet daily containing: 1 mg vit. B_1; 0.6 mg vit. B_2; 8 mg niacine and 8 mg Fe. Rose hip powder corresponding to 30 mg vit. C.
[2] ABRAMSON (1946) page 125.
[3] > 20 per cent as refined sucrose.

Carbohydrate Study Periods

The purpose of these studies was to assess the effect of the oral retention of sugar on caries activity. In order to find out whether such retention is capable of accelerating caries, large amounts of sucrose (twice the total Swedish consumption) were employed during Carbohydrate Study I. During Carbohydrate Study II the amount of sucrose was roughly the same as the average total Swedish consumption. With the exception of these quantitative discrepancies the principles in the planning of the Carbohydrate Studies I and II were essentially the same.

BASIC DIETS

The basic diet G 4 [1] during Carbohydrate Study I had a caloric value of 1800 kcal and contained all the nutritional substances generally believed to be necessary for the diet to be adequate (Table 7). The amount of sucrose used in the preparation of the

[1] Worked out by State Institute for Public Health under the supervision of Professor E. ABRAMSON and Professor E. BRUNIUS.

TABLE 12

Surface losses per period according to cause. Calculated on the 148 surfaces in the main material 1946—1951

Group	Period	Number of persons	Initially intact surfaces per person	Intact surfaces lost: Total		By primary caries			By enlargement of existing cavities	By extractions and loss of teeth because of		
										Caries	Periodontitis	Other causes [1]
				Number	Per pers.	Number	Per pers.	% of total losses	Number	Number	Number	Number
Control Group	Vit.	60	85.3	49	0.82	10	0.17	20.4	31	3	—	5
	CH I: 1		84.8	65	1.08	9	0.15	13.9	19	—	4	33
	: 2		84.3	41	0.68	8	0.13	19.5	9	4	12	8
	CH II: 1		83.8	145	2.42	36	0.60	24.8	59	—	11	39
	: 2		81.7	100	1.67	28	0.47	28.0	57	8	4	3
Sucrose Group	Vit.	57	81.8	41	0.72	25	0.44	61.0	16	—	—	—
	CH I: 1		81.4	64	1.12	28	0.49	43.8	20	1	3	12
	: 2		80.9	102	1.79	55	0.96	53.9	22	5	6	14
	CH II: 1		79.2	119	2.09	48	0.84	40.3	59	1	11	—
	: 2		77.6	138	2.42	47	0.82	34.1	79	—	4	8
Bread Group male	Vit.	41	85.0	54	1.32	23	0.56	42.6	12	4	—	15
	CH I: 1		84.0	70	1.71	12	0.29	17.1	21	3	4	30
	: 2		82.7	55	1.34	24	0.59	43.6	12	—	4	15
	CH II: 1		81.5	89	2.17	29	0.71	32.6	23	7	24	6
	: 2		79.6	186	4.54	95	2.32	51.1	54	—	27	10
female	Vit.	42	88.4	39	0.93	14	0.33	35.9	19	2	4	—
	CH I: 1		87.6	47	1.12	22	0.52	46.8	11	10	4	—
	: 2		87.3	60	1.43	16	0.38	26.7	32	3	—	9
	CH II: 1		86.5	98	2.33	27	0.64	27.6	62	2	—	7
	: 2		85.5	113	2.69	50	1.19	44.3	31	7	12	13

Chocolate Group	Vit.	47	79.0	49	1.04	21	0.45	42.9	22	6	—	—
	CH I: 1		78.7	45	0.96	11	0.23	24.4	29	3	2	—
	: 2		78.7	71	1.51	17	0.36	23.9	33	7	12	2
	CH II: 1		77.8	113	2.40	61	1.30	54.0	41	10	1	—
	: 2		75.8	127	2.70	66	1.40	52.0	50	11	—	—
Caramel Group	Vit.	62	87.3	81	1.31	31	0.50	38.3	21	—	14	15
	CH I: 1		86.0	105	1.69	26	0.42	24.8	59	6	11	3
	: 2		84.4	215	3.47	134	2.16	62.3	62	2	10	7
	CH II: 1		81.0	342	5.52	220	3.55	64.3	81	11	18	12
	: 2		75.8	151	2.44	50	0.81	33.1	71	17	11	2
8-toffee Group	Vit.	40	96.9	51	1.28	22	0.55	43.1	15	6	4	4
	CH I: 1		97.3	103	2.58	35	0.88	34.0	50	—	—	18
	: 2		95.9	209	5.23	131	3.28	62.7	46	—	21	11
	CH II: 1		92.3	273	6.83	128	3.20	46.9	112	9	14	10
	: 2		87.4	322	8.05	162	4.05	50.3	85	10	54	11
24-toffee Group	Vit.	48	88.1	58	1.21	18	0.38	31.0	13	7	4	16
	CH I: 1		87.1	254	5.29	206	4.29	81.1	20	3	13	12
male	: 2		82.5	312	6.50	220	4.58	70.5	38	8	29	17
	CH II: 1		76.6	103	2.15	16	0.33	15.5	48	14	21	4
	: 2		74.8	201	4.19	24	0.50	11.9	45	79	41	12
	Vit.	39	89.1	32	0.82	9	0.23	28.1	5	3	10	5
	CH I: 1		88.5	308	7.90	269	6.90	87.3	30	3	2	4
female	: 2		81.1	369	9.46	289	7.41	78.3	58	7	10	5
	CH II: 1		72.1	102	2.62	14	0.36	13.7	51	16	14	7
	: 2		69.9	82	2.11	17	0.44	20.7	45	8	4	8
		436		5753		2803		M = 48.7	1778	306	454	412

[1] Teeth lost between examinations mainly due to periodontitis.

TABLE 13

Caries frequency in the main material 1946—1951

DMF teeth per person at the main registrations

Group	Nr. of pers.	Vitamin Study		Carbohydrate Study I		Carbohydrate Study II	
				1st year	2nd year	1st year	2nd year
Control Group	60	15.3	15.4	15.5	15.6	16.0	16.3
Sucrose Group	57	16.4	16.4	16.7	17.3	17.6	17.8
Bread Group							
male	41	17.1	17.4	17.6	17.9	18.2	19.2
female	42	14.5	14.6	15.0	15.3	15.5	16.0
Chocolate Group	47	17.7	17.9	18.0	18.1	18.7	19.3
Caramel Group	62	15.5	15.5	15.9	16.9	18.4	18.6
8-toffee Group	40	11.7	12.0	12.3	13.8	15.4	17.1
24-toffee Group							
male	48	15.1	15.4	18.2	19.8	19.9	20.3
female	39	14.1	14.3	18.5	21.1	21.1	21.3

TABLE 14

Average weight (kg.) for the main material 1946—1951

Group	Nr. of pers.	Vitamin Study		Carbohydrate Study I		Carbohydrate Study II	
				1st year	2nd year	1st year	2nd year
Control Group	60	57.6	58.6	58.5	60.2	62.1	62.2
Sucrose Group	57	59.2	58.9	60.1	59.4	61.7	62.2
Bread Group							
male	41	64.8	65.7	67.3	69.8	71.2	74.3
female	42			59.2	58.7	61.5	60.6
Chocolate Group	47	59.2	59.4	61.7	61.5	63.9	65.8
Caramel Group	62	59.4	59.3	61.3	61.9	61.8	61.4
8-toffee Group	40	49.2	47.7	48.5	51.5	53.2	53.4
24-toffee Group							
male	48	57.3	59.3	59.8	59.6	63.7	63.0
female	39			58.1	56.0	57.9	56.8

TABLE 15

Distribution of new carious surfaces during the Vitamin Study. Westin's index-surfaces

Number of new carious surfaces	Number of persons
0	340
1	66
2	20
3	7
4	1
5	1
—	—
13	1
Total: patients	436
» carious surfaces	149
Mean	0.34
Standard deviation	0.91

in conformity with the results obtained on analysis of the error of the method (QUENSEL *et al.* 1954).

The distribution of the patients with regard to caries activity during the Vitamin Study was obviously skew. Thus a very large number, 78 per cent, of the patients had no new carious surfaces per year.

Carbohydrate Study I

The caries activity was high in groups which received sugar in sticky form between meals. In the Control, Sucrose and Bread Groups the activity was still low and of the same order as during the Vitamin Study.

TABLE 16

Statistical analysis of caries activity in the various groups during the Vitamin Study. Westin's index-surfaces. Analysis of variance

	Sum of squares	Degrees of freedom	Variance
Total	360	435	0.828
Within classes	354	427	0.829
Between classes	5.642	8	0.705

Especially remarkable was the development in the Control Group and the later 8-toffee Group, i. e. the groups that received the basic diet plus 150 grams of margarine. During this period, the activity was low in the group with the older patient material, with a mean value of 0.12 and 0.07 new carious surfaces per person. The younger group had 0.80 new carious surfaces per person. Thus, the activity did not drop to nil, despite the fact that the patients had received a diet as sugar-free as was practically possible and a large additional fat ration.

Although the patients in the Sucrose Group received 300 grams of sugar per day, caries activity did not distinctly increase in that group, or in the later Chocolate Group, which, during Carbohydrate Study I, had lived under the same conditions. In the 24-toffee Group, 8-toffee Group, and Caramel Group an increase was observed in the mean caries activity. This increase started immediately after introduction of the experimental conditions. When initial stages are not recorded as caries, there will be a retardation in the increase in dental caries activity: it will be much higher during the second year than during the first.

Carbohydrate Study II

During this period, caries activity in the Control and Sucrose Groups was on the whole on the same level as during the Vitamin Study and Carbohydrate Study I. In the Bread Groups there was a certain increase during the second year of the period.

In the 24-toffee Group, the activity returned to the same low level as during the Vitamin Study in connection with the withdrawal of the addition (and its replacement with fat). This also applies to the Caramel Group, for which the change occurred during Carbohydrate Study II.

The 8-toffee Group persisted at roughly the same activity level as during Carbohydrate Study I. In the Chocolate Group there was a moderate increase in caries activity on the introduction of the chocolate.

In some groups the activity figures recorded are high in relation to the starting values. However, in most cases they are lower than the caries activity demonstrable in school children (cf. GUSTAFSSON & WIBOM 1952).

Caries Frequency as Calculated on the Basis of DMF Teeth

The frequency of caries, based on the number of DMF teeth, is summarised in Fig. 6. The slope of the curve for this number for every patient group shows the caries activity, with the reservation that not all of the teeth were lost because of caries.

For the groups and for the times during which sugar was given with the meals, the DMF curves showed a slope, at different levels, of about 20 degrees in the scale used (exception: the Bread Group, second year of Carbohydrate Study II). Even though this slope is due partly ($<$ 10 per cent, see Table 12) to periodontitis, it is thus remarkable that the caries activity in the material was about 0.3 new DMF teeth per person per year on the whole independently of the experimental variables used when sugar was given only in a form with a slight tendency to be retained in the oral cavity.

A different and larger angle, and thereby higher caries activity, was noted when the sugar was given between meals in a form with a strong tendency to be retained. The curves recovered their usual slope as soon as the consumption of sweets between meals was stopped, as was the case in the 24-toffee Group and the Caramel Group.

Observations made in the male Bread Group during the second year of the Carbohydrate Study II were also remarkable. The slope for the DMF curve was now the same as that for the Caramel Group and greater than for the Chocolate Group, both of which had received sugar between meals. The female group studied under the same conditions showed only a tendency to a change in the slope of the curve.

Intra-Group Comparisons

The account given above can only convey a general impression of the results obtained: it tells us nothing about which changes should be attributed to the experimental variables and which changes should be ascribed to chance. Elucidation of this point requires a statistical analysis of the data.

The purpose of the statistical analysis presented was to answer the main problems of the investigation on the basis of caries activity data for the groups as entities. Where changes have been esta-

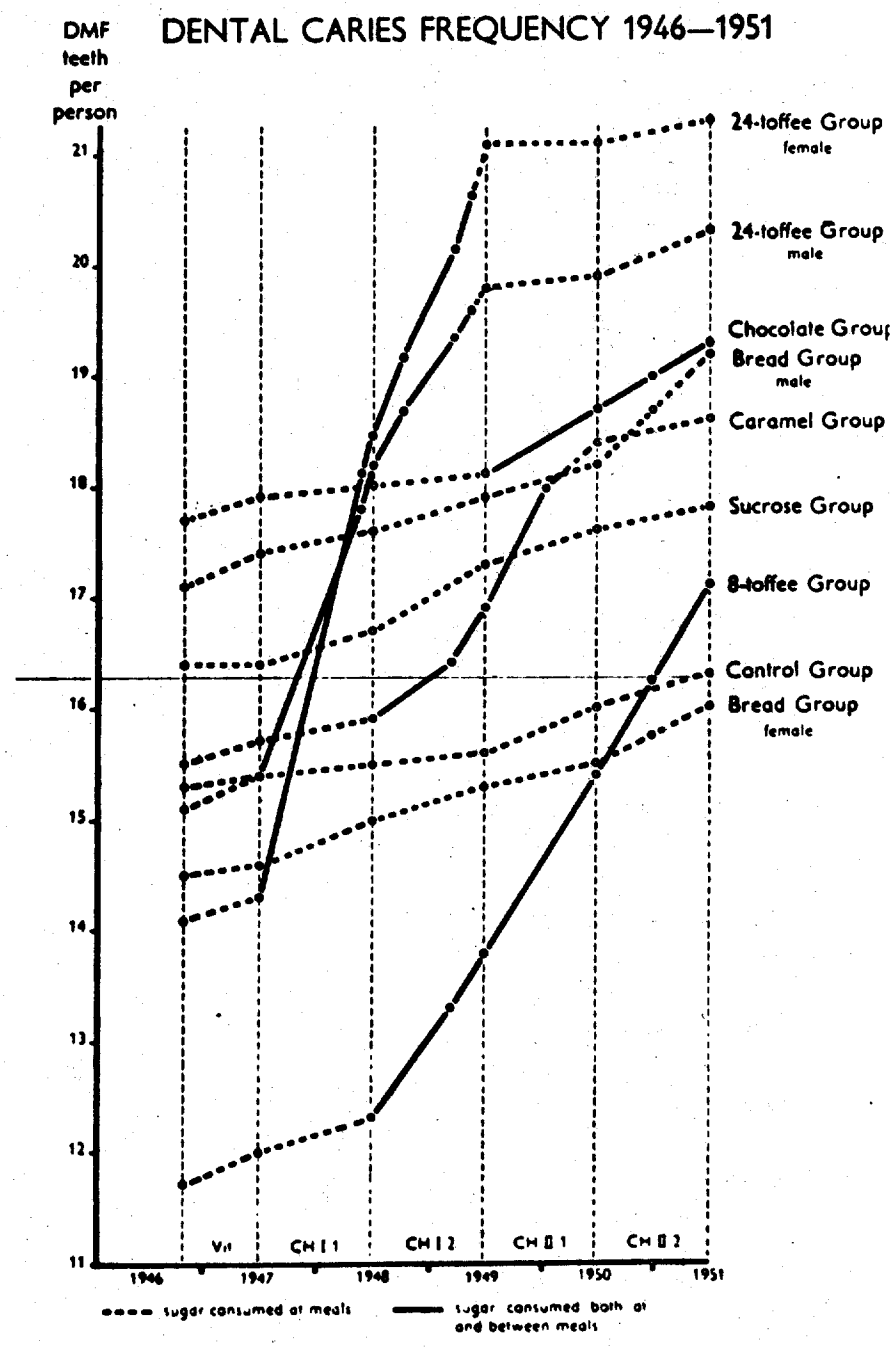

DENTAL CARIES FREQUENCY 1946—1951
DMF teeth per person
21
20
19
18
17
16
15
14
13
12
11
1946
1947
1948
1949
1950
1951
V.I
CH I 1
CH I 2
CH II 1
CH II 2
24-toffee Group female
24-toffee Group male
Chocolate Group
Bread Group male
Caramel Group
Sucrose Group
8-toffee Group
Control Group
Bread Group female
sugar consumed at meals
sugar consumed both at and between meals

Fig. 6.

blished it is planned to consider in detail activity in relation to age and dental status. Such studies cannot, however, be carried out until data have been obtained from the years following the experimental periods.

Analysis of the Data Obtained

The variation in caries activity can be analysed by the standard procedure of comparing the differences between means and their standard errors or by the analysis of variance.

If the individual observations made during a given period are grouped in classes according to the number of new carious surfaces per person per year (e. g. 0 and 1—3 and 4 or more new carious surfaces) the distribution of the patients among these classes will be very asymmetric, most patients in the present series being assignable to the class with 0 new carious surface (Table 11), but also a few cases with a large number of new carious surfaces.

Statistical analysis using the standard deviation and standard error of the mean is possible in these small groups of patients only if the distribution of the data is roughly that of the normal frequency curve, a condition that was by no means filled by the present series because of the abnormally skew distribution.

The differences in susceptibility to caries under uniform conditions (dietary, environmental) are ascribable to differences in resistance to caries, which in turn varies with age, previous caries, number of intact surfaces and a number of unknown factors. Individual differences in disposition to caries might markedly influence the mean values found for caries activity in the groups under consideration. A single individual might thus account for just as much of the numerical value of the mean, as all the other members of the group together.

The effect of these fluctuations in resistance can be compensated in the analysis by a suitable classification of the data and by making comparisons in the same group of patients during different experimental conditions. The latter is possible in such a long-term study as the present and is referred to as *intra-group comparison*. Comparisons between different groups of pa-

tients are termed *inter-group comparison*. The present investigation showed wide individual variation in caries activity under uniform conditions, this therefore increased the importance of intra-group studies.

The present material was classed as follows:

1. Patients in whom no new carious surface appeared within a given period.
2. Patients in whom 1—3 new carious surfaces were observed within a given period.
3. Patients in whom 4 or more carious surfaces appeared within a given period.

This classification reduces the effect of individual patients with a very large number of new carious surfaces. In addition, in order to avoid classes too small for statistical analysis, groups 2 and 3 were sometimes taken together as a single group; this reduced the analysis to a study of the material for the presence or absence of new carious surfaces.

Every group of patients was studied for 5 periods. For each of these periods there was thus a distribution according to the appearance of 0 and 1—3 and 4 or more new carious surfaces per person per year. The statistical analysis of the difference between these distributions was carried out in the following way. All 5 distributions were first compared simultaneously by the χ^2-test. The distributions were then taken as two groups and the new distributions thereby obtained were compared. The original distributions in each of the two subgroups were also compared.

In this way we obtained for each experimental group a longitudinal comparison giving:

1. A total χ^2-value based on differences between all periods.
2. A χ^2-value showing the differences between, for example, periods during the Vitamin Study and the Carbohydrate Study I, on the one hand, and Carbohydrate Study II, on the other.
3. Two χ^2-values for differences between distributions within these two groups of periods.

The χ^2-values given under points 2 and 3 should together roughly equal that given under point 1.

In the present investigation the analysis was complicated by the fact that two or more distributions were not based on completely different series, but on distributions of the same groups of persons during two or more different observation periods. The caries activity, as expressed by the number of new carious surfaces for each person for a given observation time must be more or less influenced by chance. But it is also dependent to no small degree on the resistance of the single individual throughout all study periods, independently of changes in the experimental conditions.

This relationship between the distributions of the same group of patients infers that the validity of any difference found on intra-group comparison is greater than that obtained from the χ^2-values and the corresponding values of probability (P), QUENSEL (1952).

In the following the results obtained in each group are summarised diagrammatically and in tabular form. Unless otherwise stated, figures given for the consumption of sugar etc. are to be understood as consumption per person per day. Caries activity figures are given as the average number of new lesions per person per given period (1 year). How the experimental conditions were filled by the groups that received additional sugar between meals and by the Bread Groups during Carbohydrate Study II are accounted for in the tables.

The tables also show losses of intact surfaces calculated on the basis of all surfaces. Here the losses have been grouped according to the cause: primary caries, loss of teeth or extension of cavities. This last mentioned cause includes the surfaces lost by the extension of an existent cavity onto a hitherto intact surface by enlargement or by conservative treatment or by fracture in connection with undermining caries.

The upper part of the diagrams indicates caries activity, as represented by the mean values. A comparison can be made between the changes in these and in the relative distributions of the patients with respect to caries activity, which are represented by the columns at the bottom of the diagram. The caries activity is also represented by the means that would have been obtained if lesions of

the type "caries without defect", roentgen caries and the initial stages of pit and fissure caries had not been included at the recording of dental caries. This also conveys an idea of the relationship between the frequency of these initial and the definite dental caries lesions.

In the statistical analysis the distributions for the various periods are grouped. In order to facilitate the description the periods 1—5 are termed 1=Vitamin Study, 2=Carbohydrate Study I, 1 year, 3=Carbohydrate Study I, 2 year and so on.

CONTROL GROUP

This group consisted of a large department and a few patients from another department. The group was made up of altogether 60 men with an average age of 34.9 years at the commencement of the study period in 1946.

During the Vitamin Study both departments received supplementary calcium and the small department received additional vitamins A, C and D (Table 17). The purpose of Carbohydrate Study I was to study the effect of reduced carbohydrate consumption on caries activity when this consumption was reduced by simultaneous

1) elimination of practically all refined sugar from the diet,

2) maximum restriction of foodstuffs naturally containing sugar, and

3) reduction of the carbohydrate ration to about half of that of ordinary Swedish standard.

These restrictions rendered the diet calorie-poor (basic diet G 4 1,800 kcal). The patients therefore received an additional ration of 150 g. margarine per day to bring up the caloric level. A reduction in caries activity can thus be attributed either to the reduction in carbohydrate consumption or to the increased fat ration (total 225 g.). This eludicates the difficulty in investigations of this type with marked carbohydrate restriction, which must be compensated by calories from some other source. This additional fat ration makes it doubtful whether this group can be considered a true control group during Carbohydrate Study I. This group is, however, classed as a control group in accordance with what was

said on page 262 because it merits this name as a basis for the conclusions drawn from the observations made in Carbohydrate Study II.

The carbohydrate-poor, fat-rich diet has been shown to cause very mild ketonuria (SWENANDER LANKE, 1952). This stresses still more the special position of this group during Carbohydrate Study I.

During Carbohydrate Study II the patients in this study group received ordinary Swedish food (basic diet G 5 2,700 kcal.) with supplementary margarine (40 g.) to bring up the caloric level. While the fat ration during Carbohydrate Study I was also used in the preparation of the food the margarine was served during Carbohydrate Study II by simply spreading it a little thicker on the bread. The total daily fat ration was 120 g., thus slightly less than what may be regarded as Swedish standard, i. e. 148 g. (Table 7). In this group the change in the conditions from Carbohydrate Study I to Carbohydrate Study II thus implied that

1) the margarine addition was decreased from 150 g. to 40 g.,

2) the total carbohydrate ration was increased from 130 g. to 370 g., partly by the increase in the bread and potato ration and partly by increasing the total sugar consumption from 30 g. to 110 g. In this group all food between meals was forbidden.

Caries Activity

The changes in the experimental conditions were accompanied by only slight changes in the average caries activity, which was low throughout all the 5 years (Fig. 7). Thus, caries activity was very low during the second year of the period with the carbohydrate-poor, fat-rich diet. Only 8 new surfaces were involved and then only by small lesions (see Moulage illustrations, Figs. 1 a—d, 2 and 3, page 244—249):

Cavity type:	Number of surfaces:
Rtg-caries	1
M 1	3
M 31	1
M 44	1
M 51	1
M 63	1

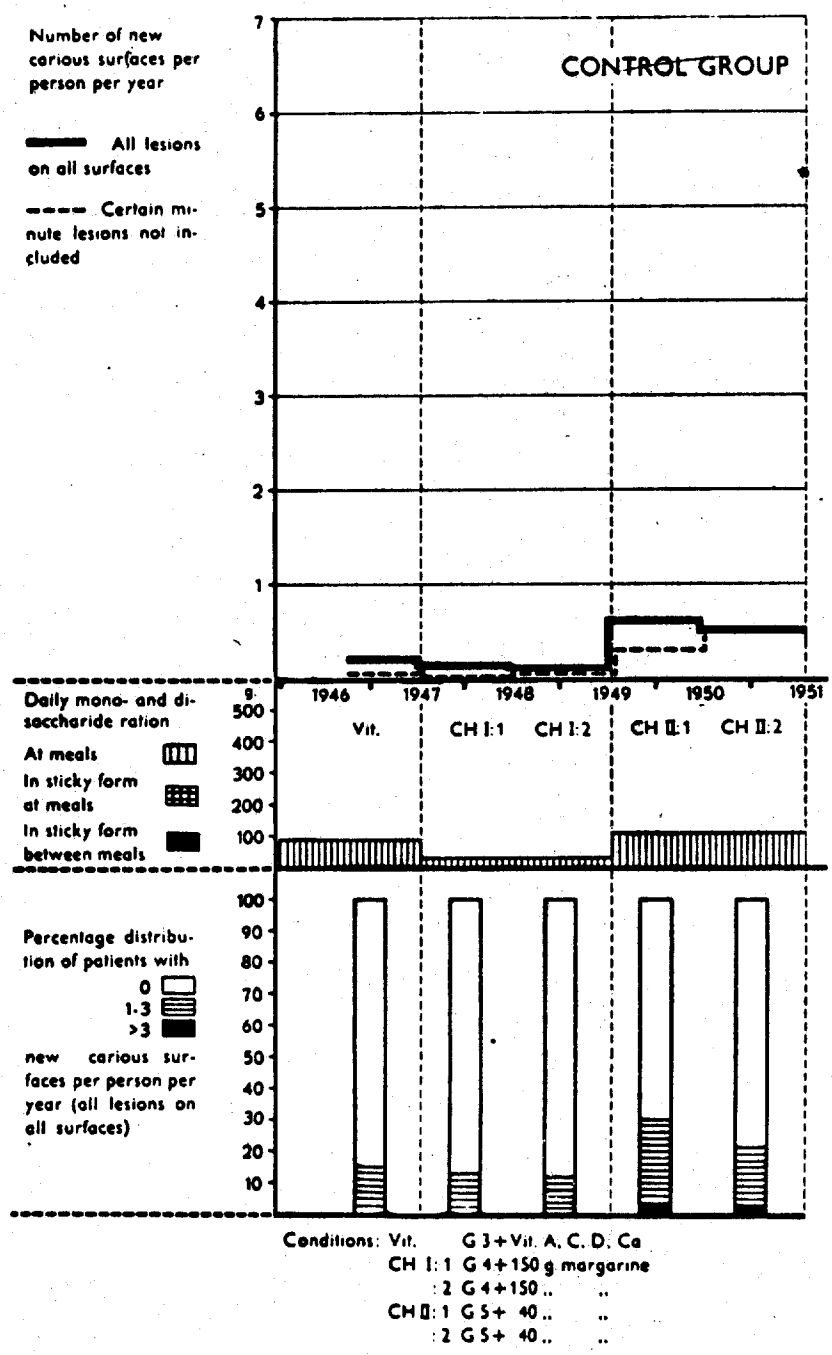

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
CONTROL GROUP
7
6
5
4
3
2
1
1946
1947
1948
1949
1950
1951
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
g.
500
400
300
200
100
Vit.
CH I:1
CH I:2
CH II:1
CH II:2
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
100
90
80
70
60
50
40
30
20
10
Conditions: Vit. G 3+Vit. A, C, D, Ca
CH I: 1 G 4+150 g margarine
: 2 G 4+150 .. ..
CH II: 1 G 5+ 40 .. ..
: 2 G 5+ 40 .. ..

Fig. 7.

TABLE 17

Survey of the results in the Control Group (60 patients)

		Vitamin Study	Carbohydrate Study I		Carbohydrate Study II	
			1st year	2nd year	1st year	2nd year
		Vit. A+C+D; Ca	No supplementary carbohydrates Calorie compensation			
			150 g margarine		40 g margarine	
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.15	0.12	0.07	0.57	0.43
	Per 148 surfaces	0.17	0.15	0.13	0.60	0.47
	Ibid. but Cwd., Rtg., Ml excl.	0.08	0.05	0.07	0.30	0.47
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.05	—	0.03	0.13	0.11
	Mandib. inc.	0.07	0.03	—	0.07	0.15
	Maxill. mol.	—	0.05	0.03	0.18	0.06
	Maxill. inc.	0.03	0.03	0.02	0.18	0.10
	Previously intact teeth	0.08	0.08	0.03	0.22	0.30
	Cementum caries	—	0.02	—	0.12	0.18
	Total surface losses per 148 surfaces					
	Primary caries	0.17	0.15	0.13	0.60	0.47
	Enlargements	0.52	0.32	0.15	0.98	0.95
	Loss of teeth					
	periodontitis	—	0.07	0.20	0.18	0.07
	other causes	0.13	0.55	0.20	0.65	0.18
	Total:	0.82	1.08	0.68	2.42	1.67

[1] Third molars not included.

TABLE 18

Statistical analysis of the changes in caries activity in the Control Group. Westin's index-surfaces

Classes: 0 and ≥ 1 new carious surface per person per period

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	12.68	4	5 % > P > 1 %
The first three periods in relation to the last two	9.86	1	1 % > P > 0,1 %
The first three periods and the last two in relation to one another separately	2.82	3	P > 50 %

The number of surfaces lost by cavities involving neighbouring surfaces was one of the lowest recorded (Table 12) during the entire investigation.

During Carbohydrate Study II caries activity increased and cases with more than 3 new carious surfaces began to occur.

Statistical Analysis

As to the number of new carious surfaces, the series was only divided into two classes — subjects with and without new carious surfaces. Statistical analysis (χ^2-test) showed a probable difference between any of the periods (Table 18). Comparison of the distribution of the first three periods taken together and the last two periods, likewise taken together, also showed a significant difference, a difference thus attributable to the change from Carbohydrate Study I to II.

On the other hand, the first three periods did not differ from one another, nor did the last two.

This difference between Carbohydrate Study I and II was, however, referable entirely to changes observed in the small department (19 patients), no changes being demonstrable in the large department with its 41 patients. The difference observed between the first three periods and the last two periods in the small department showed an χ^2-value of the same magnitude as for the whole group.

Discussion

In this group of patients low-carbohydrate, high-fat diet depressed caries activity to practically nil. It is therefore remarkable that no such depression was noted in the 8-toffee Group, who were on the same low-carbohydrate, high-fat diet during the first year of Carbohydrate Study I (Fig. 13 and Tables 36 and 37). This difference is discussed on page 324).

During Carbohydrate Study II caries activity was higher than during Carbohydrate Study I. Nothing definite can be said about the cause of this increase in caries, because several of the experimental conditions (increase in sugar, bread and potato consumption) were changed at the same time in order to secure conditions

similar to Swedish standards, i. e. conditions desired for Carbohydrate Study II.

The increase in caries activity in this group can also be attributed to a possible return to the original level on withdrawal of supplementary fat with any retarding effect it might have on dental caries. That a high-fat diet is capable of retarding caries in animals has been shown by ROSEBURY & KARSHAN (1939 a and b) and SCHWEIGERT *et al.* (1946). It might then be objected that a decrease in caries should have been recordable in association with the introduction of supplementary fat. But no such decrease was noted. It must, however, be mentioned that the figures recorded for caries activity during the Vitamin Studies were fictitiously low, because the observation time was then less than one year (cf page 262). This may have masked any difference in caries activity between the Vitamin Study and Carbohydrate Study I.

Summary

A group of 60 males with an average age of 34.9 years received a carbohydrate-poor, high-fat diet practically free from refined sugar. Slight ketonuria was noted in some of the patients. Caries activity was depressed to practically nil.

After two years this diet was replaced by an ordinary diet with a ration, at meals, of refined sugar corresponding to what may be regarded as the average Swedish household consumption in 1948. This change of diet was accompanied by a small but statistically significant change in caries activity. This change may depend either on the withdrawal of the addition of fat or to increased carbohydrate consumption. During all of the periods the patients received nothing to eat between meals.

THE SUCROSE GROUP

The group consisted of one department with 57 males. The average age of this group in 1946 was 34.7 years.

During the Vitamin Study they received a daily dose of 1 mg. fluorine as NaF in a tablet which they swallowed without chewing (Table 19). During Carbohydrate Study I the patients received an addition of 300 g. sucrose in solution. It was originally intended

to mix this sugar in beverages. However, the ration proved too large for this procedure: therefore, part of it was added to other liquid and semi-liquid food-stuffs. During Carbohydrate Study II the extra sugar ration was 75 g. The entire ration was dissolved in the beverages served at meals.

When changing over from the Vitamin Study to the Carbohydrate Study, the total carbohydrate ration was increased by the addition of sugar (Table 8), while the consumption of starch and dextrin was decreased (from 260 g. to 100 g.). The total carbohydrate consumption during Carbohydrate Study II was almost the same as during Carbohydrate Study I, but starch and dextrins were increased from 100 g. to 260 g., while the ration of sugars was decreased from 330 g. to 190 g. The sucrose consumption was equal to the average total consumption in Sweden. During all of the periods, the patients received nothing to eat between the meals.

Caries Activity

Caries activity was low throughout all the five years (Table 19 and Fig. 8). During the second year of Carbohydrate Study I the mean caries activity showed a slight tendency to increase. This increase may be partly attributed to the fact that some patients showed 3 or more new cavities (Fig. 8, Table 11). Twenty-seven (47 per cent) persons showed no new carious surfaces during either of the Carbohydrate Study Periods.

Statistical Analysis

Statistical analysis by the χ^2-test (Table 20) showed no difference between the study periods concerning the ratio between the number of patients with and without caries. During the last three periods, however, some of the patients showed more than three new carious surfaces per period, an activity never seen during the first two periods. The first two periods were compared with the last three (Table 21). The probability of the 8 patients with four new carious surfaces or more falling within *one* group of periods being due to chance is less than 2 per cent.

The analysis suggests certain true differences in the distributions which become apparent between the second and third periods, *i. e.* the first and second year of Carbohydrate Study I, during which the experimental conditions were the same.

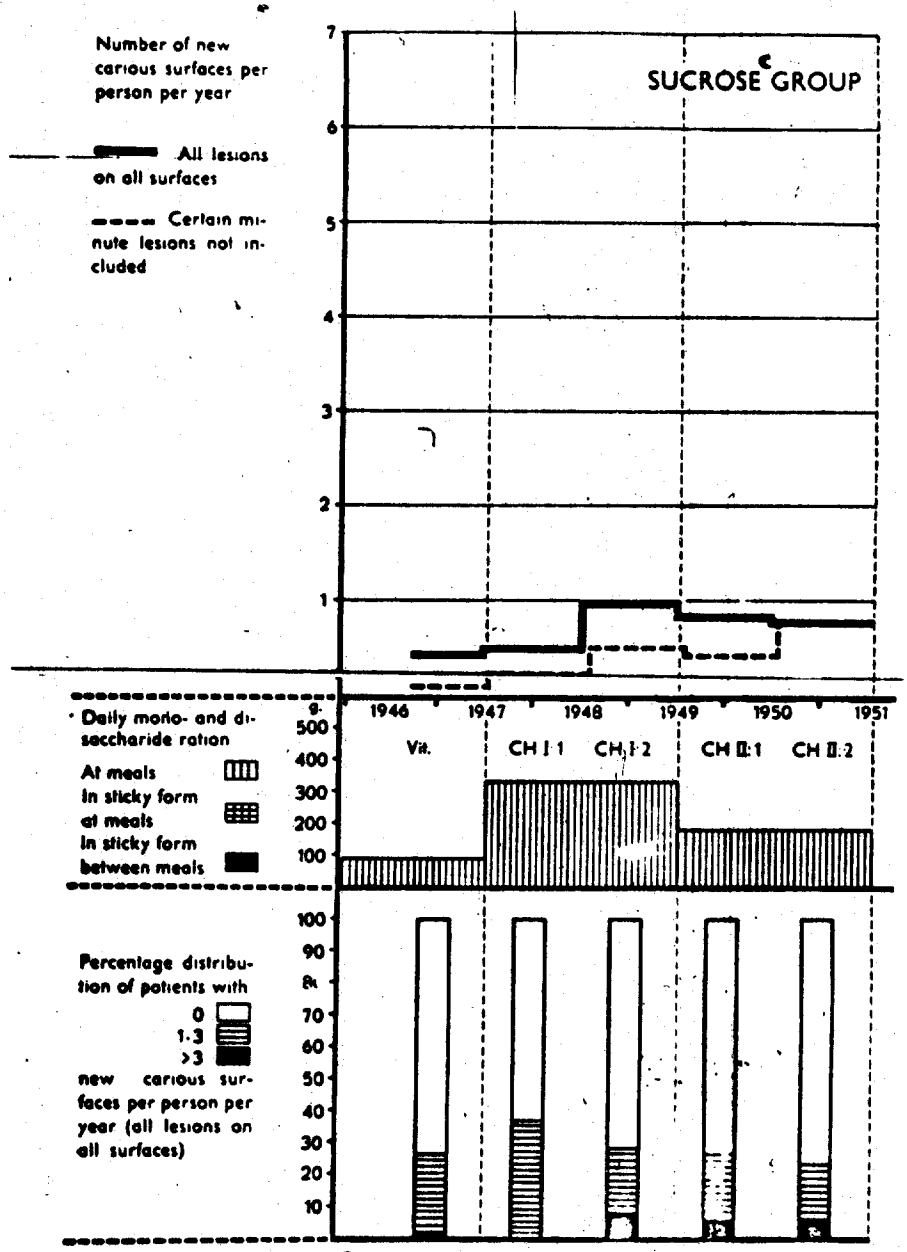
Discussion

In the search for any relationship between this change in caries activity and the experimental conditions a point deserving special attention is that during Carbohydrate Study I part of the extra sugar ration was mixed with fluid and semi-fluid food, and its tendency to be retained in the mouth thereby increased. During Carbohydrate Study I the Chocolate Group (47 patients) were on the same diet as the Sucrose Group (se main diagram, page 263). No increase in caries activity was seen in the Chocolate Group either.

This observation is also important, because otherwise the low caries activity recorded in the Sucrose Group might have been interpreted as an effect of the addition of fluorine, which was administered during the last 18 months before Carbohydrate Study I was started. It is, however, hardly likely that this addition of fluorine, which moreover had no local effect, should exert any influence on a series of patients with such a high mean age.

If the distribution of the patients with regard to caries activity in the Chocolate Group and the Sucrose Group be taken together for the Vitamin Study and for Carbohydrate Study I, during which latter period the experimental conditions were the same for both, it will provide a wider basis for judging whether any difference in caries activity occurred in connection with the addition of 300 g. sucrose (Table 22). Statistical analysis by the χ^2-test of these combined distributions of 104 patients did not show any real difference in caries activity during the three first years of the study.

So far the present analysis shows, that on consumption of 300 g. sucrose at meals mainly in the beverages there were no other changes in caries activity than those due to chance only. It ought to be stressed, however, that this 300 g. was consumed as part of a diet, which was poor in carbohydrates and differing from Swedish standards.



Conditions: Vit. G 3 + 1 mg F
CH I: 1 G 4 + 300 g sucrose in solution
: 2 G 4 + 300 ..
CH II: 1 G 5 + 75 ..
: 2 G 5 + 75 ..

Fig. 8.

TABLE 19

Survey of the results in the Sucrose Group (57 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		1 mg F (tablet, swallowed whole)	Sucrose in solution 300 g		Sucrose in solution 75 g	
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.37	0.44	0.77	0.70	0.77
	Per 148 surfaces	0.44	0.49	0.96	0.84	0.82
	Ibid. but Cwd., Rtg., Ml excl.	0.11	0.25	0.49	0.47	0.79
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.17	0.09	0.26	0.22	0.16
	Mandib. inc.	0.02	0.07	0.26	0.16	0.09
	Maxill. mol.	0.12	0.17	0.18	0.22	0.17
	Maxill. inc.	0.03	0.12	0.19	0.16	0.37
	Previously intact teeth	0.19	0.28	0.54	0.37	0.37
	Cementum caries	0.05	0.11	0.07	0.11	0.07
	Total surface losses per 148 surfaces					
	Primary caries	0.44	0.49	0.96	0.84	0.82
	Enlargements	0.28	0.35	0.39	1.04	1.39
	Loss of teeth					
	periodontitis	—	0,05	0.11	0.19	0.07
	other causes	—	0.23	0.34	0.02	0.14
	Total:	0.72	1.12	1.79	2.09	2.42

[1] Third molars not included.

TABLE 20

Statistical analysis of the changes in caries activity in the Sucrose Group. Westin's index-surfaces

Classes: 0 and ≥ 1 new carious surface per person per period

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	2.35	4	$P > 50\%$
The first three periods in relation to the last two	1.20	1	$50\% > P > 10\%$
The first three periods with one another	0.40	2	$P > 50\%$
The last two periods with one another	0.90	1	$50\% > P > 10\%$

TABLE 21

Comparison of the caries activity in the Sucrose Group before and after the increase during Carbohydrate Study I

Period	Distribution of patients according to the number of new carious surfaces		
	0	1–3	4 or more
1 + 2	81	33	0
3 + 4 + 5	130	33	8
Total	211	66	8

Summary

A group of 57 male patients with an average age of 34.7 years, who had for 1 1/2 years been on an ordinary Swedish diet and who had received 1 mg. fluorine daily in the form of a tablet, were placed on a diet in which the major part of the carbohydrate ration was consumed in the form of refined sugar mainly in solution at meals, the amount of sugar representing about twice the average total Swedish consumption. After a further two years the distribution, but not the quantity, of the carbohydrates was again changed in such a manner that the amount of refined sugar consumed at meals was slightly more than the average Swedish consumption. During these study periods all consumption of food between meals was forbidden.

These changes in the distribution of the carbohydrates produced no statistically significant changes in caries activity.

TABLE 22

Statistical analysis of the changes in caries activity in the Sucrose and Chocolate Groups taken together during Carbohydrate Study I (104 persons). Westin's index-surfaces

Classes: 0 and ≥ 1 new carious surface per person per period

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
The first three periods with one another	0.45	2	$P > 50\ \%$

BREAD GROUPS

Forty-one men and forty-two women with an average age of 30.4 and 28.0 years, respectively, at the start of the study had the same dietary regimen during Carbohydrate Study I and II. The supplement consisted of 345 g. sweet bread which contained 50 g. refined sugar. During Carbohydrate Study I the patients received this type of bread with their afternoon coffee, and most of them ate the bread before they drank their coffee. During Carbohydrate Study II this bread was served daily at all four meals and replaced the bread ration of the basic diet. During all the study periods consumption of food between meals was forbidden.

Caries Activity

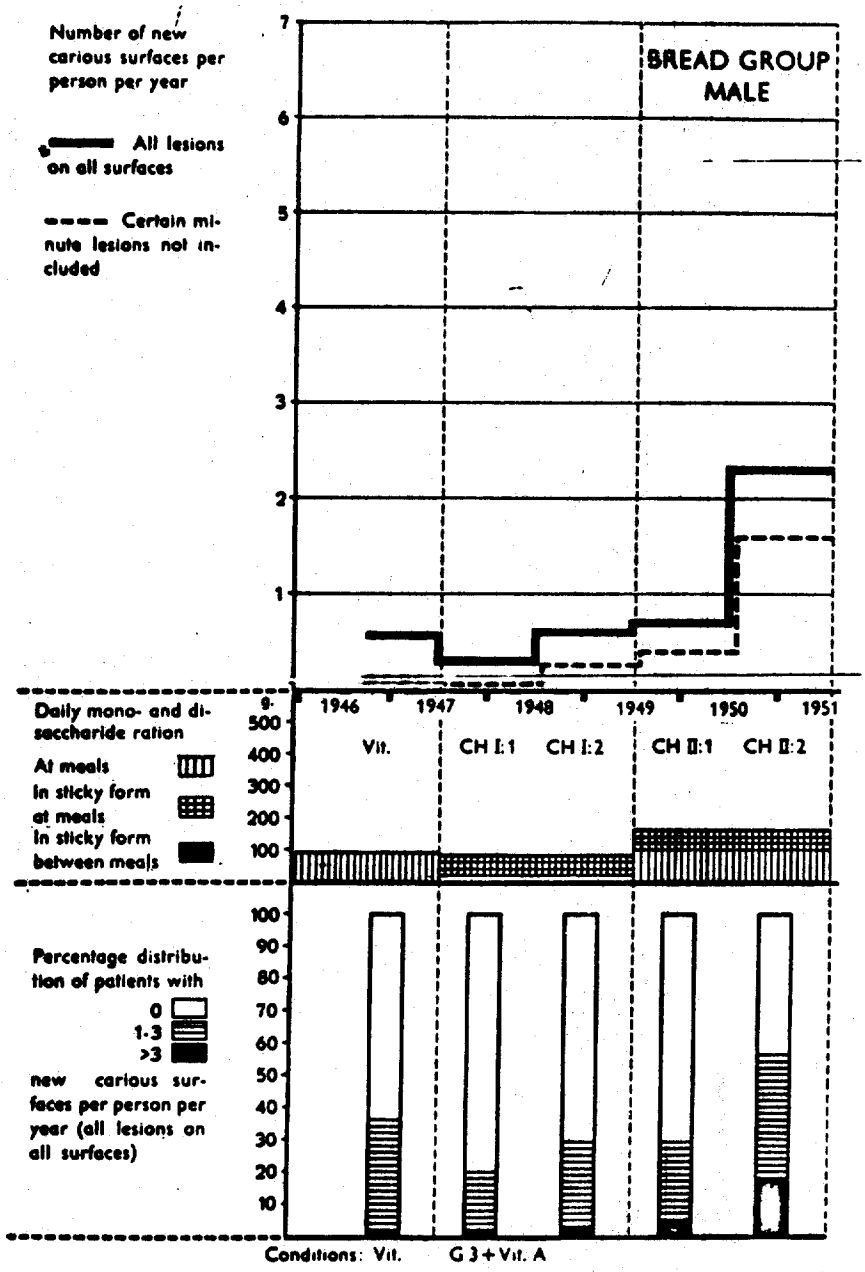
The average caries activity during Carbohydrate Study I was about the same as during the Vitamin Study Period (Tables 23, 25, Figs. 9 and 10). During the second year of Carbohydrate Study II the males showed a distinct increase in caries activity, even in the number of definite cavities. The same tendency, though less pronounced, was also seen in the female group. The change in the male group was also evident from the change in the distribution of the patients according to caries activity as shown in Table 11 and in the columns in the right hand bottom corner of Fig. 9.

Statistical Analysis

The χ^2-analysis (Table 24) showed that the increase in caries activity in the male group during the 2nd year of the Carbohydrate Study II can only be attributed to chance with a probability of less than 5 per cent. However, as far as caries activity in the females is concerned, no differences were found between the various periods, except those due to chance (Table 26).

Discussion

It is remarkable that the increase of dental caries activity does not occur until the second year of the Carbohydrate Study II *i. e.* more than one year after the experimental conditions (consumption of the sweet bread at all meals) had been altered. Further the two groups differed regarding the results despite the same dietary conditions. However, the female group differed in two important



Conditions: Vit. G 3 + Vit. A
CH I: 1 G 4 + 345 g new sugar-rich bread at 1 meal
: 2 G 4 + 345 g. .. .. 1 ..
CH II: 1 G 5 + 345 g. .. .. all meals
: 2 G 5 + 345 g. .. ..

Fig. 9.

TABLE 23

Survey of the results in the Bread Group (male, 41 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		Vit. A	New high-sugar bread 345 g at one meal		substitutes basic diet bread-	
	Number of patients who consumed					
	> the supplement				—	—
	= " "				38	41
	< " "				3	—
	0 " "				—	—
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.37	0.17	0.44	0.61	2.00
	Per 148 surfaces	0.56	0.29	0.59	0.71	2.32
	Ibid. but Cwd., Rtg., Ml excl.	0.10	0.07	0.27	0.41	1.61
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.17	0.10	0.22	0.15	0.46
	Mandib. inc.	0.02	0.02	0.12	0.05	0.46
	Maxill. mol.	0.12	0.05	0.12	0.17	0.56
	Maxill. inc.	0.05	—	—	0.27	0.56
	Previously intact teeth	0.22	0.10	0.27	0.34	0.98
	Cementum caries	—	—	0.02	0.12	0.49
	Total surface losses per 148 surfaces					
	Primary caries	0.56	0.29	0.59	0.71	2.32
	Enlargements	0.29	0.51	0.29	0.56	1.32
	Loss of teeth					
	periodontitis	–	0.10	0.10	0.59	0.66
	other causes	0.47	0.80	0.37	0.32	0.24
	Total:	1.32	1.71	1.34	2.17	4.54

[1] Third molars not included.

respects from the male group with regard to the fulfilment of the experimental conditions. First, practically all of the males ate their entire ration of sweet bread during Carbohydrate Study II, while about one third of the females consumed less than their

TABLE 24

Statistical analysis of the changes in caries activity in the male Bread Group. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	23.89	8	1 % > P > 0.1 %
The first three periods in relation to the last two	10.48	2	1 % > P > 0.1 %
The first three periods with one another	3.39	4	P > 50 %
The last two periods with one another	7.88	2	5 % > P > 1 %
The first four periods in relation to the last	20.58	2	0.1 % > P

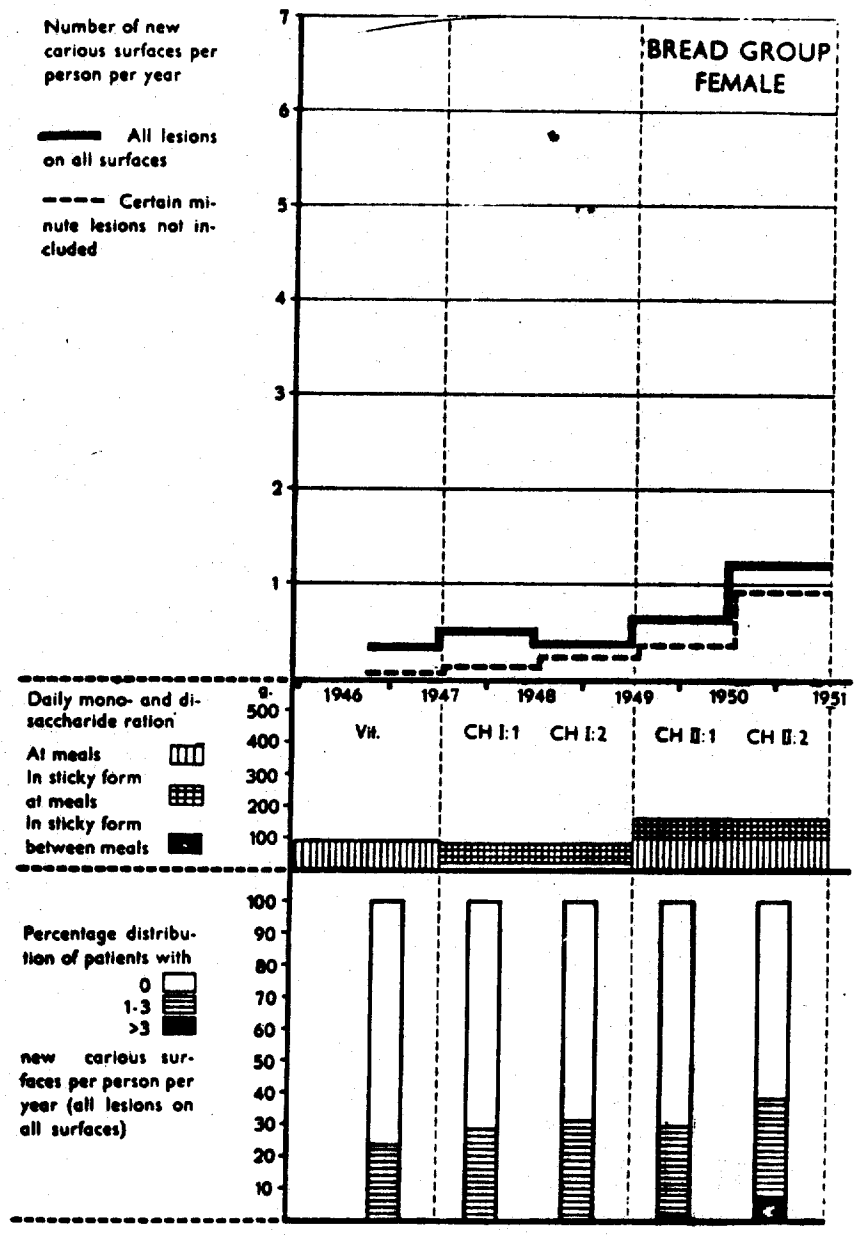
ration (Tables 23 and 25). Secondly, oral hygiene was much better in the female group. Of the 42 females, 38 had their teeth brushed regularly, as compared with only 2 of the 41 males.

Summary

A group of patients consisting of 41 males and 42 females with an average age of 30.4 and 28.0 years, respectively, received sweet bread at one meal every day for 2 years: the consumption of this bread did not produce a demonstrable increase in caries activity. During the following 2 years the same sort of sweet bread was served at all meals (breakfast, dinner, afternoon coffee and supper). During the second year of this 2-year period an increase was observed in the mean caries activity. The increase recorded was statistically significant for the males, but not for the females. The female group differed from the male group in that their oral hygiene was better and secondly they did not eat so much of their ration. Consumption of food between meals was forbidden.

THE CHOCOLATE GROUP

Forty-seven male patients with an average age of 29.1 years at the commencement of the study received an extra daily ration of 300 g. refined sugar in solution at meals during the Carbohydrate Study I i.e. the same as the Sucrose Group. At the beginning of Carbohydrate Study II the total amount of sugar served at meals



Conditions: Vit. G 3
CH I: 1 G 4+345 g. new sugar-rich bread at 1 meal
: 2 G 4+345 g. .. .. 1 ..
CH II: 1 G 5+345 g. .. .. all meals
: 2 G 5+345 g. .. ..

Fig. 10.

TABLE 25

Survey of the results in the Bread Group (female, 42 patients)

		Vitamin study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		—	New high-sugar bread 345 g at one meal		substitutes basic diet bread	
	Number of patients who consumed					
	> the supplement				—	—
	= " "				26	29
	< " "				16	13
	0 " "				—	—
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.24	0.48	0.31	0.52	0.98
	Per 148 surfaces	0.33	0.52	0.38	0.64	1.19
	Ibid. but Cwd., Rtg., Ml excl.	0.07	0.12	0.26	0.36	0.95
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.05	0.17	0.05	0.19	0.33
	Mandib. inc.	—	0.10	0.02	0.05	0.12
	Maxill. mol.	0.16	0.17	0.19	0.19	0.31
	Maxill. inc.	0.02	0.05	0.05	0.10	0.24
	Previously intact teeth	0.17	0.33	0.24	0.33	0.22
	Cementum caries	—	0.02	0.04	0.07	0.07
	Total surface losses per 148 surfaces					
	Primary caries	0.33	0.52	0.38	0.64	1.19
	Enlargements	0.45	0.26	0.76	1.48	0.74
	Loss of teeth					
	periodontitis	0.10	0.10	—	—	0.29
	other causes	0.05	0.24	0.28	0.22	0.48
	" Total:	0.93	1.12	1.43	2.33	2.69

[1] Third molars not included.

TABLE 26

Statistical analysis of the changes in the caries activity in the female Bread Group. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	5.83	8	P > 50 %

was reduced from 330 g. to 110 g. From the start of the Carbohydrate Study II the patients received 30 g. sugar between meals as 65 g. milk chocolate. This chocolate ration was served as 4 portions between the meals. The total carbohydrate consumption was almost the same during both study periods.

Caries Activity

The mean caries activity, as calculated on the basis of Westin's index surfaces and all surfaces was low during Carbohydrate Study I. The mean increased with the consumption of chocolate. This was also reflected by the distribution of the patients according to caries activity. Thus there was an increase in the number of patients with 3 or more new carious surfaces and a decrease in the number of those with no new carious surfaces. It is clear from Fig. 11 and Table 27 that the lesions occurring on introduction of the chocolate ration, were mainly of the initial types.

Statistical Analysis and Discussion

The χ^2-analysis (Table 28) of the various periods showed that there was a significant increase in caries activity during at least one of the 5 years. Further analysis showed that the difference was mainly attributable to the change in conditions between Carbohydrate Study I and II when chocolate was introduced. There was, however, an increase in caries activity also in the Control Group at the commencement of the Carbohydrate Study II which was attributed to the increase in carbohydrate consumption when the conditions were altered to resemble ordinary Swedish standards. This merited closer analysis of the activity and data in both the Control Group and the Chocolate Group in order to judge whether the greater increase in the Chocolate Group was related to a difference, in the caries tendency of the patients or to the difference in the conditions.

COMPARATIVE ANALYSIS OF THE CHANGES IN THE CHOCOLATE AND CONTROL GROUPS

As the patients have by now been studied for a further year (1951—1952) with unchanged consumption of sugar and for a subsequent year (1952—1953) since the withdrawal of chocolate,

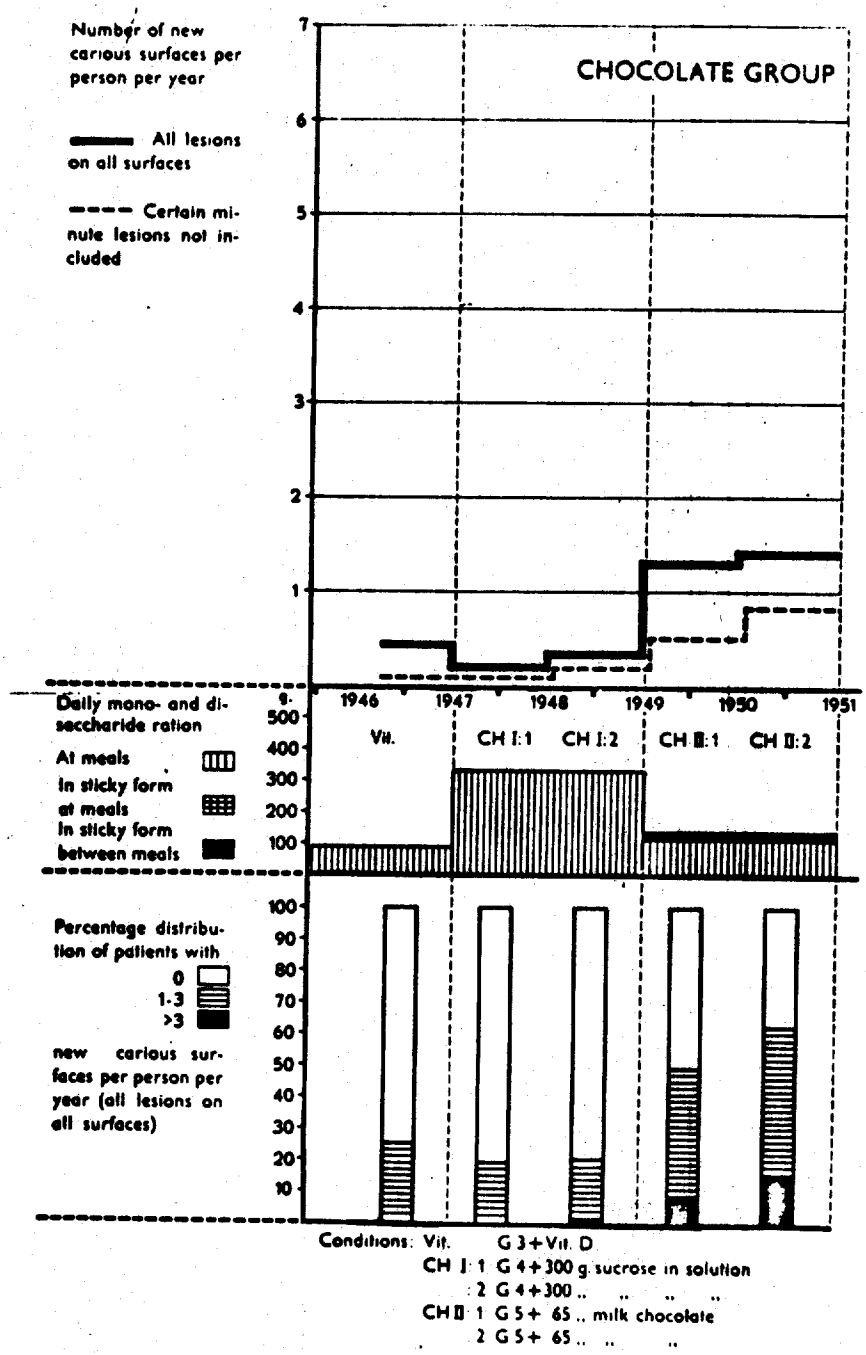

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
CHOCOLATE GROUP
7
6
5
4
3
2
1
1946
1947
1948
1949
1950
1951
Vit.
CH I:1
CH I:2
CH II:1
CH II:2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
g.
500
400
300
200
100
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
100
90
80
70
60
50
40
30
20
10
Conditions: Vit. G 3+Vit. D
CH I: 1 G 4+300 g. sucrose in solution
2 G 4+300 .. ..
CH II: 1 G 5+ 65 .. milk chocolate
2 G 5+ 65 .. .. ..

Fig. 11.

TABLE 27

Survey of the results in the Chocolate Group. (47 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		Vit. D	Sucrose in solution 200 g		Milk chocolate 65 g in 4 portions between meals	
	Number of patients who consumed					
	> the supplement				—	—
	= " "				46	47
	< " "				1	—
	0 " "				—	—
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.40	0.19	0.23	1.11	1.23
	Per 148 surfaces	0.45	0.23	0.36	1.30	1.40
	Ibid. but Cwd., Rtg., MI excl.	0.13	0.13	0.19	0.51	0.83
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.02	0.09	0.11	0.55	0.25
	Mandib. inc.	0.04	0.06	—	0.17	0.11
	Maxill. mol.	0.21	0.09	0.09	0.28	0.45
	Maxill. inc.	0.04	—	0.06	0.15	0.43
	Previously intact teeth	0.19	0.13	0.09	0.68	0.53
	Cementum caries	0.02	—	0.04	0.06	0.21
	Total surface losses per 148 surfaces					
	Primary caries	0.45	0.23	0.36	1.30	1.40
	Enlargements	0.47	0.62	0.70	0.87	1.06
	Loss of teeth					
	periodontitis	—	0.04	0.26	0.02	—
	other causes	0.13	0.06	0.19	0.21	0.23
	Total:	1.04	0.96	1.51	2.40	2.70

[1] Third molars not included.

the data obtained throw still further light on the effect of the consumption of chocolate on caries activity (Table 29). A more detailed analysis was made of the data obtained in this group. The caries activity recorded during the three years with chocolate con-

TABLE 28

Statistical analysis of the changes in caries activity in the Chocolate Group. Main material. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	31.94	8	0.1 % > P
The first three periods in relation to the last two	29.70	2	0.1 % > P
The first three periods with one another	1.42	2	P > 50 %
The last two periods with one another	1.06	2	P > 50 %

sumption was correlated with the ages of the persons and the caries activity noted during the year before the chocolate study was started, below called the pre-chocolate period. The years during which chocolate was consumed, have been called the experimental period. This applies both to the Control and Chocolate Groups.

Because of individual differences in disposition to caries, so far data have been given only for persons who partook in the entire investigation. In the analysis below, the data refer to all persons who partook in at least one of the study years and who filled the requirements given in the section "Material". This procedure was considered justified, because here the individual differences in disposition to caries was compensated in a different way. As a result of this re-grouping of the material the figures below differ, though slightly, from those given in the preceding section. However, persons for whom data were available only for the pre-chocolate period were not included.

Table 30 gives the average number of new carious surfaces per person per year and the relative number of persons who showed no new carious surfaces during the observation period.

CHANGES IN THE CONTROL GROUP

During the pre-chocolate period the caries activity in the Control Group was low, but then increased during the following four years to an average of 0.65 new carious surface per person per year. The

TABLE 29

Survey of the studies in the Chocolate and Control Groups
Total material 1948—1953

Period	Code of the periods in the extended analysis	Diet		Sugar consumption g per person per day					
		Chocolate Group	Control Group	At meals		Between meals		Total	
				Chocol.	Contr.	Chocol.	Contr.	Chocol.	Contr.
Carbohydr. Study I 2nd year	Pre-chocolate period = Pre	G_4 + 300 g sugar in solution	G_4 without carbohydrate additions	330	30	—	—	330	30
Carbohydr. Study II 1st year	Experimental period I	G_5 + 65 g milk chocolate	G_5 without carbohydrate additions	110	110	30	—	140	110
2nd year	» » II	»	»	110	110	30	—	140	110
3rd year	» » III	»	»	110	110	30	—	140	110
4th year	Post-chocolate period = Post	G_5 without carbohydrate additions	»	110	110	—	—	110	110

TABLE 30

Caries activity in the Chocolate and Control Groups
Total material 1948—1953

Period[1]	Number of persons		Average number of new carious surfaces per person per year[2]		Percentage of persons with no new carious surfaces	
	Chocolate Group	Control Group	Chocolate Group	Control Group	Chocolate Group	Control Group
Pre	54	72	0.46	0.12	70	89
I	65	82	1.58	0.57	45	70
II	74	77	1.54	0.60	41	75
III	61	66	1.20	0.83	57	67
Post	59	64	0.78	0.64	69	70

[1] Abbreviations for periods: see Table 29.
[2] Per 148 surfaces.

variation in the figures for the last four years was in line with the fact that the experimental conditions were on the whole constant during the time studied here, *except for the pre-chocolate period.*

Table 31 gives a more detailed survey of the changes. Those persons for whom data were available for two successive years were grouped according to caries activity in each period. Each person was assigned to one of the following three activity classes:

A = no new carious surfaces during the period.
B = 1—2 new carious surfaces during the period.
C = 3 or more new carious surfaces during the period.

Thus 3×3=9 combinations of caries activity classes were distinguished for two years.

On comparison of the pre-chocolate period and the experimental year I in the Control Group it will be apparent that of 72 persons, 52 belonged to the same activity class during both observation periods (AA, BB and CC). Of the other twenty, 16 were graded up from lower to higher activity classes (AB, AC, BC), and 4 were graded down. With random fluctuations only, the last two figures probably counterbalance one another. The disproportion between the number graded up and the number graded down denotes a significant change in caries activity from the pre-chocolate period

TABLE 31

Distribution of the patients according to the number of new carious surfaces during each of two consecutive years
Total material 1948—1953

Combination of activity classes[2]	Chocolate Group				Control Group			
	Consecutive years[1]				Consecutive years[1]			
	Pre I	I II	II III	III Post	Pre I	I II	II III	III Post
A A	20	14	15	25	49	47	41	30
A B	13	11	6	2	12	8	4	3
A C	5	3	1	1	3		4	2
B A	7	7	15	7	4	9	1	6
B B	4	10	8	5	3	8	7	4
B C	4	7	2	1	1	3	3	1
C A	1	3	4	3	—	1	1	5
C B	—	6	2	3	—	—	—	4
C C	—	2	6	2	—	3	3	1
Total	54	63	59	49	72	74	64	56
Unchanged	24	26	29	32	52	58	51	35
+	22	21	9	4	16	6	11	6
—	8	16	21	13	4	10	2	15

[1] Abbreviations for periods: see Table 29.
[2] A: No new carious surfaces
B: 1—2 new carious surfaces
C: 3 or more new carious surfaces.
Combination B C: Activity class B during the first, activity class C during the second of the two consecutive years.

to experimental year I. No difference in caries activity was found between experimental years I and II. From experimental year II to III there occurred a change in the Control Group, in that 11 persons were graded up to a higher activity class and only 2 were graded down. This suggests a probable difference. Between experimental year III and the post-chocolate period the activity again diminished.

In the Control Group then an increase in caries activity was recorded from the pre-chocolate period to the experimental period and among the experimental years, experimental year III might

for some unknown reason be characterised by a somewhat lower caries activity than experimental years I and II and the post-chocolate period.

COMPARISON BETWEEN THE CHOCOLATE GROUP AND THE CONTROL GROUP DURING THE PRE- AND POST-CHOCOLATE PERIODS

During the pre-chocolate period and possibly also during the post-chocolate period the Chocolate Group showed a higher caries activity than did the Control Group. During the pre-chocolate period, however, the diet was not the same for both groups, the diet of the Chocolate Group containing more sugar (300 g sucrose in solution added) than that of the Control Group.

CARIES ACTIVITY IN THE CHOCOLATE GROUP DURING THE EXPERIMENTAL PERIODS

As is apparent from the figures given above, the caries activity in the Chocolate Group increased from the pre-chocolate period to the experimental period I. Table 30 shows that the number of new carious surfaces increased from 0.46 per person to 1.58, while the number of caries inactive persons fell from 70 per cent to 45 per cent. According to Table 31, only 24 of the 54 persons remained in the same activity class, while 22 were graded up and 8 were graded down. The disproportion between these figures was in accordance with the definite difference in the experimental conditions.

As far as the Chocolate Group is concerned, no changes other than those due to chance, occurred between periods I and II. Between periods II and III there was a decrease (during which time an increase was noted for the caries activity of the Control Group). From a statistical point of view, this decrease suggests the occurrence of a probable difference, despite the absence of any changes in dietary conditions. Table 31 shows that 9 persons were graded up into a higher activity class and 21 were graded down.

A decrease was demonstrated between experimental period III and the post-chocolate period, so that the figures for the Chocolate Group were then on the same level as those for the Control Group.

Only 4 persons were graded up, but 13 were graded down, which suggests a probable difference. On closer analysis, not accounted for here, of the activity data of the experimental periods in relation to those for the pre- and post-chocolate periods, the Chocolate Group was found to differ significantly from the Control Group.

Statistically significant differences imply that the random factors, any over- or under-registration of caries and any transient fluctuation in the caries activity of the individuals, could not by themselves have produced the differences observed. But the differences might possibly have been ascribable to differences in disposition to caries in combination with any differences due to chance. As a certain difference in caries activity (after elimination of differences in age) was found between the Chocolate Group and the Control Group during the pre-chocolate period, it might be suggested that the difference found during the experimental periods was due to the persons in the Chocolate Groups really having had a greater disposition to caries than those in the Control Group. This difference would then, however, have been masked by random deviations during the pre- and post-chocolate periods.

In order to check any influence of any such objection on the results, the caries activity during the experimental periods must be studied against the background of the observed caries activity during the pre-chocolate period.

A more detailed analysis can be made on the basis of data given in Table 32, where the material is divided into two age groups, those born before respectively after 1920. In each of the age groups the patients are divided according to the presence or absence of caries activity during the pre-chocolate period. Only those persons were now included that were observed during all three experimental periods. If the caries activity in the Chocolate Group and the Control Group had been equal in all four subgroups i.e. those with and without caries in each of the age classes the distribution, according to caries activity, of the patients belonging to the Chocolate Group would have been:

Activity class no new carious surfaces: expected number 16.1 persons, observed number 8;

Activity class 1—5 new carious surfaces: expected number 17.4 persons, observed number 23;

TABLE 32

Distribution according to total caries activity during chocolate consumption.

The material is grouped by age and caries activity during the pre-chocolate period. Patients partaking in the study during the periods Pre and I—III

Group etc.		Number of patients grouped according to number of new carious surfaces during periods I—III				Total numbers of new carious surfaces during periods	
		Total	0	1—5	6—	Pre	I—III
Chocolate Group	Y —	14	1	7	6	0	86
	Y +	8	—	4	4	14	50
	O —	16	7	8	1	0	21
	O +	5	0	4	1	6	18
Total		43	8	23	12	20	175
Control Group	Y —	8	7	—	1	0	10
	Y +	3	2	1	—	3	3
	O —	40	24	13	3	0	62
	O +	5	2	2	1	6	23
Total		56	35	16	5	9	98

Y = younger, born in 1920 or later
O = olderly, born in 1919 or earlier
— = inactive during the pre-chocolate period
+ = active during the pre-chocolate period.

TABLE 33

Caries activity during consumption of chocolate
Total material 1948—1953

(Number of new carious surfaces per person per year)

Age Group	Chocolate Group	Control Group
Below 30 years	1.98	0.67
Above 30 years	0.77	0.65

Activity class 6 or more new carious surfaces: expected number 9.5, observed number 12.

These differences are too great to be ascribed to chance alone. The difference between the expected number and the number observed in the inactive class was 8.1 with a standard error of 2.2 units.

A possibly greater disposition to caries in the Chocolate Group under otherwise equal conditions can thus not have been the cause of the differences between the Control Group and the Chocolate Group during the actual experimental period, because now all comparisons took into account earlier manifest caries activity.

The increase in caries activity in the Chocolate Group during the three experimental years must thus be ascribed to the consumption of chocolate. The effect varied with the age of the patients and was only very slight in the higher age classes but distinct in the lower age classes (Table 33). Whether this difference is due to a general increase in caries resistance with age or whether only certain teeth or surfaces are involved by caries during the consumption of chocolate, and whether these teeth or surfaces are seldom intact in higher age classes, requires further investigation.

PARALLEL STUDY OF THE RELATIONSHIP BETWEEN THE CONSUMPTION OF CHOCOLATE AND CARIES ACTIVITY

A parallel study was carried out on the personnel at Vipeholm Hospital. The study covered the period from Febr. 1, 1946 to Aug. 30, 1947.

The series consisted of altogether 108 male and female volunteers with an average age of 34 years. The teeth were examined and the changes noted were recorded in exactly the same way as that used in the main investigation. The persons were examined on 5 occasions.

The series was divided into four groups:

Ordinary diet plus wheat bread
" " " Swedish hard rye bread
" " " wheat bread + chocolate
" " " Swedish hard rye bread + chocolate

Those belonging to the chocolate groups received a bar of milk chocolate (54 g.) daily, which they were allowed to eat when they liked during the day. Most of the persons reported that they ate their ration in small portions between meals.

In this connection it should, however, be stressed that chocolate was practically unavailable in the shops at that time and it is quite possible that part of the chocolate ration was consumed by other family members not partaking in the study. As in other similar investigations in non-institutionalised material it was not possible to control the conditions in an objectively satisfactory manner, especially as the experimental period was 1.5 years.

The mean number of new carious surfaces for those who received no chocolate was 0.6, the corresponding figure for those who received chocolate being 0.7. Statistical analysis showed that this difference was ascribable to chance alone. The frequency of enlargements of already existent cavities was higher in the chocolate group: this difference was statistically significant. It must be borne in mind that these figures refer to a period of the investigation, when the registration method was not stabilised. This investigation shows the difficulties in studies of dental caries activity under uncontrolled conditions. Of definite disadvantage is also the lack of a pre-experimental registration of the dental caries activity.

Summary

A male group received a diet containing an ordinary amount of carbohydrates but an amount of sugar corresponding to twice the average Swedish consumption. Caries activity, which was known to be fairly low before the commencement of the study, remained low. The following 3 years the patients were placed on an ordinary Swedish diet and received a daily ration of 65 g. milk chocolate, which they ate between meals.

This change in the experimental conditions was accompained by a statistically significant increase in caries activity. However, another change in the diet was made when the chocolate consumption started.

Therefore an extended analysis was made of all patients in the Chocolate Group. This analysis comprised also those patients not

included in the main material. The correlation between the consumption of 65 g. chocolate per person per day between the meals was studied in 87 patients for 3 years. At the same time 95 patients were studied in the Control Group. It was shown in younger (< 30 years) individuals that the consumption of chocolate was accompanied by a dental caries activity 3 times greater than before. This difference was found between the periods with and without chocolate consumption in the same individuals as well as between the Chocolate and Control Group. In older individuals (> 30 years) the increase was insignificant.

In a parallel investigation on a group of the personnel (mean age 34 years) at the hospital, no difference could be observed between a group who ate 54 g. chocolate daily and a control group who ate no chocolate.

It must be pointed out, however, that in this study of the personnel the control of the chocolate consumption was not satisfactory.

THE CARAMEL GROUP

Sixty-two patients with an average age of 35.6 years at the beginning of the study received 345 g. of stale, sugar-rich bread at one meal a day during the first year of Carbohydrate Study I. This study was, however, interrupted in order to check the preliminary results obtained in the 24-toffee Groups at the end of the first year of Carbohydrate Study I.

It was therefore not possible to draw any conclusions from this study about the effect of stale, sugar-rich bread.

During the second year of Carbohydrate study I and the first year of Carbohydrate Study II the patients received a daily ration of 22 caramels of an ordinary commercial type. It is clear from Table 34 that 3 of 62 patients consumed only part of the ration. The ration corresponds to 100 g. carbohydrates, of which different kinds of sugar represent 70 g.

During the first year of this study the caramels were served in two portions, one immediately after breakfast and one immediately after lunch. According to the personnel, two thirds of the patients consumed most of the caramels as soon as they received them. Many of them put more than one caramel at a time in

the mouth. During Carbohydrate Study II the caramels were served between meals in 4 portions of 5 to 6 caramels each.

In order to bring the total consumption of sugar up to the level in the Sucrose Group during Carbohydrate Study I, the patients received not only caramels, but also 200 g. sucrose in solution. This extra sugar ration was withdrawn during Carbohydrate Study II. The change from Carbohydrate Study I to Carbohydrate Study II thus implied no change in the caramel ration, but a decrease in the total sugar consumption from 300 g. to 170 g.

The only purpose of this study was to find out whether the consumption of caramels is followed by an increase in caries activity or not. As soon as a supplementary examination performed during the first year of Carbohydrate Study II showed that caries activity had undoubtedly increased, the caramel ration was replaced by an iso-caloric quantity of fat during the remaining part of Carbohydrate Study II. Thus the patients received caramels only during some 6 months of Carbohydrate Study II.

The data of caries activity during the first half year of Carbohydrate Study II, expressed as the number of new carious lesions per person per year, could have been obtained by multiplying the registered number of new cavities during the first half year of Carbohydrate Study II by two. Such calculations were, however, regularly avoided in view of the fairly great error of the method of registration of dental caries. The figures for caries activity, as recorded in the tables and diagrams during the first year of Carbohydrate Study II therefore indicate the caries activity for the last six months of the period during which the patients received a caramel ration, and the first six months of the postactive period.

The figures for the activity during the first year of Carbohydrate Study II are therefore lower than the figures that would have been obtained, if the consumption of caramels had continued for the whole year. Therefore such a value cannot be used for the comparison of caries activity recorded under other conditions in the other groups. This again emphasises the importance of intra-group comparisons in the analysis of the data obtained in the present investigation.

After withdrawal of the caramel ration, this group received the

same food as the Control Group, and may be classified as a post-active group.

Caries Activity

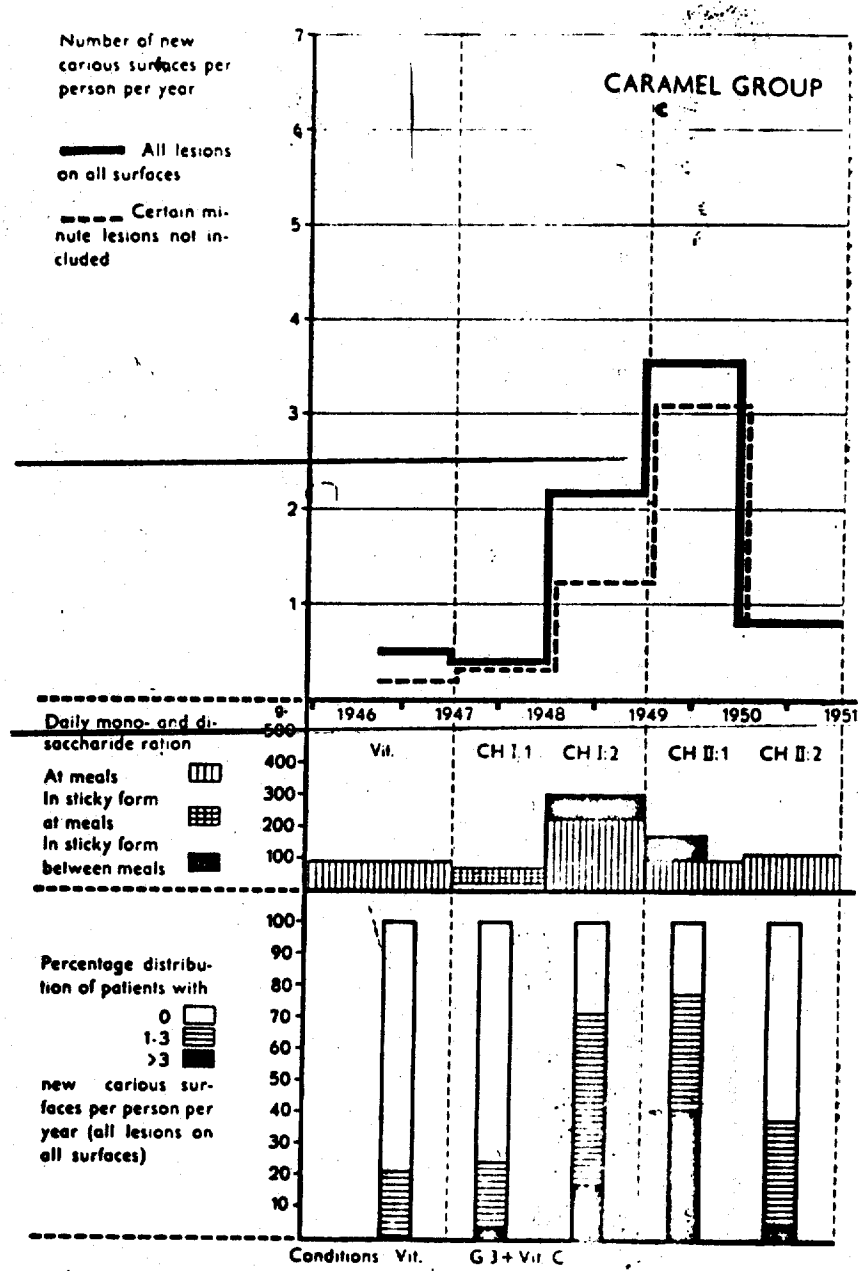
The introduction of the caramel ration was accompanied by an increase in caries activity (Table 34 and Fig. 12). This increase was, however, more gradual than in the other sweet groups. After withdrawal of the caramel ration, caries activity returned to a level corresponding to that recorded during the Vitamin Study and the first year of Carbohydrate Study I. The changes in the mean caries activity were accompanied by changes in the distribution of the number of newly carious surfaces (Fig. 12).

Statistical Analysis

The χ^2-analysis (Table 35) showed highly significant differences between the period during which the patients received caramels and the rest of the entire period of the investigation. A probable difference ($5\% > P > 1\%$) was also found between the two periods during which the patients received caramels. This latter difference was also reflected in the average caries activity and in the shape of the distribution (Table 34 and Fig. 12). The mean values for caries activity during the second year of Carbohydrate Study II (postactive period) were slightly higher than during the Vitamin Study and the first year of Carbohydrate Study I. The χ^2-analysis also showed a probable difference between these periods, depending on the conditions prevalent during the second year of Carbohydrate Study II. No difference was demonstrable between the Vitamin Study and the first year of Carbohydrate Study I.

Discussion

The statistical analysis distinctly showed that the introduction of the caramel ration was accompanied by an increase in caries activity and that this increase diminished on replacement of the caramel ration with an isocaloric amount of fat. A noteworthy observation was the delay in the rise of the curve for caries activity: a rise that appeared despite the fact that the values recorded



Conditions Vit. G 3 + Vit C
CH I: 1 G 4 + 345 g stale sugar-rich bread at 1 meal
: 2 G 4 + 22 caramels + 200 g sucrose in solution
CH II: 1 G 5 + 22 caramels
: 2 G 5 + 40 g margarine

Fig. 12.

TABLE 34

Survey of the results in the Caramel Group. (62 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		Vit.C	Stale, high-sugar bread 345 g at one meal	22 caramels 155 g between meals + 200 g sugar in sol.	155 g in 4 portions between meals (10 % red. of basic diet)	No suppl. carbo-hydrates Cal. com-pensa-tion 40 g marga-rine
	Number of patients who consumed					
	> the supplement			—	1	
	= " "			59	58	
	< " "			3	3	
	0 " "			—	—	
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.48	0.34	1.92	3.02	0.66
	Per 148 surfaces	0.50	0.42	2.16	3.55	0.81
	Ibid. but Cwd., Rtg., Ml excl.	0.23	0.35	1.24	3.06	0.84
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.08	0.12	0.57	0.71	0.16
	Mandib. inc.	0.08	0.08	0.69	1.27	0.15
	Maxill. mol.	0.22	0.08	0.26	0.46	0.13
	Maxill. inc.	0.10	0.08	0.42	0.63	0.24
	Previously intact teeth	0.13	0.18	1.05	1.60	0.21
	Cementum caries	—	0.06	0.71	1.64	0.45
	Total surface losses per 148 surfaces					
	Primary caries	0.50	0.42	2.16	3.55	0.81
	Enlargements	0.34	0.95	1.00	1.31	1.15
	Loss of teeth					
	periodontitis	0.23	0.18	0.16	0.29	0.18
	other causes	0.24	0.15	0.14	0.37	0.30
	Total:	1.31	1.69	3.47	5.52	2.44

[1] Third molars not included.

TABLE 35

Statistical analysis of the changes in caries activity in the Caramel Group. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	85.48	8	0.1 % > P
The first two periods and the last in relation to the third and fourth	64.07	2	0.1 % > P
The third period with the fourth	9.10	2	5 % > P > 1 %
The first two periods in relation to the fifth	7.11	2	5 % > P > 1 %
The first period with the second	1.04	2	P > 50 %

during the first year of Carbohydrate Study I were under-representative by the method of calculation used.

In the interpretation of this phenomenon it should be remembered

1) that during Carbohydrate Study I most of the patients consumed their caramel ration immediately after the meals, while during Carbohydrate Study II, the caramels were consumed mainly between meals,
2) that the diet prescribed for Carbohydrate Study I was not the same as that for Carbohydrate Study II, and
3) that the time necessary for the initiation and development of a carious lesion may be rather long.

Table 34 also shows interesting changes in the localisation of new carious lesions. While caries activity was relatively high in the maxillary and mandibular premolars and molars of the groups accounted for before, the highest activity recorded in association with the consumption of caramels was seen in the incisors and canines of the lower jaw. The frequency of cavities in the cementum or cemento-enamel junction was high in this group. This finding must, however, be judged in the light of the fact that in this group of patients the average age was highest.

These observations may either suggest that the type of caries observed differed from the ordinary one or that this change in the distribution is simply an accompaniment of increased caries activity. It should be pointed out that even if the analysis of the increase in activity is based only on those cavities that appeared in the enamel, the changes in this group will still be statistically significant.

Summary

The consumption of 155 g. (=70 g. sugar) of sweets between meals by 62 males with an average age of 34.9 years was accompanied by a statistically significant increase in caries activity. On withdrawal of the caramel ration without any other reduction in carbohydrates, caries activity diminished to its previous level. The decrease occurred while the patients were on a dietary regime corresponding to ordinary Swedish standard diet with a consumption of refined sugar at meals corresponding to the household consumption.

8-TOFFEE GROUP

40 male patients with an average age of 26.3 years at the beginning of the investigation, were placed on a low-carbohydrate, high-fat diet during the first year of Carbohydrate Study I. The patients then received 60 g. sweets *i. e.* 8 toffees per day for 3 years. During Carbohydrate Study I half of the ration was served at breakfast and the other half at lunch. According to the personnel, most of the patients ate their ration immediately after the meals. During Carbohydrate Study II the ration, still 8 toffees, was served only between meals. This ration corresponded to 50 g. carbohydrates, 40 g. of which consisted of different kinds of sugar.

During Carbohydrate Study I the patients also received 250 g. sucrose in solution in order to bring the sugar intake to the same level as in the other groups. For the same reason, during Carbohydrate Study II the patients received 25 g. sugar in solution at meals so that the total sugar ration was the same as the average Swedish consumption. Thus, the change from Carbohydrate Study I to Carbohydrate Study II involved a decrease in the total sugar consumption, but not of the sugar consumed between meals. The con-

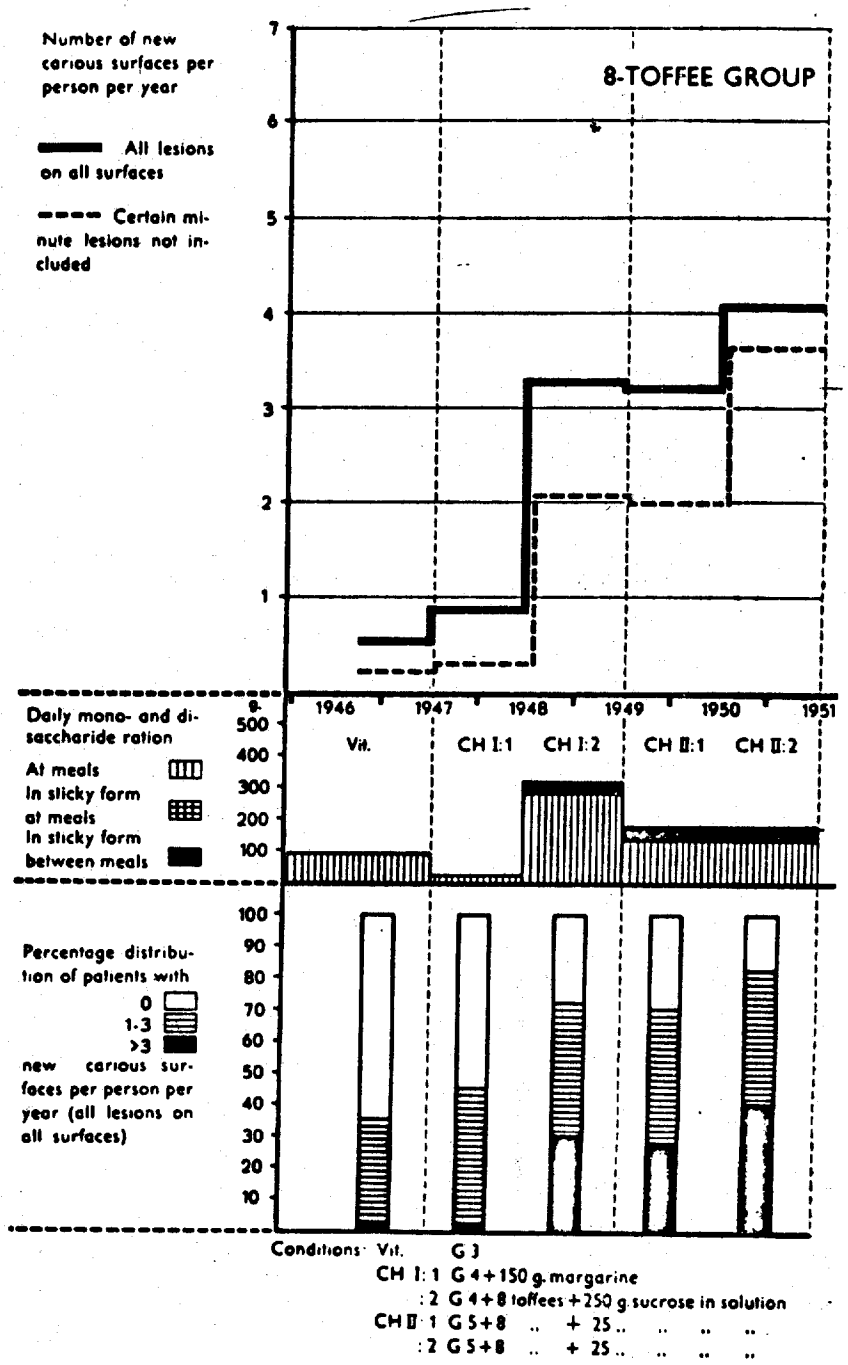

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
8-TOFFEE GROUP
7
6
5
4
3
2
1
1946
1947
1948
1949
1950
1951
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
g.
500
400
300
200
100
Vit.
CH I:1
CH I:2
CH II:1
CH II:2
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
100
90
80
70
60
50
40
30
20
10
Conditions: Vit. G 3
CH I: 1 G 4 + 150 g. margarine
: 2 G 4 + 8 toffees + 250 g. sucrose in solution
CH II: 1 G 5 + 8 .. + 25 .. ..
: 2 G 5 + 8 .. + 25 .. ..

Fig. 13.

TABLE 36

Survey of the results in the 8-toffee Group. (40 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
			No suppl. carbohydrates Cal. compensation 150 g margarine	8 toffees 60 g between meals + 250 g sucrose in sol.	60 g in 4 portions between meals + 25 g sucrose in sol.	
	Number of patients who consumed					
	> the supplement			—	—	—
	= " "			34	32	33
	< " "			5	3	1
	0 " "			1	5	6
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.50	0.80	3.03	2.78	3.58
	Per 148 surfaces	0.55	0.88	3.28	3.20	4.05
	Ibid. but Cwd., Rtg., Ml excl.	0.23	0.30	2.08	2.00	3.63
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.10	0.28	0.88	0.90	0.90
	Mandib. inc.	0.03	0.13	0.75	0.48	0.65
	Maxill. mol.	0.20	0.35	0.68	0.98	1.02
	Maxill. inc.	0.18	0.05	0.78	0.53	1.13
	Previously intact teeth	0.30	0.38	1.48	1.75	1.65
	Cementum caries	—	—	0.38	0.20	0.88
	Total surface losses per 148 surfaces					
	Primary caries	0.55	0.88	3.28	3.20	4.05
	Enlargements	0.38	1.25	1.15	2.80	2.13
	Loss of teeth					
	periodontitis	0.10	—	0.53	0.35	1.35
	other causes	0.25	0.45	0.28	0.48	0.53
	Total:	1.28	2.58	5.23	6.83	8.05

[1] Third molars not included.

trol of the conditions in this group showed that some patients ate only part of their sweet ration. Therefore the true average consumption was about 45 g. of sweets.

Caries Activity

The mean caries activity recorded with the patients on a low-carbohydrate, high-fat diet (Table 36 and Fig. 13) was, if anything, higher than during the Vitamin Study. The marked reduction in the carbohydrate content and especially the sugar content of the diet had thus not depressed caries activity to nil in these patients. The introduction of sweets was accompanied by a marked increase in caries activity, which reached a level of 3 new carious surfaces per year. It was noteworthy that this level was reached already during the first year of the trial, when the initial cavities were also taken into account. The distributions of the patients with regard to caries activity showed pronounced changes (Fig. 13 and Table 11). As in the caramel studies, the frequency of major cavities did not increase so quickly as that of the minor ones. When caries activity was evaluated on the basis of definite cavities the increase in dental caries was most pronounced during the third year of sweet consumption.

Statistical Analysis

The χ^2-analysis showed that the consumption of toffees was accompanied by a statistically significant increase in the caries activity (Table 37). The individual years before respectively during

TABLE 37

Statistical analysis of the changes in caries activity in the 8-toffee Group. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	38.02	8	0.1 % > P
The first two periods in relation to the last three	34.67	2	0.1 % > P
The first period with the second	0.92	2	P > 50 %
The last three periods with one another	2.12	4	P > 50 %

the sweet consumption did not differ statistically from one another.

Discussion

The reason why caries activity did not appreciably decrease when the patients were on a low-carbohydrate, high-fat diet might be, that the observation time was too short or that the susceptibility to caries was greater in this group than in the rest of the material. This is supported by the average age of the group being lower than that of the others. Judging by the observations made in this group, the consumption of toffees between meals was accompanied by a definite increase in caries activity. This increase was largest for the molars and premolars, although it was fairly high for the incisors and canines. This is discussed further on page 344.

Summary

40 males with an average age of 26.3 years at the commencement of the study were given a low-carbohydrate, high-fat diet for 1 year. Practically no refined sugar was used in the preparation of the food. This diet had no demonstrable effect on caries activity, which was low from the beginning. With the patients on the same basic diet, but with 8 toffees served between meals for 3 years, the caries activity increased. This increase was noted during the first year. When calculated on the basis of definite cavities, the caries activity showed a maximum during the third year (see Fig. 13).

During the last 2 years of the study period the patients were on an ordinary Swedish diet including an amount of sugar corresponding to the average Swedish consumption.

24-TOFFEE GROUPS

During Carbohydrate Study I and II, 48 males and 39 females with an average age of 31.0 and 31.1 years at the beginning of the investigation were studied under identical conditions. During the Vitamin Study the males had received a dietary supplement of 1 mg. fluorine as bone meal (1.8 g.), which was spread on bread.

The females received an addition of calcium as the lactate and vitamins A, C and D.

During Carbohydrate Study I these patients were allowed to eat toffee practically *ad libitum*. However, for purpose of standardisation and control, the daily toffee ration was limited to 24 per patient. This amount corresponds to 120 g. sugars.

In these groups, too, the toffees were consumed both at and between meals. Table 38 shows that 12 of the 48 males did not eat any of their toffee ration during the second year, while 31 ate their entire ration. Two of the patients also ate toffees they received from other patients, while 3 did not consume all their ration. During the first year the actual amount each of the patients consumed was not checked.

Half of the females ate their entire ration. The remainder ate only a varying proportion. However, on the whole, the consumption of toffees by females was greater, because only 2 of them did not eat any toffee at all (Table 40). The actual consumption of toffees during the first year was not evaluated with certainty in this group either.

At the time of these studies (1947—1949) the magnitude of the error of the method of recording caries had not been determined. Therefore the caries activity during that period was judged without regard to minor and doubtful cavities. Under these conditions it was doubtful still 1 year after the start of the study whether the increase in caries activity was statistically significant. This applies especially to the males. Therefore the study time was extended to the second year of Carbohydrate Study I.

According to the supplementary dental examinations, which were done at short intervals in these groups (Fig. 5, page 263), caries activity increased distinctly during the second year of Carbohydrate Study I. Therefore the experiment was stopped at the end of March 1949, *i. e.* earlier than originally intended. The toffees were replaced by an iso-caloric amount of fat. This additional fat ration was given until the beginning of Carbohydrate Study II, when the groups received basic diet G 5 + 40 g. margarine. During this latter period the patients were living under exactly the same conditions as the Control Group, but they have now been regarded as a special group, a post-active group.

Caries Activity

It is clear from Tables 38 and 40 and Figs. 14 and 15 that caries activity, when calculated on all types of carious lesions, increased strikingly already during the first year. As soon as the toffee ration was replaced by an iso-caloric amount of fat and the groups afterwards received the basic diet+fat during Carbohydrate Study II, the activity decreased to a level corresponding to that of the Vitamin Study. The changes in the distribution of the patients according to caries activity are striking. During the second year of Carbohydrate Study I all of the females were caries-active. The mean of the definite cavities showed an obvious lag. Caries activity calculated on definite cavities exceeds the activity as judged on all types of cavities in the female group during the latter part of the investigation. This calls for an explanation. If, for example, "caries without defect" (chalky spots) is not regarded as caries during the first year, many of these cavities enlarge and are then assignable to a type included in the Moulage System during the second year, and classed as new cavities, thus increasing the figures for the later periods.

Statistical Analysis

The χ^2-analysis (Tables 39 and 41) showed that the distributions for the two periods during Carbohydrate Study I differed essentially from the other distributions, which in turn did not differ significantly from one another.

Discussion

There is no doubt that this large consumption of toffee was associated with an increase in caries activity.

However, the two groups differed somewhat with respect to the development of this increase. The mean value for the caries activity was higher for the females; this was also apparent from the appearance of the distributions. Thus, all of the females were found to have active caries, while 10 of the males showed no new carious lesions during Carbohydrate Study I. However, 7 of these 10 patients had not consumed any of their ration during Carbohydrate Study I. It should be pointed out that during the last 18 months before the introduction of the toffee ration the males had

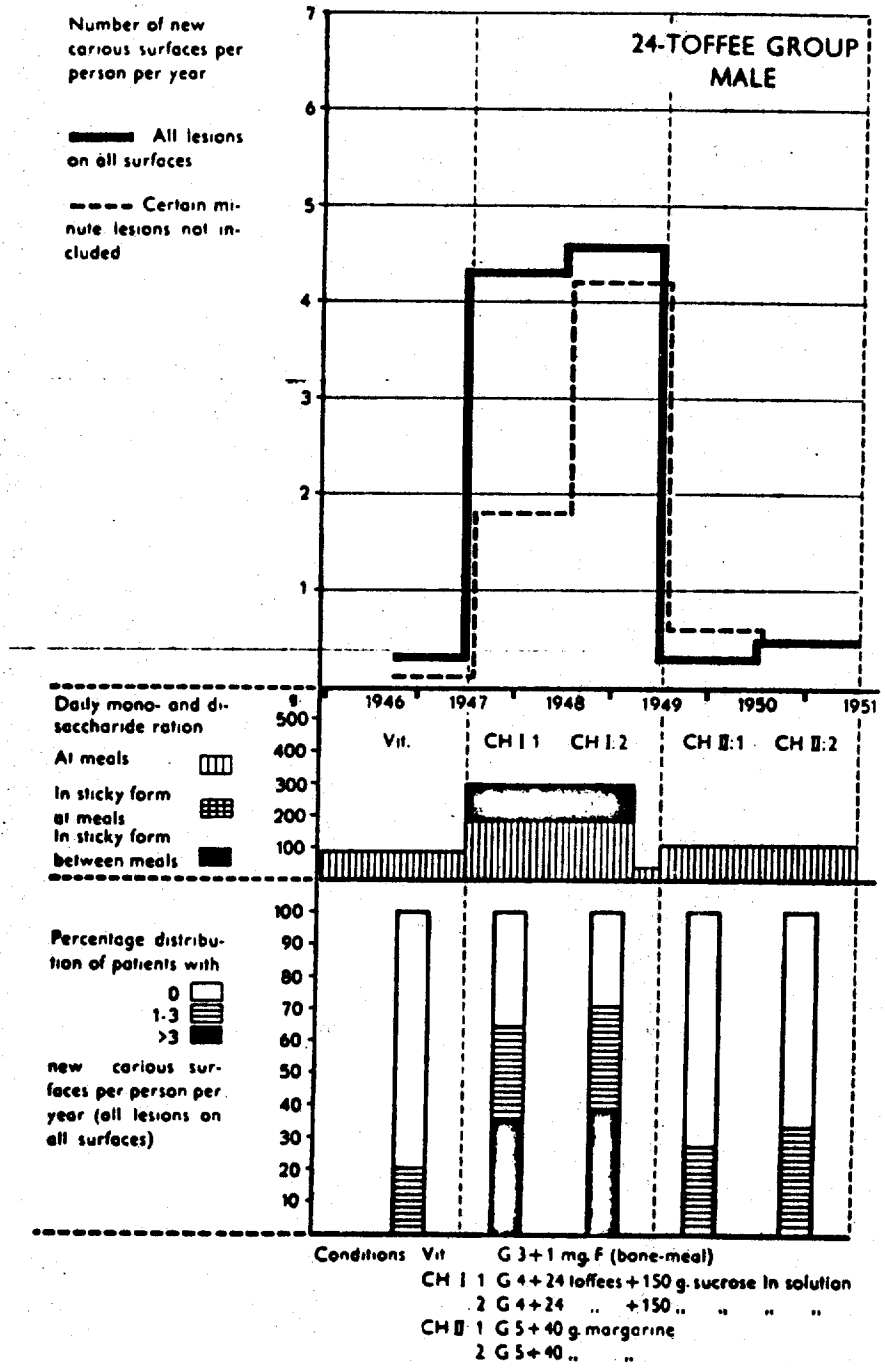

24-TOFFEE GROUP
MALE
Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
7
6
5
4
3
2
1
1946
1947
1948
1949
1950
1951
Vit.
CH I 1
CH I 2
CH II:1
CH II:2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
g
500
400
300
200
100
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
100
90
80
70
60
50
40
30
20
10
Conditions Vit G 3+1 mg. F (bone-meal)
CH I 1 G 4+24 toffees+150 g. sucrose in solution
2 G 4+24 " +150 " " " "
CH II 1 G 5+40 g. margarine
2 G 5+40 " "

Fig. 14.

TABLE 38

Survey of the results in the 24-toffee Group (male, 48 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		1 mg F (bone meal)	24 toffees 180 g between meals + 150 g sucrose in sol.		No. suppl. carbohydrates Cal. compensation 40 g margarine	
	Number of patients who consumed					
	> the supplement			2		
	= " "			31		
	< " "			3		
	0 " "			12		
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.33	3.90	4.15	0.21	0.40
	Per 148 surfaces	0.38	4.29	4.58	0.33	0.50
	Ibid. but Cwd., Rtg., MI excl.	0.08	1.81	4.19	0.58	0.50
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.15	1.13	0.95	0.04	0.13
	Mandib. inc.	0.04	1.15	1.63	0.02	0.06
	Maxill. mol.	0.15	0.67	0.85	0.11	0.11
	Maxill. inc.	—	1.02	0.79	0.06	0.10
	Previously intact teeth	0.17	2.88	1.60	0.06	0.13
	Cementum caries	—	0.69	1.17	0.02	0.06
	Total surface losses per 148 surfaces					
	Primary caries	0.38	4.29	4.58	0.33	0.50
	Enlargements	0.27	0.42	0.79	1.00	0.94
	Loss of teeth					
	periodontitis	0.08	0.27	0.60	0.44	0.85
	other causes	0.48	0.31	0.52	0.37	1.90
	Total:	1.21	5.29	6.50	2.15	4.19

[1] Third molars not included.

TABLE 39

Statistical analysis of the changes in caries activity in the male 24-toffee Group. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	66.56	8	0.1 % > P
The second and third periods in relation to the others	62.94	2	0.1 % > P
The first period in relation to the last two	0.50	1	P > 50 %
The second period with the third	0.07	2	P > 50 %
The fourth period with the fifth	2.80	1	10 % > P > 5 %

received bone meal. As this was consumed together with the bread ration, it naturally came into contact with the dental surfaces, and because of its fluorine content, it might, at least theoretically, have offered a certain amount of protection against caries.

As in the Caramel Group, caries activity was highest for the incisors and canines of the lower jaw. The preponderance was, however, slight and these teeth were by no means the only ones attacked. The frequency of cementum caries was high (25 per cent of all lesions), which may suggest the occurrence of a special type of caries. It has, however, most probably something to do with the high average age of the patients.

In the broad sense of the term, caries activity also includes loss of surfaces by enlargement of earlier existent cavities. If the process producing continual increase of the size of a cavity is of the same nature as the initial cause of the lesion, the number of intact surfaces lost because of the enlargement of existent cavities should increase with the loss of surfaces from primary caries. This should then be the case in the 24-toffee Groups, in which a great increase in the activity of primary caries was recorded. The loss of surfaces because of enlargement of existent cavities was less during the consumption of toffee than later during Carbohydrate Study II — the postactive period. This may be ascribed to the late initiation of this process, which might have become significant if the toffee consumption had not been interrupted. But the study period spanned almost 2 years, and if the consumption of sugar

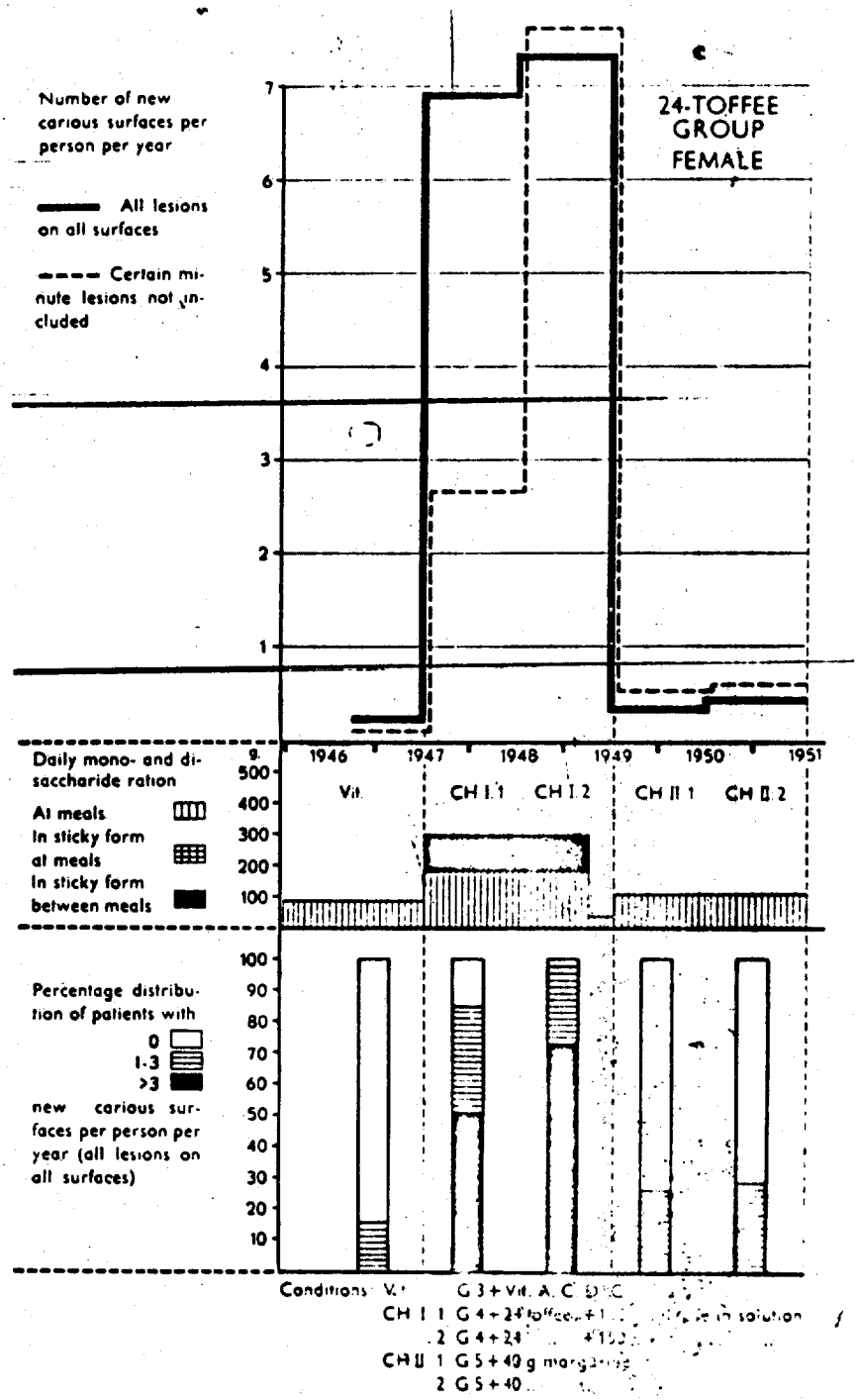

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
24-TOFFEE GROUP FEMALE
1946
1947
1948
1949
1950
1951
Vit.
CH I.1
CH I.2
CH II.1
CH II.2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions
CH I 1 G 4 + 24
2 G 4 + 24
CH II 1 G 5 + 40 g
2 G 5 + 40

Fig. 15.

TABLE 40

Survey of the results in the 24-toffee Group (female, 39 patients)

		Vitamin Study	Carbohydr. Study I		Carbohydr. Study II	
			1st year	2nd year	1st year	2nd year
		Vit. A + C + D; Ca	24 toffees 180 g between meals + 150 g sucrose in sol.		No suppl. carbohydrates Cal. compensation 40 g margarine	
	Number of patients who consumed					
	> the supplement			—		
	= " "			22		
	< " "			15		
	0 " "			2		
Surface losses per person per period	*Primary caries*					
	Per Westin's index-surfaces	0.23	6.13	6.46	0.28	0.38
	Per 148 surfaces	0.23	6.90	7.41	0.36	0.44
	Ibid. but Cwd., Rtg., Ml excl.	0.10	2.67	7.62	0.54	0.59
	Primary caries according to distribution of lesions[1]					
	Mandib. mol.	0.13	1.51	1.77	0.10	0.12
	Mandib. inc.	—	2.03	2.26	0.03	0.05
	Maxill. mol.	0.10	1.18	1.46	0.05	0.10
	Maxill. inc.	—	1.26	1.33	0.10	0.13
	Previously intact teeth	0.13	5.00	2.74	—	0.15
	Cementum caries	0.03	1.05	2.62	0.15	0.05
	Total surface losses per 148 surfaces					
	Primary caries	0.23	6.90	7.41	0.36	0.44
	Enlargements	0.13	0.77	1.49	1.31	1.15
	Loss of teeth					
	periodontitis	0.26	0.05	0.26	0.36	0.10
	other causes	0.21	0.18	0.31	0.59	0.42
	Total:	0.82	7.90	9.46	2.62	2.11

[1] Third molars not included.

TABLE 41

Statistical analysis of the changes in caries activity in the female 24-toffee Group. Westin's index-surfaces

Comparison	χ^2-value	Degrees of freedom	Probability (P) of the changes being due to chance only
All periods with one another	116.44	8	0.1 % > P
The second and third periods in relation to the others	112.92	2	0.1 % > P
The first period in relation to the last two	0.45	2	P > 50 %
The second period with the third	6.17	2	5 % > P > 1 %
The fourth period with the fifth	0.31	2	P > 50 %

TABLE 42

Frequency of enlargements of certain small carious lesions in the 24-toffee Groups.

Type of lesion	Group	Number of new lesions during CH I: 1	New lesions during CH I: 1 enlarged during CH I: 2	No enlargement	Lost by extractions	Per cent enlargements
Cwd	24-toffee Gr. male	67	37	30	—	55
	„ „ female	90	52	36	2	58
	Total	157	89	66	2	57
Rtg	24-toffee Gr. male	16	12	4	—	75
	„ „ female	18	16	2	—	89
	Total	34	28	6	—	82
M 1	24-toffee Gr. male	25	4	20	1	16
	„ „ female	33	8	24	1	24
	Total	58	12	44	2	21

plays an important rôle in the mechanism of enlargement it should have made itself felt, at least during the second year. That this was not always the case is apparent from an analysis of the subsequent development of those initial cavities of the type fissure caries (M 1), Cwd and Rtg-caries, which appeared during the first year of the large consumption of toffees in these groups. It is clear from Table 42 that only about half of these lesions

increased in size during the second year despite the great increase in the number of new carious lesions. In a study similar to the present BOYD (1950) states: "Many minor enamel lesions have appeared and then remained without advance for months or years, even though other cavities elsewhere in the same mouth or even in the same tooth may have progressed concurrently to dentinal involvement."

Summary

During a preparatory period 48 males and 39 females with an average age of 31.0 and 31.1 years, respectively, at the beginning of the study received ordinary Swedish food with an amount of sugar corresponding to the ordinary household consumption in Sweden.

During the following 2 years the total carbohydrate consumption remained unchanged, but the proportion between the different carbohydrates was varied, so that they then received refined sugar in a quantity equal to twice the average consumption. Of the daily ration of carbohydrates 160 g. was given as toffees, which was consumed mainly between meals. During the 2 subsequent years the total carbohydrate consumption remained unchanged, but then the patients received refined sugar at meals and in a quantity corresponding to the average household consumption in 1948. During these last 2 years all consumption of any food between meals was forbidden.

These changes in the carbohydrate distribution were accompanied first by a marked increase in caries activity *i. e.* during the time the patients were offered toffees, and by a marked fall to original level on withdrawal of the toffee ration.

The loss of surfaces because of enlargement of existent cavities did not significantly increase in association with the increase of caries activity.

GENERAL DISCUSSION

Some problems of importance in the evaluation of observations made in the individual groups of subjects were discussed earlier in connection with the presentation of the results. This section is concerned with more general considerations.

MATERIAL

A question that presents itself is whether the type of patients examined were suitable for the study of dental caries. The average age of the series was high, and mental development was low throughout.

In the evaluation of the general applicability of the conclusions suggested by the observations made in the present investigation it is of importance to discuss the following question.

Would the effect of any of the conditions or variables studied in the present investigation produce a stronger or weaker effect in people outside the institution, especially in the younger age groups? The answer to this question depends on how the caries susceptibility of these patients is judged.

Owing to the retraction of the gingiva with age, the cementum is more likely to be attacked than in lower age groups. The frequency of caries in the cementum or cemento-enamel junction was high in some of the groups.

For example, in the 24-toffee Group during the second year of Carbohydrate Study I and the Caramel Group during the first year of Carbohydrate Study II (Tables 38, 40 and 34) a large percentage of the new cavities were localised to the cementum or cemento-enamel junction. It is clear from table 43 that the frequency of cementum caries increased markedly with age. The relative preponderance of cementum caries in the Caramel Group, also when compared with other groups with high activity, must therefore be evaluated in the light of the fact that the average age of this group was higher than that of any of the others.

There was, however, also a correlation between the frequency of cementum caries and the experimental conditions. Thus when sugar was given between meals, the frequency of cementum caries was always higher than with any of the other experimental conditions used. See the survey tables on the opposite pages to the diagrams for the groups.

The high frequency of cementum caries might convey the impression that the changes in caries activity in this material were due mainly to this type of caries. A question that then presents itself is whether the results would be true if caries activity were calculated with the exclusion of the cementum cavities.

Such a calculation would, however, be fictitious as it would infer neglecting the important fact that gingival retraction might decrease the retention of foodstuffs on the enamel and thereby decrease its disposition to caries. Despite this objection the frequency of enamel caries was analysed for the Caramel Group. It was still found that the consumption of caramels was accompanied by a statistically significant increase of caries activity.

If the cementum is less resistant to caries than is the enamel, it might be argued that in the higher age groups the resistance in caries would be relatively low. An argument against such an assumption is, however, that when the series were taken together independently of the experimental conditions, the caries activity was twice as high in the lower age groups as in the higher (Table 43). In those individuals in whom the cementum was frequently exposed, then, caries activity was lower. Obviously this exposure of the cementum does not increase the susceptibility of the material to such an extent as to impair the applicability of the conclusions to lower age classes.

As dental caries cannot heal with *restitutio ad integrum*, the number of surfaces involved — caries frequency — increases with age. The relationship found between age and caries activity in the present material has long been known from other investigations. For example, it has been observed in large series (HOLLANDER & DUNNING 1939, KLEIN & PALMER 1941 and others) that the frequency curve shows the steepest incline during and immediately after puberty, after which it becomes flatter.

Three possible explanations of this relationship between dental caries and age may be considered.

1. Under ordinary living conditions young people may be more exposed to exogenic factors than elderly.
2. The teeth may undergo chemical and structural changes with age and thereby increase the resistance to caries, independently of the presence or absence of any earlier decay.
3. The relationship between dental caries and age may be interpreted as a sign that caries first attacks the most susceptible teeth and dental surfaces, and leaves the least predisposed intact.

The first of these possibilites, *i. e.* that the caries-producing factors are greater in young people than in elderly, cannot by itself explain the relationship between dental caries and age. In the present series, for example, on exposure of subjects to one and the same exogenous caries-producing factor the effect was greater in the lower age groups (Table 43).

TABLE 43

Relation between age, caries activity and cementum caries during all periods 1946—1951. Third molars not included

Age Group	Nr. of pat.		New carious surfaces				
		% of the whole material	Total		Cementum caries		
			Number	per person per period	Number	% of total	per person per period
Born:							
Earlier than 1910	151	34.6	719	0.95	384	53.4	0.51
1910—1919	148	33.9	525	0.71	114	21.7	0.15
1920—1929	113	25.9	923	1.63	70	7.6	0.12
1930 or later	24	5.6	326	2.79	7	2.1	0.06
Total	436		2493	1.14	575		0.26

As to the second possibility of the resistance of the dental surfaces increasing with age, depending on chemical and structural factors, suffice it here to state that if this is the case, it would infer that the experimental conditions used would have produced a still greater caries activity in a younger series than in the present one.

If the third point were the only explanation it would infer that the average resistance of a group of individuals could be expressed as the number of DMF surfaces or teeth: the higher the DMF-number the greater the resistance of the remaining surfaces..

In order to evaluate the DMF-values of the material, comparative analyses were made with other materials.

Children attending a grammar-school (*Katedralskolan*) in Lund were examined by the same dentists as performed the main registration in the Vipeholm investigation proper. The average individual number of DMF teeth recorded by them in 14-year old school children there was 15.4. The corresponding figure found by GUSTAFSSON & WIBOM (1952) in a 14-year old, public dental

clinic clientele was 11.5. This difference in frequency is probably ascribable to differences in criteria accepted in classing a tooth surface as decayed. In 20-year old men called up for military service WESTIN & WOLD (1943) found an average individual frequency of 18.44 DMF teeth.

Table 44 shows that among the 14-year old children at *Katedralskolan* the caries activity was higher than in any of the male groups in the Vipeholm material. Distribution of the children according to the number of new carious surfaces per individual per year in the *Katedralskolan* material showed the same pattern as in the active groups of the Vipeholm Hospital. Thus in some of the pupils the number of surfaces involved was markedly high, while in others there were only few or no new cavities (Table 45). Among these 14-year old children the number of surfaces lost per year was sometimes as high as 20 per individual.

In the beginning of the Vipeholm investigation the average age of the males was 32.2 years, and the average DMF-value was 15.6. At the start of the Carbohydrate Study I the DMF-values in all of the groups were not as high as that found by WESTIN & WOLD for 20-year old conscripts (18.44). This further infers that any increase in activity would not have been masked by too small a number of intact surfaces at the beginning of the investigation.

In view of the high average age of the subjects in the present material, the frequency of caries observed in the beginning of the study must be regarded as low and may be explained by any of the following possibilities.

a) The low caries frequency must be due partly to the fact that some patients (17 per cent of the total number of patients of the hospital) had less than 10 teeth at the beginning of the study and were therefore excluded from the series (cf. page 238).

b) It might be due to the fluoride content of the drinking water.

In a series consisting of 7,257 children aged 12—14 years DEAN et al. (1941, 1942) found on the average about 8.0 DMF teeth per child in districts in which the fluoride content of the drinking water was 0.0—0.1 mg./l, the corresponding figures for 0.4—0.5 mg./l and 1 mg./l being 5.0 and less than 3 DMF teeth respectively. Thus, in view of the fluoride content (0.4 mg./ml) of the drinking water used at Vipeholm, one might expect the caries frequency

TABLE 44

Caries activity in schoolboys at "Katedralskolan"

Age group	Number	New DMF surfaces per child per year
10	17	2.53
11	69	4.91
12	80	5.36
13	59	6.22
14	25	7.16
10—14	250	5.42

in the patients there to be about 30 per cent lower than in a group of subjects drinking flouride-free water.

As the patients did not enter the hospital until they were 8 years of age, before which the influence of fluorides is supposed to be of decisive importance, the fluoride content of the drinking water in Lund seems not to be the cause of the low caries frequency of the patients. RUSSELL & ELVOVE (1951), however, present results indicating that the fluorides have also a post-eruptive effect. As the school-children at *Katedralskolan* have the same drinking water, but the same caries frequency as the patients at Vipeholm Hospital despite their being 18 years younger, the fluoride content

TABLE 45

Distribution of schoolboys at "Katedralskolan" according to caries activity

New DMF surfaces per child per year	Number of children
0	18
1— 3	71
4— 6	82
7— 9	50
10—12	14
13—15	5
16—18	5
19—21	4
> 21	1

of the drinking water cannot explain why the dental caries frequency of the patients at the hospital was low.

c) The low frequency of caries may be ascribable to the assumption that mental deficiency is associated with increased resistance to caries.

This point was studied by grouping subjects according to degree of mental deficiency and comparing them with respect to caries activity and caries frequency. The patients were grouped (grades 0—6) in accordance with the method described by FRÖDERBERG (1952). The males were divided into 2 groups, those assigned to grades 0—3 forming one group, and those allotted to grades 4—6 the other, after which the frequency of caries observed at the beginning of the carbohydrate trial and the caries activity recorded during the trial were calculated (Table 46). As these values also vary with age, three age groups were distinguished.

It is clear from the table that the frequency of caries, as judged by the number of caries-free surfaces, was lower in the 0—3 grade group than in the 4—6 grade group. These two groups did not differ with regard to the average activity recorded during the study periods.

This suggests that mental deficiency is associated with a moderately decreased predisposition to dental caries, and thus that the institutionalised subjects were more resistant to caries than ordinary young people.

d) The lowness of the frequency of caries among the patients may also be explained by a possibly decreased exposure to caries-producing factors. If so, it is ascribable to the fact that most of them had spent long periods at institutions. It is known that caries activity decreases from year to year in institutionalised subjects. Readers interested in a survey of the literature on this point are referred to GUSTAFSSON, 1954.

In addition to the above aspects of resistance, certain other circumstances should be stressed concerning the question whether the caries-promoting factors under consideration are capable of exerting one and the same effect on non-institutionalised people.

As already pointed out (page 241 and 272), some of the subjects sometimes swallowed one or more toffees or caramels without che-

TABLE 46

Comparison between the intelligence groups 0—3 and 4—6 regarding caries frequency and caries activity

Born	Number of pat.		Nr. of intact surfaces per person at start of the Carbohydrate Study		Nr. of new carious surfaces per person per year. Average 1946—1951	
	0—3	4—6	0—3	4—6	0—3	4—6
Earlier than 1910	67	54	73	65	0.85	1.00
1910—1919	77	42	93	81	0.88	0.83
1920 or later	83	32	100	67	1.00	1.50

wing them. This means that the tendency of the sugar to be retained in the mouth was in reality less than that suggested by the number of caramels consumed. If caramel consumption increases caries activity, the increase seen in the present series must be smaller than it would have been if the caramels had been chewed before swallowing.

On page 240 it was mentioned that oral hygiene was poor or absent in the present material. This often resulted in massive deposits which would not be tolerated by normals. This favoured the retention of carbohydrates, and it does not sound unreasonable to suppose that any one and the same caries-accelerating factor might exert a greater effect than it would have in the presence of ordinary oral hygiene.

Summary

It is obvious that the high average age, the fairly low caries frequency observed at the beginning of the trial, the poor oral hygiene and the habit of some of the patients to swallow the sweets without chewing them, as well as the mental deficiency of the series, make it difficult to judge the caries susceptibility of the patients. However, numerous data presented in the literature show that the chemical and physical properties (e.g. increasing hardness, lower permeability) change with age in such a way as to increase the resistance to caries. In our opinion, age as such is the decisive factor and the other factors discussed above have only a

modifying influence. In view of the high average age of the patients it is therefore highly probable that the resistance to caries in the subjects studied was greater than in young non-institutionalised people. This infers that any variable producing an increase in caries activity in these patients will more than probably do so in a normal younger population. In addition, any of the factors that produced only a slight or doubtful increase in caries activity in the patients or an increase that could not be demonstrated with certainty owing to he sources of error of the method and to the smallness of the groups, might possibly cause a more distinct tendency or even statistically significant increase in caries activity in ordinary young people.

DISTRIBUTION OF CAVITIES AMONG VARIOUS TEETH AND TOOTH SURFACES

From the tables on the caries activity in the various groups (27, 34, 36, 38 and 40) it is apparent that on consumption of sugar between meals, up to 25 per cent of the new carious lesions were seen in the incisors and canines of the lower jaw, which usually show the lowest caries activity. This might suggest that the type of caries recorded differed from the ordinary type. This preponderance of carious lesions in the front teeth might, however, be ascribable to the high average age of the patients with the result that the number of hitherto intact surfaces of these teeth was much higher than for the molars, many of which had been attacked or lost already before the commencement of the investigation. Thus, at the beginning of the study there were altogether 3,327 intact incisors and cuspids, as against 2,407 intact premolars and molars. This means a ratio of 12 : 9 as against 12 : 20 in a caries-free mouth. Therefore even in groups with a low caries activity the frequency (16.7 per cent) of new carious surfaces of the incisors and cuspids of the lower jaw was higher than among 7—15 year old individuals (2.7 per cent) as calculated from data given by KNUTSSON & SCHOLZ (1950). The tables show that the preponderance of carious surfaces among the incisors and cuspids was greatest in those groups that received the largest sugar ratio between meals.

TABLE 47

Frequency of new carious lesions in incisors and canines of the lower jaw in the 8-toffee Group.

	Number of new carious lesions per person per year	Number of new carious lesions in incisors and canines per person per year	
			per cent
The year before toffee consumption (No supplementary carbohydrates)	0.81	0.13	16
During toffee consumption			
1st year	3.09	0.75	24
2nd year	2.89	0.48	17
3rd year	3.70	0.65	18

However, a survey of the results in the 8-toffee Group showed that in this group with a smaller amount of sweets between meals, the caries activity in the incisors and cuspids of the lower jaw was the same during consumption of sweets between meals as when the patients were on a practically sugar-free diet (see Table 47).

Typical of "sugar caries" is supposed to be a relative preponderance of buccal caries. Table 48 shows that the relative fre-

TABLE 48

Frequency of facial (buccal) and lingual caries in intact teeth in relation to the consumption of sweets
Main material

	Total number of new carious lesions in intact teeth	Number of facial surfaces with new lesions		Number of lingual surfaces with new lesions	
			per cent		per cent
When sweets were not consumed	380	141	37	5	3
When sweets were consumed	935	407	44	2	1

quency of new carious lesions on the facial surfaces is not significantly higher during the consumption of sweets (44 per cent) than under other conditions (37 per cent), even if the groups with the highest sweet consumption are also included.

A detailed analysis of the distribution of new carious lesions will be given in a later paper.

INDIVIDUAL VARIATION IN DISPOSITION TO CARIES

In connection with the statistical analysis of the changes in caries activity it was pointed out that the distribution of the patients according to different activity classes is markedly asymmetric. A study of the caries activity in individual patients over a number of years showed that caries activity under identical conditions varied widely, not only from one individual to another but also from one year to another in one and the same patient. Thus, in some patients who consumed large quantities of sweets no new carious surfaces were observed, while in others on a practically sugar-free low-carbohydrate diet as many as 9 new cavities per year were recorded. In the 8-toffee Group there were persons in whom active caries was noted while they were on a sugar-free diet during the first year of Carbohydrate Study I but did not show any new carious surfaces the following year when they received totally 300 g. of sugar including 60 g. of sweets between the meals. Such variations in the disposition to caries and their effect in extended studies on the averages of caries activity require further study.

REGISTRATION OF CARIES

In the registration of caries a certain amount of errors must be expected especially in materials consisting of feeble-minded patients. A study of the literature failed to reveal any information about the magnitude of the error of the method observed by earlier workers in this field. The order of the error of the method in the present investigation was assessed on the basis of special control registrations (QUENSEL et al. 1954). This made it possible to make due allowance for the inaccuracy of the method in the interpretation of the results obtained.

Pits, deep fissures, and enamel hypoplasia are sometimes mistaken for caries (over-registration) by some examiners but not by others. Sometimes, however, carious changes are not recognised or not registered as such (under-registration). This results in discrepant recordings.

In the assessment of the accuracy of the method the tendency of the individual examiners to over- or under-registration was taken into account. It was, of course, necessary to assume that the tendency of a given examiner in one direction or the other was one and the same throughout the investigation. In a long-term study of the present type it cannot be guaranteed that the examiners sooner or later do not become aware of the experimental conditions. This point calls for a few comments.

Already before the present investigation was started persuasive evidence was available that sugar is a caries-producing substance. That the dietary addition of a substance rich in sugar would increase caries activity and that a reduction in carbohydrate consumption would be followed by a decrease in caries activity was probably suspected by the examiners. It therefore sounds reasonable to suppose that the tendency of an examiner to record doubtful changes as caries, for example, would be still more pronounced in the examination of subjects receiving such dietary supplements as might be expected to accelerate caries activity. It might likewise be imagined that any tendency of the examiner to over-register would diminish or that he would even be inclined to under-register as soon as he became aware of the withdrawal of such a caries-promoting supplement. This would result in too large an increase of the values recorded for periods during which the patients received a diet rich in sugar. On the other hand, the values noted for periods during which carbohydrate consumption was restricted would be too low.

In order to check whether the examiners really were influenced by their knowledge of the experimental conditions, the following control test was performed. After registration of the state of the teeth in a group of subjects receiving a caries-producing diet, some of the members were re-examined immediately afterwards together with subjects belonging to other groups with low activity (control registrations, Tables 4 and 5 in QUENSEL et al. 1954).

If the recordings were influenced by the knowledge of the experimental conditions, the second values would be lower. However higher values were recorded, which can partly be explained by new cavities developing during the 4 weeks between the two examinations. These findings argue against over- or under-registration because of knowledge of the experimental conditions.

In both the main and the supplementary registrations the number of DMF teeth was calculated for every group of patients. These values, of course, increase according to the number of new carious surfaces.

If knowledge of the experimental conditions influenced the examiners in deciding whether a lesion should be classed as carious or not, it is difficult to understand that the 5 different DMF-values for each of the 8-toffee Groups and the two 24-toffee Groups during the sweet-consumption should form the straight lines illustrated by the curves in Fig. 6 (page 284). If the examiners had been influenced, the effect would probably have been most obvious at the first examination after the introduction of the new experimental conditions, and the curves would then have been of a different and more irregular shape.

Finally, it should be pointed out that if the examiners had overdiagnosed caries in those groups in which an increase of caries activity was expected on account of the dietary addition of sugar, such an influence would have increased the activity figures in all groups with additional sugar. An increase in the dental caries activity did, however, not appear in the group receiving the largest amount of sugar (330 g. at meals). Moreover the examiners reported that they thought it strange that the consumption of 300 g. sucrose in solution and of new, doughy bread rich in sugar during the Carbohydrate Study I did not demonstrably increase caries activity.

Summary

Despite the fact that some of the evidence presented above was indirect, it appears very improbable that the changes observed in caries activity in the present investigation should have anything to do with the examiners' having been aware of the experimental conditions.

TABLE 49

Sugar- and sweet consumption in kg. per person per year in Sweden 1939—1953[1]

Year	Total sugar	Sweets
1939	52.7	7.6
1940	43.4	7.1
1941	38.7	6.7
1942	42.2	6.5
1943	39.9	6.3
1944	38.3	7.4
1945	38.0	7.3
1946	37.0	7.5
1947	36.4	7.7
1948	40.2	7.0
1949	42.9	6.6
1950	47.1	6.5
1951	42.3	4.8
1952	42.6	6.0
1953	41.2	

[1] According to "Statens Jordbruksnämnd" (The State Agricultural Marketing Board).

RELATIONSHIP BETWEEN SUGAR CONSUMPTION AND CARIES ACTIVITY

In some of the groups the dietary supplements used during Carbohydrate Study I gave the diets the character of extreme variants. For instance, in one group the diet was poor in carbohydrates, practically sugar-free but rich in fat, while in others the sugar content was more than twice the average Swedish consumption (see Table 49).

With a mean annual consumption of 45 kg per individual in Sweden and the sugar consumption of certain groups being very low, it is quite possible that some Swedes consume 80—90 kg sugar per year.

Therefore the large quantities of sugar used during Carbohydrate Study I probably represent a level just above the highest that might be expected outside the institution. In Carbohydrate Study II some of the conditions closely resembled those met with

outside the institution. For example, the total sugar consumption was the same as the average consumption outside the institution and the percentage incorporated in the food was also about the same as in most Swedish families. In certain respects, however, the conditions differed from those outside the institution.

Thus the bread eaten at every meal in the Bread Groups was very sweet (sugar content 15 %) but still of a type consumed in Swedish households at after-noon coffee and tea. The type of bread generally consumed at every meal in the households has, however, unlike in other countries, also a rather high sugar content (about 8 %).

The consumption of sweets between meals probably bordered the upper limit of the amounts consumed outside the institution. This was shown by the investigation of the amounts of sweets consumed at the elementary shools of Lund (QUENSEL & GUSTAFSSON, 1952). If those schoolboys who consumed most sweets were to buy toffee for the total amount of money they spent on all sorts of sweets, the daily consumption would be 8—9 pieces of toffee per boy. If they spent this money on chocolate only, the amount they would eat would be about half of that received daily by the Chocolate Group.

The consumption in the sweets groups in this investigation might be compared with the average consumption in Sweden, as calculated as the total consumption distributed among all inhabitants. The consumption in the 8-toffee Group and the Chocolate Group would then be three times this figure. Such an average figure is, however, too low as a large part of the population never or very seldom consume sweets.

The consumption of sweets during the carbohydrate studies differed in other respects from that outside the institution, in that the ration did not vary from one day to another and the type of preparation was one and the same throughout the study period.

It should also be borne in mind that the toffee used in the investigation was of a type of sweets that is not generally consumed in Sweden. As the intention was to study the retention of *sugar* in the present investigation, the toffee was specially prepared to contain ingredients other than sugar in the least possible amount. The toffee, however, resembled certain types of sweets

on the market, and is very similar in consistence and taste to English toffee. The weight of each toffee was the same as that of each caramel, which was of a type very popular in Sweden. The latter can be shortly characterised as a toffee containing milk powder; therefore the caramel mass when heated takes a brown colour.

These differences between the conditions inside and outside the institution must be seen in the light of the fact that the experimental variables used in the various groups had to be studied under uniform conditions. It was never the purpose of the investigation to try to determine the minimum amount of any kind of sweets that could be consumed by an ordinary person without injury to the teeth.

The purpose of the investigation was instead to study caries activity in relation to varying sugar retention on the surfaces of the teeth under standardised conditions. Sugar retention was increased by the addition of

I. large quantities of sugar,
II. sugar on many occasions throughout the day,
III. sugar in sticky form,
IV. sugar in concentrated form.

Sometimes these methods were used separately, sometimes in combination. As to the addition of sugar, four types of experimental conditions were used:

sugar given in small quantities at meals and with only a slight tendency to be retained on the surfaces of the teeth (Control Group);

sugar given in large quantities at meals and with only a slight tendency to be retained on the surfaces of the teeth (Sucrose Group) I above;

sugar given in large quantities at meals and with a strong tendency to be retained on the surfaces of the teeth (sticky preparation, Bread Groups) I+III above;

sugar given in large quantities also between meals, the latter with a strong tendency to be retained on the surfaces of the teeth (sticky preparation with sugar in high concentration, sweet groups) I+II+III+IV above.

As far as subsequent changes in caries activity is concerned, these conditions fall into two main categories, the first comprising the first two types above, in which the sugar was served at meals and in a form with only a slight tendency to be retained on the surfaces of the teeth. As the very sweet bread was served only once a day during the Carbohydrate Study I, the conditions and caries activity recorded during this period were regarded as belonging to this main category.

Table 50 shows that in this first main category the daily total amount of sugar given varied from 30 g. to 330 g. per person. The consumption of refined sugar per person thus ranged from 0.4 kg. to 110 kg. per year. Despite this wide variation in the sugar consumption caries activity was low and constant in all groups and throughout all the periods. Comparison of the means suggested no differences. The Sucrose Group with G 5+75 g. sucrose was the only group to differ from any of the others. Statistical analysis, however, failed to reveal any difference than those due to chance only.

But the frequency of persons with more than 3 new cavities was significantly higher for the first three conditions in table 50 than for the remaining three (12 of 442, as against 5 of 740). This thus suggests that the three last-mentioned conditions in table 50 are capable of producing a slight increase in caries activity. This conclusion is based on inter-group comparison and may be due to a possible inclusion of a few subjects with high caries susceptibility in this group. Furhermore, it is probable that the larger proportion of bread and potatoes in basic diet G 5 than in G 4 also favoured caries activity.

The relationship between the sugar consumption at meals and caries activity found in the present investigation is further apparent from the diagram given in Fig. 16. The broken line in this diagram represents the relationship between sugar consumption at meals and caries activity. On account of the above described, probably slightly higher frequency of caries in those groups receiving the three types of diets with the largest amounts of sugar, the curve shows a slight slope.

Thus the consumption, at meals, of sucrose in a form with only a slight tendency to be retained on the surfaces of the teeth, with

TABLE 50

Caries activity and sugar consumption 1.
Low retention tendency. Sugar at meals

Diet	Sugar consumption		Nr. of observation units [1]	Nr. of new carious surfaces	Nr. of new carious surfaces per person per year	Nr. of observation units in activity classes		
	Total g/day	Sucrose kg/year				0	1–3	>3
G4 + 150 g. margarine	30	0 (0.4)	160	43	0.27	134	22	1
G4 + high-sugar bread at one meal	80	19	144	46	0.32	116	27	1
G3	90	21	436	149	0.34	340	93	3
G5 + 40 g. margarine	110	24	120	60	0.50	92	24	4
G5 + 75 g. sucrose in sol.	190	51	114	84	0.74	88	22	4
G4 + 300 g. sucrose in sol.	330	110	208	89	0.43	159	45	4

[1] One patient observed during one year = one observation unit.

a daily ration up to 300 g. a day and all observation time of up to 4 years, had no definite influence on the basic caries activity of 0.3—0.5 newly carious surface per year in this material.

The observations made in the subjects studied under the other main category of conditions, i.e. the consumption of sugar in a form with a strong tendency to be retained on the surfaces of the teeth were, however, altogether different. Table 51 summarises the resulting caries activity. It is clear from Table 52 that the consumption of only a fraction (13 %) of the total amount of sugar between the meals was sufficient to increase caries activity. Large differences were noted in the caries activity figures for the different conditions and periods. The differences were statistically significant: χ^2-analysis gave a value of 55.61 (12 degrees of freedom, $P < 0.1$ per cent).

TABLE 51

Caries activity and sugar consumption 2.
High retention tendency. Sugar also between meals

Diet	Sugar consumption		Nr. of observation units [1]	Nr. of new carious surfaces	Nr. of new carious surfaces per person per year	Nr. of observation units in activity classes		
	Total g/day	Sucrose kg/year				0	1–3	> 3
G5 + high-sugar bread at all meals	160	40	82	107	1.30	52	21	9
G5 + 65 g. milk chocolate	140	32	94	110	1.17	47	38	9
G5 + 22 caramels	170	33	62	187	3.02	19	20	23
G4 + 22 caramels + 200 g. sucrose in sol.	300	84	62	119	1.92	22	31	9
G5 + 8 toffees + 25 g. sucrose in sol.	180	43	80	254	3.18	23	32	25
G4 + 8 toffees + 250 g sucrose in sol.	320	102	40	121	3.03	12	16	12
G4 + 24 toffees + 150 g. sucrose in sol.	300	84	96	386	4.02	36	27	33

[1] One patient observed during one year = one observation unit.

In all the groups of this category the activity increased earlier in the subjects receiving the sugar between meals (sweet groups) than in those who received sugar at all meals (Bread Group), where the increase was not apparent until the second year. A similar tendency was in the Caramel Group, where there was a definitely higher activity during the second year of caramel consumption than during the first year. As the actual experimental period of the second year was only 6 months, the difference between the two years was in reality still greater. But also in the other sweet groups the increase in caries activity was gradual when

TABLE 52

Sugar consumption between meals and caries activity

Diet	Sugar consumption			Nr. of new carious surfaces per person per year
	Total g. per person per day	Between meals		
		g. per person per day	% of total consumption	
G5 + 65 g. milk chocolate	140	30	21	1.17
G5 + 8 toffees + 25 g sucrose in sol.	180	40	22	3.18
G4 + 8 toffees + 250 g. sucrose in sol.	320	40	13	3.03
G5 + 22 caramels	170	70	41	3.02
G4 + 22 caramels + 200 g. sucrose in sol.	300	70	23	1.92
G4 + 24 toffees + 150 g. sucrose in sol.	300	120	40	4.02

the calculations were made on the definite cavities excluding initial lesions.

This time lag between alteration in the consumption of sugar and the change in caries activity has been observed in the investigations concerning the caries activity in different countries during the two World Wars. This lag must be borne in mind when correlating the sugar consumption with caries activity. It is possible that an increase in caries activity shows up much later than generally recognised, especially if the increase in the caries accelerating factors is fairly small. In the groups studied here the time lag appears, although the consumption of sweets between meals was increased from nil to very high values.

In Fig. 17 the values for caries activity in association with the consumption of sugar with a strong tendency to be retained on the surfaces of the teeth have been plotted.

According to the above mentioned longitudinal variations, the caries activity figures for the different years of sweet consumption are not always equivalent in the same groups. Therefore the value for the second year of sweet consumption was taken as representative for the different types of conditions (chocolate, 8 toffees,

caramels and 24 toffees). In the diagram these activity figures are also connected by a thin line with the activity figures for the group during the year preceding the sweet consumption. In this way the diagram illustrates the increase in caries activity for different amounts of sugar consumed between meals. As the 8-toffee Group has a higher starting value than the other groups, the increase is of the same order for all groups, although two such different activity values as 1.23 and 2.78 (Chocolate and 8-toffee Groups) correspond to almost the same sugar consumption.

Fig. 17 directed attention to the shape of the regression line for the relation between sugar consumption between meals and caries activity. Although the distribution of the values suggest an almost linear relationship, in our opinion the number of values available is too small to merit any definite conclusion. Furthermore, no activity values are available for the important consumption values between 0 and 30 g. per day, i.e. the range within which the average consumption of the population falls. However, the observations made in the present investigation provide convincing evidence that the consumption of sugar in a sticky form between meals does favour caries.

In this connection it is also of interest to discuss certain observations made in the supplementary studies. As already pointed out, the amount of sugar consumed between meals was about the same in the Chocolate Group (30 g.) as in the 8-toffee Group (40 g.), but the increase in caries activity was much greater in the 8-toffee Group. This difference was striking, especially if only the definite cavities are taken into account (Figs. 11 and 13, pages 306 and 324).

LUNDQVIST (1952) considered the time that sugar could be demonstrated in the saliva as a measure of sugar retention. He found that the saliva contained sugar for just as long a time in the Chocolate Group as in the 8-toffee Group. This suggests that, according to the chemo-parasitic theory, both conditions should equally favour caries. Possible explanations for the differences observed between the caries accelerating potencies of chocolate and toffee are the following (GUSTAFSSON 1952 a).

1. The retention of sugar on the dental surfaces in association with the consumption of chocolate is not so high as for toffee, be-

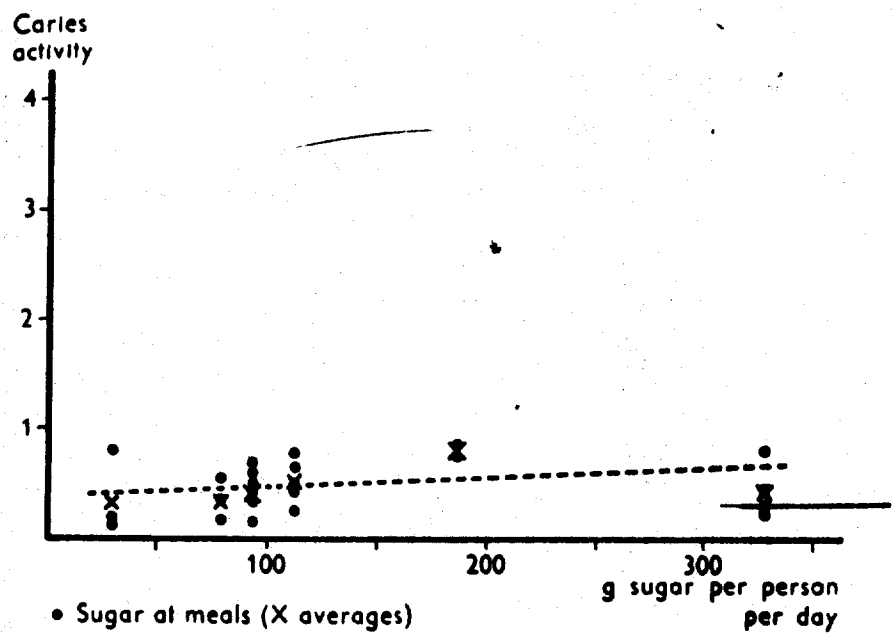


Fig. 16. The relationship between sugar consumption at meals and caries activity.

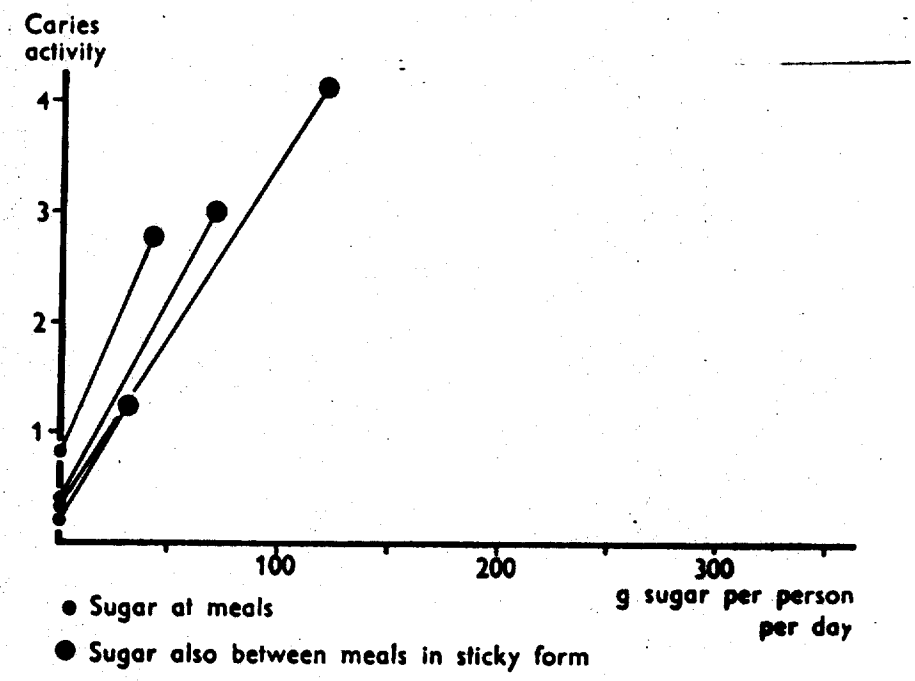


Fig. 17. The relationship between sugar consumption between meals and caries activity. Cf. p. 355.

cause the latter is "stickier". This difference in stickiness was demonstrated by the observation of LUNDQVIST that the "sugar time" for 11 g. chocolate was the same as for 7 g. toffee in the same persons.

2. Chocolate contains a large proportion of fat, which has been shown to decrease the caries-promoting effect of sugar in experimental animals (cf. page 293).

3. The concentration of sugar in chocolate is slightly lower than in toffee.

4. Chocolate might contain small amounts of anti-enzymatic or anti-bacterial substances (GUSTAFSSON 1952 b).

5. The caries susceptibility was greater in the 8-toffee Group than in the Chocolate Group.

It appears most likely that the divergence between the two groups is due mostly to the difference in susceptibility to caries, but the factors in 1—4 above may also have contributed.

Observations made in the Bread Groups also indicated that the retention factor, when measured as the time the sugar is retained in the saliva was less than might have been expected. LUNDQVIST (1952) found that the retention of sugar in the saliva of subjects belonging to these groups was on the average shorter than in any of those groups, including the Control Group, in which the members received sugar at meals and in a form with only a slight tendency to be retained on the dental surfaces. This might suggest the involvement of some other factor responsible for the difference between the bread groups and the sweet groups regarding caries activity. On the other hand it is questionable whether the "sugar time" of the saliva always reflects the actual conditions on the dental surfaces.

The course of caries activity following the withdrawal of the dietary addition of a caries-promoting factor was studied in three groups, the Caramel- and 24-toffee Groups. In all three groups caries activity diminished to the basic level within one year of the withdrawal of the dietary addition. This thus proves that it was this addition that caused the increase in caries activity.

In Carbohydrate Study I the effect of a pronounced reduction in dietary carbohydrates on existent basic caries activity was stu-

In the only study with unlimited sugar intake between meals JAY *et al.* (1936) reported an increase in caries activity, but the evidence they produced has not been generally regarded as convincing.

MACK (1949) studied the effect of the consumption of 115 g. sucrose daily (including 57 g. sweets) but was unable to demonstrate any definite increase in caries activity. Although it is difficult to judge the results of this investigation, they do support the observation made in the present investigation that caries activity is largely independent of the amount of sugar used as long as it is consumed in a form with only a slight tendency to be retained on the surfaces of the teeth. MACK's result was negative, despite the fact that part of the sugar consumed as sweets at meals had a marked tendency to be retained. This should be compared with the observations made in the Sucrose Group during Carbohydrate Study I, when a certain part of the sugar was incorporated in the food and not, as originally intended in the drinks only. This might therefore have increased the tendency to retention in the present investigation.

The experimental conditions used by BOYD (1950) and the results he obtained were roughly the same as in the present Sucrose Group.

The effect of the consumption of sugar at meals and between meals has been studied earlier but no agreement has been attained as to whether or not an increase in the sugar intake is followed by increased caries activity. In the present investigation, however, the effect of the sugar intake was studied from various angles in one and the same material, which was observed for a long time, and the observations made were able to explain the differences between the results presented by earlier workers in this field.

Summary

The fact that dental caries activity increased when the consumption of sugar in sticky form was introduced, together with the fact that it decreased when such consumption was stopped, constitutes convincing evidence that sugar increases dental caries activity in man. When sugar was consumed in solution at meals, up to more than twice the Swedish consumption, no increase in

died in two groups. In view of the error of the method in relation to the number of patients in the groups and the low basic caries activity of the present material, it can hardly be accepted as suitable for the study of the effect of maximum carbohydrate restriction on dental caries. Moreover, the reduction in calories by the withdrawal of the carbohydrates had to be compensated by an increased amount of fats, so that it is not possible to say with certainty whether any decrease in activity should be ascribed to restriction of carbohydrates, excessive fat or to a combination of both.

An observation of practical importance was, however, that new carious lesions appeared despite reduction of carbohydrate intake to a minimum. This suggests the existence of some types of caries, in which sugar and other carbohydrates play only a minor rôle as a causal factor.

Comparison Between the Present Investigation and other Similar Studies

In a survey of the literature GUSTAFSSON (1954) pointed out the lack of agreement about the relationship between dental caries and sugar consumption in man. The Vipeholm investigation clearly showed that in the evaluation of the effect of the sugar intake on dental caries it is *not only the quantity* of sugar consumed that must be considered, but also the *form* in which it is served and whether it is consumed *at* meals or *between* meals. This was shown most distinctly in the present investigation in those groups in which *a reduction in the total sugar consumption was followed by an increase in caries activity when the subjects received a small portion of the reduced amount of sugar between meals.* This was most apparent in the Chocolate Group (Fig. 11).

In studies carried out by BOYD & DRAIN (1928), TOVERUD et. al. (1942) and BOYD (1943) on the effect of sugar restriction and avoidance of sweets etc. between meals on dental caries in diabetics, the subsequently observed decrease in caries activity was probably attributable, at least mainly, to the restriction of sugar between meals. This also applies to the therapeutic reduction of sugar in the investigations performed by the Michigan Group, HOWE et al. (1933) and BECKS et al. (For survey see BECKS, 1950).

dental caries activity was observed. These results support each other to form strong evidence that sugar exerts its caries-promoting effect locally in the mouth in accordance with the widely accepted chemo-parasitic theory on the pathogenesis of dental caries.

CONCLUSIONS

Observations made in the present investigation suggested the following conclusions.

1. The consumption of sugar can increase caries activity.
2. The risk of sugar increasing caries activity is great if the sugar is consumed in a form with a strong tendency to be retained on the surfaces of the teeth.
3. The risk of sugar increasing caries activity is greatest if the sugar is consumed between meals and in a form in which the tendency to be retained on the surfaces of the teeth is pronounced with a transiently high concentration of sugar on these surfaces.
4. The increase in caries activity under uniform experimental conditions varies widely from one person to another.
5. Increase in caries activity due to the intake of sugar-rich foodstuffs consumed in a manner favouring caries disappears on withdrawal of such foodstuffs from the diet.
6. Carious lesions may continue to appear despite the avoidance of refined sugar, maximum restriction of natural sugars and total dietary carbohydrates.

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CARIOGENICITY OF SUGAR-CONTAINING DIETS[1]

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Recent studies by Sognnaes ('48) indicate that caries susceptibility in the Long-Evans strain of the Norway rat may be markedly influenced by systemic factors operating during the period of tooth development. These results have been corroborated by McClure ('51) using the National Institute of Health strain of rats.

The prenatal influence of diet on caries susceptibility, however, appears from the work of these investigators to be different with different strains of rats. In the Sherman strain McClure found that neither the incidence nor the extent of dental caries was greater when the animals were exposed to the influence of the cariogenic diet in the prenatal state than when feeding of this diet was started at birth. Sognnaes, on the other hand, observed in the Long-Evans strain a definite prenatal influence of diet.

Experiments conducted in our laboratories on the albino rat of the Wistar strain have yielded results that are in some respects similar to and in other respects unlike those obtained by Sognnaes and by McClure.

EXPERIMENTAL

The albino rats of the Wistar strain used in these experiments had been inbred in our laboratories for over 17 years. The experiment consisted of two parts. The first was designed

[1] The expense of this investigation was defrayed in large part by a grant-in-aid from the Sugar Research Foundation.

for making comparative observations on the cariogenicity of different diets; the second to determine whether these diets had a prenatal influence on susceptibility to caries.

Part I

Twenty-four groups of litter mates, of 4 animals each (a total of 96 animals), were selected at weaning from parents that had been raised and maintained on a stock diet. They were then placed on 4 different diets, which will be designated as high sucrose, high protein, high fat, and stock. The composition of the first three of these diets is presented in table 1. The stock diet consisted solely of a commercial animal food.[2] It should be noted that both the high protein and high fat diets contained over 20% sucrose.

At the end of 150 days on the experimental diets the animals were sacrificed and the teeth examined under the microscope for carious lesions. A tabulation of the observations made on these teeth is presented in the upper portion of table 2. In this table we have recorded the number and extent of the carious lesions instead of the usual caries score employed by many investigators. The caries score, obtained by multiplying the average grade of the lesions by the average number of areas affected, is undoubtedly useful for comparative purposes, but we believe a better over-all picture is obtained from more complete data as recorded in table 2.

Caries always started in the fissures and, with very few exceptions, occurred only in the lower molars. The upper molars have therefore been disregarded and the tabulation limited to data on the lowers.

Since there are three fissures in the first lower molar, two in the second and one in the third, each animal presented the possibility of 12 lesions in the lower molars; the 24 animals in each group, 288 possibilities. This should be kept in mind in evaluating the data in table 2.

[2] Purina Laboratory Chow.

A first molar tooth that was completely destroyed by caries was recorded as having three basic lesions; a second molar, two; and a third molar, one lesion. The extent of the lesion in each fissure (see table 2) was graded according to the following standards:

TABLE 1

Composition of the high sucrose, high protein and high fat diets

CONSTITUENTS	DIET 1	DIET 2	DIET 3
	%	%	%
Sucrose	64	22	24
Casein	20	62	25
Fat	8	8	43
Yeast and liver extract	4	4	4
Salt mixture	4	4	4

Salt mixture		*Vitamins*[1]	
Calcium (%)	0.60	Thiamine (p.p.m.)	24
Phosphorus (%)	0.38	Riboflavin (p.p.m.)	24
Magnesium (%)	0.053	Niacin (p.p.m.)	32
Potassium (%)	0.72	Pyridoxine (p.p.m.)	10
Sodium (%)	0.40	Pantothenic acid (p.p.m.)	25
Iron (p.p.m.)	480	Folic acid (p.p.m.)	1
Manganese (p.p.m.)	78	Biotin (p.p.m.)	0.12
Copper (p.p.m.)	10	Inositol (p.p.m.)	130
Iodide (p.p.m.)	1.5	Choline (p.p.m.)	3,000
Fluoride (p.p.m.)	0.1	Carotene (p.p.m.)	3
Zinc (p.p.m.)	4	A concentrate (I.U./gm)	10
Sulfur (p.p.m.)	310	Viosterol (U.S.P. units/gm)	3
Aluminum (p.p.m.)	0.16	α-Tocopherol (p.p.m.)	300
		Linoleic acid (p.p.m.)	500

[1] From yeast and liver extract and supplementary addition. To make the diets more palatable 1.6 p.p.m. butter flavor was added.

Grade 1. Initiation of caries, as indicated by the characteristic brown stain with or without a small cavity.

Grade 2. Cavity large enough to take an "explorer."

Grade 3. Moderate spread of the lesion involving the cusps.

Grade 4. Spread of the lesion from one fissure to another by the breakdown of the cuspal walls.

Grade 5. Spread of the lesion to the extent that the involved area (which might be the entire tooth) was lost.

Part II

Animals were selected from litters that could provide 4 males and 4 females, the parents having been raised and maintained on the stock diet. These young rats were placed at weaning on the same 4 diets as the animals in part I of the experiment. At the age of 100 days brothers were mated with

TABLE 2

Dental caries in the albino rat (Wistar strain) on a high sucrose, a high protein, a high fat and a stock diet

DIET	NO. ANIMALS	ANIMALS WITH CARIES	TOTAL NO. OF CARIOUS MOLARS	TOTAL NO. OF CARIOUS LESIONS	GRADE OF LESION				
					1	2	3	4	5
					Number of lesions				
First generation									
High sucrose	24	12	22	24	14	8	2	0	0
High protein	24	2	6	7	5	2	0	0	0
High fat	24	4	8	8	8	0	0	0	0
Stock	24	4	6	6	5	1	0	0	0
Second generation									
High sucrose	24	14	29	29	20	9	0	0	0
High protein	24	1	1	1	1	0	0	0	0
High fat	24	3	4	4	4	0	0	0	0
Stock	24	2	3	3	3	0	0	0	0

sisters on the same diet. The offspring of these animals were designated as second generation animals. When weaned they were fed the same diets as their parents for a period of 150 days. At the end of this period they were sacrificed and the teeth examined under the microscope for caries. The data on these teeth are presented in the second half of table 2.

RESULTS AND DISCUSSION

In several of the animals on the stock diet, as is shown in table 2, a few carious lesions occurred in the lower molars.

The relative frequency, however, was low and the extent of the lesions extremely small.

On the high protein and high fat diets there were a few carious lesions in both the first and second generation animals; but, both as regards incidence and extent, these were of the same order of magnitude as those of animals on the stock diet. It may, therefore, be concluded that the presence of sugar in the diet, amounting to 22% to 24% of the total food mixture, did not make the diets more cariogenic than the ordinary stock diet.

Increasing the amount of sugar to the extent that it comprised 64% of the food mixture resulted in a higher incidence of, and more extensive, dental caries. However, it is apparent from the data in table 2 that the actual number of lesions and the actual destruction of the teeth were small. In this respect our results are somewhat different from those of Sognnaes ('48), using the Long-Evans strain of rats on the Harvard high sucrose diet. In no instance did our animals develop carious lesions that were in any way comparable to the carious destruction which he observed, and reported in photographic illustrations ('49).

Since both diets contained approximately the same amount of sugar, we are confronted with some questions of basic significance. Is the difference in results on the two diets due to hereditary factors in the two strains of rats or is it due to differences in the composition of the diets other than their sugar content? Comparison of the Emory diet (table 1) with the Harvard diet (Sognnaes, '48, p. 677) reveals a number of differences in the mineral and vitamin content.

Should the difference in results be traced to a difference in hereditary factors, there would remain to be determined the nature of these factors. Conceivably there could be a difference in the composition or the apatite structure of the teeth of our Wistar strain animals and those of the Long-Evans strain. Should the difference in the cariogenicity of the two diets, however, be traced to differences in their composition, we would be driven to look for one or more specific

substances in the Emory diet that make the teeth more resistant to caries. These questions are now the subject of joint investigations in the Harvard and the Emory laboratories.

There is still another difference between our results and those of Sognnaes, the cause of which should be investigated. In the experiments of Sognnaes the prenatal diet seemed to be an important predisposing factor with respect to the subsequent dental caries. Considerably greater tooth destruction occurred on a high sucrose diet when the animals were subjected to the prenatal influence of the diet by feeding this diet to the mother before and during pregnancy. Some of the results of Sognnaes' experiments also suggested that the prenatal effects of the diet may have been more pronounced the longer the time the mother was on the diet. Our animals on the high sucrose diet in the second generation were born of parents that had been on this diet from the time of weaning, yet it will be noted from the data in table 2 that there was no greater carious destruction of their teeth than of the teeth of the first generation animals that had not been subjected to the prenatal influence of the diet. In this respect our animals resembled those of the Sherman strain studied by McClure ('51).

It is recognized that more exacting experimental conditions, as suggested by Sognnaes, would be brought about by having two successive litters born of the same parents and placing the mothers on different diets during the two pregnancies. We do not feel, however, that these stringent conditions were called for in this experiment, because of the homozygosity of the animals from which our colony originated and the constant inbreeding over 17 years. In all this time we have never had one animal born in the colony with pigmentation in the eye or colored strands of hair.

McClure found that more of his animals developed dental caries, and that the carious lesions were more severe, when the feeding of the cariogenic diet was begun at birth than when it was begun at weaning. These results are in keeping

with those of Sognnaes. In our experiments, however, using the Wistar strain, there was, as is shown in table 2, no greater carious destruction of the teeth of animals of the second generation that had been fed the high sucrose diet from birth than of those animals that had been fed the same diet from weaning. This would suggest the advisability of further investigation into the possibility of a strain difference in response to the systemic influence of cariogenic diets.

SUMMARY

The number and extent of carious lesions in the teeth of albino rats of the Wistar strain on a high protein and a high fat diet containing 22% to 24% sugar were extremely small and of the same order of magnitude as in animals fed an ordinary stock diet. Increasing the sugar content of the diet so that it constituted 64% of the food mixture resulted in some increase in the carious damage to the teeth. The actual number and the actual extent of the lesions, however, were relatively small. Exposure of the animals to the possible systemic influence of a high sucrose diet in prenatal life did not increase the incidence of dental caries.

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The Metabolism of the Oral Flora

2. THE OXIDATION OF SOME SUGARS BY MIXED HUMAN SALIVA

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The breakdown of carbohydrate by the oral micro-organisms has been the subject of numerous investigations. It is believed by many that the intra-oral production of acids from carbohydrate, arising from incomplete oxidation, is a factor in the production of dental caries. With a view to finding the agent causing acid production, specific organisms have been isolated from the mouth and their biochemical behaviour has been studied; this work has been outlined in a previous paper (Hartles & McDonald, 1950). More recently, the anaerobe *Clostridium welchii*, which can break down glucose into lactic acid more rapidly than does *Lactobacillus acidophilus* (Hartles & McLean, 1952), has been found in carious teeth (Hartles & McDonald, 1951; Evans & Prophet, 1951). Undue emphasis must not be placed on the results obtained by studying cultures of organisms isolated from the mouth, for the presence of a particular acidogenic organism may be relatively unimportant if enzymes which can complete the oxidation of the carbohydrate are contributed to the system by other organisms. For example, lactic acid may be the end point of glucose metabolism for an *in vitro* culture of *Lb. acidophilus*, but it certainly is not so in the mouth, for frequently other organisms exist there which can metabolize lactic acid (Douglas, 1950; Neuwirth & Summerson, 1951). Fosdick & Hansen (1936) were perhaps the first to realize fully the importance of this symbiotic relationship. Thus, whilst recognizing the contribution which has been made by the investigation of specific bacteria, we consider it desirable to study the enzymic properties of saliva as a whole. We have already studied the oxygen uptake of saliva samples with and without the addition of glucose (Hartles & McDonald, 1950); a similar study by Neuwirth & Summerson (1951) reached substantially the same conclusions. It seemed desirable to extend this work using other sugars, primarily to find out if there were any differences in the rates of oxygen uptake by a series of salivas when each sugar was added separately. The sugars chosen were glucose, fructose, maltose, and sucrose, since they are the most relevant to man. We found that in the saliva of all the subjects studied, fructose was metabolized less rapidly than the other three sugars.

EXPERIMENTAL

Material. Samples of saliva were obtained from ten volunteers with varying caries experience.

Collection of saliva. The method of collection of early-morning saliva was that described by Hartles & McDonald (1950).

Manometry. The saliva samples (0·5 ml. or 1·0 ml., depending on the enzymic activity) were buffered with 0·2M phosphate buffer, pH 7·4. O_2 consumption was measured in Warburg manometers at 38°. The final concn. of sugar, when used, was 0·005M. Increasing the concn. to 0·01M had no noticeable effect on respiration. The difference in O_2 uptake between saliva incubated alone and with added sugar was a measure of oxidation of the sugar.

RESULTS

The oxidation rates of the four sugars are shown in Table 1. The subjects have been arranged in two groups of five in order of increasing DMF number (i.e. the number of teeth decayed, missing or filled); those in group I have an average DMF of 8 and those in group II of 19.

In group I fructose and maltose showed a consistently significant difference in metabolic rate for each subject; the difference between fructose and sucrose was significant for two subjects, J.M. and J.R.D. ($P < 0·05$), while that between glucose and fructose was not significant at the 5% level. The analysis of variance shows that combining the data for all five subjects accentuates the difference between sugars; fructose is metabolized at a significantly lower rate than any of the other three sugars ($P < 0·001$), whilst maltose is metabolized at a significantly higher rate than either glucose ($P < 0·001$) or sucrose ($P < 0·01$). There is no significant difference in the rates of oxidation of glucose and sucrose.

Results for group II show that each subject (except M.R.W. with sucrose) metabolizes glucose, maltose, and sucrose, at a significantly higher rate than fructose; also, that the variation in the values for each sugar is greater than that displayed by the subjects of group I. This is established by an analysis of variance of the combined data for group II in which the residual variance used to assess the

Table 1. *Mean oxygen consumption of salivary samples due to the addition of different sugars*

(All values expressed as μl. O_2/ml. saliva/hr. Final concn. of added sugar, 0·005 M. Temp. 38°.)

Subject	Teeth decayed, missing, or filled (DMF)	Glucose	Fructose	Maltose	Sucrose	*S.E.	No. of days
			Group I				
S.F.S.	4	91·7	87·1	98·5	91·1	4·0	12
J.M.	6	68·5	62·9	77·5	77·8	4·0	10
J.G.	8	63·7	59·6	74·7	63·0	3·1	12
J.R.D.	10	180·4	170·2	186·7	186·3	5·8	12
A.G.L.	12	143·0	132·4	149·8	140·5	5·7	11
Mean	8	109·4	102·4	117·4	111·7	—	—
Weighted mean		110·2	103·4	118·1	112·5	—	—
			Group II				
R.L.H.	17	397·1	334·8	390·2	411·5	14·7	13
M.R.W.	17	136·4	113·5	128·9	121·1	5·1	14
L.G.	18	108·0	77·5	103·1	110·8	5·1	10
I.A.R.	20	60·6	46·8	68·0	65·0	3·9	11
P.D.B.	23	134·6	115·5	138·5	135·6	5·2	12
Mean	19	167·3	137·6	165·7	168·9	—	—
Weighted mean		173·9	143·3	172·0	175·0	—	—

* S.E. is the standard error of the difference between any two means: thus, roughly speaking, a difference of more than twice this value between any two means in a given row is statistically significant.

significance of the variance between sugars is much greater than that in the analysis for group I. Thence it can be seen from the overall analysis for group II that, again, fructose is the sugar least rapidly metabolized ($P<0·001$), but that there is no significant difference in the rates of metabolism of the other three sugars.

An analysis of variance of the whole data for the ten subjects shows that the difference in metabolic rate between fructose and the other sugars is highly significant ($P<0·001$). When deliberate selection is exercised and the subjects are arranged in two groups according to their DMF numbers, certain tendencies are observed. The differences between sugars with subjects of lower DMF (group I) are sharpened, because the general pattern of metabolism amongst these subjects is the same, that is, that fructose is metabolized less and maltose more rapidly than the others, whilst there is no significant difference between rates of glucose and sucrose metabolism. On the other hand, small differences between sugars with subjects of higher DMF (group II) are blurred because these subjects tend to behave differently toward different sugars and thus the only significant difference in this group is that between fructose and the three other sugars.

DISCUSSION

Dixon & Needham (1946) reported that yeast hexokinase had a lower affinity for fructose than for glucose. Meyerhof & Wilson (1949) obtained similar results for brain hexokinase. Slein, Cori & Cori (1950) found that yeast and brain hexokinases were similar in many respects, and they produced evidence to show that a single enzyme was responsible for the action on both glucose and fructose. Our results suggest that a hexokinase is concerned in the oxidation of glucose and fructose by the oral flora.

We found that sucrose was, in all except one of the subjects, metabolized at least as rapidly as glucose by the saliva. There is some disagreement in the literature concerning the existence of invertase in saliva (Bourquelot, 1910; Lisbonne, 1910; Tauber & Kleiner, 1933; Wohrinz, 1938; Volker, 1950). The fact remains, however, that unfiltered whole saliva can metabolize the disaccharides sucrose and maltose no less readily than glucose. Doudoroff, Kaplan & Hassid (1943) demonstrated that *Pseudomonas saccharophila* contained an enzyme which phosphorylated sucrose directly. There are, then, two possible pathways for sucrose breakdown. If sucrose is acted upon by invertase to produce glucose and fructose in equimolecular proportions, and if glucose and fructose are competing for hexokinase, it is not unreasonable to expect sucrose to be oxidized at a rate significantly less than glucose. For our group of salivas this was not so, and our results suggest that preliminary hydrolysis of sucrose may not be the first step in its metabolism. Further work is necessary to decide this point, and also whether the non-glycolytic enzymes concerned are a secretion of the salivary glands or are contributed by the oral flora. The only salivary carbohydrase which has been shown to be a product of the salivary glands is α-amylase

Roberts & Whelan (1951) have shown that this enzyme, when purified, has no action on maltose.

We are not at this stage particularly concerned with differences between 'caries active' and 'caries inactive' groups of subjects. We also realize that the present DMF number is not necessarily an indication of the present caries activity. Nevertheless, certain interesting observations can be made. In group I (low DMF) fructose is metabolized at a significantly lower rate than any of the other sugars. There are also significant differences in the rates of oxidation of maltose and sucrose, and of maltose and glucose. There is no such difference in the case of glucose and sucrose. In group II (high DMF) the only conclusion which can be drawn is that fructose is metabolized at a lesser rate than the other three sugars. Our results therefore suggest that the pattern of utilization of different sugars by group I subjects differs from that of group II subjects. It is, however, unwise to deduce more from these observations on so few subjects.

SUMMARY

1. The salivary flora metabolize fructose at a significantly lower rate than glucose, maltose, or sucrose.

2. Subjects with a comparatively low DMF number (number of teeth decayed, missing or filled) also show differences in oxidation rate between glucose and maltose, and between sucrose and maltose, but not between sucrose and glucose.

3. Subjects with a high DMF number did not show the differences enumerated in paragraph 2.

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Experimental dental caries in the albino rat

The production of carious lesions in animals maintained on a finely powdered purified diet containing 67% sucrose

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As far as we know, a caries-susceptible colony of rats has not hitherto been reared in this country. We were fortunate to receive a few animals from the stock maintained at the Harvard School of Dental Medicine. This colony has been produced over a number of years and is susceptible to the development of dental caries (Sognnaes, 1948; Shaw, 1950). All our rats were the descendants of four pairs of Harvard animals and have been multiplied by random mating. Brother-sister mating was not used. The colony is therefore more properly described as a caries-susceptible 'stock' rather than a 'strain'.

In considering experimental rat caries it is important to distinguish between the types of the disease known to occur in this animal. Hoppert, Webber & Canniff (1932) found that a diet containing coarsely ground maize (HWC diet) leads to the production of dental caries in their rats. If the maize was finely milled to pass a 60-mesh sieve and then incorporated in the diet no caries resulted. Cox, Dodds, Levin & Hodge (1948) showed that, after an initial period on the HWC diet, a high intake of sucrose (67% of the diet) causes an increased rate of progression of caries, but that a high level of sugar in the diet of animals that had not previously received the HWC diet does not lead to the production of caries. As a result of these experiments they concluded that, in their animals, caries is initiated by the fracturing of the enamel owing to the impaction of coarse maize particles in the occlusal fissures of the rat molars. King (1935) had previously put forward this view, but he studied only relatively few rats. Cox *et al.* (1948) believed that the high-sugar diet encouraged the progression of the disease in their animals but did not initiate it. This type of experimental caries is generally termed 'fracture' caries.

However, McClure (1945), Sognnaes (1948) and Shaw (1950) have described carious lesions in rats receiving a finely powdered diet containing 67% sucrose similar to that used for producing caries in the cotton rat (Shaw, Schweigert, McIntire, Elvehjem & Phillips, 1944). The experiments of Sognnaes (1948) show that, if rats are placed on the high-sugar diet at weaning, a mild caries develops. If the mother is given the sugar diet at parturition the severity of tooth decay in the offspring increases. The susceptibility to caries is greatest in the offspring of animals placed on the sugar diet throughout gestation and lactation.

The lesions described by the above workers are of the occlusal type originating in the deep fissures of the rat molars. More recently, Stephan (1951) and McClure &

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Folk (1955) have described lesions involving the buccal, lingual and proximal surfaces in addition to the occlusal fissures of the rat molars. In our animals we have observed occlusal lesions only.

The object of this preliminary study was to find out if a caries-susceptible colony could be built up from a few individuals imported from an established susceptible colony.

We were successful in producing a marked degree of caries in our animals after they had been maintained on the high-sugar diet for 6 months. This is a much longer time than is necessary in the original Harvard colony.

EXPERIMENTAL

Animals. Six pairs of rats were transported by air from Boston, Mass., U.S.A., to this country. They were from the caries-susceptible colony at the Harvard School of Dental Medicine and had been maintained on a diet containing 67% sucrose (Shaw, 1950). During their journey they had access to the sugar diet and to raw potato. On arrival in our laboratory they were placed on a stock diet of Lever cubes, known to be an adequate diet for the albino rat (Hartles, 1951). At this stage it was inadvisable to expose the animals to the risk of a synthetic diet that could not be identical with the one they had been receiving. We preferred to raise the first litters on a stock diet. The animals were allowed a period of 2 weeks in which to become acclimatized; five pairs were then mated. One of the females died before full term but the others had satisfactory litters and between them produced twenty female and fourteen male offspring. Second litters were obtained after a resting period of 1 month. All the young were maintained on the cube diet until they were 100 days old. At this age eighteen females were distributed into sets of six and mated. One set was placed on the high-sugar diet at mating, the young were weaned on to the diet and maintained on it thereafter. Seventeen of these animals formed group 1. The second set received Lever cubes until parturition, then the high-sugar diet, which the young also received thereafter. Sixteen of these offspring formed group 2. The third set received Lever cubes throughout and the young never had access to the high-sugar diet. Seventeen offspring formed group 3.

The animals of groups 1, 2 and 3 were killed when they were 119 days old.

The remaining females from the first matings were placed on the high-sugar diet and mated. Their progeny have since multiplied and the whole colony is now maintained on the sugar diet as a stock diet.

A further sixty-two animals born to parents bred and reared on the high-sugar diet were killed at intervals in an attempt to determine the time necessary to produce a degree of caries suitable for our purpose. These groups were: group 4, ten animals killed at 106 days; group 5, twelve animals killed at 149 days; group 6, twenty-four animals killed at 184 days; group 7, sixteen animals killed at 204 days. It would be wasteful to maintain the animals beyond the time after which few new lesions appear.

Housing. The animals were kept in galvanized-wire cages 12 × 12 × 6 in. with bottoms of $\frac{3}{4}$ in.^{2} mesh; four or five rats were kept in one cage. The temperature of the

animal house was maintained at 70° ± 5° F. Just before parturition the females were provided with wood-wool bedding. Litters were weaned at 21–25 days. The weight of pups at weaning was about 30–35 g.

Composition of the diet. The high-sugar diet (HS 1) was similar to the diets used by Shaw (1950), Sognnaes (1948) and Hartles (1951), but the vitamin supplements were larger, except for the omission of inositol. The diet without supplements is shown in Table 1.

Table 1. *Composition of the high-sugar diet (HS 1) without supplements (see Table 2)*

	g/kg
Sucrose (icing sugar, Tate and Lyle Ltd)	670
Casein, soluble (Evans Medical Supplies Ltd)	200
Hepamino (see below) (Evans Medical Supplies Ltd)	40
Groundnut oil (J. Bibby and Sons Ltd)	50
Salt mixture (Shaw *et al.* 1944)	40

To 1 kg of diet the supplements (Shaw's (1950) figures for 1040 g diet are included for comparison) shown in Table 2 were added.

Table 2. *Supplements added to 1 kg of diet HS 1 with, for comparison, the supplements added by Shaw (1950) to 1040 g of his diet*

	Diet HS 1 (mg)	Diet of Shaw (1950) (mg)
Thiamine hydrochloride	15	3·5
Riboflavin	15	3·5
Pyridoxin hydrochloride	5	3·5
Nicotinamide	25	25 (nicotinic acid)
Calcium pantothenate	25	20
p-Aminobenzoic acid	500	300
Choline chloride	1500	1000
Inositol	—	1000
Vitamin A acetate	20	11 (β-carotene)
DL-α-Tocopheryl acetate	300	50 (α-tocopherol)
2-Methyl-1:4-naphthoquinone	20	6
Ergocalciferol	200 μg	3000 i.u. (irradiated ergosterol)

According to the makers' specification 40 g Hepamino should include the following accessory food factors: thiamine 0·4, riboflavin 5·2, pyridoxin 0·8, folic acid 1·2, biotin 0·16, inositol 100, nicotinic acid 34 and cyanocobalamin 0·24 mg. The Hepamino also contributed one-sixth of the total protein in the diet. Shaw (1950) used equal quantities (2%) of whole liver and of a liver concentrate in his diet. He used maize oil whereas we used groundnut oil. Both oils contain adequate quantities of the unsaturated fatty acids.

Preparation of the diet. The solid components of the diet were all in a finely powdered state. The icing sugar and casein particles were sufficiently small without further treatment. The salt mixture was ground in a mechanical mortar until it all passed through a 60-mesh sieve. The Hepamino, which is a proteolysed whole-liver preparation, is in the form of friable crumbs and is easily ground to a fine powder in a hand mortar.

The groundnut oil (containing the fat-soluble vitamins) was added to the casein and thoroughly worked in. The remaining dry components were added and thoroughly mixed. The water-soluble vitamins were mixed with water so that 20 ml. contained the vitamins necessary for 1 kg of diet. The *p*-aminobenzoic acid was converted to its sodium salt with the calculated amount of sodium carbonate. The vitamin solution was added to the diet and thoroughly mixed. Water (*c.* 60 ml.) was added to the mixture so that it would just hold together when squeezed firmly in the hand. The diet was never wet or sloppy. Not more than 2 days' supply was made at a time. The animals received fresh diet daily.

Amount of food consumed. The diet was provided in 2 oz. glass pots which were clipped on to the inside of the cage. Each pot stood on a circular metal tray (diameter 4 in.) with a ¼ in. raised rim which minimized the loss of food by scattering. The animals were fed each morning and the pots replenished in the late afternoon if necessary. The rats therefore received as much food as they could eat; usually 10–15 g daily was consumed by each animal. The diet was acceptable over long periods, there being no falling off in demand.

Drinking water. Tap water was provided from drinking bottles with glass spouts.

Excreta. The faeces were small, black and dry, the diet appeared therefore to be well utilized. After 6 weeks on the régime some of the animals exhibited slight glycosuria.

Examination of the molar teeth. At the end of the experimental period the animals were killed with ether. The heads were removed and boiled for 30 min in soap solution. The soft tissues were then removed from the jaws with forceps and finally by brushing with a tooth brush. The teeth were examined under a low power (× 10) binocular microscope.

The normal rat molar (see Pl. 1, 1) exhibits on its occlusal surface several deep fissures lined with enamel. In this investigation caries appeared to begin only in the deepest part of a fissure. In most animals on the HS 1 diet, food particles, faeces and hair became impacted in the fissures. The impacted material was usually brown in colour. Where there was no caries the enamel remained unstained and retained its normal glistening appearance.

The earliest visible sign of a carious attack seemed to be a brownish staining of the walls of the fissure and a loss of translucency in the enamel without apparent loss of tooth substance (Pl. 1, 2). Ground sections were prepared from a typical lesion at this stage. These showed (Pl. 1, 3) that the enamel on the lateral walls of the fissure was affected over a considerable area and that near the base of the fissure there was a breach in the enamel communicating with the dentine. The underlying dentine showed considerable staining and, as a result of the boiling and drying process to which the teeth had been subjected, the demineralized layer adjacent to the enamel had contracted leaving a narrow space at the amelo-dentinal junction. In view of these findings it appears entirely justifiable to consider this type of lesion as being caries.

In more advanced lesions there was obvious and progressive destruction of both enamel and dentine producing large irregular cavities in the tooth (Pl. 2, 1, 2).

Scoring of the carious lesions. No elaborate scoring procedure was adopted. If more than one-quarter of the crown was destroyed the tooth received a score of 3. If the loss

of tooth substance was obvious but involved the destruction of less than one-quarter of the crown it was scored 2. If the tooth had an early lesion (as described above), not involving visible loss of tooth substance, it was scored 1. An intact tooth was scored 0. Results were expressed both as average number of carious teeth per animal and as average caries score per animal.

RESULTS

Performance of the animals

General condition. Occasional animals amongst the first litters, both on the Lever-cube diet and on the sugar diet, developed superficial abscesses in the axillary region. These, if left, burst and were cleaned up by the animal with no apparent ill effects. Examination of the thick green pus showed it to be primarily due to an infection with *Bacillus friedländer*. No such infection occurred in subsequent generations.

The general health was excellent, the animals had a good muscle tone, were lively and strong. Four animals in one cage developed a temporary baldness of the head and neck, but otherwise the coats were good. Occasionally an animal developed a sore eye which responded to treatment with boric-acid solution.

Growth. The growth of the animals was equally as good on the HS 1 diet as on the Lever-cube diet. For groups 1, 2 and 3 the mean final body-weight (119 days) and mean growth rates of the animals were as shown in Table 3. Reproduction on diet HS 1 was satisfactory, an average of six to seven pups per litter being reared.

Table 3. *Growth of animals on the HS 1 and on the Lever-cube diets*

	Females		Males	
Group	Final body-weight (g)	Gain (g/day)	Final body-weight (g)	Gain (g/day)
1 (HS 1)	170	1·4	239	2·2
2 (HS 1)	181	1·6	242	2·2
3 (Lever cubes)	191	1·6	243	2·1

Condition of teeth

Group 1. There were no obvious signs of caries, one or two of the lower first molars had slightly discoloured fissures. The teeth showed no evidence of attrition, the cusps were all intact and showed no sign of fracture (Pl. 1, 1). The fissures were often filled with debris of diet, faeces and hair. There were in some animals accretions on the smooth buccal, distal and lingual surfaces of the upper (particularly the first) molars.

Group 2. The results were similar to those for group 1.

Group 3. There was no evidence of caries but there was considerable attrition of the occlusal surfaces. As a consequence there was less adventitious material adhering to the teeth (Pl. 2, 3).

Group 4. There was no evidence of caries.

Group 5. Caries was observable in six of the twelve animals. Only one animal had grossly carious molars. Results are given in Table 4.

Group 6. There was a definite increase in both incidence and severity of lesions compared with group 5 (see Table 4); sixteen of the animals had carious teeth.

Group 7. Caries occurred in twelve rats. There was an increase in the number of lesions per animal and in their severity compared with group 6 (see Table 4).

Table 4. *Development of caries in rats on a high-sugar diet* (*HS* 1)

Group	No. of rats	Age at killing (days)	No. of rats with caries	No. of carious teeth				Caries score*			
				Total	Per rat	Per mandible	Per maxilla	Total	Per rat	Per mandible	Per maxilla
4	10	106	0	0	0	0	0	0	0	0	0
5	12	149	6	16	1·3	1·0	0·3	25	2·1	1·4	0·7
6	24	184	19	53	2·2	1·9	0·3	89	3·7	3·3	0·4
7	16	204	12	39	2·4	2·2	0·2	74	4·6	4·2	0·4

* See p. 237.

DISCUSSION

The results show that our animals maintained on diet HS 1 did not develop any measurable dental caries until they were 149 days old, the average number of carious teeth at this age being 1·3. This figure must be taken cautiously since it was raised by the inclusion of one rat with extensive caries. At 184 days of age there was a considerable increase in caries, the average number of affected teeth per animal rising to 2·2. In accordance with the results of many other workers it was found that the teeth of the mandible were very much more susceptible to decay than those of the maxilla. The first and second mandibular molars were more frequently carious than the third molars. After 204 days on the diet there was a further slight increase in the number of carious teeth per animal (2·4). It would appear, therefore, that in the conditions of this investigation the rats must be maintained on diet HS 1 until they are about 6 months old before a degree of caries adequate for experimental purposes is produced. This is a longer time than is necessary to produce caries in the parent Harvard colony. Shaw (1950) reports extensive caries after 3–4 months on a high-sugar diet.

Effect of Lever-cube diet fed to rats for one generation. In view of the work of Sognnaes (1948) it might have been expected that one result of the interruption of the high-sugar diet for one generation would be a lowered susceptibility to dental caries in the next generation (group 2). That this reduced susceptibility should persist in animals of subsequent generations (groups 4–7) was unexpected.

There is no obvious explanation why our rats should be less susceptible to caries than the parent colony. Our results emphasize, however, the dangers of comparing findings of different laboratories since small changes in the diet or a change in environment may influence the susceptibility to dental caries.

The degree of caries found in our animals is, however, sufficiently large to enable them to be used in the study of dietary factors in relation to experimental rat caries.

SUMMARY

1. Dental caries has been produced in a colony of rats bred from four pairs of animals imported from the caries-susceptible colony at Harvard University School of Dental Medicine, Boston, Mass.
2. The animals were fed on a purified diet containing 67% sucrose.
3. Growth, reproduction and lactation on the high-sugar diet were good.
4. After 184 days an average of 2·2 carious teeth per animal was observed.

The authors are greatly indebted to Dr J. H. Shaw of the Harvard School of Dental Medicine for providing the animals from which this colony has been bred.

Grateful thanks are due to Messrs J. Bibby and Sons Ltd for the gift of groundnut oil; to Evans Medical Supplies Ltd for the gift of Hepamino; and to Messrs Tate and Lyle Ltd for the gift of icing sugar.

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EXPLANATION OF PLATES

Illustrations of mandibular molars from the experimental groups of rats.

PLATE 1

1. Occlusal view of molar from group 1, deemed to present a normal picture. ×9.
2. Early carious lesion in second molar corresponding to the ground section shown in Pl. 1, 3(*b*). ×9.
3. Ground section through a fissure of molars. ×120. (*a*) Normal fissure filled with food debris and faeces. (*b*) Early caries, showing that the enamel on the lateral walls is affected; near the base of the fissure there is a break in the enamel communicating with the dentine.

PLATE 2

1. Carious lesions in the first and third molars, typical of cavities scored 2, i.e. visible tooth destruction involving loss of less than one-quarter of the crown. ×9.
2. Carious lesions in the first molar typical of a cavity scored 3, i.e. destruction of more than one-quarter of the crown. ×9.
3. Attrition: the crowns are almost completely worn away. This degree of attrition was found only in animals receiving the Lever-cube diet. ×9.

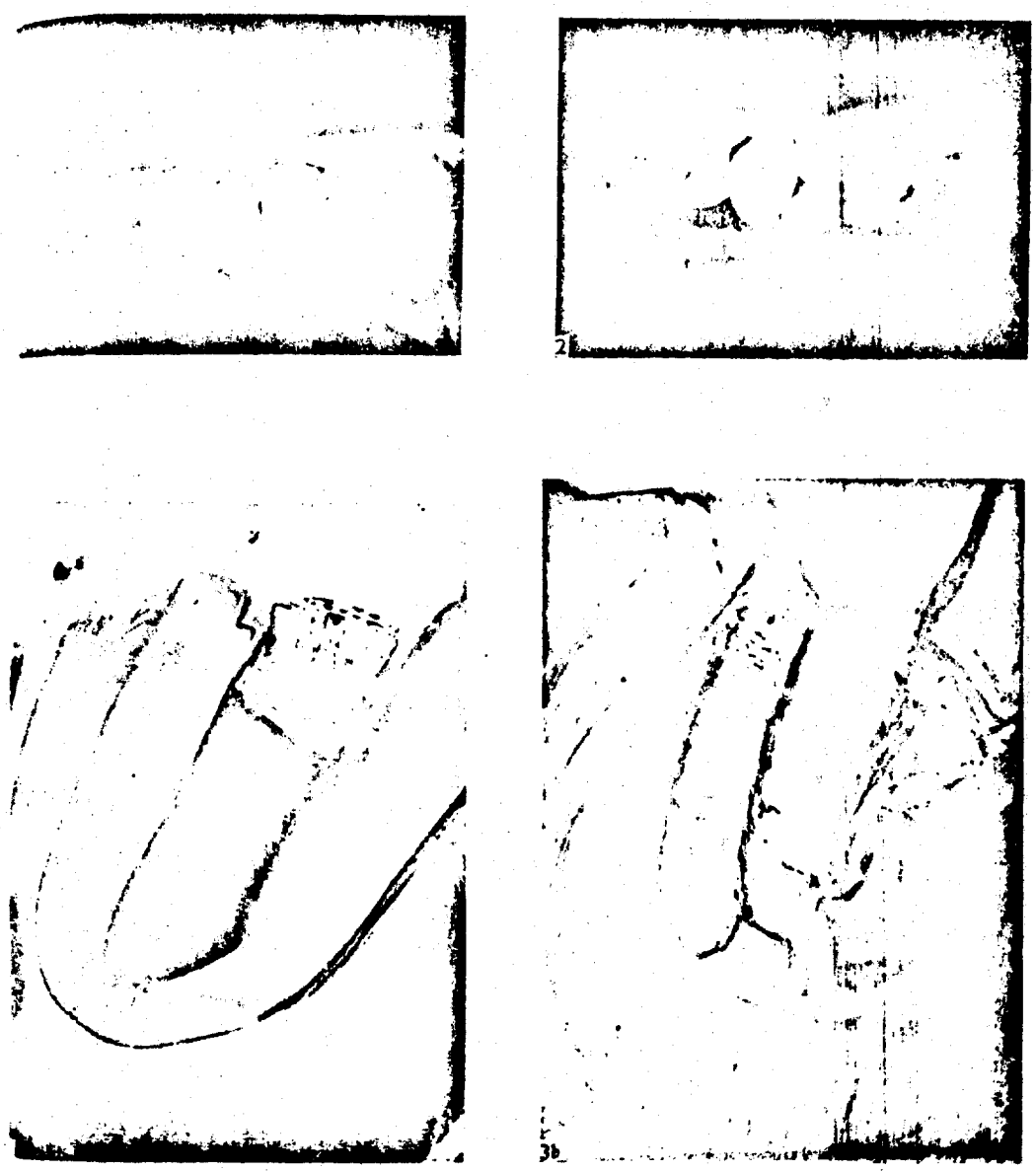
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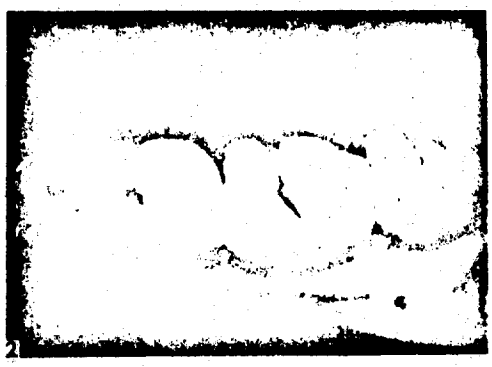
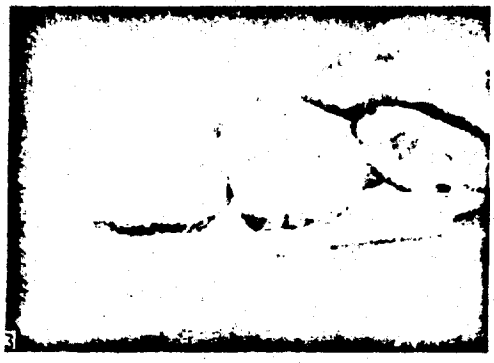
Plate 2





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Effect of Dietary Casein-Sucrose Ratios on Amino Acid Composition of Dentinal Protein

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Losee, Van Reen, Peckham, Hess, Henderson, and Gerende[1] maintained rats on diets containing 20, 30, and 40 per cent casein, designated 20P, 30P, and 40P. All the rats on all the diets developed carious lesions. While there were no marked differences in the caries responses to any of the 3 diets, there were differences in weight gain. Maximum body weight gain was on the 30 per cent casein diet. The proteins from the incisor dentin were isolated and their amino acid composition was determined by microbiological assay. The error inherent in these methods was felt to be of an order of magnitude that deprived the variations in composition of any significance.

The analyses of these proteins have been repeated by the precise chromatographic methods devised by Moore and Stein.[2] Hughston, Earle, and Binkley[3] have used one of the Moore and Stein methods for the analysis of the protein isolated from human dentin. We have included an analysis of human dentinal protein by the chromatographic method for the purpose of comparison with their results and also with the earlier results of Hess, Lee, and Neidig.[4]

MATERIALS AND METHODS

The human dentinal protein was the same sample as that previously analyzed.[4] It had been prepared by dialytic demineralization against 0.1 N HCl. Proteins had been isolated from the incisor dentin of the rats on the 3 different casein diets by demineralization with EDTA (ethylenediaminetetraacetate) at pH 7.5.[1] The proteins were hydrolyzed by 6 N HCl for 24 hours in a sealed tube.

The amino acid analyses were done by the most recent method of Moore, Spackman, and Stein,[5] using a polystyrene resin.* The hydraulic separation method of Hamilton[6] was used for the preparation of the resin particles of appropriate size.† The colorimetric ninhydrin procedure of Moore and Stein[7] was used and the optical densities were determined in a photoelectric colorimeter.

RESULTS AND DISCUSSION

The results of the analyses of human dentinal protein are given in Table 1, together with the earlier results of Hess *et al.*[4] and also those of Binkley.[3] The total nitrogen content of each protein was determined by the micro-Kjeldahl method. The values for

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* Amberlite IR-120, Fisher Scientific Co.

† This step was very kindly conducted for us by Dr. K. A. Piez at the National Institutes of Dental Research.

each protein are included, together with those for the amino acids. The recovery of nitrogen in the amino acids and ammonia estimated is given in the adjacent column. The final figures are the per cent recovery of the total nitrogen. No marked differences were noted in the values for any of the amino acids except hydroxylysine. Our hydroxylysine values are about half those of Binkley but are of the same order as previously found by Battistone and Burnett.[8] We found no evidence for the existence of the peptides in the hydrolysate of the dentin that were reported by Binkley.[3] The temperature for hydrolysis used was between 105° and 110° C. rather than their temperature of

TABLE 1*

AMINO ACID COMPOSITION OF HUMAN DENTINAL PROTEIN

	New (Per Cent)		Hess† (Per Cent)		Binkley‡ (Per Cent)
	Amino Acid (1)	N (2)	Amino Acid (3)	N (4)	Amino Acid (5)
Alanine	8.0	1.26	8.5	1.34	6.0
Aspartic	5.7	0.60	5.6	0.59	5.6
Arginine	7.8	2.50	7.4	2.38	7.5
Cystine	0.1	0.01	0.1	0.01	0.0
Glutamic	10.0	0.95	10.6	1.01	9.3
Glycine	22.4	4.18	22.5	4.20	20.2
Histidine	0.8	0.22	0.8	0.22	0.7
Hydroxylysine	1.3	0.14	1.0	0.11	2.7
Hydroxyproline	14.8	1.58	13.1	1.40	15.7
Isoleucine	1.4	0.15	1.3	0.13	1.1
Leucine	3.8	0.41	3.6	0.38	2.7
Lysine	3.4	0.65	3.3	0.64	2.6
Methionine	0.5	0.05	0.5	0.05	0.7
Phenylalanine	2.0	0.18	1.9	0.16	2.1
Proline	13.5	1.64	16.3	1.98	13.0
Serine	3.5	0.45	3.5	0.45	3.6
Threonine	2.1	0.25	3.1	0.36	1.5
Tyrosine	1.0	0.08	1.1	0.09	0.5
Tryptophan	0.0	0.0	0.0	0.0	0.0
Valine	2.2	0.26	3.0	0.36	2.3
Ammonia	0.5	0.41			0.6
Nitrogen	16.15	15.97	16.15	15.86	17.9
Per cent recovery of N		98.8		98.2	98.4

* The values in cols. 1 and 3 are expressed as grams of amino acid per 100 gm. protein. The values in cols. 2 and 4 are the amount of nitrogen in each amino acid per 100 gm. protein. The values in col. 5 are residue weights and need to be increased by 10–15 per cent to be comparable to those in cols. 1 and 3.

† See ref. 4 ‡ See ref. 3

100° C. We have never failed to obtain complete hydrolysis of the many proteins previously analyzed under our own conditions.

The data on the three proteins from the rats fed 20P, 30P, and 40P are given in Table 2. No significant differences were found in the composition of the three proteins. The values were substantially the same as those found for human dentin. It is apparent that the variations in the protein content of the diet had no effect on the composition of the dentin protein.

SUMMARY

Polystyrene resin was used for the chromatographic analysis of the proteins isolated from human dentin and from the incisors of rats fed diets containing 20, 30, and 40

per cent casein. The amino acid composition of the human dentin protein determined by this precise method was similar to that previously found by other methods of analysis and by other investigators. The rat incisor dentinal proteins from the three diets showed no differences one from the other and closely resembled the human dentinal protein in amino acid composition.

TABLE 2*

AMINO ACID COMPOSITION OF RAT DENTINAL PROTEINS

	20P		30P		40P	
	Amino Acid (Per Cent) (1)	N (Per Cent) (2)	Amino Acid (Per Cent) (3)	N (Per Cent) (4)	Amino Acid (Per Cent) (5)	N (Per Cent) (6)
Alanine	5.9	0.92	6.4	1.01	5.9	0.92
Aspartic	5.8	0.61	5.4	0.57	5.4	0.57
Arginine	6.6	2.12	6.8	2.19	7.0	2.25
Cystine	0.1	0.01	0.1	0.01	0.1	0.01
Glutamic	8.6	0.82	8.2	0.78	8.2	0.78
Glycine	20.6	3.84	20.8	3.88	21.0	3.92
Histidine	0.9	0.24	1.0	0.27	0.7	0.19
Hydroxylysine	1.4	0.15	0.9	0.10	0.9	0.10
Hydroxyproline	14.3	1.53	14.0	1.50	13.2	1.41
Isoleucine	1.1	0.12	1.1	0.12	1.2	0.13
Leucine	4.0	0.43	3.7	0.40	3.6	0.38
Lysine	4.0	0.77	3.8	0.73	3.6	0.69
Methionine	0.5	0.05	0.5	0.05	0.5	0.05
Phenylalanine	2.3	0.20	1.8	0.15	2.0	0.17
Proline	13.3	1.62	12.8	1.56	12.8	1.56
Serine	3.0	0.40	3.2	0.43	3.7	0.49
Threonine	1.8	0.21	1.8	0.21	2.2	0.26
Tyrosine	1.0	0.08	1.0	0.08	1.0	0.08
Tryptophan	0.0	0.0	0.0	0.0	0.0	0.0
Valine	2.8	0.33	3.3	0.39	3.3	0.39
Ammonia	0.5	0.41	0.5	0.41	0.5	0.41
Nitrogen	15.1	14.86	15.6	14.84	15.4	14.76
Per cent of recovery of N		98.4		95.1		95.8

* The values in cols. 1, 3, and 5 are expressed as grams of amino acid per 100 gm. protein. The values in cols. 2, 4, and 6 are amounts of nitrogen in each amino acid per 100 gm. protein.

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HAS IT BEEN PROVED THAT SUGAR IS A SIGNIFICANT FACTOR IN DENTAL CARIES?

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THERE are many important factors in dental health. The dentist should, whenever possible, advise his patients regarding them. A partial list of these factors includes heredity, environment, the general health, hygiene and nutrition. Nutrition must be subdivided on the basis of the character of food, the quality and quantity of food, the digestive processes and metabolism, the last a complex subject.

Those of us whose primary task is to teach dental health must endeavor to interpret for the people we serve the results of all the best and latest research work on dental caries. We must evaluate all the material that we can acquire and then dispense advice that will be not only sound, but also practical and usable. One needs much scientific training, much practical experience and nothing less than the judgment of Solomon to cope with this task. We study diligently the reports of this research worker and that one. We are well satisfied with the ability, honesty and scientific training of all of these workers. We can pick few, if any, flaws in their procedures or their thinking; yet they do not agree with each other. What then are we to teach?

Some workers claim that elimination of sugar from the diet reduces or entirely arrests caries.[1] Others have shown an improvement in the dental condition with an increase in the vitamin C intake.[2] Still others have stressed the importance of vitamin D in caries prevention.

Research on the influence of sugar as a factor in dental caries has had a great revival. Nina Simmonds gave a most instructive review of these activities in a paper read before the Food and Nutrition Section of the American Public Health Association at Kansas City last October. All dentists interested in this subject should read this review carefully.[3]

Most of the studies made on children to determine the relation of dietary factors have been carried on in institutions. A study of some of this work reveals that "in one of the orphanages the diet of the children was admittedly below the standards accepted as normal for children as regards protein, calcium, phosphorus and vitamins and yet during this time these children had a very low incidence of tooth decay."[4]

How much the nervous, mental or emotional state affects nutrition and health in general, we do not know. It is no doubt a factor that we cannot wholly overlook. The child in the institution is

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frequently leading a less disturbed and better regulated life than the child who must share the adult regime and the disturbances and irregularities of ordinary family affairs.

Several studies relating to the cause of dental caries have been carried on in outpatient departments or private practice on children living in average homes. A report of one study made at Forsyth Dental Infirmary stated in summary:

> In the preliminary findings of a study of 132 patients followed for dietary supervision over a period of 1.6 years, an average reduction of dental caries of 79.21 per cent was observed in 104 cooperative patients. In 28 noncooperative patients, an average increase in dental caries of 12.97 per cent occurred. This increase compares with the expectancy for the average age of the group studied, which was 6 years."[5]

In this study, the following daily diet was considered optimal for dental health: 1 quart (946 cc.) of milk; one raw vegetable (or canned tomato), with special emphasis on cabbage and tomato; at least two cooked vegetables, one of which may be potato; two servings of fruit, with special emphasis on oranges; one egg; meat or fish five times a week, and butter on vegetables and bread. An attempt was made to keep cereals and breads, because of their acid ash, as low as was consistent with the requirements for energy. Candy was allowed only at the end of the meal. Cod-liver oil was not given routinely, but was prescribed by the pediatrician in occasional cases.[5]

Another study was that of E. H. Schiotz, who was instrumental in establishing the famous Oslo breakfast for school children. He made a careful study of the 200 children in the national schools who had healthy teeth. He investigated the character of feeding from birth, the dietaries of the mothers during pregnancy, the economic status of the parents and the present height and weight of the children. He also studied the dietaries and teeth of the children in the twenty-three homes where accurate data were kept. He found that the social and economic status of the family plays an important part, well-to-do children having 13.5 times as good a chance as children from poor homes to have healthy teeth. The health of the teeth appeared to be proportional to the regular supply of protective foods. He found no established correlation between the consumption of sugar and the health of teeth in different institutions; whereas the regular supply of cod-liver oil in winter seemed to play a beneficial part.[6]

B. R. East showed that the more remote the season is from summer, the greater the caries incidence, the three winter months producing the greatest number of cases.[6]

On the Island of Tristan da Cunha, it has recently been reported[7] that the sugar consumption has lately been approximately 5 to 10 pounds per capita per year as compared with more than 100 pounds in the U.S.A. Although the diet is rather below our standard requirements, the general and dental health is remarkable. There is, however, one group of the inhabitants which differs in respect to the type and incidence of dental caries; namely, the mothers. While the decay, as a rule, is of a superficial character, affecting few teeth, most of the mothers have one or more teeth with extensive caries, which seems associated with the number of pregnancies. The small amount of sugar is divided fairly equally among all members of the community. Obviously, there are some other factors in the progress of dental caries in some of the individuals. This little island is situated in the South Atlantic, midway between the tip of Africa and South America. The isolation of this settlement not only curtails its supply of foodstuffs, but also makes for little or no strain from marital, social, financial or political upheavals such as continuously affect a large part of the population of the United States. The whole habit of life

may account for the parallelism with the institutions before mentioned as regards good teeth on a low standard diet.

A few years ago, Percy R. Howe fed a group of monkeys quantities of sugar in the form of large sticky caramel-like masses. This practice he continued for nine months. During this time, the teeth were not cleaned and the monkeys were fed a well-balanced adequate diet, which they consumed with no apparent loss of appetite. At the end of the experiment, the teeth were cleaned and carefully examined. Caries had not been produced. If a similar experiment were to be tried on human beings, the time of feeding the sugar would have to be carefully planned. Unlike the monkeys, our children, who are allowed sweets at any or all times, do not have appetites left for the foods supplying other necessary nutritional elements.

In commenting on the results of a study soon to be published by the Forsyth Dental Infirmary, Ruth White makes this very apt statement:

> The between meal consumption of sweets and adhesive carbohydrates is intimately associated with the whole field of home management and child guidance. Parents who provide carefully planned meals at regular intervals seldom allow indiscriminate between meal lunches. Conversely, regimes which permit the child to eat whatever he wishes whenever he is hungry usually involve "hand to mouth" management of the entire dietary with little regard for healthful foods.

Dr. Bundesen says that the body chemistry of a growing active child is such that he sometimes needs the readily available energy furnished by eating sweets; candy can have a proper place in the diet, as long as it does not replace the essential food substances that the child must have to be healthy, and the belief that sugar causes tooth decay has not been sustained by research work done on that subject. But the abuse of sugar if it is allowed to take the place of phosphorus, calcium and vitamin-containing foods believed to be essential to teeth may very well contribute to tooth decay.[8]

SUMMARY

1. A certain amount of sugar may be needed in the dietary.

2. Food habits of the nation, encouraged by advertising propaganda, make omission of the use of sugar impossible.

3. Good dental health and good general health are dependent on good nutrition. This involves not only eating habits, but also healthful living.

4. We do not know the cause of dental caries. Many conditions are responsible for its frequency.

5. That sugar is a significant factor in dental caries is a proved and accepted fact. Theories as to its action are still unproved. The consumption of sugar cannot as yet be identified as the real cause of dental caries.

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THE RÔLE OF SUGAR IN THE ETIOLOGY OF DENTAL CARIES

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DURING the past twenty years, considerable confusion has arisen concerning the rôle of diet in the control of dental caries. Before that time, it was quite generally agreed that caries was caused by the action of bacteria on food débris, with resultant production of acid, which was responsible for the actual decalcification of the tooth. More recently, it has been asserted by investigators in different parts of the world that caries could, in a measure, be controlled by improving the nutritional adequacy of the diet. Attempts to do this were made in several ways. May Mellanby[1] found that dogs raised on diets deficient in vitamins A and D and of high cereal content had hypoplastic, although not carious, teeth. She reported that, nevertheless, when children were fed diets fortified with vitamins A and D and low in cereal, the caries activity was appreciably reduced. It was concluded, from this study, then, that both vitamins A and D are essential for tooth protection against caries.

Other investigators stated that more important than vitamin D in fortifying the diet was vitamin C.[2] As a matter of fact, it was at one time believed that the addition of a pint of orange juice a day to the diet would reduce caries activity 50 per cent. Klein and McCollum[3] observed the occurrence of caries in rats on diets deficient in phosphorus. As a result of this and similar reports, the use of dicalcium phosphate for the control of caries became widespread, despite the fact that there is no clinical evidence of its value in this respect. A more inclusive view was taken by Boyd and Drain,[4] at the University of Iowa, in that they believed substantial control of caries to be possible on a diet adequate for good growth and development, including optimum amounts of all of the vitamins and a sufficient supply of minerals.

It was assumed, then, that these procedures necessarily prove that the structure of the tooth determines its susceptibility to caries and, secondly, that the structure of the erupted tooth can be influenced through nutrition. Both of these assumptions are debatable. We have little clinical evidence that hypoplastic teeth are any more susceptible to caries than are so-called normal teeth. Hypoplastic teeth may remain free from caries throughout the life of the individual; while so-called hard, well-formed teeth may become carious. There is even less evidence that the structure of the enamel, which is the first tissue attacked by caries, can be influenced through dietary change after the period of initial calcification.

Despite these inconsistencies, it is unquestionably true that caries activity has been reduced in children through dietary management. In seeking for a common basis for the beneficial results reported from the conflicting methods mentioned

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above, two facts have been brought out in bold relief: First, it is obvious, from a casual examination of the diets recommended by both British and American investigators for the control of caries, that, in all cases, the carbohydrate content has been reduced. This has not been done because the sugar or starch was considered to be important, but because it was hoped that by reducing the caloric intake in the form of highly sweetened foods, the children would be more apt, because of better appetites, to eat foods containing the substances considered nutritionally important. The first reports by Boyd and Drain were particularly notable in this regard. Their experimental group were children living on diabetic diets, which are, of course, low in sugar.

The relation of high sugar consumption to caries activity coincides with the indisputable relation between the presence of *Lactobacillus acidophilus* in the mouth and the activity of dental caries. Many observers are hesitant to ascribe a specific etiologic rôle to oral lactobacilli in this disease, but it is generally agreed that they bear a diagnostic relationship to caries activity. When these bacteria are constantly present in the mouth, dental caries invariably occurs. When they disappear, caries is arrested and no new lesions occur. It is well known that, under laboratory conditions, the growth of lactobacilli is enhanced by including sugar in the culture media. We know, too, that in individuals susceptible to dental caries, the number of lactobacilli in the saliva is proportionate to the amount of carbohydrate in the diet. It is perfectly obvious, therefore, that unless the carbohydrate content is carefully controlled, conclusions drawn from dietary studies are apt to be erroneous.

Rat studies have been complicated by the findings of Hoppert, Webber and Canniff,[5] who proved that caries can be produced in this animal only if the diet contains coarse particles. Conversely, regardless of deficiencies in the diet, caries will not occur in the rat if the ration is finely ground. This negates the conclusions of Klein and McCollum that caries occurred in their animals because of a dietary deficiency, particularly phosphorus, since the experimental diet also contained coarse particles. The same criticism applies to Lilly's[6] studies in which he reported no caries in rats fed diets that were high in sugar.

There is, therefore, a common denominator in the various dietary hypotheses so far advanced for the control of caries. First, it is seen that in practically all cases in which caries has been controlled by improving the nutritional adequacy of the diet, the carbohydrate content has also been reduced. Secondly, the restriction of carbohydrate in the diet always causes a decrease in the number of lactobacilli in the mouth, and since the lactobacilli are related to the activity of dental caries, we have here the distinct possibility that caries activity has been reduced through the decline in the growth of lactobacilli, a result of the restriction of sugar.

By some, it might be believed that lactobacillus counts are reduced because of the nutritional adequacy of the diet. This assumption is definitely negated by the fact that no matter how well the diets are supplemented with rich vitamin and mineral containing foods, if the carbohydrates are kept high the lactobacillus counts are not reduced and caries is not controlled. On the other hand, should both the carbohydrate content of the diet and the mineral and vitamin containing foods be restricted, even though the diet is grossly inadequate, the lactobacillus counts drop and caries is substantially controlled.

Those of us who have had the opportunity to follow the lactobacillus counts in the saliva of patients on high and low carbohydrate intake have been very much impressed by the rapidity with which the counts respond to variations

in carbohydrate intake. There are, nevertheless, many puzzling facts to remind us that this alone does not determine caries immunity. As an illustration, it has been observed by most clinicians that some persons are able to enjoy diets in which highly sweetened foods are eaten in large amounts with no apparent effect on the caries rate. It has also been observed that an individual may not react to high carbohydrate consumption in the same manner at different times. In some cases, the lactobacillus counts can be reduced from approximately 500,000 to less than 5,000 per cubic centimeter of saliva within two weeks on a diet in which the carbohydrate intake is restricted to 51 gm. daily. When starch is added to this diet in the form of bread and potatoes for a period of one week, the count may not necessarily rise. Indeed, the count may not rise in these cases even when sugar is added to the diet after the lactobacillus level has been kept low for from three weeks to a month.

We have in the past reported cases in which patients remained free from lactobacilli and caries after drinking a quart of acidophilus milk daily, and we have also failed, in some cases, to induce a rise in the lactobacillus counts of subjects who have never had caries by the feeding of huge amounts of candy. This all suggests that certain patients are periodically or permanently immune to caries. For them, the mere addition of sugar to the diet will not promote caries activity. It is encouraging to know that in the caries-susceptible, it may be possible to control the disease by restricting carbohydrate in the diet only periodically.

As was pointed out in the 1939 edition of "The Newer Knowledge of Nutrition," by McCollum, Orent-Keiles and Day, it is difficult to explain why the ingestion of starch and sugar does not always affect the growth of lactobacilli similarly in all individuals. We have been able to control the lactobacillus counts in some individuals by restricting sugar alone; while, in others, it has been necessary to restrict both sugar and starch in order to control the growth of these organisms.

For purposes of illustration, I cite here three individual cases:

Patients 1 and 2 are both over 40 years of age. Patient 1 had an original lactobacillus count of 600,000 per cubic centimeter of saliva. After two weeks on a diet in which the carbohydrate was restricted to 51 gm. daily, the count dropped to 450. After the addition of approximately four slices of bread a day to this diet, plus some potatoes, for a two-week period, the count dropped to 300, and, one month later, with no restriction of starch whatever, the count had gone down to zero.

Patient 2, on the other hand, had an original count of 500,000, which, on a low carbohydrate diet, dropped in two weeks to 5,000, but rose again to 16,000 after bread and milk were added to the diet. Patient 3, with a count of 25,000 on a diet unrestricted as to sugar intake, continued for five months on the same diet plus dicalcium phosphate and viosterol, during which time the *L. acidophilus* count rose to 75,000.

These observations serve to indicate that in Patients 1 and 2, caries may be controlled by restriction of carbohydrate. In the former, it is necessary to restrict merely the sugar; while, in the latter, sugar and starch both seem to be important factors. Case 3 serves to illustrate the hopelessness of attempting to control dental caries by the addition of minerals and vitamin D to an otherwise high-sugar diet.

Another point brought out by McCollum and his associates concerns the question as to why races who reputedly drink large amounts of sour milk have such a low incidence of dental caries, if it is assumed that the sour milk is acidophilus milk. This question may be answered in part by a table concerning per capita sugar intake in various parts of the world which appears on page 616

of "The Newer Knowledge of Nutrition." It will be noted that the per capita consumption of sugar in Bulgaria, and we assume that the Bulgarians are the milk drinkers referred to, is listed at 10 pounds, as compared to 110 pounds in the United States.

Reports from the Michigan laboratory during the past several years by Koehne,[7] Hubbell,[8] White,[9] Hadley,[10] Bunting[11] and me[12] indicate that there is no significant relation between the amounts of calcium and phosphorus in the blood and saliva in caries-free and caries-active patients. They demonstrated further that the pH of the saliva and blood is not a significant factor; while other reports by this group demonstrate definitely the relationship of high lactobacillus counts to caries activity and the possibility of controlling caries activity by restricting carbohydrate when the addition of minerals and vitamins has failed.

CONCLUSION

It may be safely said, from our observations, that dental caries is not a manifestation of malnutrition and that it cannot be controlled by adding minerals and vitamins to high carbohydrate diets. On the other hand, it has been observed that the lactobacillus counts can be controlled and caries activity can be reduced by the restriction of carbohydrate in either adequate or inadequate diets. We are fully aware of the fact that the adequacy of the diet is of primary importance for normal growth and development and for the maintenance of good health. From the standpoint of dental caries activity, however, we are obliged to state that control of carbohydrate consumption is the only dietary procedure thus far demonstrated by which the disease can be controlled.

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Sucrose and the role of saccharates in enamel caries

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The possibility that caries may be caused by a direct interaction between enamel calcium and ingested sucrose to form Ca-saccharates in alkaline conditions has been suggested by EGGERS LURA. Some evidence to support this concept has been reported in the literature but no systematic study of the subject has been published in the past.

The effect of sucrose on the solubility of whole teeth, enamel and calcium salts in saliva supernatant and a range of buffers was studied *in vitro*. Total calcium (by atomic absorption spectrometry) and ionic calcium determinations (using the Orion Ca ion electrode) showed that CaO and $Ca(OH)_2$ were solubilised by sucrose, while the solubility of $Ca_3(PO_4)_2$ and enamel powder was unaffected. The nature of the 'Ca-saccharates' was investigated and evidence was obtained to demonstrate that, contrary to previous reports, the saccharates are dissociated in solution.

It is concluded that Ca-saccharate formation is unlikely to be a factor in the initiation of caries and that the cariogenicity of sucrose cannot be ascribed to a 'direct chemical interaction' with tooth enamel.

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Effects of Sucrose on Early Dental Calculus and Plaque

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Abstract.—Sugar toffees were administered during 7-day periods and 3-day periods to ten subjects. They were without effect on the weights of deposits formed on the lingual of lower incisors as assessed by the standardized foil technique. The deposits, however, were slightly less mineralized. Plaque formation on the labial of lower incisors significantly increased in extent under the influence of sucrose treatment.

In rapid calculus formers, microbial deposits formed on previously cleaned lingual surfaces of lower incisors will mineralize to some extent within a few days. In such a group the ash content was found to be 41 percent in 3-day old deposits and 54 percent in 7-day old deposits (per dry weight, SCHROEDER, 1963). The question arose whether sucrose which is known to lower the pH of cariogenic plaques may under certain conditions have also an effect on the degree of mineralization and perhaps the rate of formation of microbial deposits forming at calculus predilection sites.

LUOMA (1965) reported that during ingestion of sugar tablets the rate of salivary secretion was increased and the pH slightly elevated; the calcium content of saliva was little influenced, however, the salivary phosphorus and potassium concentrations were strongly lowered. The pH of the plaques collected from two controlateral quadrants of the human dentition was found to decrease during sugar ingestion. The inorganic phosphorus content of plaque was reduced by 36 percent compared with presugar levels. The release of phosphate from washed resting cells of dental plaque was raised by increased acidity.

BJÖRN and CARLSSON (1964) recorded photo-

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graphically the early stage of plaque development on labial tooth surfaces in man. CARLSSON and EGELBERG (1965) reported that dogs given a protein-fat diet accumulated large amounts of bacterial plaque on the teeth. The addition of sucrose to this diet did not appear to alter the amount of plaque formed. However, CARLSSON and EGELBERG (1965) found that when young adults ingested soft protein-fat diets supplemented with frequent portions of sucrose, considerably larger amounts of plaque were formed, when compared with soft diets not containing carbohydrates and soft diets containing glucose. It was an additional purpose of the present study to investigate the effects of frequent sucking of sugar toffees on the rate of plaque formation on labial tooth surfaces.

METHODS

Subjects. Ten male subjects with healthy gingival conditions, 21 to 28 years old, known to be rapid calculus formers, were used. They had their usual diet, but were asked to avoid sweets and candies except those given for experimental purposes. They were instructed not to brush lingual and labial surfaces of the lower incisors.

Collection of deposits. Sandblasted standardized plastic foils were attached lingually to two thoroughly cleaned lower central incisors of each subject by a nylon thread. The foils were exposed to the oral environment in a first experimental series for 3 successive 7-day periods (A) and in a second experimental series for 3 successive 3-day periods (B) respectively.

Experimental treatments. The subjects received the following treatments in experiments A and B:

Period I: No treatment, control.

Period II: Sucrose treatment consisting in sucking 15 pieces of sugar toffees (Sugus®, Suchard Co.) per day, one piece every hour, starting at 7.00 a.m. The toffees had an average weight of 3.2 grams and contained 50 percent sucrose and 14 percent invert-sugar. Sugar toffees dissolved in distilled water (1 toffee per 10 ml, after 30 minutes) produced a pH of 3.3 at 37 °C. The average salivary pH during sucking and 15 minutes after ingestion of toffees was found to be 6.45 ± 0.34 (N = 5) and 6.73 ± 0.28 (N = 5) respectively.

Period III: Treatment with sugarless chewing gum consisting in chewing 10 pieces of sugarless spearmint gum per day (Trident® without enzyme). One piece was chewed for about 1.5 hours. It contained 62.2 percent hexitols, 4.2 percent carbohydrates and 0.3 percent protein.

Analysis of foil deposits. At the end of each experimental period the dry weight and ash weight of the deposits formed on the foils were determined, and subsequently the content of calcium (SCHWARZENBACH, 1960) and total phosphorus (FISKE and SUBBAROW, 1925). The chemical analyses were performed by the examiner without knowledge of the treatments. Details of the procedure have been reported previously (SCHROEDER, 1963; MÜHLEMANN and SCHROEDER, 1964).

Plaque formation rate. Color slides (Kodak, Ektachrome-X) were made under standardized conditions from the labial surface of the lower left lateral incisor at the begin and the end of the experimental periods. Prior to photography a 0.1-percent aqueous solution of basic fuchsin was applied to the tooth surface using a capillary pipet. For the evaluation of plaque extension, the slides were projected at 65 times magnification. The stained plaque and unstained tooth surface areas were traced on paper and subsequently cut out for gravimetrical determination. The percentage of stained tooth surface in relation to the total tooth surface was used as a measure of the extent and rate of sion plaque formation.

RESULTS AND DISCUSSION

Intake of sugar toffees and chewing gum. On an average, the reported daily intake of sugar toffees and chewing gum pieces for the 7-day period (Exp. A) was 14.2 ± 0.7 toffees per subject, and 9.8 ± 0.2 pieces respectively. For the 3-day experimental period (Exp. B) the cor-

* Kindly provided by American Chicle Company, Div. of Warner-Lambert Pharmaceutical Company, Long Island City, N.Y.

Table I Effects of sucrose toffees and chewing gum on 3- and 7-day old calculous deposits formed on two standardized foils attached to the lingual of the lower central incisors. Averages ($\bar{x}$) and standard deviation (s) of dry deposit weights (DDW, µg), ash weights (AW, µg), percentage ash-, calcium- and phosphorus content in dry deposits, and Ca/P ratio

		Periods	N	DDW $\bar{x} \pm s$	AW $\bar{x} \pm s$	AW/DDW % $\bar{x} \pm s$	Ca/DDW % $\bar{x} \pm s$	P/DDW % $\bar{x} \pm s$	Ca/P $\bar{x} \pm s$
Exp. 7 days	A	control	10	1689 ± 947	795 ± 575	44 ± 8	14.5 ± 3.8	8.3 ± 2.3	1.76 ± 0.13
		sucrose	10	1599 ± 1096	682 ± 714	38 ± 9	14.1 ± 3.6	7.9 ± 1.8	1.74 ± 0.15
		gum	10	1594 ± 670	815 ± 437	49 ± 10	15.8 ± 3.6	9.1 ± 1.4	1.72 ± 0.23
Exp. 3 days	B	control	10	504 ± 167	139 ± 49	29 ± 7	7.6 ± 2.6	5.8 ± 1.8	1.35 ± 0.39
		sucrose	10	467 ± 219	138 ± 105	29 ± 10	9.2 ± 3.4	6.3 ± 2.3	1.48 ± 0.25
		gum	10	483 ± 204	146 ± 76	30 ± 7	9.5 ± 3.7	6.2 ± 1.9	1.53 ± 0.37

responding figures were 14.4 ± 0.7 and 9.9 ± 0.2 respectively.

Rate of calculous deposit formation. The averages of dry deposit weights (DDW) were found to be similar for the 3 different treatments during both experiments A and B. (Table I).

Rate of deposit mineralization. The average ash weights (AW), percentage contents of ash, calcium and phosphorus of dry deposits, and the Ca/P ratios are shown in Table I. The average percentage ash content of dry deposits formed during the 7-day (but not the 3-day) sucrose treatment period was significantly smaller when compared with the control (44.1 versus 38.4 percent, $P_F < 0.05$), the Ca/P ratios of control and sucrose period being not altered. The average phosphorus content was only slightly but not significantly lower after a 7-day sucrose treatment period. The Ca/P ratios ranged between 1.72 to 1.74 in 7-day old deposits and 1.35 to 1.53 in 3-day old deposits.

Rate of plaque formation. The single and average percentages of tooth surface covered with plaque after the 3 treatment periods in experiments A and B are represented graphically in Figure 1. In experiment A, significantly more plaque was formed when administering sucrose ($P_F < 0.01$), and less plaque in subjects chewing gum ($P_F < 0.05$), the former finding being in agreement with data reported by CARLSSON and EGELBERG (1965). These differences in the rate of plaque formation were not significant in the 3-day experimental series (Exp. B). The present data indicate that frequent sucking of sugar toffees may increase the growth of non-mineralizing labial plaque. No explanation can be offered why a similar effect could not be observed with respect to the rate

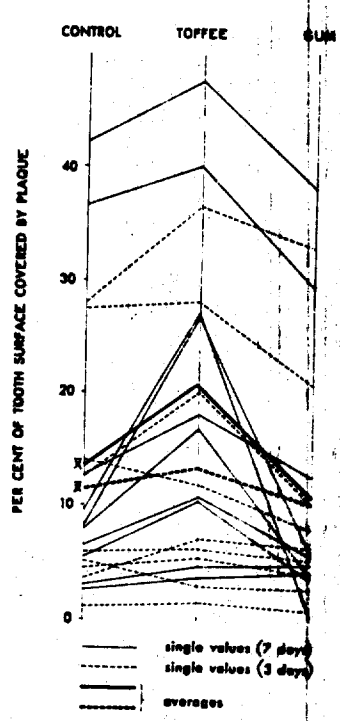

Fig. 1 Single values in 10 subjects and averages of labial surface covered by plaque in the lower left lateral incisor after 3 different treatments (control, sugar toffee and chewing gum), each lasting 3 to 7 days respectively

of microbial deposit formation on the lingual surfaces of lower incisors. These surfaces probably were also exposed to sucrose since the foil deposits were found to be slightly less mineralized at the end of the sucrose treatment (borderline significance). Chewing gum inhibited the rate of plaque formation on labial tooth surfaces, but apparently did not interfere with the lingual deposits.

Zusammenfassung. Zuckerkaramels wurden 10 Probanden ständig während einer 3- bzw. 7-tägigen Versuchsdauer verabreicht. Die Menge der an den Lingualflächen unterer Frontzähne auf standardisierten Folien gebildeten Zahnsteinablagerungen wurde durch die Saccharose nicht beeinflusst, doch waren die Ablagerungen etwas weniger stark mineralisiert. Unter der Zuckerbehandlung war die Plaqueausdehnung auf den Labialflächen unterer Frontzähne grösser.

Résumé. Durant 3, respectivement 7 jours, 10 étudiants ont sucé des caramels contenant du sucre. La quantité des dépôts de tartre déterminée par la méthode des feuilles standardisées, sur les faces linguales des incisives inférieures n'a pas été influencée par le saccharose, mais les dépôts ont été moins fortement minéralisés. Sur les faces labiales des incisives inférieures, le sucre a stimulé la croissance des plaques non minéralisées.

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THE EFFECT OF THE DIET ON THE IMPLANTATION OF CARIES-INDUCING STREPTOCOCCI IN HAMSTERS

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Summary—The implantation of streptomycin-resistant caries-inducing streptococci in the oral cavity has been studied in hamsters fed various diets. From animals fed commercial animal food, only a few such organisms could be recovered. This was the case also when the chow was given in ground or powdered form. From animals fed the standard powdered caries test diet the resistant streptococci were recovered in large numbers. When lactose or sucrose in the experimental diet were substituted by glucose the implantation was greatly impaired.

INTRODUCTION

It has been shown that caries-inducing streptococci can be implanted into the oral cavity of albino hamsters (FITZGERALD and KEYES, 1960, 1963; KEYES and FITZGERALD, 1962). The important fact is that these streptotocci can become established in the oral flora, and a search for the biochemical properties that make these organisms caries-inducing might therefore start with a study of factors which favour the implantation.

Since diet plays a decisive role in caries activity, two factors which may be of importance are its consistency and its composition. In the studies referred to above in which the streptococci were easily implanted the diet was finely powdered and somewhat dehydrated. The main ingredients were sucrose and skim milk powder.

The aim of the present investigation has been to study the effect on the implantation of the caries-inducing streptococci by variations in the consistency and the composition of the diet.

MATERIALS AND METHODS

Micro-organisms

The streptococcus (Strain 3720) was a streptomycin-resistant sub-strain of the cariogenic streptococcus (HSR-6) (FITZGERALD and KEYES, 1960) reisolated after several passages in hamsters. It had the same biochemical and serological characteristics as the HSR-6 strain.

Animals

Golden hamsters were used in most of the experiments. The animals weighed around 50 g at the start of the study. In a later part of the study albino hamsters

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weighing around 30–35 g were used. All of the animals were received from the NIH animal production station. In the various experiments four animals were used in each group and all four kept in the same cage. The experiments were repeated until eight or sixteen animals had been examined per group.

Inoculum and medium

For the inoculum 24-hr broth cultures were used. The medium consisted of horse-meat infusion thioglycollate broth with a pinch of calcium carbonate in the bottom of the tube. Approximately 0·2 ml of the culture was introduced in the mouth and the cheek pouch of each animal by means of a pipette, and about 6 ml was added to 200 ml of drinking water. The infected drinking water was provided for 48 hr. The streptococci were recovered on fuchsinazide blood agar plates containing 200 μg per ml of streptomycin (FAS medium). The methods used correspond to those reported by FITZGERALD and KEYES (1960).

Sampling and recovery

Samples of plaques and saliva were collected by means of a cotton-tipped applicator stick which was then shaken in a test tube containing 1 ml of 0·05 per cent yeast extract water. The swabs were then streaked on FAS plates (see above). The plates were incubated for 48 hr at 37°C in an atmosphere of 95% nitrogen and 5% carbon dioxide. On the medium used the cariogenic streptococci formed typical, easily identified colonies. Very seldom were atypical colonies observed. The recoveries were checked by Gram stained smears of typical or questionable colonies. Cultures of typical colonies were tested in agglutination tests. They were agglutinated in the same titre as the original strain by antiserum prepared against this strain.

The number of colonies recovered was counted and the results expressed in terms varying from − to ++++ (KEYES and FITZGERALD, 1962). The sampling and plating techniques might be subject to considerable variation but samples taken 1, 2 and 3 weeks after infection gave consistent results for the various groups. In the Tables the results after 1 and 3 weeks are given.

Diets

When studying the effect of the composition of the diet on the implantation a formula containing 56% sucrose and 28% skim milk powder was used (Diet 2000, KEYES and JORDAN, 1964). In the present study this diet was modified by changes of the main ingredients. As skim milk powder constitutes 28 per cent of this diet an artificial skim milk powder was made up in which the lactose content could be replaced by glucose (Table 1). The composition of the artificial skim milk powder was calculated from data given in "Handbook of Nutrition" (1943). The milk-salt mixture was made up from calculations of SÖLDNER (1923). The compositions of the various diets used are given in Table 1, from which it will be seen that Diets 2001 and 2002, which contain the artificial skim milk powder, are identical with the exception that Diet 2002 contains glucose instead of lactose. Likewise, Diets 2000 and 1999 are identical with the exception that Diet 1999 contains 56 per cent glucose instead of sucrose. All of the diets were finely powdered.

TABLE 1. COMPOSITION OF DIETS (per cent)

Ingredients	Diet number			
	2000*	2001	2002	1999
Sucrose	56	56	56	—
Skim milk powder	28	—	—	28
Lactose	(14)†	14	—	(14)†
Glucose	—	—	14	56
Casein	(10)†	10	10	(10)†
Lactalbumin	(2)†	2	2	(2)†
Milk salts	(2)†	2	2	(2)†
Whole wheat flour	6	6	6	6
Alfalfa	3	3	3	3
Dried brewers yeast	4	4	4	4
Liver powder	1	1	1	1
Sodium chloride	2	2	2	2

* Keyes original cariogenic Diet 2000 (KEYES and JORDAN, 1964).
† Calculated from skim milk powder content.

The experimental diets could not be satisfactorily pelleted without being mixed with water. As this treatment seemed to change not only the consistency but also the quality of the diet, the effect of the consistency on the implantation was studied by first grinding and then powdering an ordinary pelleted commercial animal formula (Purina Lab Chow) in a ball mill. The animals were given the diets for 1 week before the inoculation. During this time they were sampled for the presence of streptomycin-resistant streptococci in the oral cavity. In none of the animals were any streptococci recovered on the streptomycin containing plates before the inoculation.

RESULTS

The results of the implantation after 1 and 3 weeks in the animals fed the chow in various forms or the cariogenic Diet 2000 are given in Table 2. This table shows that in the animals on the various types of commercial laboratory foods recovery of the labelled streptococci was very low. In the animals on the experimental test diets, on the other hand, the streptococci became established in fairly large numbers.

The results of the implantation experiment in animals fed the artificial skim milk powder is shown in Table 3, which shows that the implanted streptococci are recovered in much higher numbers in the animals fed the lactose-containing diet than in those fed the diet in which lactose had been substituted with glucose. The animals fed the glucose containing diet grew more slowly than those on lactose. Some of the animals finally died following an episode of diarrhoea.

The results of the implantation experiment in albino hamsters fed the original caries test diet (2000) and the same diet with glucose instead of sucrose (1999) is shown in Table 4. This shows that the implanted streptococci are recovered in much lower numbers in the animals given the diet with glucose than in those given the ordinary test diet with sucrose. In four of the eight animals on the glucose diet no

TABLE 2. RECOVERY OF LABELLED CARIES-INDUCING STREPTOCOCCI IN GOLDEN HAMSTERS FED VARIOUS DIETS 1 AND 3 WEEKS AFTER INFECTION

	Chow		Ground chow		Powdered chow		Cariogenic diet 2000	
	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks	1 week	3 weeks
	−	±	±	−	±	±	±	−
	±	±	−	±	±	±	+++	++
	−	−	−	−	±	±	+	++
	±	±	±	±	±	±	+++	++
	−	±	±	±	±	+	+++	+++
	−	−	−	−	±	±	++++	+++
	−	+	±	+	−	±	+++	+++
	±	±	−	±	−	±	+++	+++
	±	++	±	−	−	±	+++	+++
	±	±	±	±	±	±	±	+++
	±	±	±	±	−	−	++	+++
	±	+	±	±	−	−	±	+++
	+	±	−	−	−	−	±	±
	+	±	+	±	−	−	±	±
	−	−	−	−	−	−	+++	+++
	−	−	±	−	−	−	−	++
Mean value	±	±	±	±	±	±	++	++

No colonies = −
<50 ,, = ±
50–250 ,, = +
250–500 ,, = ++
500–1000 ,, = +++
Confluent growth = ++++

labelled streptococci at all were recovered from the oral cavity 3 weeks after the infection.

In contrast to what was observed when the lactose was replaced by glucose (Diet 2002) all of the animals on Diet 1999 survived. At the end of the experimental period (56 days), however, the mean weight was somewhat lower for the glucose animals (61 vs. 68g).

DISCUSSION

From hamsters fed lab chow and infected with the streptomycin-resistant caries inducing streptococci only a few of these organisms could be recovered. This was also the case when the lab chow was given in ground or powdered form. On the regular caries test formula, on the other hand, the "labelled" organisms were recovered in large numbers. As the powdered chow and the caries test diets had the same consistency, the results indicate that the composition and not the consistency of the diet is the factor of determinative importance for the implantation. However, in order to fully evaluate the importance of the consistency of the diet the implantation ought to be studied in animals fed a pelleted diet which in powdered form supports the implantation of the caries-inducing streptococci.

In the experiment with the artificial skim milk powder the implantation was greatly impaired when glucose was substituted for lactose (Table 3). In a number of

TABLE 3. RECOVERY OF LABELLED CARIES INDUCING STREPTOCOCCI IN GOLDEN HAMSTERS 1 AND 3 WEEKS AFTER INFECTION

	Artificial milk powder diet			
	With 14 per cent lactose (2001) (56 per cent sucrose)		With 14 per cent glucose instead of lactose (2002) (56 per cent sucrose)	
	1 week	3 weeks	1 week	3 weeks
	+	+++	+++	++
	±	+++	±	±
	+++	+++	+	−
	+++	+++	+	±
	+++	+++	+++	+++
	+++	+++	+	++
	+++	+++	++	++
	+++	+++	+	++
	+++	+++	±	−
	+++	+++	±	±
	+++	+++	±	+
	+++	+++	±	−
	++++	+++	−	+
	+++	+++	+	±
	++	++++	±	±
	+++	++++	++	+
Mean value	++(+)	+++	+	+

For key see Table 2.

TABLE 4. RECOVERY OF LABELLED CARIES-INDUCING STREPTOCOCCI IN ALBINO HAMSTERS 1 AND 3 WEEKS AFTER INFECTION

	Diet 2000 (56 per cent sucrose)		Diet 1999 (56 per cent glucose)	
	1 week	3 weeks	1 week	3 weeks
	+++	+++	±	−
	+++	+++	−	−
	+++	+++	±	±
	+++	+++	−	±
	±	+++	±	−
	+	+++	±	±
	+++	++	±	±
	++	+	±	−
Mean value	++	++(+)	±	±

For key see Table 2.

studies it has been found that the addition of lactose to the diet favours the development of an aciduric type of flora in the intestine (CRUICKSHANK, 1928; PORTER and RETTGER, 1940; JOHANSSON and SARLES, 1948; NATH *et al.*, 1948). The observation made in this study that lactose favours the implantation of the caries-inducing streptococci in the oral cavity can, consequently, be interpreted with these observations in mind. Thus, lactose may favour the establishment of these streptococci as well as of other aciduric bacteria in the lower alimentary canal. By coprophagy the streptococci then might become established in the oral cavity. It is also possible that the streptococci become implanted in the mouth primarily or at least as readily as in the intestinal tract because of plaque formation and colonization on tooth surfaces. Finally, the establishment of an aciduric intestinal and oral flora may have a less antagonistic effect against the streptococci to be implanted than a more proteolytic one. This assumption needs further investigation, but in a series of experiments in which animals were fed a diet high in protein all animals died within 1–3 weeks. Consequently, the problem of whether a proteolytic flora inhibits the implantation could not be examined.

When the sucrose in the experimental diet was replaced by glucose very few of the implanted streptococci were recovered. Thus, sucrose highly favoured the implantation. This finding was of a special interest as it has been shown that sucrose favours caries activity much more than glucose in hamsters and rats (GUSTAFSON *et al.*, 1955; GRENBY, 1963). These observations motivate further investigations in respect to the way the caries inducing streptococci utilise sucrose. In this regard the studies of GIBBONS *et al.* (1962) on the intra-cellular polysaccharide formation of these bacteria are of interest.

Acknowledgements—This work was done during a stay as visiting scientist at the National Institute of Dental Research and the author wishes to acknowledge and thank especially Drs. R. J. FITZGERALD and P. H. KEYES for stimulating discussions and for facilitation of this study, Mrs. C. OVERMAN for technical assistance and Mr. R. CATLETT for taking care of the animals.

Résumé—L'implantation dans la cavité buccale de streptocoques, inducteurs de caries et résistants à la streptomycine, a été étudiée chez des hamsters soumis à différents régimes. Chez les animaux soumis à un régime préparé commercialement, seuls quelques micro-organismes ont pu être récupérés. Il en est de même lorsque la nourriture est donnée sous forme meulée ou pulvérisée. Chez des animaux soumis à un régime cariogène classique, en poudre, une grande quantité de streptocoques résistants est récupérée. Lorsque le lactose, ou le saccharose du régime expérimental est remplacé par du glucose, l'implantation est fortement diminuée.

Zusammenfassung—Es wurde die Implantation von Streptomycin-resistenten, Karies-verursachenden Streptococcen in die Mundhöhle von Hamstern untersucht, die mit verschiedenen Diäten gefüttert wurden. Von Tieren, die handelsübliches Tierfutter erhielten, konnten nur wenige solcher Organismen zurückgewonnen werden. Dies war auch der Fall, wenn das Futter in gemahlener oder gepuderter Form gegeben wurde. Von solchen Tieren, die mit der gepuderten Standard-Kariestest-Diät gefüttert worden waren, konnten die resistenten Streptococcen in großen Zahlen wiedergewonnen werden. Wenn Lactose oder Rohrzucker in der Versuchsdiät durch Glukose ersetzt wurde, war die Implantation stark vermindert.

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THE EFFECT OF CARIES-INDUCING STREPTOCOCCI IN HAMSTERS FED DIETS WITH SUCROSE OR GLUCOSE

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Summary—The effect of caries-inducing streptococci has been studied in two groups of albino hamsters fed various diets. In the group given a diet containing 56 per cent sucrose the streptococci became implanted in large numbers and the animals all showed a high caries incidence. In the group given a diet in which the same amount of glucose replaced sucrose only a few of the implanted organisms could be recovered. All the animals in this group had very low caries activity and several of them remained completely free from caries.

INTRODUCTION

CERTAIN strains of streptococci have been found to induce dental caries in albino hamsters fed a diet high in sucrose and low in fat (FITZGERALD and KEYES, 1960; KEYES and FITZGERALD, 1962). The implantation of these organisms in the oral cavity is greatly impaired when glucose is substituted for sucrose in the experimental diet (KRASSE, 1965). As it has been observed that both hamsters and rats showed a considerably higher incidence of caries on a diet containing sucrose than on one containing glucose (GUSTAFSON *et al.*, 1955; GRENBY, 1963), it seemed worthwhile to investigate whether this was also the case in the streptococcus-hamster model demonstrated by FITZGERALD and KEYES.

MATERIALS AND METHODS

The cariogenic streptococcal strain (3720) was a streptomycin-resistant substrain of HSR-6 (FITZGERALD and KEYES, 1960) with the same biochemical and seriological characteristics as the original strain. The albino hamsters, which were received from the NIH animal production station, weighed approximately 30 g at the start of the study. Animals from the same litter were randomly distributed into the different dietary groups. As a rule four animals were kept in the same cage. After the animals had been on the test diet for 2–3 days, they were infected with the streptomycin-resistant (labelled) streptococci. For the inoculum 24-hr broth cultures were used; approximately 0·2 ml of the culture was introduced in the mouth and the cheek pouch of each animal, and 6 ml was added to about 200 ml of drinking water. The

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infected drinking water was provided for 48 hr. After the animals had been on the diet for 3 weeks, samples of plaques and saliva were collected by means of a cotton-tipped applicator stick which was then shaken in a test tube containing one ml of 0·05 per cent yeast extract water. The swabs were then streaked on fuchsin-azide blood agar plates containing 200 μg/ml of streptomycin. The plates were incubated for 48 hr at 37°C in an atmosphere of 95% nitrogen and 5% carbon dioxide. The number of colonies recovered on the plates was expressed in terms varying from − to + + + + (KEYES and FITZGERALD, 1962).

The diet containing sucrose was Keyes diet 2000 (KEYES and JORDAN, 1964). It had the following composition (per cent): sucrose 56, skim milk powder 28, whole wheat flour 6, alfalfa 3, dried brewers yeast 4, liver powder 1, sodium chloride 2. The glucose diet had the same composition with the exception that it contained 56 per cent glucose instead of sucrose. The animals were kept on the test diet for 56 days. They were then sacrificed and the teeth were scored according to the method of KEYES (1959).

RESULTS AND DISCUSSION

When the teeth of the living animals were inspected under the microscope the uninfected controls and the infected animals on the glucose diet did not show any gross plaque formation. The infected animals on the sucrose diet, however, developed heavy plaques within 1–2 weeks after their inoculation.

The samples of saliva and plaque material from the uninfected controls did not give a growth on culture of any streptomycin-resistant streptococci during the total experimental period. From the infected animals on glucose the recovery was very low (see Table 1), whilst the implanted streptococci were recovered in large numbers from most of the animals fed on the sucrose diet.

It is evident from Table 1 that all the noninfected controls had low to negligible caries activity, and the same was the case with the infected animals on the glucose diet. On the contrary, the animals on the sucrose diet, which had been infected and carried the "labelled" organisms in large numbers, showed a high degree of caries activity.

These results show that on a diet with glucose as the main carbohydrate source the streptococci do not become implanted in great numbers, and caries is not a highly active process. It has been stated by KEYES (1962) and by FITZGERALD (1963) that the development or caries is affected by the interaction of three main determinants: a suitable diet, a cariogenic microflora, and a susceptible host. They have also pointed out that unless all three of these determinants are simultaneously present and operative, caries does not develop. The present study illustrates how a variation in a single dietary factor can affect the system. This finding suggests that further research ought to be concentrated on the metabolic action of the caries-inducing streptococci in the presence of sucrose.

TABLE 1. RECOVERY OF STREPTOMYCIN-RESISTANT STREPTOCOCCI AND CARIES SCORES IN ALBINO HAMSTERS FED DIETS CONTAINING SUCROSE OR GLUCOSE

Sampling 3 weeks after inoculation. Experimental period 56 days.

Diet with sucrose						Diet with glucose					
Uninfected controls			Infected animals			Uninfected controls			Infected animals		
Animal number	Recovery	Caries score	Animal number	Recovery	Caries score	Animal number	Recovery	Caries score	Animal number	Recovery	Caries score
329	–		334	+++	42	313	–	0	317	–	3·0
330	–	<1	335	+++	48	314	–	1	318	–	<1
331	–		336	+++	41	315	–	0	319	±	0
332	–					316	–	<1	320	±	0
401	–	<1	405	+++	49	417	–	2·5	421	–	0
402	–	1	406	+++	59	418	–	0	422	±	0
403	–	<1	407	++	92	419	–	1	423	±	0
404	–	1	408	+	40	420	–	1	424	–	<1
			4573:1	+++	18				4573:4	++	<1
			„ :2	++++	65				„ :5	++	1
			„ :3	++++	51				„ :6	–	<1
51	–	<1	55	++++	15						
52	–	0	56	++++	45						
53	–	1	57	++++	43						
54	–	1	58	++++	55						

No colonies = –
50 „ = ±
50– 250 „ = +
250– 500 „ = ++
500–1000 „ = +++
Confluent growth = ++++

Acknowledgements—This work was done during a stay as visiting scientist at the National Institute of Dental Research and the author wishes to acknowledge and thank especially Drs. R. J. FITZGERALD and P. H. KEYES for stimulating discussions and for facilitation of this study, Mrs. C. OVERMAN for technical assistance and Mr. R. CATLETT for taking care of the animals.

Résumé—L'action de streptocoques, inducteurs de caries, est étudiée dans 2 groupes de hamsters albinos, soumis à différents régimes. Dans le groupe, ayant reçu un régime contenant 56% de saccharose, les streptocoques se sont implantés en grand nombre et les animaux montrent tous un grand nombre de caries. Dans le groupe, ayant reçu un régime dans lequel le saccharose est remplacé par une quantité équivalente de glucose, seuls quelques rares micro-organismes implantés ont été récupérés. Tous les animaux de ce groupe ont une activité carieuse très basse et plusieurs d'entre eux sont resés indemnes de caries.

Zusammenfassung—Bei zwei mit verschiedenen Diäten gefütterten Gruppen von Albinohamstern wurde die Wirkung von Karies-verursachenden Streptococcen untersucht. In derjenigen Gruppe, die eine Diät mit 56% Rohrzucker erhielt, wurden die Streptococcen in großen Zahlen implantiert, und alle Tiere zeigten einen hohen Kariesbefall. In der anderen Gruppe, in deren Diät die gleiche Menge Glukose den Rohrzucker ersetzte, konnten nur wenige der implantierten Mikroorganismen wiedergewonnen werden. Alle Tiere in dieser Gruppe wiesen eine sehr geringe Kariesaktivität auf, und mehrere von ihnen blieben vollständig kariesfrei.

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Lauterstein-'61
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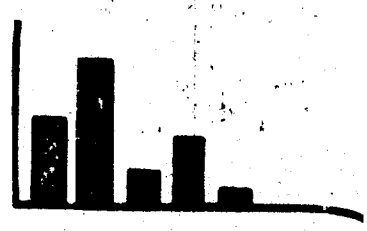
This investigation was undertaken primarily to study the relationship between sugar intake during pregnancy and infancy and the caries attack later during childhood. The children were from a middle-income group who were being treated in private practice



SUGAR INTAKE AND CARIE

By Aubrey M. Lauterstein and Maury Massler

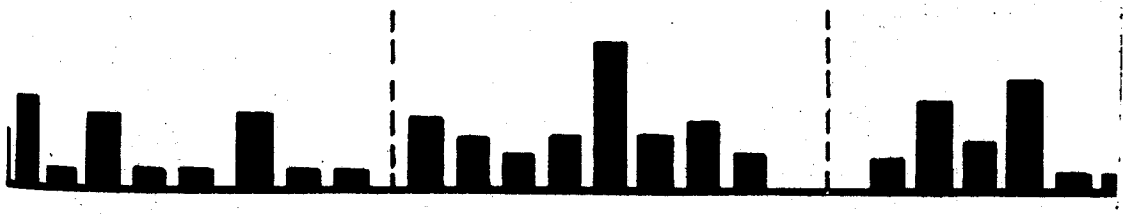
The relation between sugar ingestion and caries-attack rate has been shown many times in animals and in man. An increased sugar intake can be associated with an increased caries-attack rate, and, conversely, reduced sugar intake is usually followed by a reduction in caries attack (4, 5, 6, 13, 14). There is little doubt that the physical character of the sugar and its retention time on the tooth surface is more important than the actual amount of sugar ingested (1, 4, 7). Thus caramels and cookies which stick to the teeth are far more cariogenic than beverages with equivalent amounts of sugar.

Evidence shows different sugars have different caries-producing potentials, at least in experimental animals. In general, starches and dextrins have low cariogenic potential; sucrose has very high caries-producing effect (3, 4, 12).

A study done on rats (15, 16) indicates that sugar ingestion by the mother during pregnancy and the offspring's sugar intake during early growth years may have a distinct effect on the caries-attack rate later. And the implications to human caries are well made by these investigators.

Ninety Children Studied

Ninety healthy white children (50 males and 40 females, aged 4–14 years), their parents, and siblings were studied to investigate the relation between sugar ingestion of the child during his infancy and at present and of the mother during pregnancy and at present. The present caries-attack rate of these children was also investigated. Five visits per child were required to collect the data on (1) caries experience of patient, siblings, and parents; (2) nutritional status of patient and



N CHILDREN

mother; (3) sugar-intake levels at various periods in life of patient and mother; (4) physical character of diet, salivary volume and viscosity, and patient's oral hygiene. The results are herewith presented in graphs.

Our case-history form contained questionnaires on diets, suggestions to the examiner on interviewing, records for keeping diets, infant formulae, medical and dental findings. Averaging five conferences with each of the ninety children, the following data were obtained from questionnaires and clinical examinations.

I. Caries Experience
   A. *Patient and siblings.* Clinical examinations with complete intra-oral X-rays available. Results rated as follows:
      1. Zero caries: no carious teeth.
      2. Low caries: 1–2 DMF and def permanent and deciduous tooth surfaces.
      3. Moderate caries: 4–8 DMF and def tooth surfaces.
      4. High caries: 12 or more DMF and def tooth surfaces. At least 2 anterior teeth involved.
   B. *Mother and father.* Based on clinical examinations only, without X-rays.
      1. Zero caries: no carious teeth visible.
      2. Low caries: 1–6 DMF permanent tooth surfaces visible.
      3. Moderate caries: 12–16 DMF permanent tooth surfaces visible. No more than 1 anterior tooth involved.
      4. High caries: 22 or more DMF surfaces visible. 2 or more anterior teeth involved.

II. Age, Sex, Nutritional Status, and Health of Patient

III. Sugar Intake
   A. *By patient during infancy.* Rated into categories according to amount of sugar contained in nursing milk, first six months.

1. None: no more than the basic 9 per cent sugar in the average daily milk intake.
2. Low: 1 tablespoon of sugar added to daily milk intake (in excess of the basic 9 per cent level).
3. Moderate: 2–3 tablespoons of sugar added to the basic daily formula.
4. High: 4 or more tablespoons of sugar added to the basic daily formula.

B. *By mother during pregnancy and at present.*
1. Low: weekly average intake of 10 or less sugar items.
2. Moderate: weekly average intake of 15–25 sugar items.
3. High: weekly average of 30 or more sugar items.

C. *By patient at present.* Weekly dietary records analyzed and estimates made of number of *teaspoons of sugar consumed in one week.* (Not included: natural sugars in fresh fruits, fresh vegetables, meats without sauces, white milk, and dairy products.)
1 = 15–30 teaspoonfuls
2 = 35–58 teaspoonfuls
3 = 60–75 teaspoonfuls
4 = 80–100 teaspoonfuls
5 = 120–40 teaspoonfuls
6 = 150–200 teaspoonfuls

IV. Physical Character of Food. A detailed list of adherent and detergent foods was derived from the weekly dietary record. (5)

A. *Adherent foods* (sticky—pasty foods consumed per week).
1. High: 40 or more
2. Moderate: 25–39
3. Low: 10–24
4. Very low: 0–9

B. *Detergent foods* (stringy—pulpy foods consumed per week).
1. High: 15 or more
2. Moderate: 9–14
3. Low: 4–8
4. Very low: 0–3

V. Patient's Salivary Volume and Viscosity of Saliva

A. *Volume.* Accumulated salivary volume measured in sitting position with the occlusal plane parallel to the floor, mouth open for two minutes.
1. High: saliva filled the entire floor of mouth, spilled over lips.
2. Moderate: saliva accumulated to approximately the middle third of the lingual surfaces of lower incisor teeth.
3. Low: little or no saliva accumulated within the two-minute period.

B. *Viscosity.* Determined by dipping finger and thumb into the puddle of saliva to examine consistency.
1. Ropy: ropy, thick strands of saliva.
2. Stringy: wispy, thready saliva.
3. Thin: little or no texture, almost like distilled water at body temperature.

VI. Patients' Oral Hygiene

A. *Tooth-brushing habits.*
1. Very good: two minutes brushing after every meal.
2. Good: morning and evening for at least two minutes.
3. Moderate: once a day for less than two minutes.

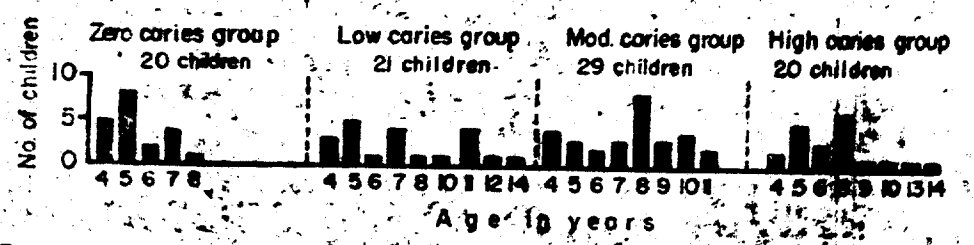


FIG. 1.—Relation of caries experience to age. 8-year-olds were the only age group showing marked tendency to cluster in one of the categories. Fourteen of the sixteen 8-year-old children showed moderate and high-caries experience. Other ages were evenly distributed throughout the four caries-experience classes (none, low, moderate, high). This suggests a peak in caries experience just before 8 years of age with decrease thereafter (due to loss of deciduous teeth).

4. Poor: two to four times per week.
5. Very poor: once a week or less.

B. *Tooth cleanliness.*

1. High: clean, no plaques or calculus present. Little or no discoloration revealed by disclosing solution.
2. Moderate: some plaques present but no calculus. Three to six tooth surfaces discolored by disclosing solution.
3. Low: most tooth surfaces discolored by disclosing solution. Calculus present.

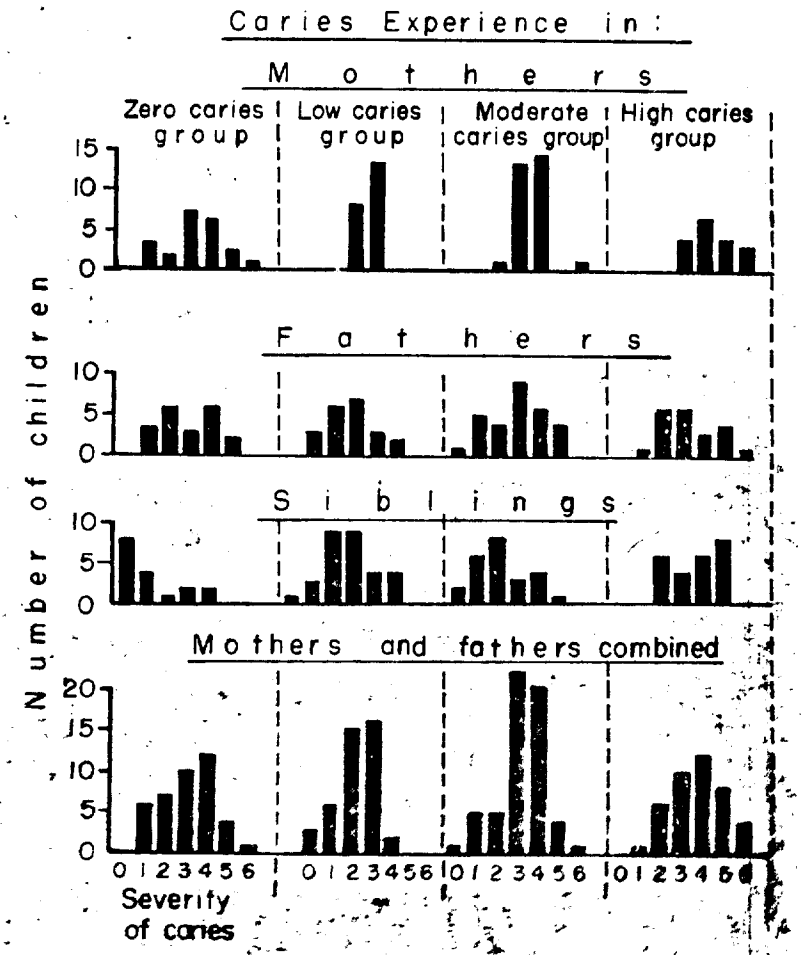
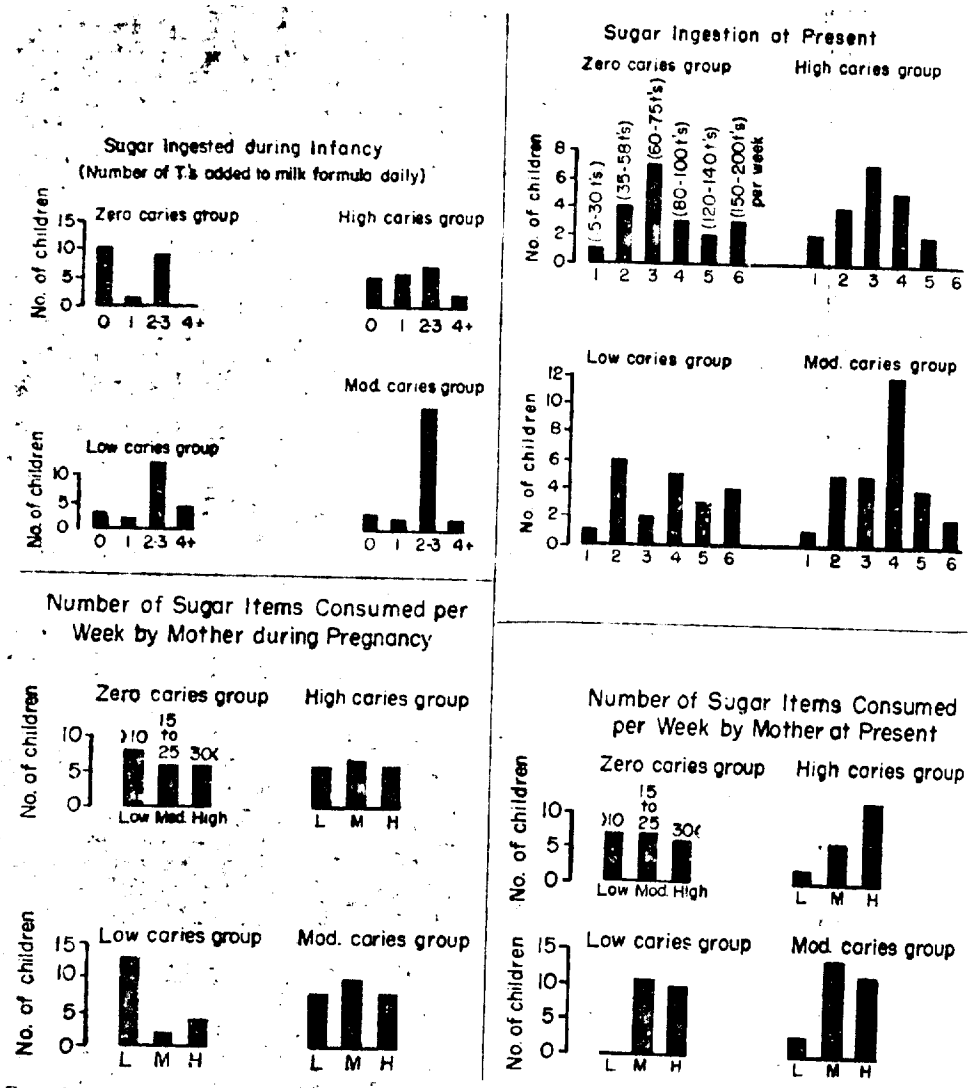


FIG. 2.—Relation of caries experience in patient to siblings and parents. No significant relationship was found between caries experience of children studied and their parents; distinct relation was seen between caries experience of patients and their siblings. The 20 caries-free children had 12 siblings with zero- to low-caries rating, 4 siblings with moderate-caries rating. The 20 children with high-caries experience had 18 siblings with moderate- or high-caries rating, 6 siblings with low-caries experience, no siblings caries-free.



FIGS. 3-6.—Relation of caries experience to sugar ingestion. No distinct relationship could be seen between caries experience of child and his ingestion of sugar during infancy (birth to 6 months) or at the present time. Also, no relationship could be established to sugar ingestion by the mother during pregnancy or to sugar ingestion by the mother at present.

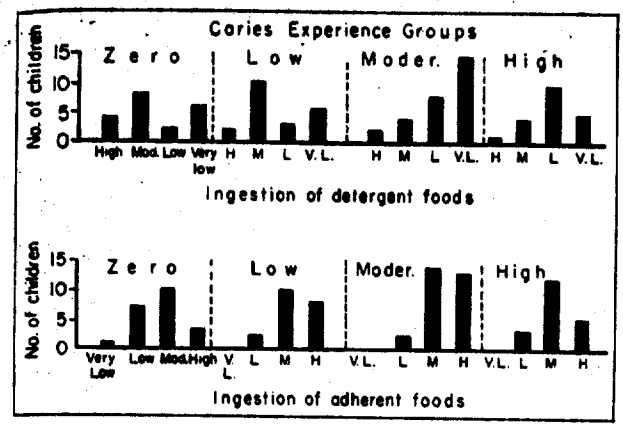


FIG. 7.—Relation of caries experience to physical character of diet. No relationship could be seen between caries experience and the amount of detergent or adherent foods ingested by the child.

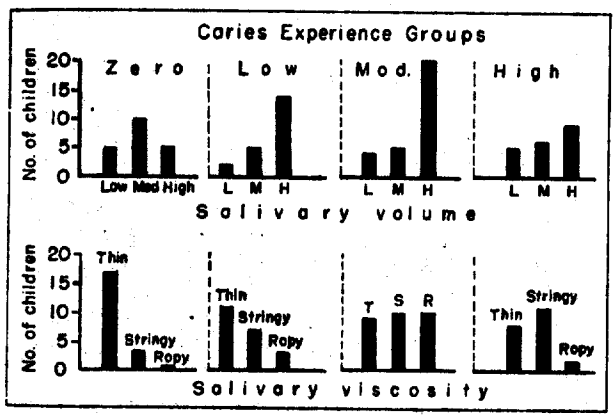


FIG. 8.—Relation of caries experience to volume and viscosity of saliva. No relationship was observed between caries experience and salivary volume, but there was a distinct relationship between caries experience and viscosity of saliva. The caries-free and low-caries groups totaled 28 children with thin saliva, 7 with stringy saliva, 3 with ropy saliva. The moderate- and high-caries groups totaled 17 children with thin saliva, 21 with stringy saliva, 11 with ropy saliva. The 20 children in the caries-free group had 17 members with thin saliva and 3 with stringy saliva. The 20 children in the high-caries group had 8 members with thin saliva, 11 with stringy saliva, and 1 with ropy saliva.

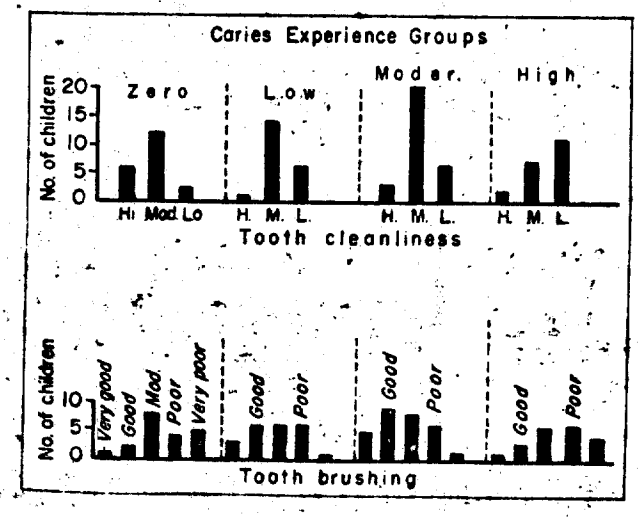


FIG. 9.—Relation of caries experience to tooth cleanliness. Distinct relationship was found between cleanliness of teeth and lack of caries. In the caries-free group, 6 children showed very clean teeth compared with 2 in the high-caries group. The caries-free group had 12 members with moderate and only 2 with low cleanliness ratings. The high-caries group had 7 members with moderate and 11 with low cleanliness ratings. The low- and moderate-caries groups were approximately equal in the degree of their cleanliness.

No relationship could be established between tooth-brushing habits and caries experience.

Discussion

Surprisingly, in this group of ninety children no relationship could be established between dental-caries experience and sugar consumption by the patient during infancy or at present. Also, sugar ingestion by the mother during pregnancy or at present could not be correlated with the child's caries experience. Children with rampant caries showed no history of significantly greater sugar intake during any period of their development than children with no caries. These findings do not coincide with the studies of some researchers (15, 17) but do concur with others (10) who found no relation between sugar ingestion and dental-caries experience in large groups of children. Some (10) found that a relatively great increase in the sugar content of the diet had no significant effect on the incidence of dental caries over a two-year period. A recent study (21) on the relation between such dietary carbohydrates and dental-caries experience found that, although between-meal sugar consumption correlated well with DMFS (+0.77), there was very little relationship between the total amount of sugar consumed and DMFS (+0.10).

Some researchers (15, 16) found the dental-caries incidence in mice, rats, and hamsters significantly *increased* in progeny from parents receiving a high-sucrose diet during and after pregnancy—particularly when the progeny continued on a high-sucrose diet. In a similar experiment (20), a significant *decrease* in dental-caries incidence was obtained.

While no relationship was found between the dental-caries experience of these children and their parents, there was a definite relation between patients and siblings. Caries-free patients had a higher percentage of siblings with a low-caries experience. Children with a high-caries experience had more siblings with medium- to high-caries rating. These findings concur with a study (2) of forty 20-year-old males, which showed that caries-active members usually had caries-active siblings, and caries-inactive members had siblings resistant to caries.

We could establish no relation between dental-caries experience and the physical character of the diet, salivary volume, and tooth brushing. There was a slight relation between caries experience and salivary viscosity. Patients with more viscous saliva tended to have more caries than patients with less viscous saliva. This supports findings (8) that an increased incidence of caries was related to increased salivary viscosity. However, our data did not support the observation that low salivary volume and excessive carbohydrate consumption were related to increased viscosity of saliva and increased incidence of caries. The degree of viscosity of saliva also bore no relationship to the cleanliness of the teeth.

There was a distinct inverse relation between cleanliness of the teeth and dental caries. This has previously been observed by a number of investigators (7) who, in studying cariogenic food clearances from tooth surfaces, found clearance of adherent sugar foods more important in determining caries experience than sugar clearance from the saliva.

These findings do not necessarily contradict the well-established relationship between caries experience and sugar ingestion, physical character of the diet, character of the saliva or oral hygiene. This relatively small sample of ninety children could show no one factor most important in determining caries susceptibility. The findings, however, suggest that, of all the factors observed, only three had a significant relationship to dental-caries experience: natural cleanliness of teeth, salivary viscosity, and some caries resistance or susceptibility factor that is familial in nature.

The pedodontist is apt to consider only current sugar ingestion as most important in determining caries susceptibility. Perhaps he should also consider other factors, including natural hygiene, quality of the saliva, and caries experience in the siblings.

Conclusions

1. Children with low-caries experience generally had siblings with little or no caries. Children with high-caries rating generally had siblings with a high-caries experience. A relationship to the caries experience of the parents could not be established (Fig. 2).

2. No significant relationship could be established between caries experience in the ninety children and sugar ingestion of the child during infancy or at present (Figs. 3 and 4). No relationship could be established with the mother's sugar ingestion during pregnancy or at present (Figs. 5 and 6).

3. No relationship could be established between dental-caries experience and the adherent and detergent character of the diet (Fig. 7), tooth-brushing habits (Fig. 9), or salivary volume (Fig. 8).

4. There was a distinct relationship between clean teeth and low-caries experience (Fig. 9). Unclean teeth had a high-caries-attack rate.

5. There was a slight relation between dental-caries experience and salivary viscosity (Fig. 8). There was a slight tendency for the children with more viscous saliva to have a higher caries index than the children with thin saliva.

6. These findings do not deny or negate the well-established relationship between sugar ingestion and caries, between caries and adherent foods and tooth-brushing habits. This study merely points out that these relationships are difficult to demonstrate clinically and that the causes of caries are multiple and not solely a function of sugar ingestion.

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THE ROLE OF SUCROSE AND OTHER SUGARS IN THE DEVELOPMENT OF DENTAL CARIES: A REVIEW

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Introduction

Numerous reviews have been published on nutrition and dental caries. Nizel (1972) has recently published a valuable course book on nutrition in preventive dentistry, where he draws attention to the early finding of Aristotle that soft, sweet figs produced damage to teeth. In his review on nutrition and dental health Shaw (1970) recommended that for optimal oral health, foods which are readily cleared from the mouth and which contain low amounts of readily fermentable carbohydrates should be used. Consumption between meals of candy, cookies, sweet rolls, etc., should be discouraged. If carbohydrate-free diets are fed to animals, dental caries does not occur. In various laboratory conditions, sucrose is the most readily utilized substrate for bacterial enzymes responsible for the formation of dextrans (polyglucans) by cariogenic streptococci, but in some cases other sugars (monosaccharides and disaccharides) caused carious lesions.

Nikiforuk (1970) stated that the food item in human nutrition which significantly increases the rate of caries formation is sucrose. He suggested, among other things, that research in food processing should be directed to reduce the cariogenicity of human diets by means of inhibition of glycolysis and of synthesis of carbohydrate polymers (dextrans, levans, etc.), and alteration of the sucrose molecule. In his review, Pigman (1970) suggested that sucrose may be the most cariogenic of all commercial sugars and stated that scientific knowledge is currently such that the cariogenicity of carbohydrate products can be evaluated reasonably well. Cooperation between industry and dental and health agencies may result in an effective programme to limit sucrose consumption. According to Pigman it may be possible to substitute a less cariogenic substance for a more cariogenic component. Winter (1968) mentioned that complete replacement of sucrose by other monosaccharides or disaccharides would not necessarily result in a reduction of the incidence of caries. Winter points out that a return to high levels of caries would occur through microbial adaptation.

Bibby (1970) reviewed methods for comparing the cariogenicity of foodstuffs. Information was provided on such methods which could yield knowledge on the caries-producing potential of a great variety of carbohydrate-containing foods. Bodden et al. (1972) developed another method for the evaluation of cariogenic potential of liquid foodstuffs. The method was based on microhardness measurements.

According to Navia (1968) it still remains to be firmly established if a direct cause-and-effect relationship can be demonstrated between sugar consumption patterns and disease. Yudkin (1969) comes to a similar conclusion. Harris (1963) established that the absence of sucrose limited the caries formed to a fraction of that which occurs in the overall population. Goldsworthy et al. (1953) and Sullivan and Harris (1958), all working on Australian populations, had earlier come to the same conclusion. The well-known Vipeholm study (Gustafsson et al., 1954) and the study of Sognnaes (1948) provided further support of a circumstantial nature for a relationship between sucrose consumption and human caries prevalence. Von der Fehr et al (1970) showed that it was possible to produce detectable carious alterations within 23 days by total neglect of oral hygiene and frequent sucrose additions. These alterations were reversible.

The importance of the synthesis of extracellular polysaccharides (such as levans and dextrans) by cariogenic micro-organisms in the etiology of caries has become generally accepted. Newbrun (1967, 1969) stated that the

ability of certain micro-organisms to synthesize these polysaccharides is the most important property which determines their cariogenicity. Berman and Gibbons (1966) showed that organisms with intracellular polysaccharide also continue acid production after the exogenous carbohydrate supply in the oral cavity is depleted. Such organisms synthesize polysaccharide equally well when grown on sucrose, glucose, or maltose. Baastaed and Rølla (1970) recently reviewed the literature concerning the topic of sucrose and dental caries. They came to the conclusion that the availability of easily fermentable carbohydrates for cariogenic streptococci metabolism resulting in plaque formation is the key to caries.

The cariogenicity of sucrose in the light of some earlier animal studies

In the early studies comparisons between different carbohydrates were carried out with rats kept on semi-synthetic diets (Schweigert et al., 1945). The carbohydrates used (sucrose, glucose, maltose, fructose, and certain sugar mixtures) did not differ from each other in their ability to induce or maintain caries, but dextrinized starch caused lower caries activity. In another study with hamsters (Shafer, 1949) 61 per cent starch or 12 per cent lactose as the components of the diet caused less caries than sucrose or glucose. Gustafsson et al. (1955) obtained with hamsters most caries activity with sucrose, less with glucose, fructose or lactose, and least with maltose.

Some recent animal tests

Grenby (1963) showed that sucrose was more cariogenic than glucose, and that raw wheat starch produced practically no lesions in rats. Guggenheim et al. (1966) observed with rats, treated with erythromycin and inoculated with a strain of streptococcus producing extracellular polysaccharides, that sucrose and lactose were most cariogenic at the fissures of occlusal surfaces. Fructose, maltose and starch yielded least caries and glucose caused an intermediate caries activity. At other tooth surfaces sucrose was more cariogenic than the other sugars mentioned. In a similar study König and Mühlemann (1967) showed the more potent cariogenicity of sucrose in comparison with glucose. Frostell et al. (1967) showed with hamsters and rats that sucrose produced more plaque and caries than fructose, glucose, maltose, or hydrogenated starch. In a study of Shaw et al. (1967) sucrose was found to be more cariogenic in rats than glucose and maltose, but the results were somewhat different on different strains of rats. The test animals were selected to represent caries activity characteristic to a particular tooth surface.

Green and Hartles (1969) stated that sucrose, glucose, fructose, galactose, lactose, and maltose were all cariogenic in rats. Grenby and Hutchinson (1969) also came to the conclusion that the difference in the ability between sucrose and glucose to promote caries was not great. Glucose and fructose were almost equally cariogenic. Rosen (1969) obtained results with the diet 2000 of Keyes and Jordan (1964) showing that glucose was more cariogenic than sucrose in gnotobiotic rats infected with various micro-organisms. Larje and Larson (1970) tested the following sugars in comparison with sucrose to induce caries in rats infected with cariogenic micro-organisms: glucose, fructose, mannitol, sorbitol, potato starch, hydrogenated starch, and certain sugar mixtures. All these sugars produced less plaque than sucrose, but the ability to reduce caries depended on the tooth surface involved. Woodell and Kreitzman (1971) have shown with rats that prenatal sugar type did not influence the ultimate caries experience of the animals.

It has become evident that in the animal studies described different results were obtained depending on which animal strain, diet and microbial flora were used. However, sucrose was almost unequivocally considered the most cariogenic simple sugar (one study with glucose and fructose yielded the opposite result). It is not known if all animals possess an inherent physiological need for sweet products, although bears have been occasionally shown to like honey (Disney, 1901-1966). Although the results of animal studies cannot be directly applied to man, they have been necessary in obtaining a better understanding of the biology of caries in general. However, the need for long term caries studies on man, comprising the use of sucrose and all the other sugars which have occasionally been claimed to be less cariogenic than sucrose, is great. One such study is being

carried out at the present time in the University of Turku, Institute of Dentistry. This study utilizes dental students in a two year investigation, where the effects of sucrose, xylitol, and fructose are recorded in several ways, including attempts to exploit the microscopic technique of von der Fehr et al. (1970).

The dextran era

Fitzgerald et al. (1960) found that when a strain of streptococcus, isolated from a caries lesion, was used to inoculate germ-free rats, the caries activity increased. Caries-inactive hamsters developed carious lesions when infected with a streptococcus isolated from a hamster caries lesion (Fitzgerald and Keyes, 1960). Krasse (1965a, b) observed that sucrose played a role in the manifestation of streptococci in the hamsters oral cavity. The same was proved to be true with man (Krasse et al., 1967). Carlsson and Egelberg (1965) showed the high plaque-forming property of sucrose. In the presence of sucrose cariogenic streptococci produced great amounts of extracellular dextran (Gibbons and Banghart, 1967), which is the most dominant polysaccharide in human dental plaque (Critchley et al., 1967). Dextran was not synthesized from glucose or fructose.

Dextran was estimated to account for 20 per cent of the plaque carbohydrate and from 1 to 3 per cent of the plaque dry weight (Minah et al., 1971), or no more than 1.6 per cent (Gold et al., 1971). Low molecular weight dextrans prevented caries activity (Gibbons and Keyes, 1969). Maltose and dextran (MW 2000), in addition to α-methyl glucoside and rabbit antisera directed to *Streptococcus mutans*, inhibited plaque formation by the organism (Genco et al., 1970). Lactose and starch did not inhibit or inhibited only little. Maltose inhibited the polysaccharide synthesis of a cariogenic streptococcus (Knuuttila and Mäkinen, 1972). More polyglucose and polyfructose synthesis occurred with sucrose than with glucose, fructose or glucose-fructose (1:1) in a salivary sediment system (SSS), where the degradation of dextrans was slower than that of levans (Halhoul and Kleinberg, 1969). Grenby (1969) showed that persistent dental plaque can be formed from certain carbohydrates at least as readily as from sucrose.

Also other organisms than streptococci produce extracellular polysaccharides. An oral strain of *L. casei* has been shown to produce a dextran-like extracellular polysaccharide from glucose or sucrose by a constitutive enzyme. Furthermore, the synthesized polysaccharides may serve as substrates in other reactions. Creamer et al. (1969) showed that dextran- and levan-forming streptococci of human origin utilized levan.

Dextran formed clusters of numerous molecules that clumped together (Newbrun et al., 1970). This property may be significant in the formation of dental plaque. It should be emphasized, however, that the synthesized polysaccharides are not completely homogenous products. Halhoul and Kleinberg (1970) showed that the type and heterogeneity of polyglucose and polyfructose is determined by the concentration of substrate (sucrose) and probably pH. Halhoul and Kleinberg (1972b) showed that an acidic pH and the continual presence of sucrose would favour accumulation of extracellular glucose and fructose polymers by reducing their rates of degradation.

At pH 4-5 the glucose utilization in the SSS-system (suspended salivary sediment) was nil or slow, being at a maximum at pH 7, and decreasing at pH up to 8.5, above which pH value evidently keto acids were formed. The formation of the latter was considered to represent a homeostatic means of returning the pH towards neutrality (Korayem and Kleinberg, 1970). The same homeostatic mechanism also applies to plaque (Korayem and Kleinberg, 1971). The carbohydrate metabolism of the SSS and plaque was considered to be essentially the same (Halhoul and Kleinberg, 1972a). The presence of extracellular dextran imposes diffusion limitation upon growth (Tanzer et al., 1969). On the other hand, the high content of fermentable carbohydrates in dental plaque suggests that microbial activity is not limited by the supply of fermentable substrates (Hotz et al., 1972).

Hageage et al. (1969) showed with diphtheroids that no evidence was obtained to indicate that recognized pathogens produced more plaque than those strains whose pathogenicity has yet to be established. The composition of extracellular products germane to the process of adhesion in the diphtheroid organisms cannot be simply ascribed to the

synthesis of extracellular dextrans or levans.

The soluble sucrases from a strain of *Streptococcus mutans* (grown on glucose) produced both glucans and fructans when incubated with sucrose. The small amount of cell-bound sucrase present in the strain synthesized only glucans (Schachtele, 1972). The glucosyltransferase of *Streptococcus sanguis* did not act reversibly to form sucrose from fructose and dextran (Newbrun, 1971). The same has been shown in this laboratory with a human dental plaque sample.

Dextranase has been suggested to play a role in the removal of dental plaque (Fitzgerald et al., 1968; Block et al., 1969). Several authors have not verified this, because controversial results were obtained (König and Guggenheim, 1968; Guggenheim et al., 1969; Caldwell et al., 1971; Lobene, 1971; Keyes et al., 1971). It seems that while the dextranase effect is a most interesting phenomenon from a biochemical or scientific point of view, the use of dextranase in practice is perhaps not well-grounded, due to several reasons (different dextrans may have slightly varying structure, which is not attacked by more or less specific mould enzymes, enzyme stability in storage may be too low, contact with the substrate is for too short a time, etc.). The use of preparations obtained from cariogenic streptococci themselves might also be a promising approach. Cariogenic streptococci display dextranase activity (Mäkinen and Paunio, 1971). Levanase and dextranase is present in dental plaque samples (Manly et al., 1971). Crude dextranase produced fructose from sucrose and levan (Gold et al., 1970).

Leach (1970) has recently argued that the inert extracellular polysaccharide component from sucrose reduces rather than promotes dental caries and that the levan component, which is labile in the plaque, might be more significant in the carious process. Small amounts of levan were found in the dental plaque if the plaque was examined several hours after its last exposure to sucrose (Critchley et al., 1967). If the plaque was examined immediately following its exposure to sucrose, much more levan was found. This was metabolized during the following two hours (Leach, 1970). However, some levan-forming streptococci are weakly cariogenic. One explanation of this is that these strains produce, in addition to levan, a small amount of dextran which is responsible for their cariogenicity (Delisle and Lee, 1972). Several

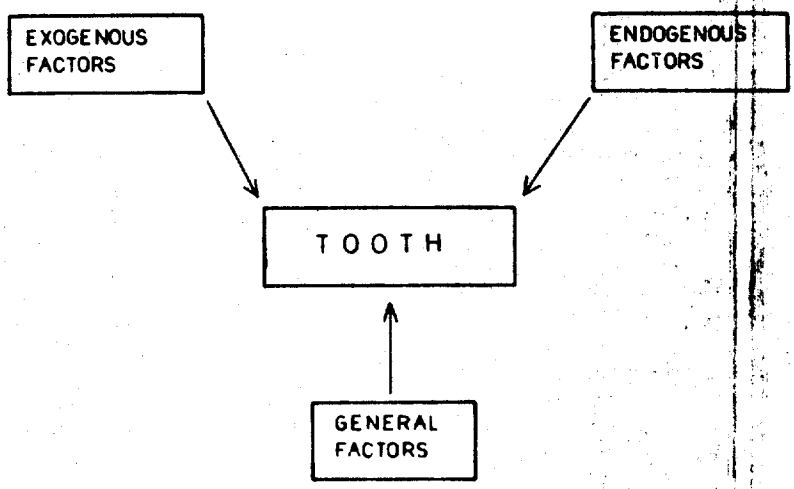


FIG. 1

The three main factors governing dental caries.

Les trois principaux facteurs gouvernant la carie dentaire.

Die drei Hauptfaktoren der Kariesentstehung

Los tres factores principales en la regulación de la caries dental.

levan negative strains of *Str. salivarius* were isolated which displayed essentially identical sugar fermentation pattern with their parent strains. Their cariogenicity remains to be elucidated.

Critchley and Bowen (1970) showed with monkeys that sugar is vital for the normal metabolism of the plaque bacteria. The organisms starved of sugar had thin, convoluted walls separated from the cell cytoplasm and less plaque was formed. Glucose facilitated the recovery of bacteria more rapidly than sucrose. Both sugars gave rise to extracellular polysaccharides which were morphologically and chemically different. Saxton and Critchley (1970) showed that in the deeper region of the plaque the organisms were more closely packed, had thicker cell walls, exhibited less cell division and synthesized more glycogen than organisms at the plaque saliva interface.

Caries theories

Dental caries is a multicausal disease which is governed by three different types of factors: exogenous, and general. This situation is described in Fig. 1 where the factors mentioned can be seen affecting the tooth. Without looking further into the other two factors, the exogenous ones can be examined in greater detail, because the intake of carbohydrates can be included with these, as is shown by the list below.

Exogenous factors:

Easily fermentable carbohydrates:
Sucrose
Glucose
Maltose
Lactose
Fructose
Many oligo- and polysaccharides, etc.

Specific micro-organisms:
Cariogenic streptococci
Cariogenic lactobacilli, etc.

The list presents specific micro-organisms as exogenous factors, but it must be questioned whether all or most oral micro-organisms should be regarded as cariogenic, each contributing to some phase of the carious process (Larmas and Mäkinen, 1971). Some micro-organisms may, however, be regarded as more potently cariogenic than others.

When evaluating the role of sucrose and other simple sugars in the carious disease, three possibilities can be suggested for the action of these carbohydrates: first, the sugars promote caries, secondly, they reduce caries incidence, and thirdly, they do not affect the process in any way. It is almost unequivocally maintained that at least sucrose ingestion can be related to caries formation, but authors disagree as to the theory of the mechanism of the process. Table I summarizes perhaps the most important caries theories, which all suggest sucrose or other sugar intake as being the necessary factor. The theories are numbered from 1 to 7 and although they partly overlap each other the different factors are presented separately, because they may act at different stages of the process and also independently of each other.

The versatile and complex basis of the biology of caries has been elucidated by several recent findings. A 'fructose-lethality' effect was found by Creamer et al. (1971) in an oral strain of *Streptococcus salivarius*. Rapid death of the strain resulted when fructose was the sole exogenous sugar source present in an otherwise adequate medium. The effect was also seen when culturing was done in mixtures of fructose and glucose. The pH of dental plaque *in situ* can vary between approximately pH 4.0 and 9.5 (Kleinberg, 1961, 1967). The release of the high calcium and phosphorus levels in plaque (Dawes and Jenkins, 1962) over this pH range has been examined by Reddy and Kleinberg (1971) in a plaque-saliva mixture containing glucose or urea. Low pH favoured the release of calcium while a high pH favoured release of phosphorus. The authors suggested that suppression by F^- ions of the solubilization of calcium and phosphorus in plaque may have an opposite effect on the caries process than suppression of the release of these elements from enamel.

The secret of sucrose

As stated by Newbrun (1967, 1969), sucrose can be considered to be the arch criminal of dental caries. The particular chemical properties which make sucrose especially cariogenic are in part related to the special energy of hydrolysis of this disaccharide when compared to some others (Table II). The enthalpy of the reaction, $\triangle H$, expressed as calories per mole, is about -6700 for sucrose, whereas the

TABLE I

Factors which can be considered as caries theories. The ingestion of sucrose (and evidently some other sugars as well) may lead to caries through the factors listed

1. Enamel decalcification by the acids produced by some micro-organisms. Such acids were lactic acid, citric acid, and perhaps some others. Hamilton et al. (1972) suggested that the mechanism of acid attack upon human enamel is primarily by a penetration through the interface between the rod-core and the surrounding rod-sheath. The acid formed may also undergo further transformations. Hu and Sandham (1969) showed that under certain conditions plaque streptococci utilized lactic acid, converting it to acetic acid, ethanol, and CO_2. Komiyama et al. (1972) have demonstrated a pH-rise factor which was considered to be a major regulator of the carbohydrate metabolism of the oral microorganisms. The factor was fractionated on Bio-Gel P-4 into components with intermediate molecular weight. The exclusion limit of P-4 is approximately 3600.
2. Hydrolysis of peptide bonds of the organic matrix of teeth by microbial enzymes. The hydrolysis of peptide bonds would involve the action of collagenases and more unspecific proteinases, as well as aminopeptidases and carboxypeptidases. A number of such enzymes have been demonstrated in dental plaque and carious dentine (*cf.* Mäkinen, 1966a,b, 1969, 1970).
3. Chelation of tooth calcium with products of microbial digestion or products of microbial fermentations. Such products would include amino acids liberated during peptide hydrolysis, and citric acid, lactic acid, and related acids. The complex-forming property of sucrose itself is to be noticed (the complexing non-acid theory; Schatz and Martin (1962).
4. The transfer of tooth phosphate and other material into bacterial cells of plaque at the enamel interface (*cf.* Luoma, 1972; Eggers-Lura, 1949, 1958, Keyes and Jordan, 1963; König, 1965). According to Howell et al. (1972) it is clear that tooth phosphate is utilized by *Streptococcus mutans* for growth. The ability of cariogenic organisms to utilize phosphate from the tooth may be in part responsible for their ability to colonize the tooth, resulting in decay.
5. Action of phosphatases and phosphoprotein phosphatases on the phosphate ester bonds of the tissue (Kreitzman et al., 1969; Paunio et al., 1968; Mäkinen and Paunio, 1969, 1970).
6. Action of all other enzymes and enzyme toxins on the tissue, which have not yet been discovered. Bacterial endotoxins have been demonstrated in plaque.
7. Increase of the solubility of human enamel and dentin by certain salts (for example, ammonium sulphate and related salts; *cf.* Paunio and Mäkinen, 1971).

TABLE I

Facteurs pouvant être considérés comme théories de carie. L'ingestion de sucrose (et bien sûr d'autres sucres également) peut conduire à la carie à travers les facteurs énumérés.

Faktoren, die als Kariestheorien betrachtet werden können. Der Konsum von Saccharose (und offensichtlich ebenso einiger anderer Zucker) kann aufgrund der aufgeführten Faktoren zu Karies führen.

Factores que pueden ser considerados como teorias de la caries. La ingestion de sacarosa (y evidentemente de algunos otros azucares) puede conducir a la caries a traves de los factores enumerados.

TABLE II

The energy of the hydrolysis of some simple carbohydrates (Forlot, 1971)

Sugar	Enthalpy of the reaction, ΔH, cal $moles^{-1}$
Sucrose	From -6600 to -6700
Lactose	-3000
Maltose	From -3000 to -3200
Cellobiose	-3100

TABLE 2

Energie de l'hydrolyse de plusieurs hydrates de carbone simples (Forlot 1971).

Hydrolyse-Energie einiger einfacher Kohlenhydrate (Forlot, 1971).

La energia de la hidrolisis de algunos carbohidratos simples (Forlot) 1971.

other disaccharides listed yield considerably lower values. For example, glucose 1-phosphate, which is a very important phosphorylated intermediate in carbohydrate metabolism, has a value of -5000, and other important compounds, *i.e.* glucose nucleotide diphosphates, may have a value close to -7600. The latter compounds are usually regarded as rather energy rich phosphate esters. This shows that sucrose differs from other usual disaccharides and other sugars. The energy mentioned is built in the structure of sucrose

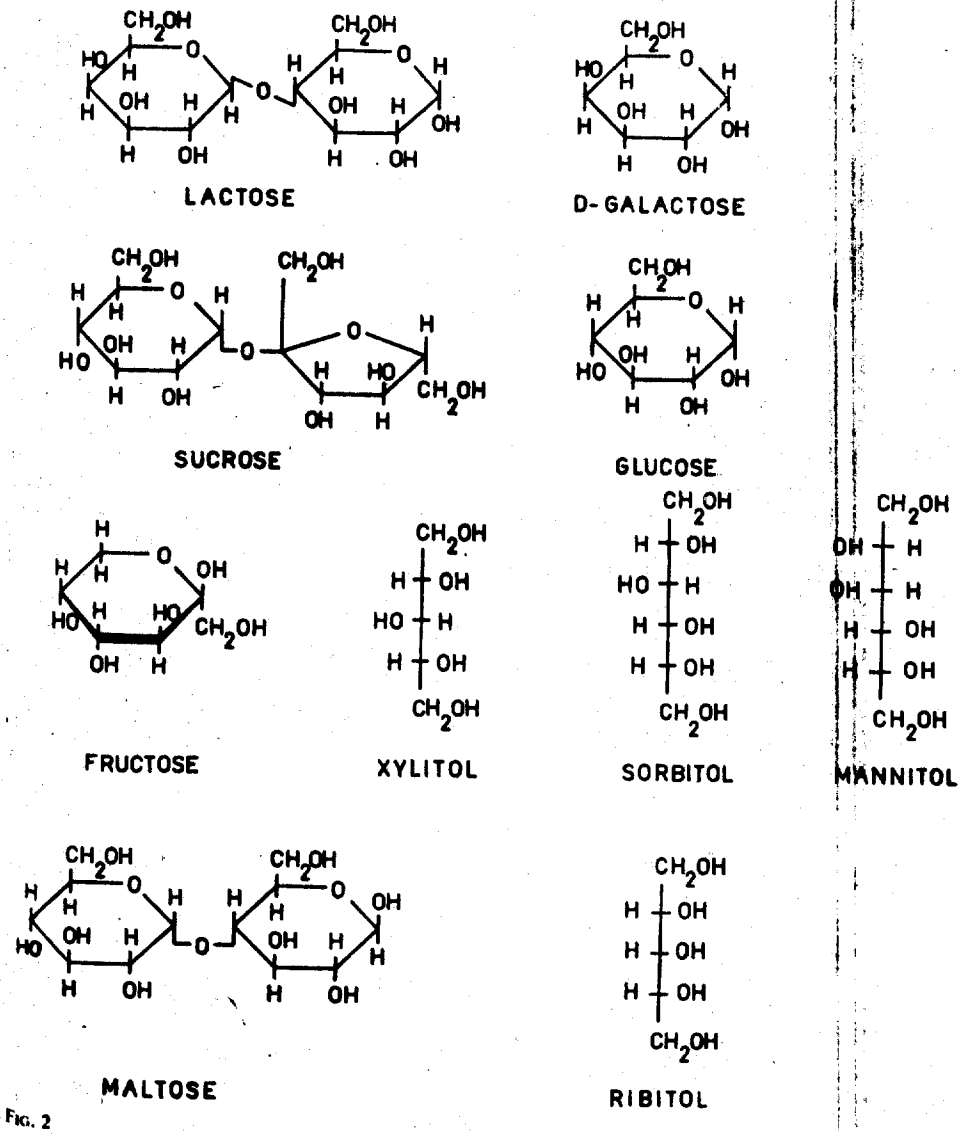


FIG. 2

Structural formulae of some common sugars.

Formule de la structuration de plusieurs sucres communs.

Strukturformel einiger verbreiteter Zucker.

Formula estructural de algunos azucares corrientes.

and makes this sugar suitable in the microbial production of extracellular polysaccharides, such as dextrans and levans, which are considered important factors in the etiology of caries. All that has been said against sucrose has led to the search for proper substitutes which could partly replace sucrose as a sweetener. Some alternatives are shown in Fig. 2.

The possibility offered by polyols

Polyols are polyhydric alcohols and those most frequently used in dental caries research have included mannitol, sorbitol and xylitol, of which xylitol is the sweetest. In effect these compounds are sugars (the term sugarless candy containing, for example, sorbitol, is a false expression). They very closely resemble other simple carbohydrates in their chemical structure and they can be obtained by known chemical reactions from hexoses and other means. The mammalian and microbial metabolism of polyols is rather well known due to certain recent advances made in the field.

Sorbitol and particularly xylitol inhibited the growth of certain micro-organisms, for example, that of a cariogenic streptococcus (Mäkinen, 1972) grown in the presence of 2.5 per cent glucose. The cells were adapted to utilize these polyols, or the growth inhibiting effect of the polyols otherwise disappeared, if the cells were stored for a few months in the presence of 5 per cent xylitol or sorbitol. Shockley et al. (1956) stated that sorbitol can be shown to be attacked by an adaptive enzyme or enzymes. Sorbitol is slowly fermented by oral micro-organisms (Grubb 1945; Shaw, 1954; Crowley et al., 1956) It has been shown that sorbitol does not cause a pH-decrease in the dental plaque in contrast to sucrose (Fosdick et al., 1957; Frostell, 1956; Mühlemann, 1969). It has been claimed

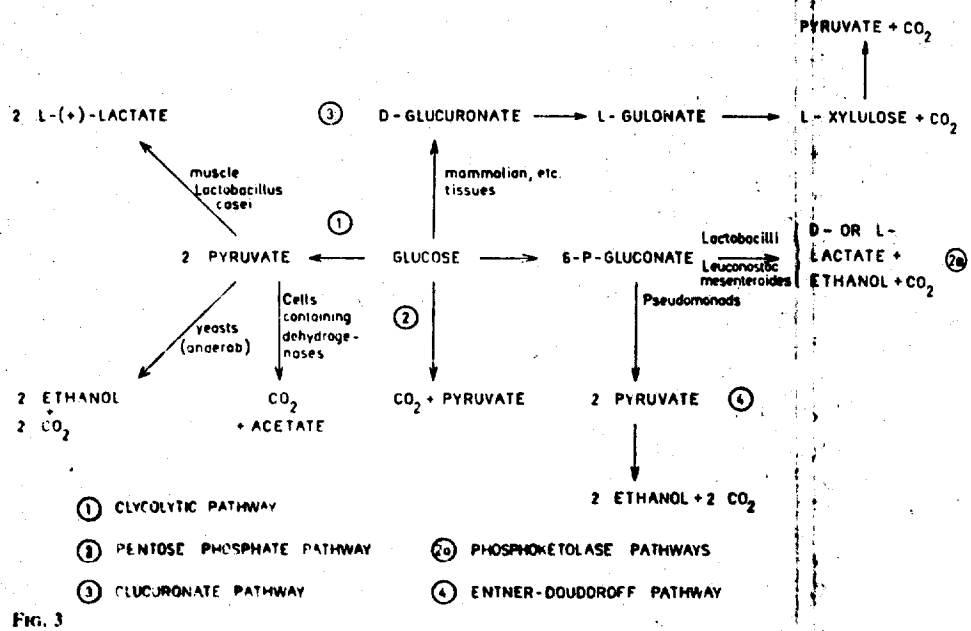


FIG. 3

Reaction sequences of glucose catabolism. Note the pathway No. 3 which includes the metabolism of xylitol (see Fig. 4).

Séquences de réaction du catabolisme du glucose. Noter le tracé n° 3 qui comporte le métabolisme du xylitol (voir Fig. 4).

Reaktionsfolge des Glukose-Katabolismus. Zu beachten ist der Weg Nr. 3, der den Metabolismus von Xylitol (s. Fig. 4) umfaßt.

Reacciones del catabolismo de la glucosa. Observese que el N° 3 incluye el metabolismo del xylitol (ver Fig 4).

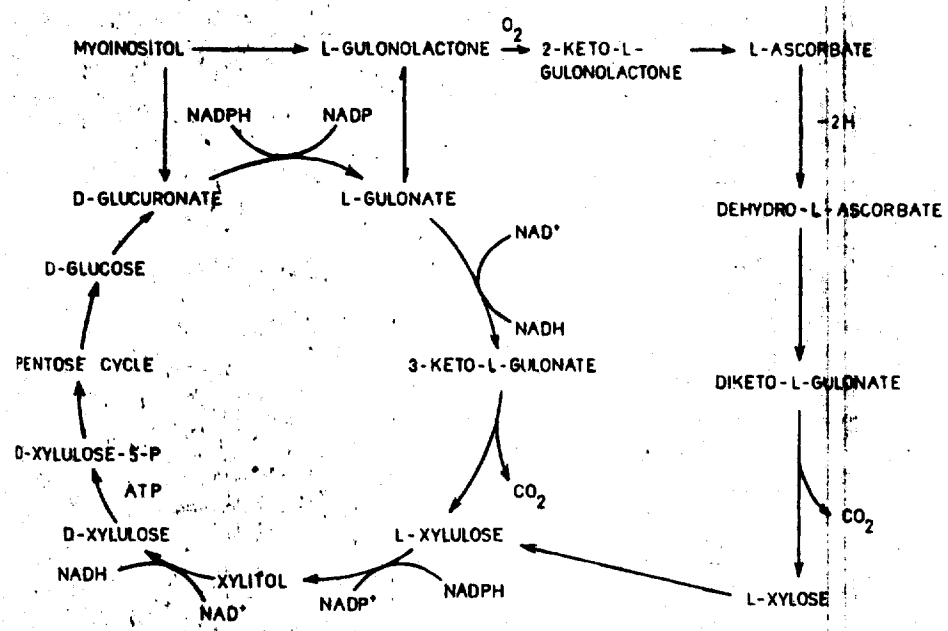


FIG. 4

The D-glucuronate-L-gulonate pathway. Note that the metabolism of xylitol is linked to that of ascorbic acid and glucose.

Tracés du glucuronate D et gulonate L. Noter la liaison existant entre métabolismes du xylitol, de l'acide ascorbique et du glucose.

Die D-Glucuronat-L-Gulonat-Entwicklung. Es sei darauf hingewiesen, daß der Xylitol-Stoffwechsel mit dem von Askorbinsäure und Glukose verbunden sit.

Los pasos del D-gluconat ó-L'gulonate'. Notese que el metabolismo del xylitol esta unido al acido ascorbico y glucosa.

to be less cariogenic in animal experiments than sucrose (Klapper and Volker, 1954, 1955, Shaw and Griffiths, 1960; Frostell et al., 1967). Mühlemann et al. (1970) have shown the low cariogenic effects of xylitol. Frostell (1964) and Gülzow (1968) have come to the conclusion that acid production from sorbitol may increase with time in the oral cavity and that sorbitol will be broken down in the mouth. The acid production by cariogenic streptococci was almost normal in the presence of xylitol and sorbitol after an adaptive period of several months (Mäkinen, 1972).

Mammalian and microbial metabolism of polyols

During the first stages of catabolism large molecules are broken down to yield, apart from carbon dioxide and water, a restricted group of small organic molecules. These product molecules for carbohydrates are shown below.

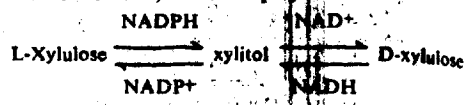
$$\text{Carbohydrates} \xrightarrow{\text{Catabolism}} \left\{ \begin{array}{l} CO_2 + \\ H_2O + \\ \text{Triose phosphates and/or} \\ \text{pyruvate} \end{array} \right.$$

The catabolism shown above includes the formation of glucose as a very important metabolite. The most important patterns of the catabolism of glucose are shown in Fig. 3. The reaction sequences shown include a special route (No. 3) which comprises the metabolism of a polyol, i.e. xylitol. In Fig. 4 this route is described in more detail. The set of conversions shown in Fig. 4 has been

demonstrated in animals and higher plants. Evidence is accumulating that these reactions (Fig. 4) may play an important role in the metabolism of L-ascorbate, of *myo*inositol and of hexoses. In man, primates, and guinea pig, a genetic block makes these species unable to synthesize L-ascorbic acid, which is therefore an essential vitamin. In this glucuronate-gulonate pathway the key reductive step in the biosynthetic sequence to L-ascorbate between glucuronate and gulonate and both the reductive steps for the whole cycle involve NADPH, a nicotinamide dinucleotide coenzyme. These reactions include the reaction from L-xylulose to xylitol. The corresponding oxidative reactions (gulonate → keto-gulonate → L-xylulose and xylitol → D-xylulose) both utilize NAD^+.

A hereditary abnormality known as idiopathic pentosuria in humans is most likely caused by an inability to synthetize the NADPH-dependent xylitol dehydrogenase. Large amounts of L-xylulose are found in the urine of such individuals, especially on a diet which is rich in carbohydrates and particularly of D-glucuronate.

There is a set of reversible pyridine nucleotide-linked dehydrogenations that generate sugar alcohols (polyols), which affect various isomerizations, for example,



These enzymes are located in mitochondria in mammalian liver. An analogous and ubiquitous set of enzymes catalyzes reactions involving sorbitol (Fig. 5). Like most enzymes involved in carbohydrate metabolism, the $NADP^+$-specific D-hexitol dehydrogenase is

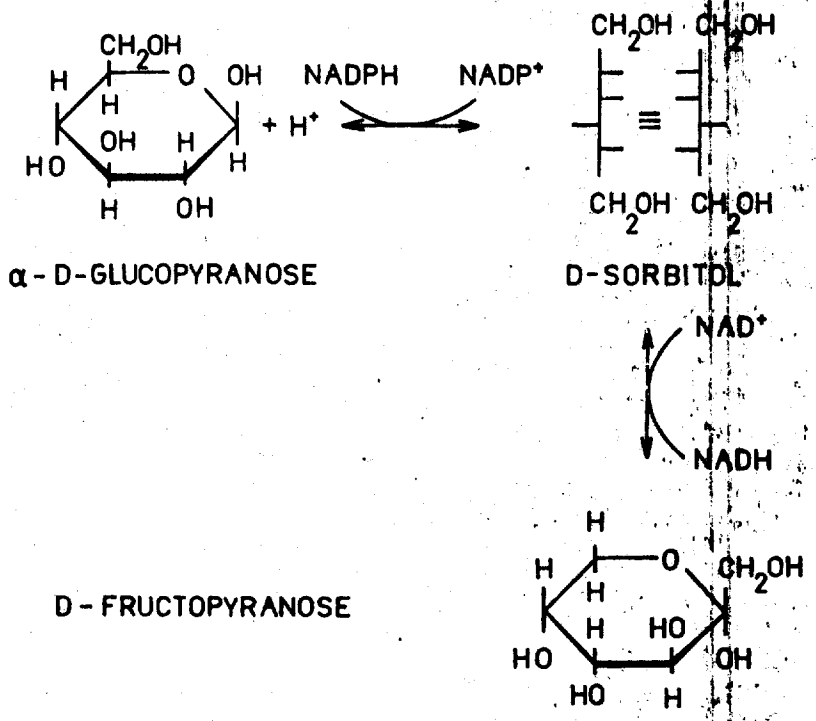


Fig. 5

The oxidation and reduction of sorbitol.

Oxydation et réduction du sorbitol.

Oxidation und Reduktion von Sorbitol.

Oxidación y reducción del sorbitol.

specific for the α-D-form. The second enzyme is NAD^+-specific hexitol dehydrogenase, which leads to the formation of the corresponding ketose. This enzyme is also widely distributed in mammalian tissues. It is very similar to that catalyzing the reaction between L-xylulose and xylitol, but its origin is probably extramitochondrial.

Xylitol metabolism in humans and test animals has been investigated in numerous papers. Because it is a normal intermediary product in the glucuronate-xylulose cycle (Touster, 1960), it is utilized both by man and animals (McCormick and Touster, 1957; Schmidt et al., 1964; Bässler et al., 1962). Because the structural configuration of xylitol differs from D-glucose at the first three carbon atoms, its transport into muscle is not facilitated by insulin (Goldstein et al., 1953; Bässler and Prellwitz, 1964). Xylitol is well metabolized by diabetic subjects and patients with renal disease and xylitol stimulates the release of insulin, although the levels attained are less than after glucose (Mehnert et al., 1964; Spitz et al., 1970). Xylitol should prove to be a useful source of calories in uremia and other conditions characterized by carbohydrate intolerance and insulin resistance (Spitz et al., 1970). Xylitol is a normal metabolite in man and a normal ingredient of many natural products. Certain mushrooms contain xylitol and there is plenty of xylitol in bananas. It is almost sure that it will be later shown to be a rather widely distributed sugar in the plant kingdom.

Numerous micro-organisms can be made to utilize polyols (mainly hexitols and pentitols) as the sole carbon source. Under these conditions a variety of induced polyol dehydrogenases have been demonstrated and purified enzymes isolated (Horwitz and Kaplan, 1964). Among them are D-sorbitol (L-iditol) dehydrogenase similar to that described above, isolated from D-sorbitol grown *Bacillus subtilis;* a D-mannitol 1-phosphate dehydrogenase, isolated from the cells of the same organism, from *E. coli* or *A. Aerogenes*, etc., all grown on D-mannitol, and so on. Table III lists most of the enzymes known to participate in polyol metabolism. Many of them or related enzymes will certainly become important research objects in dentistry when polyols are used as a substitute for sucrose. The genetic trait to form the inducible mannitol dehydrogenase can be transferred from a donor strain of *Diplococcus pneumoniae (Pneumococcus)* to one not possessing this capacity (acceptor strain) by means of pure DNA ('transforming principle'), a process known as 'transformation'. Once 'transformed', the acceptor cells become capable of synthetizing the enzyme and of transmitting this capability to its daughter cells and of acting as donor in subsequent transformations (Austrian and Bernheimer, 1959; Fox and Hotchkiss, 1960; Fox, 1962). It is not known if this type of a mechanism can occur in oral bacteria grown on xylitol or sorbitol, but the possibility should be kept in mind.

Xylitol metabolism in perfused rat liver was studied by Jacob et al. (1971) and by Williamson et al. (1971). In perfused liver xylitol was converted mainly to glucose, after phosphorylation of D-xylulose to D-xylulose 5-phosphate and metabolism via the pentose phosphate pathway. Xylitol metabolism was not associated with any increased rate of respiration, showing that reoxidation of NADH produced by xylitol dehydrogenase caused an inhibition of the citric acid cycle. The xylitol metabolism was not controlled by the activity of NAD-xylitol dehydrogenase but by the rate of reoxidation of NADH.

The routes by which xylitol and sorbitol are metabolized in the oral cavity in principle resemble those found in the study of pure microbial cultures. Dallmeier et al. (1970) have studied the breakdown of glucose and sorbitol by plaque streptococci. The streptococci tested metabolized glucose, sorbitol, and mannitol almost exclusively by the glycolytic pathway (Embden-Meyerhof). Glucose was chiefly catabolized to lactate (80-90 mole %). In the breakdown of sorbitol less lactate was produced, but considerable quantities of formiate and ethanol were formed. This may be a common pathway for the metabolism of hexitols in the oral cavity. The breakdown of pentitols (like xylitol) evidently involves the reactions already mentioned above for inducable polyol dehydrogenases. In a recent study (Mäkinen, 1972) no xylitol dehydrogenase activity in cells maintained in the presence of xylitol was observed. The cells were, however, not 'xylitol cells', because the broth also contained 2.5 per cent glucose. According to Gehring (1968) no sorbitol

TABLE 3

Some microbial and other enzymes acting on polyols and related compounds. Compiled from Methods in Enzymology (Vol IX, Academic Press, New York, 1966, pp. 143-193)[1]

Enzyme	Reaction	Source
Mannitol dehydrogenase	D-Mannitol + NAD ⇌ D-fructose + NADH (D-sorbitol and xylitol are not oxidized and D-glucose and D-xylulose are not reduced)	*Lactobacillus brevis* (may also occur in other *Lactobacillus species*)
Mannitol (cytochrome) dehydrogenase	D-Mannitol + 0.5 O_2 = D-fructose + H_2O (A wide range of acylic polyols containing the D-erythro configuration is oxidized)	*Acetobacter suboxydans*
D-Mannitol 1-P dehydrogenase and D-sorbitol 6-P dehydrogenase	D-Mannitol 1-P + NAD ⇌ D-fructose 6-P + NADH D-Sorbitol 6-P + NAD ⇌ D-fructose 6-P + NADH (The enzymes are specific for D-mannitol 1-P and NAD, and D-sorbitol 6-P and NAD, respectively)	*Aerobacter aerogenes*
D-Mannitol 1-P dehydrogenase and D-sorbitol dehydrogenase	D-Mannitol 1-P + NAD ⇌ D-fructose 6-P + NADH D-Sorbitol + NAD ⇌ D-fructose + NADH (The first enzyme is highly specific for D-Mannitol 1-P and NAD. The second enzyme oxidizes D-sorbitol and xylitol)	*Bacillus subtilis*
Sorbitol dehydrogenase	Sorbitol + NAD ⇌ fructose + NADH (D-Sorbitol and xylitol are oxidized)	Spermatozoa
Polyol dehydrogenase	NAD-linked enzyme: H_2COH — $CHOH$ — $(CHOH)_n$ — H_2COH + NAD ⇌ H_2COH — $C{=}O$ — $(CHOH)_n$ — H_2COH NADH (Most active with xylitol, but also other polyols react) NADP-linked enzyme: $HC{=}O$ — $HCOH$ — $(CHOH)_n$ — H_2COH + NADPH ⇌ H_2COH — $HCOH$ — $(CHOH)_n$ — H_2COH + NADP (Xylitol and sorbitol are oxidized)	*Candida utilis* *Candida utilis*
Polyol dehydrogenases	L-*erythro* polyols are oxidized to the corresponding ketoses. Pentitols and hexitols react depending on coenzyme and enzyme fraction.	*Gluconobacter oxydans*
Pentitol dehydrogenases	I. Ribitol dehydrogenase Ribitol + NAD ⇌ D-ribulose + NADH Xylitol + NAD ⇌ D-xylulose + NADH L-Arabitol + NAD ⇌ L-xylulose + NADH (I oxidizes xylitol, ribitol, and arabitol) II. D-Arabitol dehydrogenase (II is induced by growth on arabitol, xylitol, etc.)	*Aerobacter aerogenes*
Pentitol dehydrogenases	D-Ribulose + NADPH → D-arabitol + NADP D-Xylulose + NADH → xylitol + NAD (The enzymes are specific for the respective substrate)	*Sacharomyces rouxii*
D-Xylose reductase and D-xylitol dehydrogenase	D-Xylose + NADPH + NAD^+ ⇌ D-xylulose + $NADP^+$ + NADH (The enzymes are not specific)	*Penicillium chrysogenum*

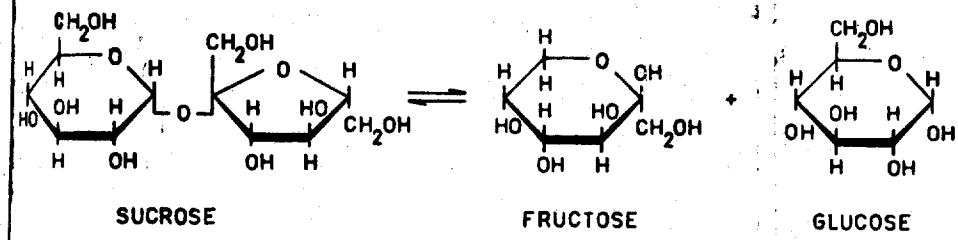


FIG. 6

The invertase reaction, when sucrose is the substrate.

Réaction d'invertase lorsqu'il y a du sucrose dans le substrate.

Invertasereaktion mit Saccharose als Substrat.

Reacción invertida cuando la sacarosa es el substrato.

dehydrogenase activity could be shown in plaque streptococci. It seems obvious that such enzymes will be demonstrated in cariogenic streptococci when grown on xylitol and sorbitol.

In most cases polyols serve as usual energy sources for the growth of oral bacteria, but in some cases special functions in other organisms have been reported. In *Aspergillus clavatus* the predominant function of mannitol is that of a storage compound, possibly connected with conidiation, while ribitol appears to be primarily involved in hydrogen-acceptor mechanisms (Corina and Munday, 1971). Much interest has recently centered round the enzymology of polyhydric alcohols in fungi (Spencer and Sallans, 1956; Lee, 1967; Strandberg, 1969). Mannitol has been implicated in sporulation of higher fungi (Rast, 1966) and also as a carbon source in germinating *Aspergillus oryzae* conidia (Horikoshi et al., 1965). Mannitol and other polyols in micro-organisms have been considered either to be involved in hydrogen-acceptor mechanism (Helle and Klungsoeyr, 1962) or to act as reserve carbohydrates (Birkinshaw et al., 1931; Lee, 1967). Certain polyols (at least glycerol and ribitol) are components of teichoic acids which are polyolphosphate polymers found in bacteria, usually in close association with the cell wall.

Because certain fungi contain xylitol, it could be suggested that this polyol is utilized by the organism in hydrogen-acceptor or related mechanism. The next suggestion would involve a similar role for polyols in bacteria.

The significance of invertase activity

Invertase (ß-fructofuranosidase) is an enzyme which catalyzed the following reaction:

A ß-D-Fructofuranoside + H_2O = an alcohol + D-fructose

The substrates of this enzyme include sucrose (Fig. 6). ß-Fructofuranosidase from yeast is the best known invertase. Yeast invertase resembles the ß-glucosidase of sweet almond emulsin and the ß-galactosidase of *E. coli* in respect to its ability to tolerate considerable variations of a glycon structure while manifesting a much stricter specificity requirement in relation to the fructon moiety (Hestrin et al., 1965). The cleavage catalyzed

TABLE 3

Action de certaines enzymes microbiennes et autres sur les polyols et composés qui y sont liés. Compilation d'après 'les Méthodes d'Enzymologie' (Vol IX Academic Press New York 1966 pp. (143-193) [1]

Einige bakterielle und andere Enzyme, die auf Polyole und hiermit verwandte Verbindungen wirken. Entnommen aus 'Methods in Enzymology' (Vol. IX, Academic Press, New York, 1966, pp. 143-193)[1]

Algunos microbios y otros enzimas actuando sobre los polioles y otros compuestos relacionados. Recopilados de Metodos en Enzimologia (Vol. IX, Academic Press, Nueva York, 1966, pp. 143-193)

by invertase occurs at the bond between the fructose carbon and the bridge oxygen (Koshland and Stein, 1954).

The invertase activity of the human oral cavity (Mäkinen and Scheinin, 1971, 1972) most likely consists of several enzymes formed by several oral micro-organisms. There was an indicative correlation between lactobacillus count and invertase activity of oral fluid (Mäkinen and Scheinin, 1972), but this result should be verified. No oral invertase has been studied or purified in greater detail. It has been suggested that invertase activity could be employed as a caries test (Mäkinen and Scheinin, 1971, 1972). This suggestion was in part based on the observation that test persons kept on a sucrose diet developed considerable invertase activity in plaque and oral fluid, whereas people kept on either glucose, fructose, xylitol, fructose-glucose, fructose-sorbitol and fructose-xylitol diets exhibited much weaker oral invertase activity.

Experiments carried out in this laboratory indicate that invertase activity increases in oral fluid within a few hours after sucrose intake. When schoolchildren had consumed sucrose-containing candies in the morning prior to the collecting of saliva, the activity of invertase was considerably higher than in cases where no sucrose or fructose was consumed. The formation of invertase was considered to have taken place in bacterial cells due to enzyme induction. The presence of the substrate of invertase (sucrose) in the oral cavity was thought to cause the induction. On the other hand, xylitol, for example, did not induce the formation of invertase, because it cannot be considered as an invertase substrate (Mäkinen and Scheinin, 1971, 1972). It was also suggested that the assay of salivary invertase would reflect the cooperation between dentist and patient (children), because sucrose intake leads to increased invertase activity. The only difficulty is that the 'normal' enzyme activity of the particular patient should be known. It is possible that oral invertase activity also reflects the activity of sucrose-utilizing bacteria in the mouth.

Gibbons (1972) found that the cells of a strain of *Streptococcus mutans* contained 'sucrase' activity which was distinct from that of the glucosyl and fructosyl transferases. The enzyme appeared to be identical or similar to invertase. The growth of the organism was not considered to be solely dependent upon the glucosyl and fructosyl transferases for the utilization of sucrose. According to Tanzer et al. (1972) a sucrose-dissimilative system(s) exists in *Streptococcus mutans*, independent of glucosyl and fructosyl transferases systems. This system was suggested to be the predominant means of degrading sucrose. The system was induceable in glucose grown cells.

Enzymic breakdown of enamel and dentine

The intake of sucrose and other carbohydrates leads to bacterial growth and synthesis of extra- and intracellular enzymes in the oral cavity. These enzymes have occasionally been implicated in the development of dental caries, by direct hydrolysis of important covalent bonds of the tooth structure. The enzymes suggested as playing a role in these reactions include phosphatases (Mäkinen, 1970a; Paunio, Mäkinen and Scheinin, 1968; Mäkinen and Paunio, 1969; Kreitzman et al., 1969). It was also thought that aminopeptidase (Mäkinen, 1966a, b, 1969) and proteinases like collagenases (Mäkinen, 1970b) would play a similar role. Although it is evident that the close proximity of plaque enzymes and their suggested substrate (enamel, dentin, etc.) almost certainly results in hydrolysis of some of the bonds mentioned above, the significance of these reactions in relation to dental caries remains to be elucidated more thoroughly. However, a considerable part of the ability of the bacterial phosphatase preparations used to release phosphorus from enamel and dentin powder was due to the presence of ammonium sulphate in the enzymes (Mäkinen and Paunio, 1970; Mäkinen, 1970a; Paunio and Mäkinen, 1970; Paunio and Mäkinen, 1971) Yet the problem cannot be considered as solved. It is now known that enamel proteins contain chemical bonds that may be hydrolyzed by plaque enzymes. A submicroscopic injury, whether mechanical or chemically caused, may initiate an enzymic reaction chain which, in the presence of fermentable carbohydrates and acid production, leads to caries.

Collagen fibrils provide the matrix on which the hydroxyapatite crystals of bone and dentin are deposited (Glimcher and Krane, 1967). Dentin consists of a phosphoprotein, covalently attached to collagen, that serves as a nucleation site for mineralization (Veis and

Perry 1967). The chemical character of proteins in rat incisors was elucidated by Butler et al. (1972). Three protein fractions were demonstrated. The findings obtained supported those presented by Veis and Perry. Glimcher and Krane (1967) speculate that the presence of organically bound phosphate in collagen and enamel proteins may play a role in the mineralization process. Seyr and Glimcher (1969) determined the amino acid sequence of two O-phosphoserine-containing tripeptides which were isolated from embryonic bovine enamel. Kreitzman et al. (1972) have studied strains of *Streptococcus mutans* which inducively forms high levels of a phosphatase by adaptation of the cells to phosphate depleted medium. The organisms were able to utilize the phosphate from organic substrates such as phosphoserine and phosphoproteins. A soluble non-collagenous phosphoprotein was isolated from the EDTA extracts of unerupted bovine dentin. The protein contained plenty of aspartic acid, serine, and phosphoserine (Veis et al., 1972).

The above information on the organic structure of tooth may also support the idea of a possible enzymic reaction in the degradation of dentin (and enamel) at some stage of caries. Consequently, the consumption of sucrose and other sugars may lead in alternative ways to caries (Table I).

The results of two recent studies

The results obtained in two recent short term sugar studies on man showed that even four or five days sugar diet was enough to obtain clearly detectable differences in the quality and quantity of dental plaque (Scheinin and Mäkinen, 1971, 1972; Mäkinen and Scheinin, 1971, 1972). In these studies the effect of the consumption of different sugars on the formation and properties of dental plaque and oral fluid was studied using dental students as research subjects. The sugars included sucrose, glucose, fructose, xylitol, fructose-glucose (1:1), fructose-sorbitol (1:1), fructose-xylitol (1:1), and sucrose-maltose (9:1). The most important results are summarized.

1. The deliberately mild dietary regime produced the most distinct changes in the rate of plaque formation and less significant or undetectable changes in its chemical composition.

2. The least amount of plaque (four or five days old) and lowest plaque index were found in the xylitol, fructose, and fructose-xylitol groups.

3. The activity of salivary or plaque invertase (ß-fructofuranosidase) was clearly highest in the sucrose group. When test persons were transferred from sucrose diet to diets containing other sugars, the activity of invertase decreased noticeably. High invertase activity was considered to reflect the metabolism of cariogenic micro-organisms. It was suggested that a special caries test could be developed which exploits the determination of invertase activity.

4. The activity of plaque dextranase was higher in the sucrose group than in other groups.

It was also found that the consumption of xylitol seems to be periodontologically acceptable (Paunio et al., 1972). The earlier study showed that the activity of aminopeptidase B (an arginine aminopeptidase which is believed to play a role in inflammation) in gingival fluid was increased in the xylitol group. This was later found to have been due to mechanical irritation of oral epithelium, because the xylitol-containing candies used were hard (Paunio et al., 1971).

A similar study to that described above was carried out by Drummond et al. (1972) with various sugar diets. In the high sucrose group more plaque was developed during a four day period than in the control group. More streptococcal micro-organisms were found in the plaques of the sucrose group than in the control group. Naylor et al. (1970) also used some of the sugars listed above. They showed that sucrose, glucose, fructose, glucose syrups and a fructose-glucose mixture supported plaque growth *in vitro*. The protein content and total dry weight of plaque was in all cases almost similar, but hexose was more abundant in sucrose plaques. In sucrose plaque approximately 23 per cent of the dry weight was represented by hexoses. The corresponding value in the fructose group was only approximately 8.7 per cent. Also in the above mentioned study (Scheinin and Mäkinen, 1971) it was found that the amount of hexosamines and sugars (the latter determined by the anthrone method) was higher in sucrose plaque than in the plaque of other sugar groups.

SUMMARY

1. It is possible to regard many oral micro-organism species as cariogenic, although it is evident that some species or strains on certain tooth surfaces may be more cariogenic than others. Anyhow, the disease can be regarded as a mixed infection.

2. Somewhat different and even controversial results have been obtained in animal experiments. This is most probably due to the difficulties in standardizing the methods. It is evident that in human experiments the difficulties will be even more pronounced.

3. It can be regarded as proved that sucrose is the most cariogenic common sugar. The cariogenicity is evidently mostly related to the rather high energy of hydrolysis of the covalent bond between the monosaccharide moieties. In this respect sucrose is in a special position among other disaccharides.

4. The energy content of sucrose is utilized by oral micro-organisms to synthesize, among other things, hydrophilic polysaccharides, which may form a bulk of the extracellular compartment of dental plaque. The polysaccharides (such as dextrans and levans) may be adsorbed on crystal surfaces in enamel, thereby partly promoting the growth and manifestation of cariogenic and other bacteria, creating diffusion barriers, acting as inflammatory agents, and providing strating material for the formation of simple cariogenic monosaccharides (glucose, fructose, etc.) and acid.

5. Although the role of polysaccharides in the etiology of caries is strongly emphasized at the present time, their significance should not be overestimated. Some polysaccharides may turn out to be more harmful than others and the ultimate effect also depends on which tooth surface is involved. The bacterial polysaccharides in plaque most probably represent a very heterogenous group of polymers which deserve intensive study.

6. The cariogenicity of some common sugars decreases roughly in the following order (on the basis of both animal and human studies): sucrose > glucose > maltose, lactose, fructose > sorbitol > xylitol. Invert sugar (fructose-glucose, 1:1) has not been found to be advantageous from the dental point of view. Certainly, other sugars or their derivatives will be found which may be effective in reducing caries. However, before becoming accepted, they should be shown to occur normally in usual natural products and as normal intermediates in the intermediary metabolism. Xylitol seems to fulfill these requirements, although controversial opinions exist concerning the insulin increasing effect of xylitol. The effect of xylitol on intestinal flora should be thoroughly studied. It is obvious that pentitols are more effective in reducing caries than hexitols.

7. Xylitol and other polyols act in certain fungi as hydrogen acceptors (i.e. the acceptor causes a metabolite to become oxidized; in many other cases molecular oxygen acts as a hydrogen acceptor). Although they may serve as the usual energy source in bacteria, they may also be shown to act as hydrogen acceptors in some bacteria.

8. Bacteria grown on polyols normally develop inducible polyol dehydrogenases and related enzymes which render the exploitation of these sugars possible as an energy source. This bacterial adaptation is a normal consequence of the presence of polyols in growth media. In effect, bacterial adaptation and enzyme induction are conditions for the evolutionary life of cells. Consequently, bacterial adaptation as regards polyols in the oral cavity is not a disadvantage, because polyols (particularly xylitol) do not promote the growth of plaque as effectively as sucrose, glucose, or fructose.

9. There are several interesting new findings which will receive attention in future, for example: a fructose lethality effect against an oral micro-organism strain, the utilization of lactic acid *in vivo* in plaque by certain bacteria, the catabolism of plaque polysaccharides to yield cariogenic sugars, the inducive formation of plaque invertase during high sucrose diet, the production of bacterial endotoxins in plaque, the proposed more cariogenic nature of levans when compared to dextrans, the utilization of tooth phosphorus by cariogenic streptococci, etc.

RÔLE DU SUCROSE ET AUTRES SUCRES DANS LE DÉVELOPPEMENT DE LA CARIE DENTAIRE REVUE

RÉSUMÉ

1. Il est possible de considérer beaucoup d'espèces de micro organismes buccaux comme cariogènes bien qu'il soit évident que certaines espèces ou colonies sur les surfaces dentaires soient plus cariogènes que d'autres. En tout cas, cette affection peut être tenue pour infection mixte.

2. Des résultats quelque peu différents et même controversables ont été obtenus lors des expériences sur des animaux. Cela est très probablement dû aux difficultés de standardisation des méthodes. Il est évident que pour des expérimentations humaines, les difficultés seront encore plus grandes.

3. On peut tenir pour prouvé que le sucrose est le sucre commun le plus cariogène. La cariogénicité est de manière évidente, principalement liée à l'énergie hydrolytique relativement élevée du lien covalent entre les portions de monosaccharides. A cet égard, le sucrose se trouve occuper une position spéciale au sein des autres disaccharides.

4. Le pouvoir énergétique du sucrose est utilisé par les micro organismes buccaux pour, entre autres choses, synthétiser les polysaccharides hydrophiles qui peuvent former une partie importante de la portion extracellulaire de la plaque dentaire. Les polysaccharides (tels que dextrans et levans) peuvent être adsorbés sur les cristaux de la surface de l'émail, favorisant ainsi la croissance et la manifestation d'autres bactéries, parfois cariogènes, créant des barrières de diffusion, agissant comme agents inflammatoires et fournissant les matériaux de départ nécessaires à la formation de monosaccharides simples cariogènes (glucose – fructose – etc.) et d'acide.

5. Bien qu'à l'heure actuelle, on mette beaucoup l'accent sur le rôle des polysaccharides dans l'étiologie de la carie, il ne faut pas surestimer leur signification. Certains polysaccharides peuvent devenir plus nocifs que d'autres et leur effet ultime dépend de la surface dentaire impliquée. Les polysaccharide bactériens de la plaque représentent très certainement un groupe très hétérogène de polymères qui justifient une étude intensive.

6. La cariogénicité de certains sucres communs décroît, en gros, dans l'ordre suivant (d'après des études effectuées tant sur l'animal que sur l'homme): sucrose > glucose > maltose, lactose, fructose > sorbitol > xylitol. Les sucres inversés (fructose- glucose I=I) ne se sont pas révélés avantageux au point de vue dentaire. Certainement on trouvera d'autres sucres ou leurs dérivés qui peuvent effectivement réduire la carie. Cependant, avant de les accepter, il faudra démontrer qu'on les rencontre normalement dans les produits naturels usuels et comme intermédiaires normaux dans le métabolisme intermédiaire. Le xylitol semble remplir ces critères encore que des divergences d'opinion existent à propos de l'effet d'accroissement de l'insuline par le xylitol. Il faudrait également étudier soigneusement l'effet du xylitol sur la flore intestinale. Il est certain que les pentitols sont plus efficaces pour la réduction de la carie que les hexitols.

7. Le xylitol et autres polyols agissent sur certains fongi comme des accepteurs d'hydrogène (c'est à dire que l'accepteur entraîne l'oxydation d'un métabolite). Dans beaucoup d'autres cas, l'oxygène moléculaire agit comme source usuelle d'énergie dans les bactéries. On peut aussi montrer qu'ils agissent comme accepteurs d'hydrogène dans certaines bactéries.

8. Les bactéries nées sur des polyols développent normalement des déhydrogénases provoqués et des enzymes correspondantes qui permettent l'exploitation de ces sucres comme cource d'énergie. Cette adaptation bactérienne est une conséquence normale de la présence de polyols dans le milieu de croissance. En effet, l'adaptation bactérienne et l'induction enzymaire sont les conditions d'une vie évolutive des cellules. En conséquence, l'adaptation bactérienne à l'égard des polyols dans la cavité buccale n'est pas un désavantage parce que les polyols (et en particulier le xylitol) ne facilitent pas le développement de la plaque aussi sûrement que le sucrose, le glucose ou le fructose.

9. On a fait plusieurs découvertes intéressantes qui seront exploitées dans l'avenir, par exemple, un effet léthal du fructose sur certaine colonie de micro organismes buccaux, l'utilisation de l'acide lactique in vivo dans la plaque par certaines bactéries, le catabolisme

des polysaccharides de la plaque qui donne naissance à des sucres cariogènes, la formation inductive d'invertase de la plaque au cours d'un régime riche en sucrose, la production d'endotoxines bactériennes dans la plaque, la proposition d'une nature plus cariogène des levans par rapport aux dextrans, l'utilisation du phosphore de la dent par les streptocoques cariogènes, etc . . .

DIE BEDEUTUNG VON SACCHAROSE UND ANDEREN ZUCKERN FÜR DIE KARIESENTWICKLUNG EINE ÜBERSICHT

ZUSAMMENFASSUNG

1. Es ist möglich, viele Mikroorganismen der Mundhöhle als kariogen zu betrachten, obwohl evident ist, daß einige Arten oder Stämme auf bestimmte Zahnflächen kariogener wirken als andere. In jedem Fall kann die Karies als eine Mischinfektion betrachtet werden.

2. Irgendwie unterschiedliche und sogar widersprüchliche Ergebnisse wurden in Tierversuchen erzielt. Dies ist sehr wahrscheinlich den Schwierigkeiten in der Normung der Methoden zuzuschreiben. Es ist offensichtlich, daß bei Versuchen mit Menschen die Schwierigkeiten sogar noch stärker hervortreten.

3. Es kann als bewiesen gelten, daß Saccharose unter den verbreiteten Zuckern die größere kariogene Wirkung aufweist. Die Kariogenität steht offensichtlich meist mit der ziemlich hohen Hydrolyseenergie der atomaren Bindung zwischen den Monosaccharidenteilen im Zusammenhang. In dieser Hinsicht nimmt die Saccharose eine besondere Stellung unter den Disacchariden ein.

4. Der Energiegehalt der Saccharose wird von Mikroorganismen der Mundhöhle dazu verwendet, u.a. hydrophile Polysaccharide zu synthetisieren, die wahrscheinlich die Masse des extrazellulären Anteils der Zahnplaque bilden. Die Polysaccharide (wie Dextrane und Levane) können sich an kristallinen Schmelzflächen anlagern und dadurch zum Teil das Wachstum und Auftreten kariogener oder anderer Bakterien fördern, Diffusionsbarrieren schaffen, als Entzündungsagens wirken und das Ausgangsmaterial für die Bildung einfacher kariogener Monosaccharide (Glukose, Fruktose usw.) und Säure liefern.

5. Obwohl die Rolle der Polysaccharide in der Kariesätiologie zur Zeit stark hervorgehoben wird, sollte ihre Bedeutung nicht überschätzt werden. Einige Polysaccharide mögen schädlicher sein als andere; im Endeffekt hängt die Wirkung auch davon ab, welche Zahnflächen befallen sind. Die bakteriellen Polysaccharide in der Plaque stellen sehr wahrscheinlich eine sehr heterogene Gruppe von Polymeren dar, die eine intensive Untersuchung verdienen.

6. Die Kariogenität einiger verbreiteter Zucker nimmt grob betrachtet in der folgenden Reihenfolge ab (aufgrund von Tier- und Menschenversuchen ermittelt) Saccharose > Glukose > Maltose, Laktose, Fruktose > Sorbitol > Xylitol. Invertzucker (Fruktose und Glukose, 1:1) stellt sich vom zahnärztlichen Standpunkt aus nicht als vorteilhaft heraus. Gewiß werden noch andere Zucker oder deren Derivate gefunden, die sich als Karies verringernd erweisen. Bevor sie jedoch akzeptiert werden, sollte man zeigen, daß sie normalerweise in unseren üblichen Nahrungsmittelprodukten und als normale Zwischenglieder im intermediären Stoffwechsel auftreten. Xylitol scheint diese Voraussetzungen zu erfüllen, wenn auch hinsichtlich der Insulin steigernden Wirkung von Xylitol gegenteilige Auffassungen vertreten werden. Die Wirkung von Xylitol auf die Darmflora sollte gründlich untersucht werden. Es ist offensichtlich, daß mit Pentitolen eine wirksamere Kariesreduktion erreicht wird als mit Hexitolen.

7. Xylitol und andere Polyole wirken auf bestimmte Pilze als Wasserstoffakzeptoren (d.h. der Akzeptor veranlaßt ein Stoffwechselprodukt zur Oxydation); in vielen anderen Fällen wirkt molekularer Sauerstoff als gewöhnliche Energiequelle der Bakterien. Man kann selbst nachweisen, daß sie auch bei einigen Bakterien als Wasserstoffakzeptoren wirken.

8. Die auf Polyolen gewachsenen Bakterien entwickeln normalerweise induzierbare Polyoldehydrogenasen und entsprechende Enzyme, die die Ausnutzung dieser Zucke als Energiequellen ermöglichen. Diese bakte elle

Anpassung ist eine normale Konsequenz beim Vorhandensein von Polyolen im Wachstumsmediam. In der Tat gehört die bakterielle Adaption und die Enzymproduktion zu den Bedingungen für das evolutionäre Zelleben. Infolgedessen bedeutet die bakterielle Adaption in bezug auf Polyol in der Mundhöhle keinen Nachteil, weil Polyole (besonders Xylitol) das Plaquewachstum nicht so wirksam fördern wie Saccharose, Glukose oder Fruktose.

9. Es liegen einige interessante neue Forschungsergebnisse vor, die in Zukunft Beachtung finden werden, z.B.: eine Fruktoseletalitätswirkung auf einen Mikroorganismenstamm der Mundhöhle, die Verwendung von Milchsäure durch bestimmte Plaquebakterien in vitro, der Katabolismus von Plaquepolysacchariden zur Erzeugung kariogener Zucker, die induzierte Bildung von Plaqueinvertase bei einer stark saccharosehaltigen Diät, die Produktion bakterieller Endotoxine in der Plaque, die vermutlich stärkere kariogene Eigenschaft von Levanen im Vergleich zu Dextranen, die Utilisation von Zahnphosphor durch kariogene Streptokokken usw.

EL PAPEL DE LA SACAROSA Y OTROS AZUCARES EN EL DESARROLLO DE LA CARIES DENTAL; REVISIÓN

RESUMEN

1. Es posible observar muchas especies de micro-organismos bucales con actividad cariogenica, si bien es evidente que algunas especies o tensiones en algunas superficies de los dientes pueden ser más cariogenicas que otras. De todas formas la enfermedad puede ser considerada como una infección mixta.

2. En los experimentos animales se han encontrado resultados diferentes y contradictorios. Lo más probable es que sea debido a la dificultad de estandarización de los metodos. Esta claro que en los experimentos humanos las dificultades seran mucho mayores.

3. Se puede considerar como demostrado que es la sacarosa es el azucar comun más cariogenico. La cariogenicidad esta en relación con el grado de energia de hidrolisis del enlace covalente entre los monosacaridos.

4. El contenido energetico de la sacarosa, es usado por los micro-organismos para sintetizar entre otras cosas, polisacaridos hidrofilo los cuales pueden formar una masa a partir del compartamento extracelular de la placa dental. Los polisacaridos (tales como las dextrosas y levalosas) pueden ser absorbidos en la superficie el esmalte, favoreciendo asi el crecimiento y actividad cariogenica de otras bacterias, creando unas barreras de difusión que actuan como agentes inflamatorios, suministrando asi el material inicial para la formación de monosacaridos cariogenicos simples (glucosa, fructosa) asi como de acidos.

5. Sie bien hoy dia se ha insistido mucho en el papel de los polisacaridos en la etiologia de la caries, su importancia no debe ser sobreestimada. Asi algunos polisacaridos pueden ser menos nocivos que otros, y a fin de cuentas el ultimo efecto depende tambien en la superficie dental que este involucrada. Los polisacaridos bacterianos en la placa representan el grupo más hetereogeneo de polimeros que merecen un estudio intensivo.

6. El grado de cariogenicidad de los azucares comunes, disminuye en el siguiente orden: (segun los estudios animales y humanos) sacarosa, glucosa, maltosa, lactosa, fructosa, sorbitol, xylitol. Los azucares invertidos (fructosa-glucosa, 1:1) no se ha visto que tengan ninguna ventaja desde el punto de vista dental. Ciertamente que otros azucares o bien sus derivados se ha encontrado que peuden ser efectivos y reducir el numero de caries. Pero antes de que esto sea aceptado, esto debe ser demostrado en los productos corrientes de uso, como intermediarios normales en el metabolismo intermediario. Parece ser que el xylitol cumple estos requisitos, si bien existen opiniones contradictorias concernientes al aumento de la insulina debido al xylitol. Por esto el efecto del xylitol en la flora intestinal debe ser estudiado. Esta claro que los pentoles son más efectives, como reductores de la caries que los hexoles.

7. El xylitol y otros polioles en ciertos hongos como receptores de hidrogeno, ocasionando la oxidacion de los metabolitos; en muchos otros casos las moleculas de oxigeno actuan como fuente de energia en las bacterias,

pudiendo tambien demostrarse como receptores de hidrogeno en algunas bacterias.

8. El crecimiento de bacterias en polioles normalmente conduce al desarrollo de dehidrogenasas y enzimas que contribuyen a explotar la energia de estos azucares. Esta adaptación bacteriana es consecuencia de la presencia de polioles en el medio de crecimiento. En efecto, la adaptación bacteriana y la inducción bacteriana son condiciones para el desarrollo e la vida celular. En consecuencia, la adaptación bacteriana con arreglo a los polioles en la cavidad bucal no es una desventaja, ya que los polioles no favorecen (particularmente el xylitol) no favorecen el crecimiento de la placa de una forma tan efectiva como la sacarosa, glucosa, o fructosa.

9. Ha habido ultimamente unos descubrimientos muy interesantes que recibiran gran atención en un futuro inminente. Por ejemplo: una fructosa tiene un efecto letal contra los micro-organismos bucales, la utilización in vivo de acido lactico por ciertas bacterias en la placa, el catabolismo de los polisacaridos de la placa y su influencia en la producción de azucares cariogénicos, la inversión en la producción de la placa durante la dieta pronunciada de sacarosa, la producción de endotoxinas bacterianas en la placa, la ya propuesta mayor actividad cariogénica de las levulosas en conparación con las dextrosas, la utilización del fosforo dentario por el estreptococo cariogenico, etc.

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Dental Caries in Rats Fed a Diet Containing Processed Cereal Foods and a Low Content of Refined Sugar

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There has been slow progress in formulating dietary regimens on which laboratory animals develop dental caries, which may be regarded in all respects as a satisfactory analog of human dental caries. The coarse particle diets of Shibata (*1*) and Hoppert, Weber, and Canniff (*2*) and the refined diets containing an excessive amount of sugar (*3–5*) produce carious lesions which occur almost exclusively on occlusal surfaces and originate, with very few exceptions, only in the deep fissures. The identity of this type of caries with human dental caries has been questioned for numerous reasons (*6, 7*). One of the most disconcerting facts of such experimental caries is the general failure of caries to develop on buccal, lingual, or proximal surfaces, whereas these surfaces in human dentition are highly susceptible to caries. During the course of our present studies, however, Stephan (*8*) reported that low-fat, high-sugar diets, consisting chiefly of skimmed milk powder and cane sugar supplemented with cod liver oil, induced buccal and lingual surface caries as well as proximal and occlusal surface caries in white rats. It has been observed also that the Syrian hamster may develop a cervical type of surface caries in addition to buccal caries, on a finely powdered diet consisting chiefly of powdered corn meal and whole milk powder (*9*) and also on a diet of whole wheat flour, cornstarch, confectionary sugar, whole powdered milk, and alfalfa (*10*). In one other species (the cotton rat), caries is produced under a number of dietary regimens but presumably occurs only "deep in the occlusal fissures of the molars" (*11*).

The cariogenic properties of the following basal diet (586) are being studied:

Ingredients	Percentage
Cereloso (commercial glucose)	18.0
Enriched rye bread (dry)	25.0
Enriched white bread (dry)	25.0
Cooked rolled oats (dry)	15.0
Cooked yellow corn grits (dry)	15.0
Sodium chloride	2.0

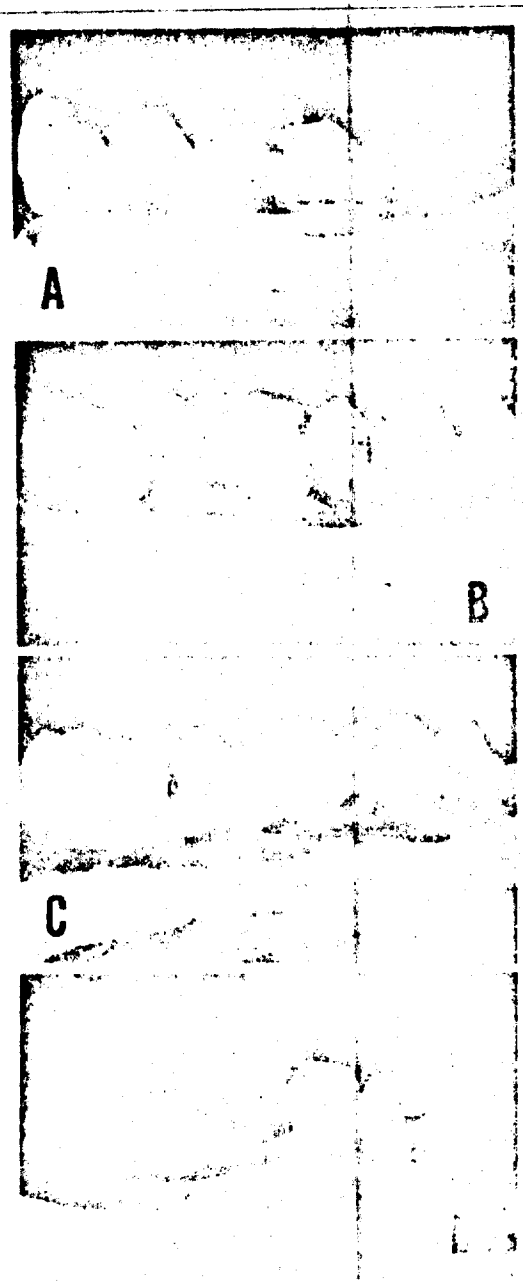

FIG. 1. *A*, lower buccal surfaces showing "orange peel" appearance of extensive white opaque area. At this stage, enamel will crumble easily on probing. Initial white areas may be smaller and more sharply defined. *B*, enamel is destroyed, exposing areas of dentin. *C*, further destruction on interproximal and distal areas. *D*, caries below gingival line and extensive destruction of occlusal areas.

The rolled oats and corn grits were cooked about 1 hr, dried at 100° C, and ground to a fine powder. The bread was dried at 100° C for a period of approximately 8 hr to a golden-brown color and ground fine. Vitamin B concentrates were added to provide thiamine 2.0 ppm, riboflavin 3.0 ppm, pyridoxine 2.5 ppm, calcium pantothenate 20.0 ppm, choline hydrochloride 1000 ppm, and inositol 25.0 ppm. Vitamin A and D concentrate (Natola) was given in separate feed cups

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(or directly by mouth) twice weekly to provide approximately 4000 units of A and 800 units of D weekly. The rats were housed, usually in pairs, in screen-bottomed cages, and the diet was started at weaning. In other experiments rats were given the diet from birth, and were on a sawdust surface prior to weaning. In this case the litters remained intact until the end of the experiment. All the rats were of the Holtzman strain and were obtained from the stock colony at the National Institutes of Health. This colony is maintained on a stock diet consisting of Purina lab chow and greens given twice weekly. Bread and milk are given daily during the first 2 weeks of life. The rats were sacrificed after about 90 days on the diet, the heads were autoclaved, and the flesh was removed. Prior to examining the teeth, the maxillae and mandibles (teeth *in situ*) were kept under water and in a refrigerator. The teeth were allowed to dry while being examined for caries under low power magnification. During this slow drying the initial white opaque areas, a characteristic effect of this diet, appeared easier to detect.

The gross appearance of the carious lesions produced by feeding Diet 586 is shown in Figs. 1 and 2. Initially, the carious process appeared as an opaque elongated area in the enamel, which progressed through various stages as shown in the photographs to an involvement of the dentin and loss of the entire tooth. The carious areas most frequently follow the gingival outline. Caries occurred mostly on buccal surfaces of lower molars, but was not limited to lower teeth or to buccal surfaces entirely. Lingual and mesial surfaces were sometimes involved. Occlusal caries was present but, unlike the occlusal caries previously referred to (*1–5*), did not appear to originate consistently deep in the sulci of the tooth.

Diet 586, which appears instrumental in producing this type of caries, is deficient in a number of dietary essentials. Growth is much below normal, and mortality may be high, particularly if the diet is started at birth. It should be noted, however, that this diet does not involve a coarse particle factor and that the content of sugar (18%) is relatively low. The obvious inadequacy of the cereal protein in the diet, particularly the lysine content, and the fact that the cereals have been heat-processed, have suggested a somewhat new approach to problems of nutrition in relation to dental caries. Much information has accumulated concerning the effects of heat-processing and storage on the nutritive properties, particularly the protein value, of foods (*13–15*). These effects are being investigated specifically in connection with the caries potential of Diet 586. The results with the diet are of special interest also because the initial appearance and general development of the carious process and the involvement of the lingual, buccal, and proximal molar tooth surfaces are strikingly similar to human dental caries.

Our past experiences in encountering unexplained variations in caries production among groups of rats have been duplicated in our current studies with Diet 586. These variations relate both to the number of animals in a group which will develop caries, as well as the number of teeth involved and the extent of the carious process in each individual tooth. Under seemingly identical conditions, groups of rats given Diet 586 may show a disconcerting variability in caries production. However, over a period of 2 years, and in studies on approximately 500 rats, the above diet has continued to produce caries of the type described in relatively large numbers of animals.

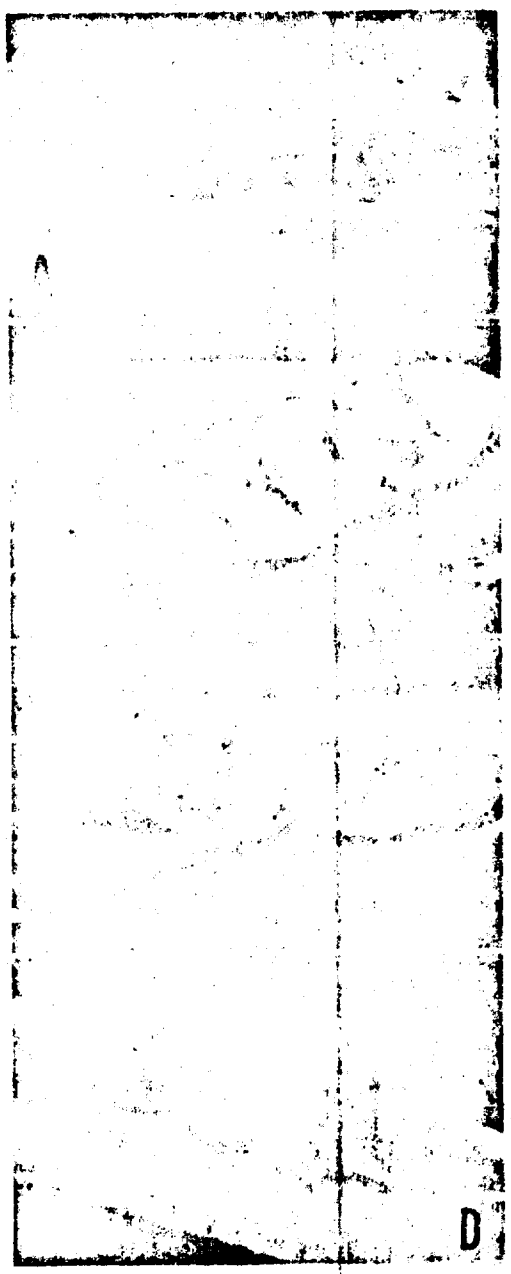

FIG. 2. *A*, upper buccal surfaces showing typical carious areas; *B*, destruction in mesial, interproximal and occlusal areas of lower molars; *C*, advanced caries in first and second lower molars; *D*, complete loss of second lower molar, advanced caries in first and second lower molars.

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Millican-'64
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Are Candy Sales in Schools Justified?

CARLENE MILLICAN

To the question "Are candy sales in schools justified?" another question may be asked: "Will the elimination of candy sales in schools effectively reduce the problem of dental caries?

The answer, I believe, depends upon the assumption you support. If your thesis is that tooth decay is the most prevalent chronic disease in the United States, and since it has been demonstrated that the restricted use of refined carbohydrates—especially sugar and sugar products—will reduce the prevalence of tooth decay, then there is a justifiable reason for banning candy sales in schools. However, your thesis may be that the taste for sweet is the most highly developed taste in the infant, that cultural factors are important determinants in influencing the child's food choices, and that, regardless of whether schools do or do not sell candy, children are conditioned to candy and will have it.

Support continues for the latter thesis, unfortunately. It is documented as contributing a sizeable source of revenue to schools for financing such worthwhile projects as educational trips for students and purchase of athletic equipment, band uniforms, and library books. This has occurred despite the action of professional and community groups which have taken a stand against the sale of soft drinks and candy in schools. Such action has taken the form of resolutions or other official statements indicating the grounds on which the organization takes exception to the practice of selling soft drinks and concentrated sweets on school premises, particularly in the school lunchroom.

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The Council on Foods and Nutrition of the American Medical Association takes the position that the purpose of the school lunch is to provide training in good food habits and that the serving of sweets and sweetened beverages does not promote that purpose. The Council statement concludes: "Expenditure for carbonated beverages yields a nutritional return much inferior to that from a similar sum spent for milk or other staple foodstuff. Furthermore, when given a choice between carbonated beverages and milk to accompany a meal, a child may frequently choose the less nutritious beverage. In view of these facts the Council believes that carbonated beverages should not be sold on school premises."

The American Dental Association has for many years warned against the hazards to dental health from consumption of sugar. The Association's house of delegates passed a resolution in September 1953 recommending that dental societies call to the attention of school administrators the need for eliminating from the schools the sale of sweetened beverages and confections.

The State of Washington has taken the lead among the states in urging the ban on sale of soft drinks and confections in schools. In a policy statement from the offices of the State Department of Health and the Superintendent of Public Instruction, the Joint Committee on School Health recommended to school administrators and county and city health officers that: "The sale of carbonated beverages, candy, and other sweets be eliminated entirely, inasmuch as scientific evidence shows that: (a) they often replace the basic seven foods necessary for normal growth and development; (b) they are important contributing factors to dental caries."

The New York State Education Department has issued a similar policy statement to district, city and village school superintendents and supervising principals calling attention to "the relationship between the consumption of candy and soft drinks and good nutrition and the relationship of both to the control of dental caries . . . .Since dental caries among school children outranks all other defects, consumption of sweets presents a serious related problem. The protection of children against the sale of candy and sugared soft drinks is an important aspect of this problem."

The American Dietetic Association has also gone on record in support of the above recommendations. In addition, it declines to accept advertising in its official journal or exhibits at its annual meeting from companies selling candy or soft drinks.

The major question with regard to candy sales is: Are we relying on a hand extinguisher to put out a fire, when, in reality, the fire department should be called? To put the question another way, should we not get out our bifocals and take a closer look at the child consumer of today, his environment, and some of the cultural factors which influence his food choices?

The social scientists—the "people watchers"—supply us with bifocals. They report some very interesting observations on cultural changes in the United States and their probable effects on children. One such factor is the vending machine, which during the past 15 years has shown more than a 400 percent increase in volume of business. In 1946, the total volume vended (which does include items other than food) amounted to $600,000,000; in 1960, the amount had grown

(*Continued on page 65*)

Candy Sales in Schools?

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to $2,586,000,000. One effect of the vending machine, as well as all types of readily available service, is that they encourage piecemeal eating. Coffee breaks and snacks have become part of our life. With the aid of the refrigerator, the vending machine, and other newer gadgets, we are becoming like the simpler, less organized societies in this custom of frequent, unplanned eating.

Children, it seems, are growing up with a new tradition: the family doesn't just stop for gas—it stops for gas and pop. A person doesn't just bowl or skate—he bowls and has a cup of coffee or eats a hot dog. People now eat and drink when they attend sports events, the theater, concerts, and at home while being entertained by TV. People eat and shop in what were formerly variety stores, apothecary shops, and ladies' apparel shops; people even find time to eat or drink while doing the wash at the corner laundromat.

The vending machine represents impersonal feeding. It may not always suit our food preferences, but we like the convenience of it. Stokers of vending machines cannot afford to cater to the gourmets and a few nationality groups by serving pumpernickel sandwiches or pepperoni sausage. Choices must be confined to the few most generally acceptable foods and beverages. Also, the machine is not so well adapted to serving such items as soufflés, baked potatoes, and carrot sticks, but it can serve either relatively dry things like nuts, chewing gum, candy, cookies or else fluids or semi-fluids like soda pop, fruit drinks, coffee, and soft ice cream.

Another cultural trend is the tendency to remove farther and farther from the consumer's experience the whole complex business of food production, processing, and marketing. City children, and many farm children as well, have no experience of the labor and organization required to bring food to them. Hence they do not appreciate the intrinsic value of food unless they happen to have been genuinely deprived and hungry, either physically or psychologically. Today, we need do very little work in food preparation; we do not need to wait for a food product while it goes through a series of processes, nor need we be confined to one food for long periods. One wonders, then, whether a child can readily understand or sense that any specific food, or nutrient, is vital to him, if he does not feel, emotionally, that food is precious.

Dentists, dental hygienists, and nutritionists, as well as other health workers, are united in their teaching that children should not only eat less sweets, but eat sweets less frequently and that they should not consume sweets to the exclusion of more protective foods. The question is, how do we go about the education and influencing of the child on the subject of eating?

One sure way of eliminating the sweet goodies would be control over the source, but no one is so naive. The reformers would have to "take on" the soft drink, confectionery, and baking industries, and the first two alone are reported to represent a $2 billion wholesale business. Then there is the advertising business—a $10 billion enterprise—which would have to change the content of its advertising. Also, there is retail merchandising, only one of which is the automatic vending industry, and that already amounts to more than $2½ billion. To accomplish any significant change in dietary practices at the merchandising sources, the educators would have to tackle all these giants.

Banning the sale of candy in schools would contribute in some degree toward a reduction in dental caries. The effectiveness of this method, however, in altering either the rate or extent of dental caries to a significant degree is in my opinion a moot question.

What, then, is the alternative? It is to teach, to educate, the most vulnerable unit—the individual child. Ultimately, the burden rests on the consumer to make the necessary choice. It is his choice which affects the producer. Children must learn to understand and deal with the many inducements to buying food and drink.

A recent study of preschool children shows how much children influence the foods selected for purchase: 91 percent of mothers reporting stated that their children requested the purchase of certain food items regardless of whether or not the child accompanied the parents on shopping trips. Preschool children most commonly requested that cereal be purchased. Seventy percent of the children either asked select the cereal at the store or asked their parents to buy a certain cereal. Candy ranked as the second most popular item requested with fruits and cookies both ranking third. Among other foods requested were crackers, potato chips, milk, milk-flavoring, sweets, and desserts.

The mothers were asked what they thought prompted their child's request for the food item he asked to be purchased. About three-fourths of the mothers indicated that their children asked them to buy products which they had seen advertised on TV. Free gifts, prizes, puzzles, and the like were felt to be contributing factors in influencing the child's food requests.

It is questionable whether the banning of candy sales in schools will accomplish, on a long-term basis, a measurable or significant reduction in the prevalence of dental decay in children. Cultural factors are important determinants in influencing the food choices of children. Generally speaking, children are exposed and conditioned to a preference for candy and concentrated sweets long before entering school. Whether schools sell candy or not, many children will have it. However, children should be educated in the essentials of nutrition so that they will be able to make wise choices in their selection of food.

It would seem that the thesis is self-evident: every child has the right to know what to eat and why, and how it affects his health. Since nutrition is an essential element of health, nutrition education should be a definite and planned part of the total school health program. The first essential of success in any school undertaking is the support and understanding of the administrators. The effectiveness of the program obviously increases as it becomes integrated into an all-school activity, with each member of the staff carrying his share of the responsibility.

Schools, as well as parents, share a major responsibility for fitting the child for society and helping him to be responsible for himself and his health. The extent to which both parents and the school recognize the role and importance of nutrition and dental health education in equipping the child for society and its culture is, to me, the critical issue at hand. When this issue can be resolved, we shall certainly not need to be concerned with whether candy sales in schools are justified. ■

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The Influence of Sugar Content and pH on *In Vivo* Decalcification of Rat Molar Teeth by Acid Beverages

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Introduction

It has been previously reported (1,2,3,4,5) that a wide variety of acid beverages are capable of grossly eroding the molar teeth of white rats *in vivo*. Using a cola-type beverage containing 10% sucrose and 0.055% phosphoric acid, having a pH of about 2.6, Restarski and coworkers (2) observed that marked destruction of the lingual enamel resulted when rats consumed 20 ml. of this beverage daily for one week; when saccharin replaced the sucrose, the extent of destruction was considerably less even though the pH of the solution remained essentially the same. The conclusion was reached that sucrose somehow augmented the decalcifying porperties of the phosphoric acid. Further studies (3) indicated that glucose and maltose behaved as did sucrose, whereas lactose had no such effect. With acids other than phosphoric (*i.e.*, lactic, citric, sulfuric) no difference in the extent of molar damage was noted (3) whether sucrose of saccharin was used as the sweetening agent. Accordingly, the present research was undertaken to ascertain the mechanism by which certain sugars apparently increased the tooth-destructive properties of phosphoric acid solutions.

Procedure

Animals

Young albino rats (Sherman strain) 7–8 weeks of age and weighing 150 g. or more were used in all experiments. They were grouped comparably according to sex and litter and kept in separate cages so that the intake of fluid could be accurately controlled and recorded. All rats received commercial dog feed pellets *ad libitum* and 20 ml. daily of the appropriate test solution.

At the end of each 7-day experimental period the animals were sacrificed, their jaws removed, and the teeth examined and graded as previously described (6). According to this system of assessing tooth destruction, the individual upper and lower molars were given scores ranging from 0 (normal) to 6 (very severe destruction), depending on the extent of damage to the enamel and dentin.

Solutions

The concentrations of the sugars and the phosphoric acids in solutions fed to rats were the same as those previously employed by Gortner *et al.* (3), *i.e.*, 10% sugar and 0.055% phosphoric acid. In the sugar-free acid solutions, except in the initial experiment, 37.5 mg.-% of pure saccharin was added to make the solutions comparable in sweetness to the sucrose solution so that the rats would drink the full 20 ml. of solution given to them daily. When glucose or lactose was substituted for sucrose, saccharin was also added at the lower levels of 6.0 and 31.5 mg.-%, respectively.

In experiments concerning the *in vivo* decalcifying effects of phosphoric or lactic acid solutions of different pH, varying amounts of approximately 0.1 *N* NaOH were added by burette to 0.055% phosphoric acid-saccharin or 0.45% lactic acid-saccharin solutions to bring the initial pH (2.5–2.6) up to the desired level, as determined with a Beckman pH meter.

Glass drinking tubes having a J shape were used exclusively; by nature of their construction they permit maximum consumption of the solution with a negligible loss due to spillage and evaporation.

Results and Discussion

To check earlier observations (2), an initial experiment was performed on the relative decalcifying effects of phosphoric acid-sucrose and phosphoric acid-saccharin solutions. To our surprise, typical etching of the molars resulted from the sugar-acid solution, but *no effect whatever* was noted with the saccharin-acid solution. On checking the pH of the two solutions, the former was found to be 2.5–2.6, while the latter was in excess of pH 4. The saccharin used in this experiment was a commercial type sold in tablet form; obviously something in the saccharin preparation had partially neutralized the acid and thus prevented tooth decalcification. This buffer substance proved to be sodium bicarbonate. The earlier studies of Restarski *et al.* (2) had also been carried out with a commercial saccharin preparation, but these workers had reported no significant change in the pH of their saccharin-containing solutions. Nevertheless, it appeared probable that an interfering substance of this type was responsible for the results which they observed. Likewise, the observation (3) that lactose behaved differently from sucrose, glucose, and maltose, might be explained on

the basis that a considerable amount of the saccharin preparation was added to the lactose solution to make it comparable in sweetness to the sucrose solution.

In all of our later studies chemically pure saccharin was used. Table I summarizes the results obtained when phosphoric acid solutions containing sucrose, glucose, lactose, and/or c.p. saccharin were consumed by rats for one week. It is clearly evident that all of the sugars behave similarly and that none of them has any significant influence on the extent of tooth decalcification by the acid.

TABLE I

In Vivo Decalcifying Effects of Phosphoric Acid-Sugar and Phosphoric Acid-Saccharin Solutions at pH 2.5–2.6 on Rat Molar Teeth

Solution	No. of rats	Average molar scores		
		Lower jaw	Upper jaw	Whole head
Acid-sucrose	13	3.7	2.7	3.2
Acid-lactose	6	3.6	2.8	3.2
Acid-glucose	4	3.8	3.0	3.4
Acid-saccharin	10	3.2	2.9	3.1
Sucrose (controls)	6	0.0	0.0	0.0

Two questions still presented themselves: (a) How can these results be reconciled with those of Restarski and coworkers (2), who noted no appreciable change in pH of their saccharin-containing solutions? (b) Why should the buffered saccharin tablets counter decalcification by phosphoric acid solutions but not that by other acids such as lactic and citric? The answers to both questions clearly lie in the titration curves of the various acids. Fig. 1 shows such curves obtained with phosphoric and lactic acids. It can be seen that with the former acid in the pH range of 2.3–3.0 there is little change in pH with a relatively large change in titratable acidity. Above pH 3.0, however, there is a large change in pH with only a small change in titratable acidity. As much sodium hydroxide is required to change the pH from 2.3 to 2.7 as is needed to change the pH from 2.7 to 6.2. Thus, although the saccharin preparation used by Restarski *et al.* (2) did not contribute enough buffer to materially increase the pH of their phosphoric acid solutions, it did apparently decrease the titratable acidity of such solutions to the

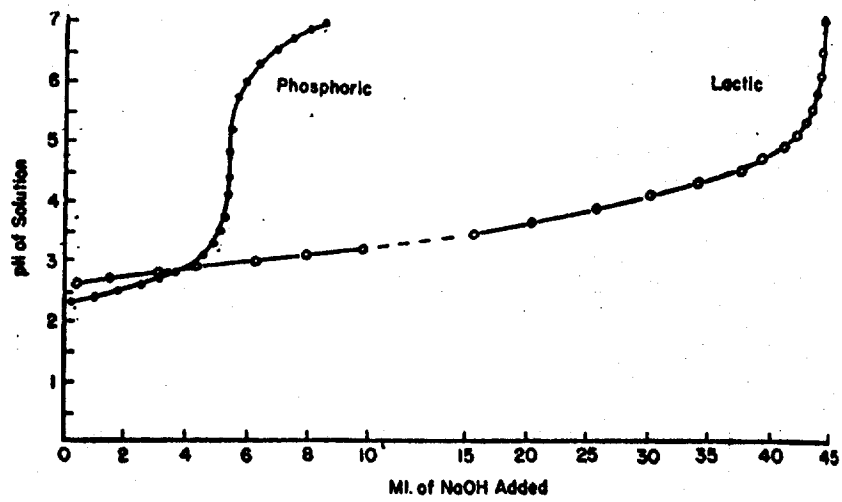

FIG. 1. Titration curves of phosphoric and lactic acids, showing the changes in pH accompanying the titration of (a) 50 ml. of 0.11% phosphoric acid and (b) 100 ml. of 0.45% lactic acid with 0.0987 *N* sodium hydroxide.

point where they no longer caused as severe tooth decalcification *in vivo*.

Lactic acid, being a weaker acid, requires a much greater concentration than phosphoric acid to give a solution of pH 2.5–2.6; consequently, far more base is needed to cause neutralization. The titration curve of lactic acid (Fig. 1) shows a gradual rise up to about pH 4.5, followed by a more rapid rise to the equivalence point, which is at a higher pH than that of phosphoric acid. It can be seen, therefore, that the small amount of buffer contained in commercial saccharin tablets would not appreciably modify the pH or titratable acidity of 0.45% lactic acid solutions. Thus, no differences in molar decalcification would be noted between rats receiving lactic acid-sucrose on the one hand, and lactic acid-commercial saccharin on the other.

On the basis of these titration curves, one would predict that, *in vivo*, a rapid decrease in enamel destruction would occur as the phosphoric acid solutions approached pH 3.0 and as the lactic acid solutions approached pH 4.5. This has been confirmed by feeding to rats phosphoric and lactic acid solutions of known pH, ranging from pH 2.5 to pH 4.5. The results, shown graphically in Fig. 2, demonstrate conclusively that the *titratable acidity* of the solution consumed, rather

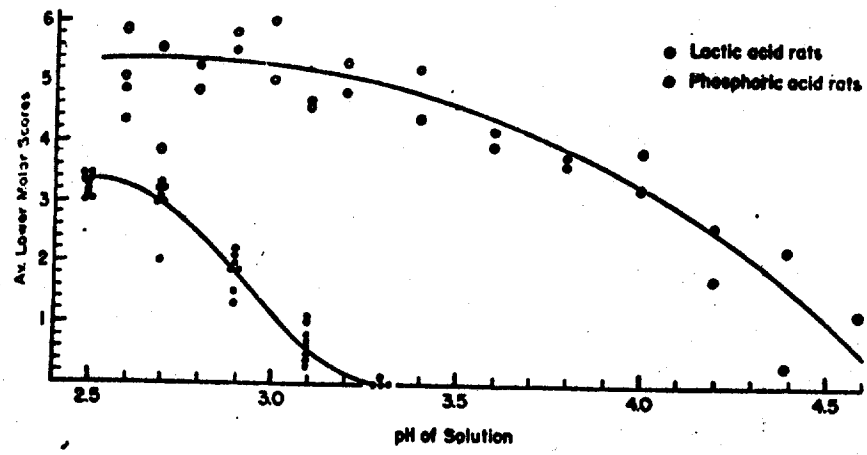

FIG. 2. The decalcifying effects of lactic acid and phosphoric acid solutions, containing saccharin, on the lower molars of albino rats. Each dot represents the average lower molar score for one rat.

than its pH, is the more important consideration in tooth decalcification. It is the final pH of the solution *in the mouth*, rather than the pH of the solution ingested, which determines the extent of enamel destruction; this, in turn, is a function of the buffering capacity of the saliva and of the properties characteristic of the acid under observation.

SUMMARY

1. The addition of 10% of sucrose, lactose, or glucose to a 0.055% phosphoric acid solution does not significantly alter the extent of decalcification, *in vivo*, of rat molars above or below that caused by a 0.055% phosphoric acid-saccharin solution.

2. The extent of enamel dissolution caused by phosphoric acid solutions *in vivo* drops rapidly when the pH of such solutions exceeds 2.7. At pH 3.3 no observable decalcification occurs during a period of one week.

3. The extent of *in vivo* tooth decalcification caused by lactic acid solutions remains fairly constant with solutions up to about pH 3.4, then decreases gradually until, at pH 4.6, enamel erosion is essentially absent.

4. The degree to which acid solutions attack the teeth is a function not of the pH of the solutions ingested, but of the titratable acidity of such solutions and the buffering capacity of the saliva.

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Certain cariogenic strains of microorganisms possess the ability to synthesize extracellular levans and dextrans from sucrose. Evidence to support the hypothesis that this is the most important property determining cariogenicity is presented.

Sucrose, the Arch Criminal of Dental Caries

Ernest Newbrun, D.M.D., Ph.D.*

The dental literature is replete with studies relating caries prevalence to human dietary habits. Because of the difficulty of establishing and enforcing scientifically acceptable control and experimental groups of human volunteers on a dietary regimen for any extended period, most studies have relied on information obtained from the patient's dietary history.[1-5] The reliability and accuracy of such a procedure of measuring the contribution of diet to the caries prevalence is open to question. Fortunately there have been a few studies such as at Vipeholm and Hopewood House where the human diet has been carefully controlled.[6-9] These latter have established clearly that the absence of sucrose *per se* or in the form of cakes, cookies and candies can limit the caries to a fraction of what occurs in the general population. The Vipeholm investigation has shown further that deliberate addition of sucrose to the food or drinks or as chocolate, caramel, or toffee can increase caries activity, especially if consumed between meals. The epidemiological observations of Hardwick, Toverud and Sognnaes provide further circumstantial evidence showing a correlation between sucrose consumption and human caries prevalence.[10,11,12]

The clinical studies noted above have been reviewed elsewhere and shall not be considered further.[13,14] There has emerged recently a considerable body of laboratory evidence specifically implicating sucrose rather than any of the other carbohydrates as the "arch criminal" of dental caries. These findings will be discussed in light of the hypothesis that it is only sucrose which serves as a substrate for extracellular polysaccharide synthesis by cariogenic microorganisms. Extracellular polysaccharides function in a dual role as a structural matrix of the dental plaque, and as a reservoir of substrate for the plaque organ-

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isms in between meals. Two case reports of patients suffering from hereditary fructose intolerance will be presented to further substantiate this hypothesis.

Biochemical Evidence

It has been known for a long time that growing cultures of certain organisms (*Leuconostoc mesenteroides, Leuconostoc dextranicum, Streptococcus bovis* and *Betabacterium vermiforme*) can synthesize dextrans, polymers of glucose. Other organisms (*Bacillus megaterium, Streptococcus Salivarius, Aerobacter levanicum, Bacillus subtilis, Bacillus polymyxa* and *Pseudomonas prunicola*) form levans, polymers of fructose (Fig. 1). Biosynthesis of these polysaccharides occurs by the agency of extracellular enzymes which show a high specificity for sucrose (and sometimes raffinose) as a substrate. Unlike intracellular bacterial, mammalian or plant polysaccharide synthesis which involves sugar-1-phosphate or nucleoside diphosphate-sugar intermediates, this extracellular synthesis appears to transfer glucose or fructose units directly to the growing polymer. The precise mechanism involved is not yet known. Somehow the enzyme conserves the relatively high energy of the link between the two anomeric carbons, C1 of glucose and C2 of fructose, found in sucrose (Figure 2). Disaccharides, other than sucrose such as maltose and lactose, are hemiacetals not dihemiacetals. Therefore their free energy of hydrolysis is significantly less (Table 1) and they cannot serve directly as glycosyl donors in this system. Sucrose on the other hand has a free energy of hydrolysis of -6,600 cal/mole which is in the same range as the nucleoside diphosphate sugars, -7,600 cal/mole and higher than glucose-1-phosphate.[15]

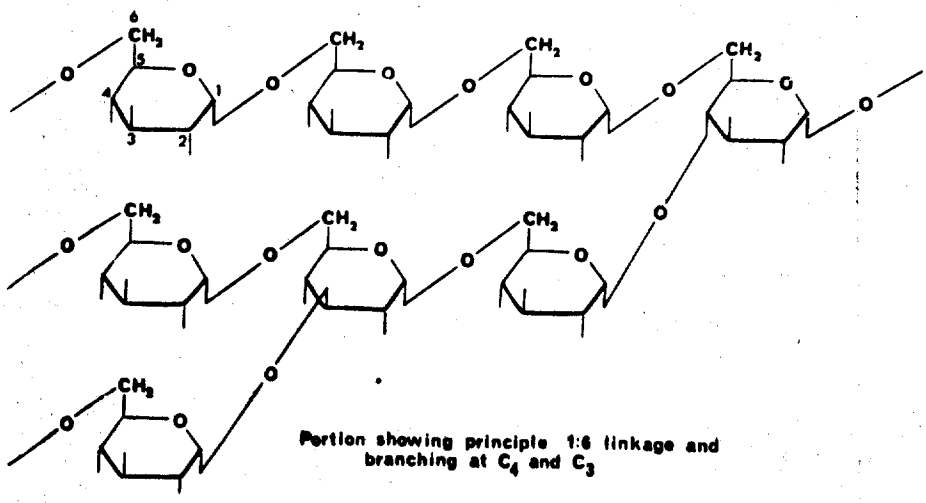
The enzymes involved in the synthesis, dextransucrase and levansucrase or more correctly sucrose 6-glucosyl-transferase and sucrose 6-fructosyltransferase have been partially purified from some of the organisms mentioned.[16,17] They are highly specific for the glycosyl donor which must have a fructofuranosidic group linked to the anomeric Carbon (i.e. potentially aldehydic carbon) of an aldose, e.g. in sucrose, raffinose. Compounds with the same group linked to a carbinol carbon (i.e. one with a hydroxyl group) proved ineffective glycosyl donors.

Microbiological Evidence

Most of the bacteria referred to in the previous section, with the exception of *Streptococcus salivarius*, are not normal inhabitants of the mouth. However, recent studies on rodent and human cariogenic streptococci have reported a gelatinous or slimy emanation, presumably polysaccharide, around the periphery of the colony when grown on solid medium. Large amounts of polysaccharide surround the colony if grown on mitis salivarius agar containing sucrose.[18,19] In liquid culture the amount of nondialysable extracellular carbohydrate formed by cariogenic streptococci has been measured. Significantly more polysaccharide was formed when the medium contained sucrose, than an equivalent amount of glucose, maltose, lactose or fructose or glucose and fructose combined.[20,21] The polysaccharides formed by the oral streptococci have not as yet been well characterized. Most of the streptococcal strains studied so far

form a dextran which on hydrolysis yields mostly glucose and only minor amounts of fructose; at least one cariogenic streptococcal strain, however, produces extracellular levan.[21,22] *Streptococcus salivarius*

BACTERIAL DEXTRAN



BACTERIAL LEVAN

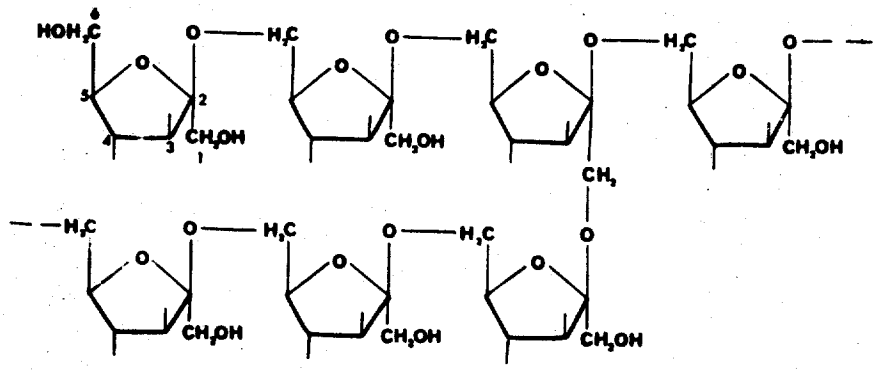


Figure 1. Chemical structure of a portion of the bacterial dextran and levan molecules. Hydroxyl groups are represented by a straight line and hydrogens have been omitted. The dextran is a homologous polymer of D-glucopyranose with predominantly L—(1→6) linkages. The levan is a homologous polymer of D-fructofuranose with predominantly B—(2→6) linkages. The frequency of branching and overall size of these polysaccharides varies with the organism from which it is obtained.

synthesizes a levan of extremely high molecular weight, approximately 25 x 10^6, which although soluble could diffuse only slowly from the dental plaque.[23]

Sucrose has been incriminated further in the caries process by some important animal studies.[24,25] When hamsters were fed lab chow and infected with streptomycin resistant caries-inducing streptococci only a few of these organisms could be recovered from their mouths after 3 weeks. On the other hand, recovery of the "labelled" organisms from hamsters maintained on a sucrose containing diet, was consistently high. If sucrose was replaced by glucose in the cariogenic diet very few of the implanted streptococci were recovered. An extension of these studies has demonstrated that frequent chewing of sucrose favours the implantation of cariogenic-streptococci in the human oral cavity.[26]

Evidence from Animal Caries Studies

As in the case of human dietary studies there have been a multitude of animal experiments concerning the relation of diet to caries development. Only a few specifically concerned with sucrose will be considered here. Not only has the diet and the rodent species tested varied but also these observations represent the efforts of independent laboratories.[14,25, 27-32] In all cases the conclusion has been the same: a sucrose containing diet is markedly more cariogenic than a similar diet in which sucrose has been substituted by glucose, lactose, maltose, maize, raw wheat starch or flour.

In a comparison of a wide range of human foods fed to rats in addition to their base diet, sucrose gave by far the highest caries score.[33]

It should be pointed out also that all gnotobiotic experiments to date which have been able to induce caries in monoinfected animals have included sucrose in the diet or the drinking water.[34]

Evidence from Studies on Plaque Formation

There can be little doubt that the dental plaque plays an integral role

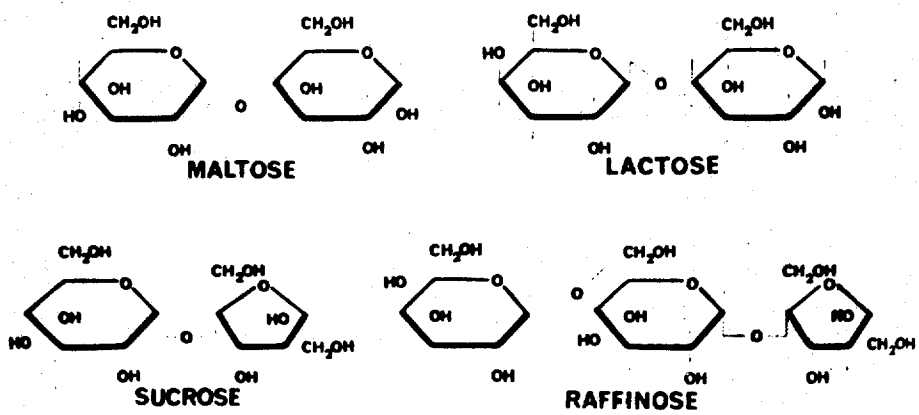


Figure 2. Chemical structure of maltose, lactose, sucrose and raffinose. The potentially high energy-dihemiacetal linkages between the aldehyde and ketone carbons of glucose and fructose respectively, in sucrose and raffinose are shown by stippling. In maltose, lactose, and between glucose and galactose in raffinose the glycosidic link is between an aldehyde and hydroxyl carbon and is of much lower energy.

TABLE 1
Energies of hydrolysis of glycosyl donors and some polysaccharides[15]

Compound	Standard free energy of hydrolysis $\Delta F H$ (cal/mole)
Glucose — 1 — phosphate	—5000
UDP — glucose	—7600
Sucrose	—6600
Maltose	—3000
Lactose	—3000
Glycogen	—4300
Dextran	—2000
Levan	—4600

in the carious process. Both *in vitro* and *in vivo* experiments on plaque formation confirm the hypothesis that sucrose is the specific substrate for extracellular polysaccharide biosynthesis by plaque microorganisms. Using an artificial mouth system it has been possible to produce heavy plaque growth *in vitro* with certain cariogenic strains of oral microorganisms.[35] Those strains capable of plaque formation did so only when sucrose was provided in the system. No plaque growth was observed with the carbohydrates glucose, fructose, galactose, lactose, sorbitol or a mixture of glucose and fructose. Similar findings have been reported for *in vivo* plaque growth although obviously the flora can not be controlled in such cases.[36] Patients on a soft protein-fat diet developed a thin structureless plaque on their teeth after a few days, with little increase in amount during the course of a week. When this diet was supplemented with frequent portions of glucose or fructose there was no appreciable difference in amount or appearance of the plaque. However, if sucrose was ingested at half-hourly intervals, a striking difference in the appearance of the plaque became noticeable. It attained a considerable size and grew to form a voluminous, turgid mass.

Hereditary Fructose Intolerance

Hereditary fructose intolerance (H.F.I.) is an uncommon metabolic disorder believed to be due to an absence of hepatic fructose-1-phosphate aldolase. It is characterized by symptoms of nausea, vomiting, malaise, excessive sweating, tremor, coma and convulsions following the ingestion of foods containing fructose.[37] Most of these symptoms can be attributed to a secondary hypoglycemia that occurs after fructose ingestion. Fructosemia and fructosuria are found following a fructose tolerance test.

Patients suffering from this genetic defect learn to avoid all forms of sweets, chocolates, candies, cakes, cookies, pastries and other sucrose and fructose containing foods. Of the cases described in the literature only nine provide a sufficiently detailed description of the caries prevalence to express as a DMFT score.[37-39] Others have commented on the "excellent" or "good dental health" of these patients.[40] Table II summarizes the caries prevalence of

TABLE 2

Dental Caries Experience of Patients with Hereditary Fructose Intolerance

Case	Age	Sex	D	M	F	DMFT	Comment	Reference
RKC	7	M	0	0	0	0	dark stain in groove of 2nd primary molar	
DFC	15	M	0	0	5	5	deep development grooves prophylactic fillings?	37
JC	9	F	0	0	0	0	———	
MJD	26	F	0	0	0	0	———	
JAC	18	F	0	0	4	4	———	
KE	39	F	0	0	2	2	teeth surprisingly intact	
EP	33	M	0	0	1	1	no active caries	38
P	33+	M	0	0	1	1	no active caries	
	39	M	0	0	2	2	very good dental health	39
IG	18	F	?	?	?		excellent dental health	
FS	54	F	?	?	?		good dental health	
OS	63	M	?	6	?		teeth in good condition	40
BB	50	M	?	?	?		excellent dental health	
EB	57	M	?	?	?		good dental health	
AEH	41	M	0	0	0	0	calculus present	Case 1
LR	32	F	0	0	0	0	calculus present	Case 2

these patients and of the two case reports to follow. Although there are too few for any adequate form of statistical treatment, it is readily apparent that in those few patients where any filled teeth have been observed, the score is extremely low compared to the general population in an equivalent age group. Furthermore, the number of caries free individuals, 5 out of 11, is vastly higher than in a random group of Naval recruits, 1 out of 4,887.[41] Surprisingly, several recent reviews concerning the influence of genetic factors on dental caries failed to mention H.F.I., the one genetic disease where an unequivocal effect on the caries prevalence can be demonstrated.[42-44] There is little doubt that the genetic effect can be attributed to a strict elimination of all sucrose containing foods from the diet. Glucose, galactose and lactose containing foods such as milk, dairy products, bread, potatoes, noodles and rice are well tolerated by these individuals. In contrast, siblings unaffected by H.F.I. do not show the same freedom from dental caries.

Case Reports

Case 1. A.E.H., a 41 year old Caucasian male was treated at U. C. Medical Center for high circulating triglycerides. A careful history revealed the characteristic symptoms of H.F.I. namely nausea, sweating and tremor on eating any sweets, candies, or fruits. He stated that his biggest disappointment as a child was his inability to tolerate watermelon. The patient was born at Dayton, Texas, which at that time contained 0.3-0.4 ppm fluoride in the drinking water. A dental examination revealed 28 permanent erupted teeth, caries free and with no restor-

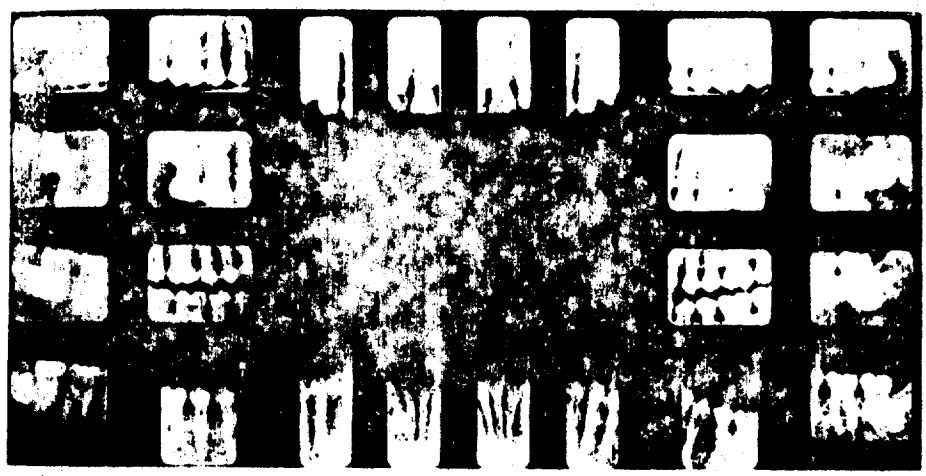


Figure 3. Full mouth X-rays of Case 1. Showing total absence of caries in 41 year old patient with hereditary fructose intolerance.

ations (Fig. 3). The four 3rd molars were unerupted and x-rays disclosed appreciable amounts of subgingival calculus.

Case 2. L.R., a 32 year old Caucasian female was the sister of the proband A.E.H. The patient had difficulty eating anything sweet as a baby and remembered frequent vomiting when fed fruits or sweet foods. In grade school she would become nauseated, weak, sweaty, and tired if she ate candy.

An examination of her dentition indicated 32 permanent teeth present, with no restorations or caries although some staining of fissures (Fig. 4). Her lower anterior teeth overlapped, and both supra and subgingival calculus could be detected.

Discussion

The prevention of dental caries presents a tremendous challenge to the dental profession. The greatest progress has been made by increasing the resistance of the tooth by systemic or topical fluoride. Successful efforts to control the local oral environment by altering the patients dietary habits have proved frustratingly elusive. Perhaps one of the reasons has been the indiscriminate attempt to limit drastically all dietary carbohydrate consumption.[45-47]

In light of the foregoing evidence a more specific elimination of sucrose or sucrose containing foods is indicated, rather than a total carbohydrate prohibition. From the point of view of enlisting the necessary patient cooperation, such a regimen is more likely to be followed, if suitable alternatives are presented to the patient with a predeliction for sweet foods. The burgeoning market in sugar substitutes, saccharin, cyclamate and sorbitol reflects some progress in this direction.[48] Another possible alternative is the development of phosphorylated sucrose, although experiments with these are sparse and their routine use would be premature.[49,50]

Certain strains of oral microorganisms have the ability to store carbohydrates as intracellular io-

dine-staining polysaccharide[51-57]. These intracellular polysaccharides are polymers of glucose linked by predominantly d 1-4 bonds as shown by their susceptibility to cleavage by d-1, 4-glucan hydrolase (d amylase).[52] They resemble the starchs of plants and the glycogen of mammals. It has been proposed that the capacity of microorganisms to form intracellular polysaccharide is an important criterion in distinguishing between potentially cariogenic and non-cariogenic strains. Organisms with intracellular polysaccharide could continue acid production after the exogenous carbohydrate supply is depleted. This hy-

Figurre 4. Full mouth X-rays of Case 2. Showing total absence of caries in 32 year old patient with hereditary fructose intolerance.

pothesis, however, does not fit in with available thermodynamic, microbiological and dietary observations. For example, it has been found that oral micro-organisms capable of synthesizing intracellular iodophilic polysaccharide do so equally well whether grown on glucose, maltose or sucrose.[55] It is not known whether sucrose is hydrolysed into its component monosaccharides prior to or after entering these bacterial cells. In either case the potentially high free energy of the dihemiacetal linkage would be lost, and the individual glucose and fructose molecules would be indistinguishable from those derived from dietary precursors other than sucrose.

The intracellular polysaccharide could not contribute, furthermore, to the structural matrix of the plaque in the same way as do the extracellular dextrans and levans. The plaque is made up in part of an acquired cuticle bound to the enamel surface and apparently derived from the saliva. The bulk of the plaque, however, consists of bacteria embedded in a homogenous matrix, which is not salivary in origin but formed by the bacteria themselves. Not only are these extracellular polysaccharides important in enabling cariogenic organisms to colonize on the enamel surface, but they also appear to play a significant role in the periodontal plaque at the gingival sulcus. An aerobic, gram-positive, filamentous bacterium, *odontomyces viscosus*, isolated from subgingival plaque, can cause periodontal disease, when inoculated into uninfected hamsters.[58] In pure culture, *odontomyces viscosus* produces an extracellular levan if grown on a medium containing sucrose or raffinose, but not with arabinose, fructose, galactose, xylose, lactose, maltose or glucose plus fructose.[59] ■

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A COMPARISON OF CRUDE AND REFINED SUGAR AND CEREALS IN THEIR ABILITY TO PRODUCE IN VITRO DECALCIFICATION OF TEETH[1, 2]

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In another paper (1) which deals with the correlation between the diet and dental caries in the South African Bantu the proposition is advanced that refined sugar and/or refined flour cause dental caries, but that these carbohydrates, as they occur in nature, are accompanied by a "protective agent" which is removed, to a greater or lesser degree, in the course of refining—that, in effect, these carbohydrates are a danger to the teeth, that nature makes them up with an antidote, and that refining removes this antidote. A series of *in vitro* experiments were carried out to test the validity of the propositions.

The authors were faced with the ubiquitous problem of how far *in vitro* results may be trusted as an index of *in vivo* processes. In the case of teeth they were more hopeful of their reliability than they would have been in the case of other organs. The fact that the enamel is laid down before the eruption of the tooth, gives added confidence in *in vitro* experiments. It is probable that there are several reasons for dental decay. It is to be expected that a severe disorganisation of the calcium and phosphorus metabolism of the individual would cause decay and that other forms of malnutrition may leave the teeth an easy victim to the forces of attack. The work of Mellanby and her collaborators has shown the importance of vitamin D in the development of teeth. It is equally certain that, even where there is no deeply hidden constitutional sabotage at work, teeth decay because

[1] Presented at the 15th Annual Meeting of the International Association for Dental Research, Baltimore, March 13-14, 1937.

[2] Most of the expense of this work was defrayed by a grant from the Research Grant Board.

they are assailed on the frontiers they expose to the food in the mouth. Both the following propositions may be put with full justification: a. that some teeth decay because they were poorly made or because they are weakened from within, b. that some teeth decay because their exposed surfaces are attacked by acid-producing bacteria. They are complementary and not contradictory. It is probable that some teeth are so weak that the most innocuous food debris will rot them; it is probable that the strongest teeth will succumb to some surface attacks; and it is probable that the two factors often work together. The authors, in concerning themselves mainly with the second proposition, do not wish to deny the validity of the first. Being concerned principally with events occurring on the surface of the tooth, they feel they have further reason to expect events *in vivo* to follow the line indicated by *in vitro* experiments.

In spite of this the authors emphatically do not advance dogmatic theories on the basis of *in vitro* results. Any speculative or tentative propositions may be advanced require the fullest possible *in vivo* testing before they can be accepted as anything more than theories.

EXPERIMENTAL

Teeth were induced to decalcify by incubation in mixtures of sugar and saliva or cereals and saliva. In the early experiments the teeth were cut into discs so that parts of the same tooth could be put into the control and experimental mixtures. The cut surfaces were sealed with cement, only the surface normally exposed in the mouth being in contact with the solution. The media were changed daily and were agitated by a stream of air bubbles. An attempt was made to discover the decalcification by measuring the tooth and by X-rays. These refinements of technique were discarded as they were found to give no advantage over the simpler technique subsequently adopted.

Undecayed extracted teeth were immersed in media obtained by chewing or rinsing the mouth with: a. normal saline, b. crude sugar-cane juice,[8] c. a 12 percent solution of refined white sugar, d. a cooked 15 percent whole cereal "porridge" (the whole meal was made by grinding the cereal grain in a coffee mill), e. a cooked 15 percent "porridge" made from best white flour or mealie meal. The strength of 15 percent was chosen for the cereals as this gave a "porridge" of a normal consistency. A pinch of common salt was added to the "porridge" because there is some idea that sodium chloride assists the amylolytic action of the ptyalin.

In each experiment the subject rinsed his mouth thoroughly with water, took a comfortable mouthful of the solution or porridge, chewed it or rinsed with it for two minutes

[8] Obtained through the courtesy of the research laboratory of the S. A. Sugar Association and containing about 12 percent sucrose. Since this is a highly fermentable material it was boiled immediately after being drawn off and was subsequently kept in a refrigerator.

and spat out the mixture. He then washed his mouth out again, waited ten minutes and repeated the performance with the next solution or porridge. In case the sequence should have an effect, in alternate experiments the solutions or porridges were taken in the reverse order (e.g. in one experiment they chewed in the order saline, whole wheat porridge, fine flour porridge, in the next they were taken in the reverse order).

The teeth used were undecayed, normal teeth and were obtained from patients who had been advised to have multiple extractions. Special attention was paid to the distribution of the teeth in the different media in each experiment. As far as was possible each tube contained the same number of each sort of tooth i.e. incisors, canines, premolars and molars.

Each tooth was examined independently by two of the authors (J. N. N. and J. S.) before the experiment commenced and a record was made of its main features. After incubation at 37°C. for periods of from two to eight weeks it was examined again by the same observers, independently. To eliminate the possibility of bias the observers were given the teeth at random and they were given no information as to what media the teeth had been in. Using a sharp probe they looked for softening of the enamel and, by holding the tooth in a good light, they were easily able to see the white patches characteristic of decalcification when present. On probing, these patches were found to be friable. In the later experiments a groove was ground in the enamel, because it was found decalcification took place sooner and could be observed more easily on a worn surface. In some cases interim examinations were made during the course of the experiments. Finally the teeth were stained with Grenacher borax carmine. The decalcified areas were dull and stained, the healthy enamel remained glossy and unstained.

The observers reported, in the case of each individual tooth, either that: a. there was no decalcification, (−) b. there was a doubtful change, (±) or c. there was a definite change, (+). In most cases their findings agreed; where they differed the following system was used:—one + the other ± counted as change, one ± the other − counted as no change, one + the other — counted as no change. It was decided, for the purposes of the present work to divide the teeth into two classes only, the decalcified and the unchanged (the doubtful cases, as indicated above, being put into the second class). An attempt to record the degree of decalcification was abandoned.

The authors do not imagine, that they are dealing with *in vitro* caries. They go no further that to expect that that which produces decalcification in an extracted tooth is a likely caries-producer *in vivo*, and that conditions which fail to produce decalcification *in vitro* are not likely to cause decay in the mouth.

RESULTS AND DISCUSSION

The results obtained are recorded in Tables I, II and III. In the case of sugar they lend strong support to the proposition that refined sugar causes decay but that sugar, as it occurs in the cane, is nearly harmless.

The similar proposition that had suggested itself for the cereals—"that the process of refining removes some protective agency"—is

supported by Tables II and III. The naturally occurring carbohydrates of sugar-cane, of the mealie and, more especially of wheat, do produce decalcification. Therein lies a problem for some future year. The significant question which arises out of this work is why do the refined products produce the decalcification more rapidly than the crude. An obvious suggestion was that the refined developed more

TABLE I

Crude cane juice vs. sucrose solution

EXP. NO.	3	4	5	10	11	28	29	TOTAL
Saline and saliva:								
No. teeth	4	2	3	4	4	—	—	17
No. decalcified	0	0	0	0	0	—	—	0
Crude juice and saliva:								
No. teeth	4	3	3	5	5	6	6	32
No. decalcified	0	1	2	0	0	0	0	3
Sucrose and saliva:								
No. teeth	3	3	4	5	5	6	6	32
No. decalcified	3	3	2	3	3	0	1	15

TABLE II

Whole wheat meal vs. flour (60–70% extraction)

EXP. NO.	7	8	9	21	22	23	24	TOTAL
Saline and saliva:								
No. teeth	4	4	4	—	—	—	—	12
No. decalcified	0	0	0	—	—	—	—	0
Whole wheat meal and saliva:								
No. teeth	4	4	4	3	4	2	3	24
No. decalcified	4	3	4	1	0	2	0	14
Flour and saliva:								
No. teeth	4	4	4	5	4	3	4	28
No. decalcified	4	4	3	2	3	3	4	23

acid than the crude, but this soon had to be discarded since, in numerous pH determinations in the course of the experiments, it was found that all the mixtures reached an acidity of the order of pH 4–5 within twenty-four hours of the commencement of the incubation and remained at this point for the duration of the experiment. It therefore becomes incumbent upon the authors to explain different rates of decalcification in the same order of hydrogen ion concentration. As

a tentative hypothesis it is postulated that naturally occurring carbohydrate is accompanied, in sugar-cane and in the cereals, by a protective factor which exerts its effect otherwise than by controlling pH. They are not prepared to speculate as to the identity of this "protective agent."

The cane-sugar used in the experiments is of the order of 99.95% pure. The flour and mealie meal were the most refined obtainable,

TABLE III

Whole mealie meal vs. highly refined mealie meal (60–70% extraction)

EXP. NO.	2	19	20	25	26	27	TOTAL
Saline and saliva:							
No. teeth	4	—	—	—	—	6	10
No. decalcified	0	—	—	—	—	0	0
Whole mealie meal and saliva:							
No. teeth	4	3	5	4	4	6	26
No. decalcified	3	1	0	0	0	0	4
Refined mealie meal and saliva:							
No. teeth	4	3	5	3	3	5	23
No. decalcified	3	2	4	0	1	2	12

TABLE IV

Whole mealie meal vs. 90 per cent extraction mealie meal

EXP. NO.	41	42	43	44	45	TOTAL
Whole mealie meal and saliva:						
No. teeth	8	10	6	6	9	39
No. decalcified	0	1	3	3	0	7
90% extraction mealie meal and saliva:						
No. teeth	8	10	6	6	10	40
No. decalcified	0	2	3	4	0	9

in each case between thirty and forty percent of the grain was eliminated in the process of milling. Another series of experiments was performed to discover the properties of less highly refined mealie meal, a meal from which only about ten percent of the grain had been eliminated. The results of these are shown in Table IV and they indicate that this degree of refinement does not significantly increase the decalcifying potency of the mealie.

It must be stressed that the data reported in Tables I, II, III, and IV

do not permit of a comparison of the relative decalcifying potency of sugar, flour and refined mealie meal. There is great variation between salivas from different persons and between the resistence of teeth from various cases. In addition, there has been no standard time for the incubation in the different experiments; investigation is still in progress and experiments have been terminated in an arbitrary manner to test the effects of shorter and longer exposures. It is clear, therefore that comparisons can only be made between different tubes of the same experiment, i.e. between tubes containing saliva from the same donor, teeth from the same subjects and incubated for the same period. To make a comparison between refined sugar, flour and mealie meal a

TABLE V

Sugar vs. flour vs. refined mealie meal

EXP. NO.	46	47	48	49	50	51	52	53	54	TOTAL
Sugar and saliva:										
No. teeth	3	2	2	3	4	3	3	2	3	25
No. decalcified	0	2	1	0	1	0	1	0	3	8
Flour and saliva:										
No. teeth	3	2	3	3	4	4	4	2	3	27
No. decalcified	2	2	1	2	1	3	1	2	2	16
Highly refined mealie meal and saliva:										
No. teeth	3	3	3	3	4	3	3	2	3	27
No. decalcified	0	2	0	1	2	1	2	2	3	13

separate series of experiments was set up the results of which are reported in Table V. It appears that flour and highly refined mealie meal, are rather more potent as decalcifiers than sugar under conditions obtaining in our experiments.

The authors are now attempting to obtain some information as to the nature of the protective substance which they postulate.

SUMMARY

1. By incubation in media containing sugar or cereals mixed with saliva for periods of, from two to eight weeks, healthy teeth were induced to decalcify. 2. Refined white sugar produced this change in a high percentage of teeth, crude cane juice caused very few to

decalcify. 3. This strengthens the proposition that refined sugar causes caries but that sugar, eaten as it occurs in nature, does not. The suggestion that there is a "protective agent" in crude sugar-cane juice, which is removed in the course of refining, is advanced with the added assurance. This agent does not depend on an ability to prevent or reduce acid fermentation. 4. Whole mealie meal causes very few teeth to decalcify, highly refined mealie meal (60–70% extraction) produces this change in a high percentage of teeth. There is no significant difference between 90% extraction mealie meal and whole mealie meal. 5. Whereas whole wheat caused a high percentage of teeth to decalcify, highly refined white flour (60–70% extraction) attacked a significantly greater proportion of teeth. 6. By analogy with the proposition put forward for crude cane juice, it is suggested that the carbohydrate in those cereals occurs in nature with a similar "protective agent," which tends to inhibit its injurious action on the teeth. The fact that this is no more than a partial protection on the case of wheat does not affect the theory. 7. Of the highly refined substances dealt with, flour and mealie meal appear to be more potent in producing *in vitro* decalcification than sugar.

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PATHOLOGY

Cariogenicity of Common Sugars as evaluated in the Artificial Mouth

THE artificial mouth is a device for simulating *in vitro* the principal oral conditions presumably involved in dental caries[1]. Qualitatively, the *in vitro* lesions produced in human teeth are similar to those produced naturally[2-4]. The method has been used for the quantitative evaluation of anti-caries agents and dentifrices[5-7].

The procedure consists of exposing extracted human teeth to a mixed culture of oral micro-organisms[5,6]. A bacteriological medium is dripped continuously on the tooth, which is covered with a mixed bacterial plaque derived from oral micro-organisms. The rate of softening of the external enamel is followed by

measurements of the change of hardness. The sugar used in the medium has generally been D-glucose, the concentration of which has been shown to be very important in determining the type of attack[8]. When the sugar was present in low concentrations (< 0·10 gm./100 ml.), the teeth were not attacked unless decalcified dentin was present. For concentrations of 0·5 gm./100 ml., the attack was mainly a decalcification, and the dentinal matrix was not attacked. For concentrations of about 0·2–0·3 gm./100 ml., both decalcification and dentinal matrix destruction occurred.

The present work was done in order to determine whether other common sugars can be used instead of D-glucose and to compare their cariogenicity under these conditions.

The effect of the following sugars in the bacteriological medium used in the artificial mouth was studied: D-glucose, D-fructose, D-mannose, D-galactose, lactose, maltose and sucrose. Soluble starch was also included in the investigation. The original plaques were grown in the presence of these sugars, and they were also present during the tooth-softening period. Some difference in amount of bacterial plaque formation was noted, but the amount was always sufficient to cover the experimental tooth surfaces. The amount of sugar (0·3 gm./100 ml. of hexose equivalent) was the same as the optimal amount found previously for D-glucose to produce maximal decalcification and dentinal matrix destruction.

The average softening-rates are given in Table 1. The rate for D-glucose was similar to that previously reported[6] and was used as the standard for comparisons. For D-fructose, D-mannose and sucrose, the softening rates were similar to those for D-glucose. Those for D-galactose, maltose and lactose seemed to be less than for the other sugars. However, analysis by the Student *t* test indicated that only maltose was significantly different from D-glucose at the 0·01-level of confidence.

When soluble starch was present in the medium, the teeth showed no change during the experimental period.

Table 1. COMPARISON OF DIFFERENT SUGARS* ON THE RATE OF SOFTENING OF ENAMEL SURFACES IN THE ARTIFICIAL MOUTH FOR 8 HR.

Sugar	No. of teeth	Softening-rate† (percentage per hr.)	*S.D.*
D-Glucose	23	6·1	2·4
D-Fructose	12	6·2	3·3
D-Mannose	12	7·3	3·1
D-Galactose	10	4·8	2·2
Lactose	12	4·9	2·3
Maltose	12	3·8	1·8
Sucrose	16	5·2	3·8
Soluble starch	12	0·0‡	—

* Present as hexose equivalent of 0·3 gm./100 ml. of culture medium.

† $\frac{\text{Decrease in Knoop hardness No.} \times 100}{\text{Time (hr.)} \times \text{initial hardness}}$

‡ The teeth of this group all showed a slightly greater hardness than the initial value.

The pH and titratable acidity of the effluent media were measured after the 8 hr. operation of the artificial mouth. The absolute values have no significance, since they are dependent on the rate of flow. For all the effluent media which contained sugars, the pH was in the range 5·0–6·0, whereas that of the original media was 6·4–6·7. The production of acids was also shown by an increase of the titratable acidity to pH 8·0 of the medium, but no consistent pattern was evident for the sugars. For the soluble starch solutions, the pH was 6·3–6·4, and some titratable acid was formed.

The two most common dietary sugars, D-glucose and sucrose, thus produced similar rates of softening of teeth in the artificial mouth when incorporated in the bacteriological medium. This result is not surprising since many bacteria can use either interchangeably. Slower rates produced by galactose and lactose, if significant, might be explained by their utilization by fewer micro-organisms; actually the oral micro-organisms did utilize them and produced enamel softening.

The lack of any softening by soluble starch is probably related to the slight ability of the oral micro-organisms to break down the starch to fermentable sugars. This particular result has no direct relation to oral conditions, however, since saliva contains amylases which hydrolyse starches at least partially to fermentable oligosaccharides. The low rate produced by maltose is interesting in this connexion since its rate was the lowest of the sugars, and it is a major product of amylase action.

As shown earlier[9], the softening produced by the medium containing D-glucose was completely prevented in the presence of calcium phosphate solutions which will rehardden softened surfaces. Presumably, the effects of other sugars would be similarly inhibited.

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COMPARISON OF SUCROSE AND GLUCOSE IN THE CAUSATION OF DENTAL CARIES IN GNOTOBIOTIC RATS

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Summary—Complete replacement of sucrose by glucose resulted in a higher incidence of dental caries in gnotobiotic rats inoculated with *Lactobacillus casei*, *Streptococcus salivarius*, *Streptococcus faecalis*, or *Streptococcus FA-1*. Caries was observed only in the occlusal sulci and there were no observable accumulations of dental plaque.

A series of thirteen experiments was performed. There was considerable variation from one experiment to another. Mean scores of 18·0 were obtained in one experiment only to be followed by a mean score of zero although the same strain of rats, the same diet, and the same micro-organism were used in a repeat experiment. Biologic variation is a factor which must be considered even in gnotobiotic experiments.

EVIDENCE that sucrose promotes greater caries activity than other carbohydrates has been reported by GRENBY (1963), GUGGENHEIM *et al.* (1966) and by HALDI *et al.* (1967) for rats, and by SHAFER (1949), GUSTAFSON *et al.* (1955), KRASSE (1965), and EDWARDSSON and KRASSE (1967) for hamsters. SHAW *et al.* (1967) found that the replacement of sucrose by glucose did not induce as great a reduction on the initiation and progression of dental caries in white rats as had been reported previously. STEPHAN (1966) found that 10 per cent dextrose water induces slightly greater caries activity than 10 per cent sucrose water in rats fed a non-cariogenic diet.

Cariogenic streptococci become established and induce plaque formation and caries in hamsters maintained on a diet containing sucrose, but not in hamsters maintained on diets containing certain other carbohydrates (KRASSE, 1965a, 1965b; EDWARDSSON and KRASSE, 1967). Plaque formation is promoted by sucrose and not by other carbohydrates according to CARLSSON and EGELBERG (1965) and JORDAN and KEYES (1966). In contrast to the above findings, SHAW *et al.* (1967) reported that albino rats developed masses of bacterial plaque and dental caries when fed diets containing glucose or maltose, as well as when fed sucrose.

Effects of diet on plaque formation and on dental caries may be better studied if variability of the oral flora is controlled. This study was designed to compare the effects of glucose and sucrose on dental caries in gnotobiotic rats inoculated with cariogenic bacteria.

MATERIALS AND METHODS

Germfree Sprague–Dawley, germfree caries-resistant, and germfree caries-susceptible rats were used. The caries-resistant and caries-susceptible strains were derived from the colonies developed by HUNT, HOPPERT and ERWIN (1944). The stock rats of

all strains were maintained in flexible plastic isolators on Purina 5010 diet. Entire litters of animals used in the experiments were transferred into other isolators at weaning (21–25 days old).

The experimental rats were fed diet L-2000 fortified with 1 per cent Gustafson's vitamin mixture (General Biochemicals, Inc., Chagrin Falls, Ohio). Diet L-2000 contains 56 per cent sucrose. The glucose diet used in this study contained all the ingredients of diet L-2000 except that cerelose (commercial glucose) was used instead of sucrose. The sucrose and glucose diets were sterilized by exposure to 5 Mrads of cobalt 60 radiation.

The organisms used were: *Lactobacillus casei* (ATCC 4646), *Streptococcus salivarius* (H-1) isolated from a caries-susceptible human, *Streptococcus salivarius* (ATCC 13419), rat cariogenic streptococcus FA-1 of Fitzgerald, and *Streptococcus faecalis* (ND 547) of Orland.

Strep. salivarius H-1 and *Strep. salivarius* ATCC 13419 were identical as judged by colonial morphology and biochemical reactions.

Active cultures of one or another of these bacteria were used to inoculate the animals at the time of transfer into the experimental isolators. The establishment of the culture was checked by oral swab and faecal cultures. All isolators were checked for contamination by removing samples of faeces and drinking water about every 2 weeks. Some of this material was plated on Brain Heart Infusion Agar (Difco Laboratories, Detroit, Michigan) containing 5 per cent human blood and about 1 ml was inoculated into test tubes containing Fluid Thioglycollate Medium (Difco Laboratories, Detroit, Michigan). Enough plates and tubes were inoculated so that the plates were incubated aerobically and anaerobically and the plates and tubes were incubated at 50°, 37°C and room temperature. The carcasses were not checked for contamination at the termination of the experiment.

At the conclusion of the experimental period (about 100 days) the animals were sacrificed by chloroform inhalation and the jaws removed and scored for caries by a modified method of Cox *et al.* (1939).

In addition to caries evaluation, the jaws were examined for alveolar bone loss and dental plaque accumulation. Alveolar bone loss was evaluated by cutting away the gingiva to expose alveolar bone and roots of the molars. Dental plaque accumulation was determined by scraping the tooth surfaces with a dissecting needle.

Thirteen experiments were performed: six with *Lact. casei*, five with *Strep. salivarius*, one with cariogenic streptococcus FA-1 and one with *Strep. faecalis* ND 547. The experiments are listed in Table 1. Each horizontal line indicates that a single experiment was conducted within one isolator. For example, in experiment 6, all 4 groups (2 strains of rats and both test diets) were contained in the same isolator. From two to four experiments were conducted simultaneously. The lower numbered experiments preceded the latter.

In experiments 1–4 and 7, only the sucrose diet was used. These experiments are included to indicate the cariogenic quality of the test organism and to show the variability obtained in caries from one experiment to another. In experiments 5 and 6 and 8–13, groups of littermates were divided and fed either a sucrose or a glucose diet.

TABLE 1. EFFECT OF SUCROSE AND GLUCOSE ON CARIES INCIDENCE

		Sprague–Dawley				Resistant			
		Sucrose		*Glucose*		*Sucrose*		*Glucose*	
	Experiment No.	No. of rats	Mean caries score	No. of rats	Mean caries score	No. of rats	Mean caries score	No. of rats	Mean caries score
Lactobacillus casei ATCC 4646	1	4	4·8 (1–9)	—	—	3	1·0 (1–1)	—	—
	2	5	16·0 (12–19)	—	—	—	—	—	—
	3	9	15·1 (4–22)	—	—	—	—	—	—
	4	3	0·0	—	—	3	0·0	—	—
	5	5	1·6 (0–5)	5	2·0 (0–4)	—	—	—	—
	6	4	0·0	4	1·2 (0–4)	5	0·0	5	3·8 (0–10)
Streptococcus salivarius (H-1)	7	4	15·0 (12–17)	—	—	—	—	—	—
	8	3	0·0	3	18·0 (8–30)	—	—	—	—
	9	4	0·0	5	0·0	4	0·0	4	0·0
Streptococcus salivarius ATCC 13419	10	3	6·0 (0–17)	2	10·0 (9–11)	3	0·0	5	0·0
	11	5	0·0	6	0·0	5	0·0	4	0·0
FA-1	12	3	2·7 (0–4)	3	12·0 (0–18)	3	12·0 (11–13)	3	12·0 (8–14)
Streptococcus faecalis ND 547	13	4	0·0*	4	3·0* (0–8)	3	0·0	3	0·0

* Caries-susceptible instead of Sprague–Dawley rats were used.

Numbers in parentheses are ranges of mean values.

RESULTS

The data are presented in Table 1. There are 14 comparisons of mean caries scores between littermates fed either sucrose or glucose. Seven of these comparisons are with Sprague–Dawley rats, one with caries-susceptible rats, and six with caries-resistant rats. In seven of these comparisons, a higher mean caries score was obtained in rats fed the glucose diet; in six comparisons, no caries developed; and in one comparison a mean score of 12·0 was found both in rats fed sucrose and in rats fed glucose. In some of the seven comparisons in which a higher mean caries score was obtained in rats fed the glucose diet, the difference is small (e.g. experiment 5), but in other comparisons, the difference is substantial (e.g. experiment 8). Sucrose did not induce a greater mean caries score than glucose in any of the experiments.

Caries developed in the fissures and not on any of the buccal or lingual surfaces of the molars. There was no observable alveolar bone loss and no accumulation of gelatinous plaque.

DISCUSSION

At first glance, the results obtained in this study appear different from those investigators who have reported that sucrose is more cariogenic than glucose. Sucrose appears to greatly enhance or be required for coronal plaque formation, and plaque accumulations are necessary prerequisites for carious lesions on smooth surfaces of teeth. It has been suggested that certain cariogenic streptococci have the ability to synthesize extracellular polysaccharides from sucrose capable of forming a gelatinous plaque on the tooth surface (WOOD and CRITCHLEY, 1966; GIBBONS and BANGHART, 1967; DAHLQVIST *et al.*, 1967). Since the strains of bacteria used in this study evidently lacked plaque-forming potential under the conditions of the experiment, one could not expect them to initiate smooth surface caries, and consequently, one could not expect sucrose to be more cariogenic. Many of the studies indicating sucrose to be highly cariogenic have utilized hamsters. The teeth of these animals do not contain deep fissures. If caries is initiated in hamsters, it must be considered to be of the smooth surface type, and as such, it is plaque-dependent. In this study, there was no gelatinous plaque accumulation and caries originated in the fissures and not on any of the smooth surfaces. Caries must have resulted from the activity of the micro-organisms on the food accumulated in the fissures. The physical and chemical properties of the material in the fissures was not determined.

The establishment of *Strep. salivarius* (ATCC 13419) as a cariogenic organism indicates that a stock strain of a levan-producing organism can induce caries. GIBBONS and BANGHART (1968) also have shown that a levan-producing streptococcus induces caries. In their study the streptococcus produced a plaque which adhered to the smooth surfaces of the molars whereas the streptococcus reported in this study produced no apparent plaque on the smooth surfaces.

The data in this study support the view that fissure caries is more easily induced by glucose than by sucrose. This view is limited by the conditions used in these experiments such as rat strain, diet, and specific micro-organisms.

These data suggest certain interrelationships between diet, micro-organisms and host susceptibility in the causation of dental caries. There were two instances when the caries-resistant strain of rats showed greater susceptibility to caries than the Sprague-Dawley strain; when rats were inoculated with *Lact. casei* and fed the glucose diet (Table 1, experiment 6), and when the test organism was rat cariogenic streptococcus FA-1 and sucrose diet was used (Table 1, experiment 12).

Caries experience varied considerably from experiment to experiment. Mean scores as high as 18·0 were obtained in one experiment only to be followed in another experiment by a mean score of zero for the same strain of rats, the same diet, and the same micro-organism. Litter variation, certain changes in diet composition (especially if the fluoride content of the diet used when the rats were weaned varied), and microbial variation could explain the highly variable results. Since the experiments were not performed simultaneously, it is possible that subsequent batches of diets varied in composition from previous batches. Also, each time an experiment was set up, the test micro-organism was selected from a culture maintained on ordinary laboratory media. This could have resulted in certain subtle variations which could have affected its cariogenicity.

Résumé—Le remplacement total de saccharose par du glucose provoque une fréquence carieuse plus élevée chez des rats gnotobiotiques inoculés avec du *Lactobacillus casei*, du *Streptococcus salivarius*, du *Streptococcus faecalis* ou du *Streptococcus FA-1*. Des lésions carieuses sont observées uniquement dans les sillons et il n'y avait pas d'accumulations visibles de plaques dentaires.

Une série de treize expériences a été entreprise. Des différences considérables ont été observées d'une expérience à l'autre. Des scores moyens de 18·0 ont été obtenus dans une expérience, suivis d'un score moyen de zéro, bien que la même espèce de rats, le même régime, et le même micro-organisme aient été utilisés dans cette expérience de contrôle. Des variations biologiques doivent être prises en considération, même dans des expériences gnotobiotiques.

Zusammenfassung—Der vollständige Ersatz von Rohrzucker durch Glukose führte bei Gnatobiose-Ratten, die mit *Lactobacillus casei*, *Streptococcus salivarius*, *Streptococcus faecalis* oder *Streptococcus FA-1* inoculiert worden waren, zu höherer Kariesfrequent. Die Karies wurde lediglich in den okklusalen Fissuren beobachtet; es gab keine feststellbaren Ansammlungen von Zahnplaques.

Dreizehn untersuchungen wurden durchgeführt. Von einem Experiment zum andern gab es erhebliche Abweichungen. Mittelwerte von 18,0, die von einem mittleren Befund 0 gefolgt waren, wurden lediglich in einem Versuch erzielt, obwohl beim Wiederholungsversuch derselbe Rattenstamm, dieselbe Kostform und derselbe Mikroorganismus benutzt worden waren. Bei allen Gnatobioseversuchen müssen biologische Variationen als Faktoren berücksichtigt werden.

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The Effect of Sugar Solutions on pH of Dental Plaques from Caries-susceptible and Caries-free Individuals

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The etiology of dental caries may be better understood when the factors for natural resistance to caries are determined. In an effort to determine some of these factors, several investigators have studied the effect of refined carbohydrates on acid production of dental plaques from caries-susceptible and caries-free individuals. Stephan[1] has found that, in individuals with differing degrees of caries activity, the pH of dental plaques dropped from one to two units following an oral rinse with 10 per cent glucose. The decrease in pH following the rinse was related to the pH before the rinse, which was progressively lower in five groups of individuals with increasing caries activity. Moore, Carter, Dunn, and Fosdick[2] have reported that the pH in the plaques of caries-active individuals decrease from 6.3 to 5.6 following an oral rinse with 50 per cent sucrose. Clement, Plotkin, and Fosdick[3] have found that the pH in the dental plaques of caries-free primitive Bushmen dropped from 6.9 to 6.5 following an oral rinse with 50 per cent sucrose. Englander, Carter, and Fosdick,[4] using plaque obtained from caries-free naval recruits, have reported a mean pH change from 6.8 to 6.7 following a 50 per cent sucrose rinse.

Stephan[1] has determined the pH of dental plaques *in situ*, using an antimony electrode. Moore *et al.*,[2] Clement *et al.*,[3] and Englander *et al.*[4] have determined the pH of dental plaques by removing plaque material and measuring the pH in a glass electrode described by Fosdick, Campaigne, and Fancher.[5] Forscher and Hess[6] have compared measurement of plaque pH *in situ*, using an antimony electrode, with measurement of plaque pH by removing plaque material and placing it in either the glass electrode of Fosdick *et al.*[5] or the Beckman one-drop assembly-glass electrode. They found that the Beckman electrode gave results similar to the other glass electrode and to the antimony electrode, so suggested its use because of its durability and ease of handling.

Miller, Muntz, and Bradel,[7] Neuwirth and Summerson,[8] Manly,[9] and Manly, Shiere, O'Brien, and Harrington[10] have found that dental-plaque material from both caries-free and caries-susceptible individuals produced acid from carbohydrates in vitro, but they could not demonstrate any difference between the two groups in the amount of acid produced.

The diversity of results in these reports indicates that further experimentation is necessary in order to determine the relationship of pH in dental plaques to caries. In the study reported here, a comparison was made between the pH of plaques of caries-susceptible and caries-free individuals before and after exposure to sugar solutions.

Materials and Methods

Caries-susceptible subjects who had a minimum DMFS score of 25, including 6 or more open carious lesions, were selected. The caries-free individuals who were selected had neither carious lesions nor restorations, as determined by radiographic and clinical examinations. The selection of subjects was based only on the above criteria. The subjects ranged in age from seventeen to forty. About half the caries-free individuals showed evidence of very mild mottling, and none of them restricted refined carbohydrates from their diets. All the caries-free subjects had 0 or counts of less than 1,000 for salivary lactobacilli on Rogosa SL agar.*

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* Difco Laboratories, Inc., Detroit, Mich.

Most of the caries-susceptible subjects had a lactobacillus count ranging from 5,000 to 20 million per ml.

Subjects were asked not to brush their teeth for 24 hours and not to eat or smoke for 1 hour prior to reporting for the experiments.

The subjects were seated in a dental chair and a sample of plaque was removed with a dental scaler generally from the gingival areas of non-carious buccal surfaces of the upper molars.

After the pH of the plaque was determined, the subject rinsed his mouth for 30 seconds with 20 ml. of a 50 per cent (w/w) sucrose solution. Following the rinse, samples of plaque were removed at different time intervals, and the pH of each plaque sample was determined.

Each sample of plaque was placed on the tip of a calomel electrode,* which was then set into a one-drop assembly-glass electrode.* The plaque was homogenized gently between the two electrodes for 3–5 seconds, and the pH was read on a Model G pH meter.*

For this study, plaque was the organic coating of the tooth, regardless of its physical properties or derivation. This would include film, materia alba, and any matter that is consistently found on the tooth surface. Loose accumulations and samples contaminated with blood or excessive saliva were not used.

* Beckman, South Pasadena, Calif.

Results

Figure 1 shows the mean pH of plaque before and at 30 seconds and 5, 10, and 15 minutes after a sucrose rinse. The mean pH at each time interval for the caries-susceptible and the caries-free groups was determined by converting individual pH values into hydrogen ion concentration, averaging arithmetically, and then converting back into pH. The mean pH of caries-susceptible individuals is consistently from 0.1 to 0.2 unit lower than caries-free individuals from before the rinse to 15 minutes after the rinse. A statistical analysis of the data, using the *t*-test, is presented in Table 1. The difference is not significant between mean (H^+) values for caries-susceptible and caries-free groups before the sugar rinse and for values at 30 seconds and at 5 minutes after the rinse ($p > 0.05$); but it is significant for values at 10 and 15 minutes after the rinse ($p < 0.05$).

From the above data, the mean minimum pH values were determined by selecting the lowest pH attained by a particular individual at any time interval after the sugar rinse and averaging as described previously. These data are given in Table 2. The data were analyzed statistically by the *t*-test for the (H^+) values and by the Mann-Whitney *U*-test for the pH values. The difference between means is significant ($p < 0.05$ for both tests). The minimum pH data are also presented in Figure 2 as frequency-distribution curves.

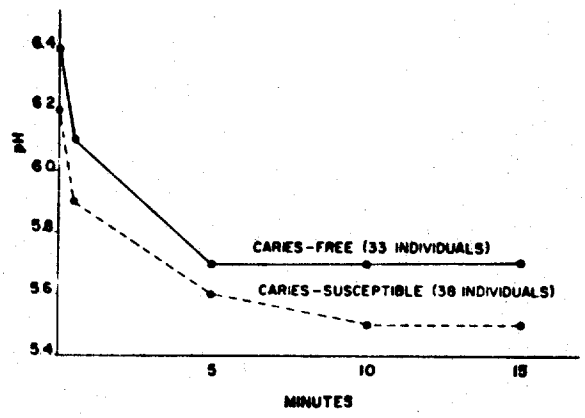

FIG. 1. Mean pH of dental plaque before and after a sucrose rinse

TABLE 1

STATISTICAL COMPARISON BETWEEN CARIES SUSCEPTIBLE AND CARIES FREE INDIVIDUALS FOR DATA PRESENTED IN FIGURE 1

Time Interval	Mean (H+) and Standard Error (No. × 10⁻⁷) Caries-susceptible	Caries-free	p
Before sugar rinse	6.21 ± 1.28 (6.2)*	3.75 ± 0.42 (6.4)*	>0.05
30 Seconds after rinse	12.4 ± 1.88 (5.9)*	8.33 ± 4.03 (6.1)*	>.05
5 Minutes after rinse	27.2 ± 2.91 (5.6)*	20.7 ± 3.27 (5.7)*	>.05
10 Minutes after rinse	34.2 ± 4.57 (5.5)*	18.9 ± 2.36 (5.7)*	<.05
15 Minutes after rinse	29.3 ± 3.51 (5.5)*	18.3 ± 2.18 (5.7)*	<0.05

* Equivalent pH values.

Figure 3 shows the mean pH of plaque before and at 5, 30, 60, 90, and 120 minutes after a sucrose rinse. These data show a drop in pH to its lowest point at the end of 5 minutes following the rinse, but at each of the subsequent time intervals there is a consecutive increase toward the original pH. This gradual return to the initial pH is similar in both groups. The mean pH is lower for caries-susceptible than for caries-free subjects at all time intervals except at 30 minutes.

The authors are cognizant that certain variables may affect the results of this study. Therefore, such variables as the amount of time allowed for plaque to accumulate, the area from which plaque was collected, and the method of plaque collection were considered. Separate experiments were performed to determine the effect of these variables, and the results are given in Table 3. The first line shows data taken from Figure 1. In the second line, plaque was removed from interproximal areas of upper molar teeth. For these areas, the method of plaque collection was altered by clearing the area of debris with a jet rinse of water and drying with cotton. The mean initial pH is 6.3 for both caries-free and caries-susceptible subjects. The mean pH at 10 minutes after the sugar rinse is 5.5 for the caries-free individuals and 5.6 for the caries-susceptible individuals. In the third line, data are given on plaque pH for subjects who were asked to brush their teeth on the day before reporting. Plaque was removed as in earlier experiments. The mean initial pH is 6.4 for caries-free subjects and 6.1 for caries-susceptible subjects. The mean pH at 10 minutes after the sugar rinse is 5.6 and 5.5, respectively. In the fourth line, data are given on plaque pH for subjects who were asked not to brush their teeth for 2 days prior to reporting. The mean initial pH is 6.4 for caries-free subjects and 6.5 for caries-susceptible subjects. The mean pH at 10 minutes after the sugar rinse is 5.4 for both groups.

TABLE 2

MEAN MINIMUM (H+) AND EQUIVALENT pH OF CARIES SUSCEPTIBLE AND CARIES FREE INDIVIDUALS

Group	Mean (H+) and Standard Error (No. × 10⁻⁷)	Equivalent pH
Caries susceptible	45.9 ± 4.44	5.3
Caries free	25.7 ± 2.97	5.5

Discussion

The pH of the plaques is 0.1–0.2 higher in caries-free than in caries-susceptible individuals, both before and after a sugar rinse, in most of our experiments. On the basis of this small but statistically significant difference, it is difficult to explain the wide difference in caries experience between the two groups of subjects used in these experiments. The use of caries-susceptible subjects, even with open lesions, does not necessarily mean that caries is progressing in these subjects. It was not observed whether or not the subjects developed new carious lesions or if caries progressed after the plaque pH measurements were made. Therefore, the interpretation of these data should

TABLE 3

EFFECT OF CERTAIN VARIABLES ON PLAQUE pH BEFORE AND 10 MINUTES AFTER A SUGAR RINSE

Plaque Collection Area	Days without Brushing	Caries-free Mean pH before Rinse	Caries-free Mean pH 10 Min. after Rinse	Caries-free No. Subjects	Caries-susceptible Mean pH before Rinse	Caries-susceptible Mean pH 10 Min. after Rinse	Caries-susceptible No. Subjects
Buccal upper molar	1	6.4	5.7	33	6.2	5.5	38
Interproximal upper molar	1	6.3	5.5	12	6.3	5.6	12
Buccal upper molar	0	6.4	5.6	21	6.1	5.5	7
Buccal upper molar	2	6.4	5.4	23	6.5	5.4	9

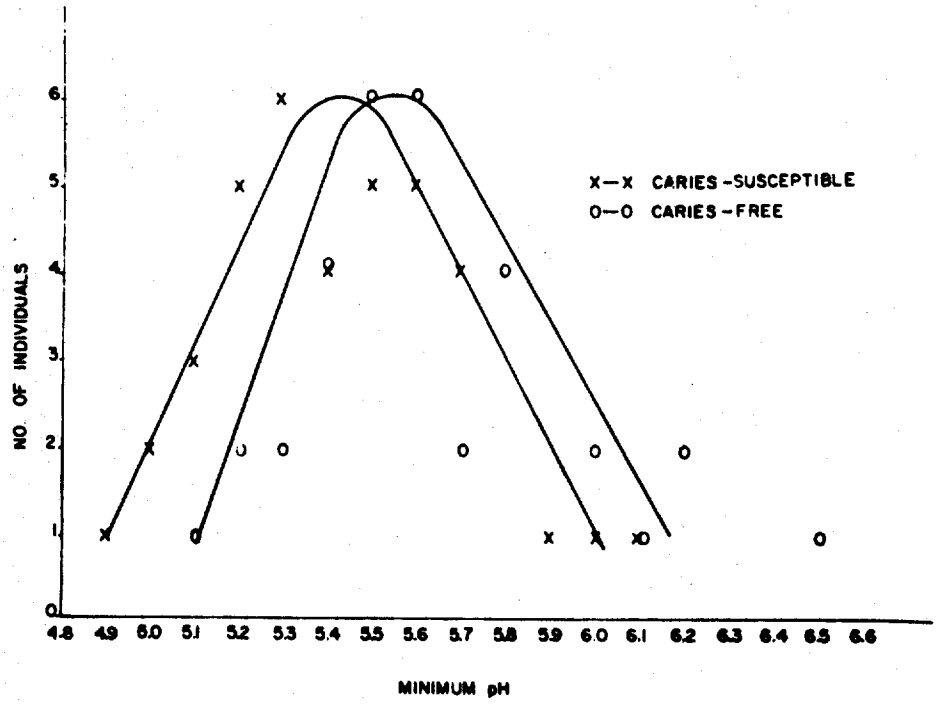

FIG. 2. Frequency-distribution curves for the minimum pH obtained in the dental plaques following a sugar rinse in caries susceptible and caries-free individuals.

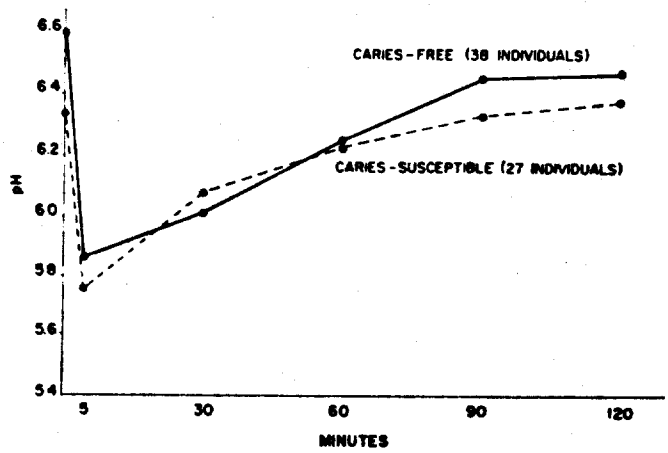

FIG. 3. Mean pH of dental plaque before and after a sucrose rinse

apply only to two groups of individuals, namely, caries-free and caries-susceptible.

Caries-susceptible individuals should be distinguished from caries-active individuals. The former may or may not have teeth developing caries but show a caries history; the latter have teeth that are either developing new lesions and/or have caries that is progressing in lesions already present.

The distribution curves (Fig. 2) for minimum pH for caries-susceptible and caries-free subjects overlap considerably. Under the conditions of our experiment, this indicates that the concentration of acid in plaques before and after a sugar rinse is similar for many individuals regardless of their caries history.

Summary

In a series of experiments using caries-susceptible and caries-free individuals, the pH of dental plaque removed from the mouth was determined before and after an oral rinse with sugar solutions. The pH decreased following the rinse. The difference in pH levels in the plaques of caries-susceptible individuals generally was from 0.1 to 0.2 unit less than in the plaques of caries-free individuals. Thirty minutes after the rinse, the pH of the plaques started to rise and gradually approached the original level. The length of the period of time without brushing preceding the pH determinations did not markedly affect the pH reading either before or after the sugar rinse.

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Effects of Ready-to-Eat Breakfast Cereals on Dental Caries Experience in Adolescent Children: A Three-Year Study

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The 375 adolescent public school children who participated in a permissive three-year breakfast consumption study showed no differences in dental caries experience whether they ate ready-to-eat breakfast cereals or other dietary regimens.

The influence of dietary composition on dental caries experience has been well documented in experimental animals[1,2] and in man.[3-5] Conflicting reports exist regarding the role of cereals in the genesis of, or protection against, dental caries.[6-8] It would be helpful for dietary counseling of the adolescent child if information could be generated to determine whether consumption of modern ready-to-eat breakfast cereals should be avoided, ignored, or encouraged. While conducting a clinical trial on adolescent children to determine the effect of phosphate enrichment of ready-to-eat breakfast cereals on dental caries experience, we had an opportunity to analyze whether the incorporation of ready-to-eat cereal into the breakfast regimen altered dental caries experience from that of children who consumed other foods for breakfast.

Materials and Methods

Ann Arbor seventh grade public school children of both sexes were enrolled in a permissive dental caries prevention study by parental consent. Participants were provided at monthly intervals, ad libitum, toothbrushes,[a] dentifrice,[b] and seven varieties of ready-to-eat breakfast cereal delivered to their homes. Cereals were produced and packaged for the study by General Mills, Inc. (Minneapolis, Minn). Cereals were not identified for the participants. Cereals marketed under the names Wheaties, Cheerios, Corn Flakes, Trix, Cocoa Puffs, Frosty O's, and Corn Bursts were dispensed in plain white 7 ounce boxes labeled simply wheat flakes, corn cereal, oat cereal, fruit-flavored corn puffs, cocoa-flavored cereal, sugared oat cereal, and corn puffs (in addition to federally required information). The last four cereals were presweetened, whereas the first three were not.

Participants were instructed that adherence to a rigid schedule for eating the study cereals was not required. Parents were requested to have the study cereals available, but the participant was free to eat as much or as little of them as he desired. Some advised us that after a brief "novelty period," bacon and eggs, cooked cereal, and so on were preferred to the study cereals, and as a consequence the participant no longer ate the study cereal. Children were reminded that participation in the study did not require them to eat the study cereal in lieu of a preferred or customary breakfast regimen. This provided us with the opportunity to examine dental caries increments in ready-to-eat breakfast cereal eaters vs those who ate other foods for breakfast.

Complete three-year data for 375 children were available for analysis at the conclusion of the study. These children of both sexes had been an average of 13 years of age (standard deviation, 0.38) at the beginning of the study period. Cereal was dispensed after an initial six-month delay (to comply with a United States Food and Drug Admin-

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[a] Oral B30, Oral B Company, Wayne, NJ.
[b] Crest, Procter & Gamble Co., Cincinnati, Ohio.

istration directive). Dental caries experience was determined initially and annually thereafter.

Examinations were conducted by two experienced dentists. One conducted all the clinical examinations; the other conducted all the radiographic examinations at the 0-, 12-, 24-, and 36-month intervals. Participants were instructed individually in oral hygiene by a hygienist; instruction was followed by supervised brushing. The participant was seated in a head-rest-equipped portable dental chair and teeth were examined with a mirror and explorer. High intensity light was carried uniformly to each of the participant's teeth by a fiber optic light catheter within a hollow-handled mouth mirror. Verbalized observations were recorded (by another dentist). Seven radiographs (bitewing and anterior) were taken per individual per year.

Careful records were maintained by one person throughout the study period; the amount and type of cereal supplied to each participant were listed. Of the cereals requested, 60% were presweetened (sugar-coated). For the purpose of this analysis, a participant was assigned to the "noncereal eater" group if he consumed less than 28 boxes during the three-year study period. This amounted to less than one box per month, which was, at the most, less than one serving every fourth day. This is an arbitrary categorization, the effect of which was checked statistically by reassignment on the basis of 50 boxes per child. Results were similar; thus, assignment error is probably negligible. Considerable variation in the amount of cereal requested by the various participants was observed as expected (Table 1).

TABLE 1

FREQUENCY DISTRIBUTION OF PARTICIPANTS ACCORDING TO THE TOTAL NUMBER OF BOXES OF CEREAL SUPPLIED DURING THE THREE-YEAR STUDY PERIOD

Boxes	No. of Participants	Percent
0– 20	51	8.5
20– 40	48	13.6
40– 60	46	13.0
60– 80	33	9.3
80–100	25	7.1
100–120	21	5.9
120–140	21	5.9
140–160	13	3.7
160–180	5	1.4
180–200	13	3.7
200–220	15	4.2
220–240	15	4.2
240–260	7	2.0
260–280	6	1.7
280–300	8	2.3
300–320	3	.8
320–340	4	1.1
340–360	5	1.4
360–380	7	2.0
380–400	6	1.7
400–500	16	4.5
500–600	6	1.7
600–700	1	0.3

Results

Only three-year data (30-month cereal use) are analyzed here. This was done to discriminate against (1) the novelty effect (which probably did occur in the early months), that is, sampling the test cereals and then quickly reverting to previous dietary regimens with the uneaten portion discarded, and (2) questionable examiner judgment in the case of incipient lesions, which are known to plague short-term dental caries studies.[9]

Similarity of the two groups with respect to age, sex, and previous and incremental dental caries experience is evident (Table 2). When the null hypothesis (no difference exists between those who ate the study cereal and those who did not) was applied, rejection was not accomplished. Statistical analysis of the incremental dental caries data by stepwise multiple regression and analysis of variance including age, sex, and initial caries experience plus eaters-noneaters as factors, also showed no difference between cereal eaters and noneaters.

In view of the great variation in the number of boxes of cereal supplied to the participants during the course of the study, an additional analysis was made in which those who received more than 200 boxes ("heavy eaters") were compared with the noneaters. Data revealed no difference in caries increments between the heavy eaters and the noneaters.

Discussion

Positive and negative group assignment errors are possible. The participant could have eaten other ready-to-eat breakfast cereals as a substitute for the study cereal;

TABLE 2

COMPARATIVE DENTAL CARIES EXPERIENCE OF ADOLESCENT PUBLIC SCHOOL CHILDREN WHO ATE READY-TO-EAT BREAKFAST CEREALS AND THOSE WHO DID NOT

	Mean		Standard Deviation				
	Eaters (302)	Noneaters (73)	Eaters	Noneaters	Difference	Percent	t
Age (in years)	13.10	13.05	0.37	0.40	0.05	0.4	0.98
Sex	0.53*	0.45*	0.50	0.50	0.08	15.4	1.24
Initial DMF teeth	4.55	5.11	2.92	3.27	−0.56	−12.3	−1.34
Initial DMF surfaces	6.12	7.25	4.41	5.67	−1.12	−18.3	−1.58
Incremental DMF teeth	3.24	3.63	2.69	2.90	−0.39	−11.9	−1.04
Incremental DMF surfaces	5.20	6.41	4.67	5.62	−1.21	−23.2	−1.70

* Male = 1; female = 0.

thus, from shipping records he would have been recorded as a noncereal eater. Because of the economics of the situation (the study cereal was free and ordered by the parent), it is unlikely that this situation occurred. Interviews by telephone with parents at various times during the study, as well as direct interviews with each participant at the three annual examinations, strongly reinforced this belief.

The opposite error of the participant's family consuming the cereal, while the subject himself ate alternate foods, also was possible. Telephone interviews with parents would be less likely to reveal this source of error because of fear that the free cereal supply would cease if the situation were discovered. Again, the rules encouraged frankness. It had been stated at the outset that supplies would be provided to each participant for the duration of the study regardless of consumption. Even so, a small amount of this type of error probably did occur. Good rapport with the participants who were interrogated annually and the candidness of children at this age (especially when they knew that judgment was not made whether they responded affirmatively or negatively with regard to preference of cereal variety, or whether they ate it or not) leads us to believe that the magnitude of this error was small.

Although absolute homogeneity of either sample cannot be completely assured, the practical question of dietary counseling with regard to incorporation or avoidance of ready-to-eat breakfast cereals can be approached in general terms. In the present study, in which conditions more or less approximated those of the average American adolescent child, factors other than incorporation or avoidance of ready-to-eat breakfast cereals completely dominated dental caries causal relationships. Under conditions of water fluoridation (the Ann Arbor municipal water supply has contained 1.1 ppm fluoride since 1952) and oral health enlightenment (dental care and consciousness enjoy a relatively high level in this community), recognizable benefit or harm from consumption of ready-to-eat breakfast cereals in place of other breakfast regimens could not be identified.

Conclusions

Under the conditions of this study, adolescent children who consumed ready-to-eat breakfast cereals did not differ with respect to dental caries activity from classmates who consumed other breakfast dietary regimens.

We thank P. Carrigan, director, and C. Towers of the Ann Arbor Public Schools System, Department of Research, for assistance in coordinating the clinical phase of this study with the pupils, teachers, and administrators involved. Examiners during the field study were J. A. Regezi, USAF, Travis Air Force Base, California, and J. P. Sapp, Department of Pathology, The University of Western Ontario, London, Canada. We acknowledge the interest and cooperation of the principals, teachers, and participants and their parents.

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BODY FLUID GLUCOSE LEVELS AS RELATED TO DENTAL CARIES STATUS IN MAN

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Summary—Parotid fluid samples were collected without exogenous stimulation from 507 systemically healthy young adult males. Blood was drawn from each participant and subjects were classified as to dental caries experience (DMFS). Parotid fluid flow rate was recorded, and serum and parotid fluid were analysed for glucose concentration by the glucose oxidase procedure. The presence of glucose in parotid fluid was confirmed. Neither parotid fluid flow rate, parotid fluid glucose level, nor serum glucose concentration was found to be related to caries experience. For all participants, the mean for each of the three variables was 0·042 (S.D. ± 0·036) ml/min, 0·75 (S.D. ± 0·68) mg%, and 84·8 (S.D. ± 7·76) mg%, respectively.

INTRODUCTION

THE IMPORTANCE of intraoral carbohydrates in the caries process has been one of the most frequently discussed subjects in the dental literature. BUNTING, NICKERSON, HARD and CROWLEY (1928), BUNTING, CROWLEY, HARD and KELLER (1928), BUNTING (1934) and BUNTING (1935) reported many years ago that the institution of a low-sugar diet arrested caries in the majority of cases. These studies led to the conclusion that diet controlled caries by altering the environment of teeth rather than by making them more resistant to caries (BUNTING, 1935). BECKS (1948) and BECKS and JENSEN (1948) initiated carbohydrate restriction in 1250 subjects with rampant caries and found that, in about two-thirds of the cases, no new lesions developed in the year during which the restricted diet was followed. While there is convincing evidence that the production of acid from carbohydrate is not the entire explanation of the caries process, the majority opinion is that the primary lesion is produced by acid decalcification of the inorganic portion of the enamel and that the degradation of carbohydrates to lactic acid is of primary importance in this regard.

The development of an enzymatic laboratory method with a specificity for glucose has made it possible to establish that this substance is actually present in parotid fluid in measurable amounts (SHANNON, PRIGMORE and BROOKS, 1960; ESGLAVATH, JEFFAY, FULLER and CHAUNCEY, 1962). It was of interest to determine whether or not levels of this biologically important endogenous carbohydrate in body fluids were related to dental caries status.

* Opinions expressed herein are those of the authors and are not to be construed as official USAF policy.

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MATERIALS AND METHODS

Subjects were 507 males between the ages of 17 and 22 years. Each had been proved physically fit for military service by recent medical examination. Environmental conditions and dietary and emotional exposure were similar for all subjects. The selection of subjects was completely random, and oral health status was not a consideration in selection.

Collection of parotid fluid was initiated between 7.30 and 8.00 a.m., this being 2–2½ hr after breakfast. A parotid cap (SHANNON, PRIGMORE and CHAUNCEY, 1962) was placed over the orifice of the duct of the right gland with an absolute minimum of manipulation, and parotid fluid was collected over a period of at least 2 hr. No exogenous stimulants of any type were employed; on the contrary, every precaution was taken to minimize stimulation as much as possible. During the sampling period the patients were seated comfortably in a semi-isolated section of an air-conditioned laboratory that was kept free from extraneous disturbances. Subjects were allowed to write letters and to peruse selected reading material in an effort to minimize psychic glandular stimulation. All participants were carefully supervised to prevent talking and unnecessary movement and to assure that they remained awake and alert. Parotid fluid was collected in graduated tubes and volume was read to the nearest 0·05 ml. A venous blood sample was collected from each participant between 10.00 and 10.30 a.m. and, as soon as a clot was formed, the serum was separated and retained. The glucose concentration of both fluids was determined enzymatically with glucose oxidase by methods based on the contributions of KESTON (1956) and of SAIFER and GERSTENFELD (1958). Serum, 0·2 ml, was taken to 4·0 ml with water and the proteins were precipitated by the addition of 2·0 ml each of 2% zinc sulphate and 0·12 N NaOH. This mixture was filtered and 1·0 ml of filtrate was exposed to 4·0 ml of Glucostat (Worthington Biochemical Corp., Freehold, New Jersey) reagent at 37°C for 1 hr. The reaction was halted with acid, and the developed colour was read at 400 mμ in a Beckman DU spectrophotometer. In the parotid fluid determination, 0·5 ml of the sample was added to 0·5 ml of 2% zinc sulphate, and 0·5 ml of 0·12 N NaOH was added to this mixture. Filtrate and Glucostat were mixed in equal portions in the saliva procedure and colour was read in microcuvettes. The performance of each method was evaluated as to reproducibility and recovery ability.

Subjects were divided into six dental status groups based upon the sum of decayed, missing, and filled tooth surfaces (DMFS) noted. Thorough clinical examinations were supplemented by bitewing roentgenograms. Third molars were excluded in all instances. Each subject possessed at least 20 teeth and none presented evidence of advanced periodontal disease.

RESULTS

The ability of the enzyme procedure to recover glucose added to serum is outlined in Table 1. The mean recovery rate was 103 per cent. Duplicate glucose determinations were performed on 20 sera (mean glucose, 82·3 mg%) to test method

TABLE 1. RECOVERY OF ADDED GLUCOSE (mg)

Flask No.	(a) From serum pool	(b) Added from stock solution	(c) Found	(d) Recovered (c–a)	(e) Difference (d–b)	Percent recovery (d/b × 100)
1	41·250	—	41·25	—	—	—
2	40·425	10·00	51·10	10·675	+0·675	107
3	38·775	30·00	70·00	31·225	+1·225	104
4	37·125	50·00	88·35	51·225	+1·225	102
5	35·475	70·00	105·55	70·075	+0·075	100

reproducibility. The average difference between duplicates was 0·96 mg%, the standard deviation of this difference was 0·81 mg%, and the coefficient of variation for the method was 0·98%.

When 20 parotid fluid samples were analysed in duplicate for glucose content a mean of 0·80 mg% was found. The average difference between duplicates was 0·04 mg%, and the standard deviation of this difference was 0·033 mg%. The coefficient of variation for the salivary procedure was 4·1%. The method performed well in recovering added glucose. Glucose was added to portions of a parotid fluid pool that contained 0·77 mg/100 ml. To 0·3773, 0·3465, and 0·3080 mg of glucose from the pool, 0·20, 1·00, and 2·00 mg of glucose, respectively, were added from a 20 mg/100 ml stock solution. Glucose in the amounts of 0·570, 1·345, and 2·305 mg was found in these three solutions with a resultant mean added glucose recovery rate of 99·7%.

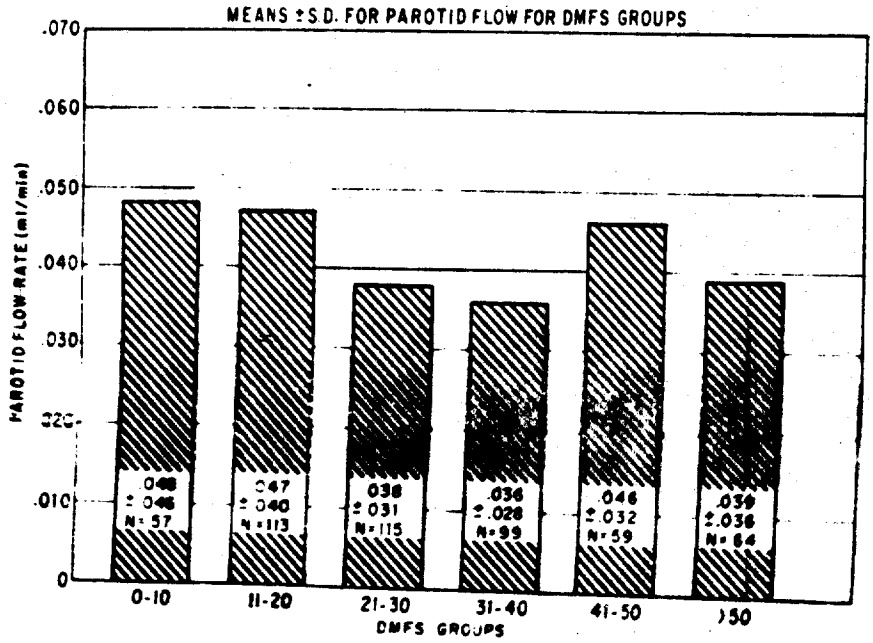

FIG. 1. Means ± S.D. for parotid flow for DMFS groups.

Fig. 1 demonstrates that parotid fluid flow rate was independent of DMFS status. This confirms our past observations under similar experimental conditions (SHANNON and ISBELL, 1962; SHANNON, ISBELL, GIBSON and O'LEARY, 1962). The mean of 0·036 ml/min found for the 31–40 DMFS group differed from the means for the 11–20 and 41–50 groups but, since these differences were significant only at the 0·05 level and since no pattern of differences was evident, they were held due to chance. The mean for all participants was 0·042 (S.D. ± 0·036) ml/min.

For serum glucose, there were no significant differences between DMFS groups (Fig. 2). The serum glucose mean for the 507 subjects was 84·8 (S.D. ± 7·76) mg%. For parotid fluid glucose, the mean of 0·57 mg% for the 41–50 DMFS group was significantly lower than the means for the 0–10, 11–20 and 21–30 DMFS groups (Fig. 3). As with the flow rate results, no pattern was discernible, and this observation was held probably due to chance. The overall mean for all subjects was 0·75 (S.D. ± 0·68) mg%.

DISCUSSION

Many investigations have been conducted to ascertain the presence or absence of glucose in saliva. The investigators who have claimed its presence have attempted to determine the mechanisms responsible for the control of the salivary sugar level primarily by altering the blood level and following the response in saliva. The extensive disagreement in past results has been primarily due to the lack of an analytic method with a specificity for glucose. In 1960, SHANNON and PRIGMORE evaluated the performance of an automatic method of parotid fluid glucose determination and reported a glucose mean of 11·14 mg%. In the same year SHANNON, PRIGMORE and BROOKS found that this automated ferricyanide method gave falsely high results when compared to the enzymatic procedure which takes advantage of the exquisite specificity of glucose oxidase. These authors concluded that there was a measurable level of glucose in human parotid fluid and that the level was sensitive to the intravenous administration of glucose. In 1962, ENGLANDER *et al.* reported measurements of parotid fluid glucose by the glucose oxidase procedure in both normal and diabetic subjects. WOLCOTT and WEBER (1960) studied parotid fluid by the anthrone procedure in an attempt to develop a glucose tolerance procedure. WEBER, LUX and SHKLAIR (1960) found three times as much carbohydrate in the parotid fluid of caries-rampant individuals as in caries-immune and implied that this finding also held true for glucose by stating that glucose oxidase/anthrone ratios compared favourably. No glucose data were presented.

The present study confirms the presence of glucose in parotid fluid. Although the mean for this variable was only about 1 per cent of the serum glucose mean, there was a measurable level in the parotid fluid of each participant. It seems quite clear that the endogenous salivary glucose is not a fermentable carbohydrate of importance in the decay process. The concentration of glucose is so low that its presence is of little importance when compared to the high level of intraoral carbohydrate that is produced by oral intake of these substances. This is in agreement with the observation of AFONSKY (1961) that salivary carbohydrates, because of the

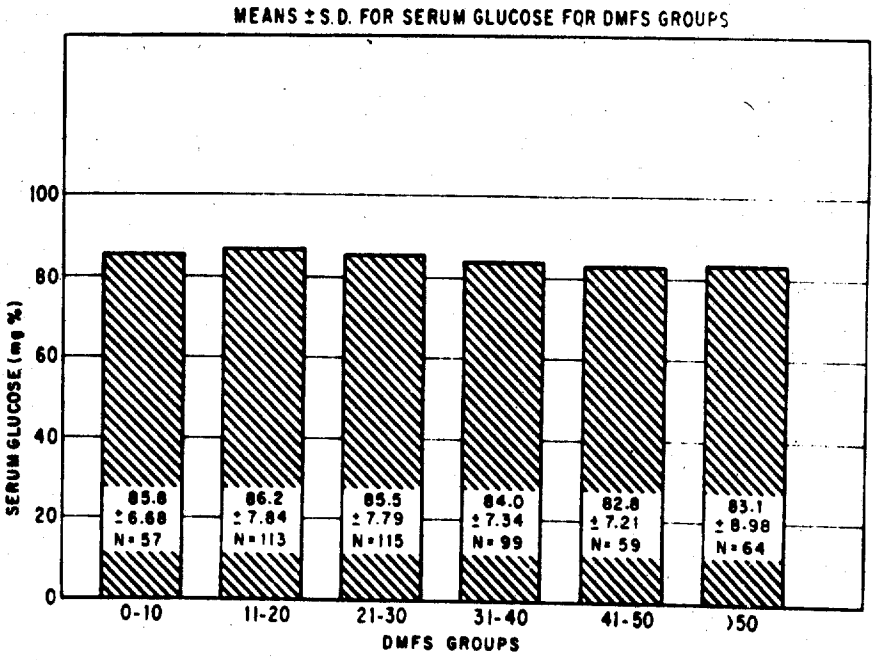

FIG. 2. Means ± S.D. for serum glucose for DMFS groups.

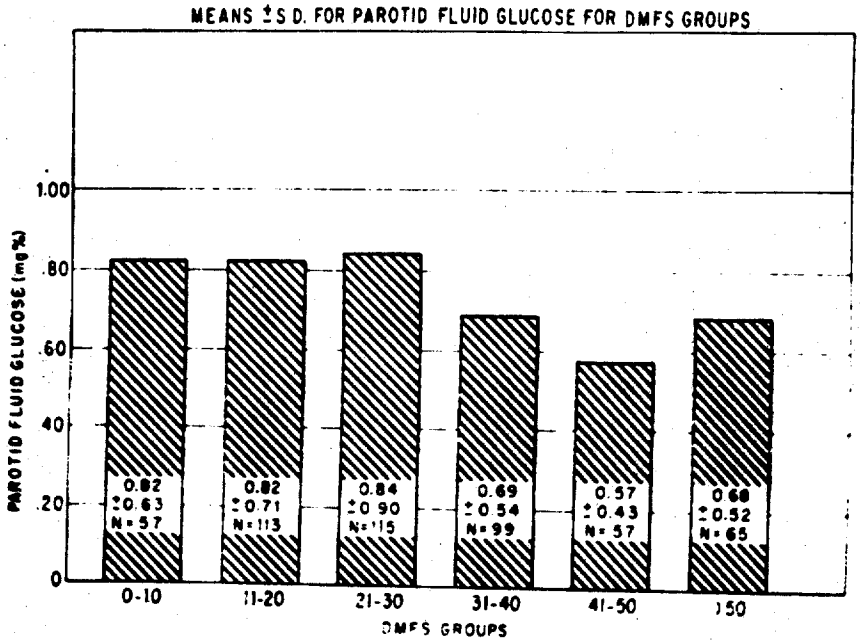

FIG. 3. Means ± S.D. for parotid fluid glucose for DMFS groups.

small amount present, probably play an insignificant role in acid formation in the saliva or in the plaque.

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Partial Substitution of Hexitols for Sucrose and Dextrin in Caries-producing Diets

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The hexitols, sorbitol and mannitol, have been used by bacteriologists for many years as test substrates in systematic fermentation trials for the classification and identification of microörganisms. Various strains of *Lactobacillus acidophilus* have been reported to have some ability to ferment sorbitol and mannitol.[1-3] Later, more exhaustive comparisons were made on the ability of a wide variety of strains of oral microörganisms to ferment sorbitol, glycerol, dextrose, and sucrose.[4-6] Numerous strains of common oral microörganisms were able to ferment sorbitol. However, routinely when fermentation of sorbitol occurred, it was found that the rate of acid production was slow, the total amount of acid produced was low, the final pH of the culture medium was rarely low enough to be capable of causing enamel decalcification, and the conditions for fermentation had to be optimal. Each microörganism that had any ability to ferment sorbitol could ferment dextrose or sucrose more rapidly than sorbitol. Furthermore, no greater ability to ferment sorbitol could be acquired readily.[6]

In vivo tests have been conducted in human subjects, where the pH of carious lesions was determined before and after sorbitol and sucrose rinses.[7, 8] In both studies the pH values after the sorbitol rinse did not decrease, in contrast with 5 minutes after the sucrose rinse, when readings of 6.0 or lower were observed. In one study[8] small increases in the pH values were observed after the sorbitol rinse instead of decreases; in addition, when a sucrose rinse was given 1 hour after a sorbitol rinse, the depression in pH values was not so great as when the sucrose rinse was used without the preliminary sorbitol rinse.

These studies suggested the need for trials with caries-susceptible animals to determine whether the partial replacement of sucrose or dextrin by sorbitol or mannitol in the caries-producing diet would alter the initiation and progression of carious lesions. In order for adequate tests to be conducted, relatively large amounts of sorbitol and mannitol would have to be incorporated into the diet; in so doing, extensive diarrhea could be expected to be produced in the experimental subjects. In addition, sorbitol—and mannitol to an even greater extent—are slowly absorbed from the intestinal tract and are not completely metabolized by the rat.[9-13] While these factors mitigate against an ideally designed and executed series of experiments, they were not believed to be sufficient to obscure the interpretation of the results from such experiments.

These studies were supported to a large extent by a grant-in-aid from the Atlas Powder Company, Wilmington, Delaware.

We are indebted to Dr. Lyon P. Strean, Merck, Sharp & Dohme, for the generous amounts of B-complex vitamins provided for use in these experiments.

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EXPERIMENTAL METHODS

Three experiments were conducted to test the influence of partial substitution of sorbitol or mannitol for sucrose and dextrin in the diet upon the initiation and progression of carious lesions. The subjects used were offspring of the Harvard caries-susceptible strain of rats; the breeding animals of this strain have been maintained without interruption for several generations on purified diet 2700 to continue a uniform and predictable dietary background throughout tooth development and maintenance. For these experiments the rats were weaned at 21 days of age and maintained for 7–14 days on diet 2700 prior to the initiation of the experiments. In preliminary trials it had been found necessary to use rats that were 28–35 days of age because of their greater ability to withstand the extensive diarrhea caused by all the diets containing 20 per cent or more of sorbitol or mannitol. In each of the three experiments, littermates were distributed as uniformly as possible, as a means to control any inherent variation from litter to litter. Throughout the experimental periods the rats were maintained in individual wire-bottom cages with food and water supplied ad libitum. After periods of 140 days in experiments 1 and 2 and 91 days in experiment 3, the rats were sacrificed and the heads preserved in 95 per cent ethanol preparatory to skinning and evaluation of the dental caries incidence by the procedure of Shaw, Schweigert, McIntire, Elvehjem, and Phillips.[14]

The first experiment was designed to test the influence of partial substitution of sorbitol for sucrose in a caries-producing diet on the ability of the rat to survive and grow and on the initiation and progression of carious lesions. This experiment was composed of 10 groups of subjects which were fed several modifications of our basal caries-producing diet 700[15] which are presented in Table 1. The experimental design is given in the first three columns of Table 2. The rats in the first group served as controls and received ration 700. The rats in the second group received ration 711, which is identical with ration 700 except that 200 of every 670 gm. of sucrose were replaced by an equal weight of powdered crystalline sorbitol.* The subjects in the third group received ration 2711, which is ration 711 to which 15 per cent Cellu flour has been added. The fourth group received ration 713, which is identical with ration 700 except that 450 of every 670 gm. of sucrose were replaced by sorbitol. Groups 5 and 6 received rations 2713 and 3713, respectively, which are the same as ration 713 except that 15 per cent and 45 per cent Cellu flour have been added. In the seventh and eighth groups, efforts were made to determine whether the sorbitol levels could be increased as the rats grew older, with less penalty than the younger rats incurred. In group 7, ration 700 was fed for 3 weeks, followed by 11 weeks on ration 713, followed by 6 weeks on carbohydrate-free ration 2717, in which all sucrose had been replaced by sorbitol and a 15 per cent Cellu flour supplement had been included. In group 8, ration 711 was provided for 3 weeks, followed by ration 2713 for 11 weeks and ration 2717 for the remaining 6 weeks. In the ninth group, ration 4700 was fed, which is equivalent to ration 700 except that a 100 per cent supplement of Cellu flour was added. In group 10, ration 4700 was fed for 3 weeks to accustom the rats to handling very large amounts of indigestible material, followed by ration 2713 for 11 weeks and ration 2717 for 6 weeks.

The design of experiment 2 is shown in the first three columns of Table 3 and is identical with that of experiment 1 except that a comparable series of diets was used in which the same amounts of powdered mannitol* instead of sorbitol were incorporated in substitution for sucrose.

The third experiment was designed to test the partial substitution of sorbitol or mannitol for dextrin in a series of diets in which all the sucrose had been replaced by dextrin. This change in design was made in the belief that the lower caries-initiating

* Atlas Powder Company, Wilmington, Delaware.

TABLE 1

COMPOSITION OF RATIONS IN GRAMS

Ration No.	Sucrose	Dextrin	Sorbitol	Mannitol	Vitaminized Casein*	Vitaminized Corn Oil*	Salt Mixture*	Whole Liver Substance	Cellu Flour
700	670	.....	.....	.....	240	50	40	40	.....
2700	670	.....	.....	.....	240	50	40	40	150
4700	670	.....	.....	.....	240	50	40	40	1,000
711	470	.....	200	.....	240	50	40	40	.....
2711	470	.....	200	.....	240	50	40	40	150
713	220	.....	450	.....	240	50	40	40	.....
2713	220	.....	450	.....	240	50	40	40	150
3713	220	.....	450	.....	240	50	40	40	450
2717	.....	.....	670	.....	240	50	40	40	150
712	470	.....	.....	200	240	50	40	40	.....
2712	470	.....	.....	200	240	50	40	40	150
714	220	.....	.....	450	240	50	40	40	.....
2714	220	.....	.....	450	240	50	40	40	150
3714	220	.....	.....	450	240	50	40	40	450
2718	.....	.....	.....	670	240	50	40	40	150
2720	.....	670	.....	.....	240	50	40	40	150
2721	.....	470	200	.....	240	50	40	40	150
2723	.....	220	450	.....	240	50	40	40	150
2722	.....	470	.....	200	240	50	40	40	150
2724	.....	220	.....	450	240	50	40	40	150

* J. D. Res., 26:47, 1947.

TABLE 2

INFLUENCE OF PARTIAL SUBSTITUTION OF SORBITOL FOR SUCROSE ON EXPERIMENTAL DENTAL CARIES

Group No.	Ration	No. Rats Begun	No. Survivors	Av. Weight Gain of Survivors (Gm.) ♂	Av. Weight Gain of Survivors (Gm.) ♀	Av. No. Caries Molars	Av. No. Caries Incisors	Av. Extent of Caries Incisors
1	700	5	4	215	156	9.5	21.8	89+
2	711	5	3	190	189	8.0	16.0	59+
3	713	6	4	250	134	7.8	15.8	60+
[illegible]	[illegible]	10	0	.....	.....	.....	.....	.....
[illegible]	2713	10	2	73	69	8.0	14.0	48+
[illegible]	3713	10	1	.....	39	6.0	12.0	41+
[illegible]	700-713-2717	5	3	81	46	9.0	16.3	63+
[illegible]	711-2713-2717	9	8	70	23	6.4	12.3	46+
[illegible]	4700	6	4	145	91	8.0	15.3	86+
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

influence of dextrin than sucrose would be less likely to obscure any changes in dental caries experience that occurred as a result of the inclusion of sorbitol or mannitol in the diet. This experiment consisted of 8 groups of rats and lasted for 91 days. The first group of rats received ration 2700. The rats in the second group can be considered as the controls against which the others will be compared. They received ration 2720, which is the same as ration 2700 except that all sucrose had been replaced by the same weight of dextrin. The subjects in group 3 received ration 2721, which was similar to ration 2720 except that 200 of every 670 gm. of dextrin were replaced by sorbitol. In group 4, the rats were fed ration 2723, which was similar to ration 2720 except that 450 of every 670 gm. of dextrin were replaced by sorbitol. The rats in group 5 were fed ration 2721 for 4 weeks followed by ration 2723 for 9 weeks. The rations fed

TABLE 3

INFLUENCE OF PARTIAL SUBSTITUTION OF MANNITOL FOR SUCROSE ON EXPERIMENTAL DENTAL CARIES

Group No.	Ration	No. Rats Begun	No. Survivors	Av. Weight Gain of Survivors (Gm.)		Av. No. Carious Molars	Av. No. Carious Lesions	Av. Extent of Carious Lesions
				♂	♀			
1	700	5	5	221	144	7.6	16.2	62+
2	712	6	4	252	129	7.5	14.0	46+
3	2712	5	2	184	63	7.5	12.5	41+
4	714	10	1	.....	90	4.0	8.0	32+
5	2714	10	2	.....	97	4.0	5.5	15+
6	5714	10	0	.....	.....	.....	.....	.....
7	700-714-2718	5	5	76	57	6.2	12.0	43+
8	712-2714-2718	9	6	115	48	5.3	8.4	26+
9	4700	5	5	147	94	8.6	16.7	68+
10	4700-2714-2718	5	3	.....	69	5.0	8.3	25+

groups 6, 7, and 8 were identical with those fed groups 3, 4, and 5, respectively, except that mannitol was used instead of sorbitol.

RESULTS

The data for experiments 1 and 2 are summarized in Tables 2 and 3, respectively. Relatively small numbers of rats were used in these experiments, as they were preliminary trials to determine the levels of sorbitol and mannitol that could be tolerated by our strain of caries-susceptible rats for the length of assay period required. Moderate to extensive diarrhea was observed in almost every rat that consumed a diet containing 20 per cent or more of sorbitol or mannitol. From these data it can be concluded that levels of 20 per cent sorbitol or mannitol, as in rations 711, 2711, 712, and 2712, can be tolerated reasonably well for prolonged periods by rats when begun at 28–35 days of age. Although about one-third of the rats on the 20 per cent levels died in the early days of the experiment, the remainder grew well and remained reasonably healthy, [illegible] diarrhea was moderate to extensive throughout the experimental period. In all [illegible] four rations, modest reductions in tooth decay were [illegible]

713, 2713, 3713, 714, 2714, and 3714, 80–100 per cent of the rats died in the early weeks of the experiments. The caries experience among the scattered survivors from these groups tended to be slightly lower than for the rats in the groups fed diets containing 20 per cent sorbitol or mannitol. Increases in the amount of the Cellu flour supplement from 0–15 to 45 per cent in the attempt to control the diarrhea did not appear to aid in the survival of these rats. Beginning the diets containing 45 per cent sorbitol or mannitol at later ages, as in group 7 of each experiment, increased the rate of survival, although the rats grew very poorly throughout the experimental period. Stepwise increases in the amounts of sorbitol and mannitol, as attempted in group 8 of each experiment, tended to permit increased survival. In groups 7 and 8 the surviving rats after 14 weeks on lower levels of sorbitol or mannitol were able to exist for 6 weeks on carbohydrate-free diets 2717 and 2718, which contained 67 per cent of one of the hexitols. Modest reductions in caries resulted from these procedures.

The addition of 100 per cent Cellu flour to the cariogenic diet in group 9 of each experiment did not alter the initiation and progression of carious lesions from that observed among the control rats in group 1, although their rate of growth was materially reduced. The beginning of the 45 per cent sorbitol and mannitol diets in group 10 of each experiment after a period of accommodation to diet 4700 containing the 100 per cent Cellu flour supplement permitted good survival. However, part or all of this effect may have been a result of the increased age and weight of the rats, as in group 7 of experiments 1 and 2. The rats in group 10 of both experiments had substantially less caries than did the control rats. The carious lesions in the rats of groups 7, 8, and 10 of both experiments where carbohydrate-free diets 2717 and 2718 had been fed for the 6 weeks preceding sacrifice were stained and hard, with no evidence of active progression of the carious process.

No statistical comparisons of these data are justified in view of the small number of rats. However, from inspection of the data in Tables 2 and 3 it can be concluded that wherever 20 per cent or more of sorbitol or mannitol was used in substitution for sucrose in the diets fed the groups of rats in these experiments, modest and consistent reductions in tooth decay were observed. These data strongly suggest that neither sorbitol nor mannitol supports the caries process under the conditions of these experiments. The amounts of sucrose in all diets except 2717 and 2718 are sufficient to promote a major rate of initiation and progression of carious lesions. This caries-promoting effect of the sucrose could overshadow any minimal initiation and progression caused by the hexitols.

The data from the third experiment are presented in Table 4. The rats in group 1 maintained on the sugar-containing cariogenic diet 2700 grew well and had a typical incidence of carious lesions. The control rats in group 2, which received diet 2720, where sucrose had been replaced by dextrin, grew almost as well as those fed diet 2700 and had, on the average, 11–23 per cent less caries. Moderate to extensive diarrhea was observed throughout the experimental period in practically every rat in groups 1–3. In group 3, where ration 2721 with approximately 20 per cent sorbitol was fed, the rats grew poorly, 6 of the 17 died, and significant reductions in caries were observed, varying from 43 per cent for the average number of carious molars to 53 per cent for the average extent of carious lesions. In group 4, where ration 2723 contained [illegible] per cent sorbitol was fed, only 2 of 17 rats survived the

full experimental period. These rats grew very slowly and had experienced an incidence of dental caries very little lower than the rats fed ration 2720. In view of the few survivors, no fair comparison can be made with respect to the influence of diet 2723 on experimental dental caries. In group 5, where ration 2721 was fed for 4 weeks followed by ration 2723 for 9 weeks, 9 of the 17 rats survived and grew relatively slowly. These rats had amounts of tooth decay which differed highly significantly from the rats fed ration 2720 and which were slightly, but insignificantly, lower than for the rats fed ration 2721 throughout the experimental period.

In group 6, where the rats were fed ration 2722 containing 20 per cent mannitol, 16 of the 17 survived and grew moderately well, although not so well as those fed ration 2720. These rats had less tooth decay than those fed ration 2720, with the re-

TABLE 4

INFLUENCE OF PARTIAL SUBSTITUTION OF SORBITOL AND MANNITOL FOR DEXTRIN ON EXPERIMENTAL DENTAL CARIES

Group No.	Ration No.	No. Rats Begun	No. Survivors	Av. Weight Gain of Survivors (Gm.)		Av. Weight of Crown and Contents (Gm.)	No. Carious Molars			No. Carious Lesions			Extent of Carious Lesions		
				♂	♀		Av.	SEM*	CR†	Av.	SEM*	CR†	Av.	SEM*	CR†
1	2700	18	18	199	117	3.4	7.3	0.5	1.4	13.3	1.3	1.2	44+	5+	1.3
2	2720	17	15	185	105	3.1	6.1	.7	4.0	10.9	1.5	3.8	34+	6+	4.0
3	2721	17	11	119	82	6.3	3.5	.8	2.5	5.4	1.6	2.5	16+	5+	2.3
4	2723	17	2	89	38	8.0	5.5	...	2.9	9.5	...	3.5	29+	...	3.3
5	2721–2723	17	9	104	66	10.9	3.0	.6	3.3	3.9	1.3	3.4	10+	4+	3.5
6	2722	17	16	134	92	7.4	4.5	.7	1.6	6.8	1.4	2.0	19+	5+	1.9
7	2724	16	1	142	...	17.0	4.0	...	0.7	5.0	...	1.8	13+	...	1.7
8	2722–2724	17	11	131	62	10.9	5.4	0.7		7.5	1.2		22+	4+	

* SEM = Standard error of mean.

† CR = Critical ratio. The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Wherever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; when from 2.0 to 2.9, moderately significant; when 3.0 or higher, highly significant.

ductions varying from 26 per cent for the average number of carious molars to 44 per cent for the average extent of carious lesions. These reductions were consistent but were just below the borderline of statistical significance. In group 7, where the rats were fed ration 2724 containing 45 per cent mannitol, only 1 rat survived, which had a very low dental caries experience. In group 8, where the rats were fed ration 2722 followed by ration 2724, 11 of the 17 survived; the males grew fairly well, although the females did much more poorly. These rats had about the same amount of tooth decay as those in group 6 fed ration 2722. Although there were consistent reductions in dental caries experience from the rats fed ration 2720, these reductions again were just below the borderline of statistical significance.

The caries and its contents were weighed together at the time the rats in experiment 3 were sacrificed. In the rats of groups 1 and 2 without sorbitol or mannitol in the diet, the caries and its contents weighed an average of 3.4 and 3.1 gm., respectively. The [illegible] in groups [illegible] were heavier. The rats in [illegible] and [illegible]

the diets containing 20 per cent sorbitol and mannitol were fed, respectively, had average weights for the cecum and its contents of 6.3 and 7.4 gm. Little data are available for groups 4 and 7 because of the few survivors: the cecum and contents of the two survivors in group 4 averaged 8.0 gm., while the cecum and contents of the one survivor in group 7 weighed 17.0 gm. In groups 5 and 8, where low levels of sorbitol were followed by high levels, the average weight of the cecum and its contents was 10.9 gm. for each group.

DISCUSSION

From these data the conclusion can be drawn that neither sorbitol nor mannitol supported the initiation and progression of carious lesions at a rate comparable to sucrose or dextrin under the experimental conditions in these trials. If these hexitols supported the caries process at all, their influence must have been completely obscured by the much greater influence of the amounts of the carbohydrates in the various diets. In those few experimental subjects to which carbohydrate-free diets containing 67 per cent sorbitol or mannitol were fed during the last 6 weeks of experiments 1 and 2, the impression was gained that caries activity had ceased in the lesions which had been initiated in the first 14 weeks on experiment and which were undoubtedly active at the time the feeding of the carbohydrate-free diets was begun.

Although sorbitol and mannitol have little or no ability to support the caries process, their use in human nutrition must be limited by reason of their limited tolerance. For example, Ellis and Krantz[10] found that very soft watery stools were produced by the daily consumption of 10–20 gm. of mannitol, 20–30 gm. of sorbitol, or 50 gm. of crystalline sorbitol by 12 normal individuals. In other studies, Peters and Lock[16] fed 25 gm. of sorbitol per day in 2 doses to 84 volunteers for 1 week. Discomfort and laxation were experienced by 5 subjects, 2 of whom did not complete the week. The remainder encountered no significant problem except for a slight tendency to flatulence after sorbitol ingestion. The amounts of sorbitol used as a sweetening agent and humectant in various confections are likely to be less than enough to cause problems unless excessive amounts of the confections are consumed. On the basis of the present experiments, it would seem likely that the use of sorbitol instead of sucrose or glucose in these confections would reduce their caries-producing ability.

SUMMARY

Three experiments were conducted with caries-susceptible white rats in which sorbitol or mannitol were used in partial substitution in cariogenic diets for sucrose or dextrin. Wherever 20 per cent or more of sorbitol or mannitol was present in the cariogenic diets, moderate but consistent reductions in caries experience were noted. Although an absolute statement cannot be made about the ability of sorbitol and mannitol to initiate or maintain the caries process because of the need for the simultaneous presence of some caries-initiating carbohydrates in the diets, it appears that these hexitols have little or no capacity to promote the caries process.

The tendency of sorbitol and mannitol to produce diarrhea among the experimental animals prohibited the feeding of high amounts for prolonged periods or their complete replacement for carbohydrates except under unusual circumstances. However, 20 [illegible] of the hexitols could be tolerated in [illegible]

to 140 days. Higher amounts could be tolerated, provided that the rats were older at the beginning of the feeding trials or that they had been accustomed to lower levels previously.

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Caries in Rats with Starch as the Only Dietary Carbohydrate

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Starch replacement for all monosaccharides or disaccharides in cariogenic diets has resulted in major reductions or prevention of caries in rodents (W. G. SHAFER, *Science* **110**: 143-144, 1949; G. FROSTELL, P. H. KEYES, and R. H. LARSON, *J Nutr* **93**:65-76, 1967). However, in experiments in this laboratory (unpublished results) some rats maintained on diets with corn starch as the only carbohydrate developed extensive carious lesions in the occlusal sulci and on the smooth surfaces. An experiment was conducted to determine if continuous use of corn starch as the only dietary carbohydrate for several generations leads to a greater frequency of rats with carious lesions and higher caries activity. The rats were descendants of stock obtained 20 years ago from the Wistar Institute's mutant albino strain. Throughout life rats were fed diet 2718, Harvard cariogenic diet 2700 in which all sucrose had been replaced by corn starch (J. H. SHAW, *J Nutr* **88**:419-426, 1966). Breeders were selected from each generation on the basis of grossly observable carious lesions. The remaining rats were killed to evaluate the extent of the carious process at 84 days of age in the first four generations, and at 150 days in the fifth through seventh generations. None of the rats, breeders or experimentals, throughout the seven consecutive generations consumed any monosaccharides or disaccharides. The indigenous flora of these rats was not modified by inoculation with known cariogenic flora. Comparable populations were maintained for the first four generations on diets that contained only sucrose or glucose as the carbohydrate.

Offspring of parents fed the starch diet grew less rapidly and were subject to higher neonatal mortality than offspring of parents fed the same diet with sucrose or glucose as the carbohydrate. The results for carious lesions in the occlusal sulci and on the smooth surfaces are shown in the table. Throughout the seven generations the percentage of cariesfree rats was lower and the extent of carious lesions at both types of sites in the molars was higher than would be expected from previously published data where starch was the only carbohydrate. Although the breeders were selected to emphasize the development of carious lesions, considerable variation was observed in the expression of caries from generation to generation. No trend was observed toward a higher frequency or more rapid progression of the lesions as a result of genetic selection. Among comparable rats fed sucrose or glucose only, an occasional rat was cariesfree. For the 69 rats fed the sucrose diet, caries activity in the occlusal sulci was 34.1+ ± 1.9+ and on the smooth surfaces it was 5.0+ ± 0.8+. For the 79 rats fed the glucose diet, caries activity in the occlusal sulci was 26.6+ ± 1.8+ and on the smooth surfaces it was 4.4+ ± 0.6+.

Typical carious lesions in a rat of the sixth generation are shown in the illustration.

Typical carious lesions in sixth generation rat.

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Additional information available on request to authors.

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TABLE
INFLUENCE OF STARCH DIET ON CARIES ACTIVITY

Generation	Age	No. of rats	Carious Lesions in Occlusal Sulci: Percentage of Cariesfree Rats	Carious Lesions in Occlusal Sulci: Extent of Carious Lesions	Carious Lesions on Smooth Surfaces: Percentage of Cariesfree Rats	Carious Lesions on Smooth Surfaces: Extent of Carious Lesions
1	84	8	37.5	12.4+ (5.1+)	12.5	10.1+ (2.6+)
2	84	5	60.0	10.6+ (3.0+)	0	5.9+ (1.5+)
3	84	6	66.7	2.2+ (1.5+)	50.0	0.7+ (0.4+)
4	84	12	33.3	10.4+ (3.3+)	33.3	2.1+ (0.6+)
5	150	26	73.1	5.5+ (2.4+)	46.2	3.5+ (1.0+)
6	150	27	22.2	12.0+ (4.8+)	18.5	8.3+ (1.4+)
7	150	47	38.3	16.7+ (2.9+)	25.5	5.7+ (0.6+)

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COMPARISON OF SUCROSE, LACTOSE, MALTOSE AND GLUCOSE IN THE CAUSATION OF EXPERIMENTAL ORAL DISEASES

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Summary—Complete replacement of sucrose in the periodontal syndrome-producing diet by glucose or by confectioner's sugar or one-half replacement by lactose did not have any significant effect on the prevalence and severity of the periodontal syndrome in the rice rat. Replacement of sucrose by maltose caused a significant reduction in the periodontal syndrome; replacement by whole wheat flour or white flour resulted in still greater reductions in the syndrome.

Three experiments with different combinations of strains of rats and diets were used to compare the caries-producing ability of sugars. Complete replacement of sucrose by glucose in diet 2700 did not have any effect on incidence of dental caries in rats of the mutant albino strain but did cause moderate reductions in rats of the Harvard caries-susceptible strain. Complete replacement of confectioner's sugar by glucose in diet 2000 caused highly significant reductions in initiation and severity of carious lesions, both in the occlusal sulci and on the smooth surfaces. However, replacement of granulated sugar, sucrose, for confectioner's sugar also caused significant reductions in both types of lesions. Maltose supported as rapid development of carious lesions as sucrose in all three experiments, but a lower rate of lesions on the smooth surfaces in one out of two experiments. Lactose replacement for half of the sucrose in one experiment led to a lower incidence of carious lesions in the sulci and on the smooth surfaces in one experiment but not in a second experiment.

Studies on mixed cultures from rats fed diets containing sucrose, glucose or maltose in one experiment led to the observation that maltose and sucrose seemed to promote somewhat greater synthesis of extracellular material than glucose. Some dextran has been synthesized from each of the carbohydrates. Comparison studies with plaque samples from five humans and oral swabbings from five hamsters led to the observation that extra-cellular slime was synthesized from sucrose alone.

INTRODUCTION

STUDIES along several lines have suggested that sucrose might support caries initiation and progression and plaque formation in experimental animals preferentially to glucose. SHAFER (1949) observed in hamsters that the caries-producing capacity of a diet containing 61 per cent sucrose was much higher than that of a comparable diet containing the same level of glucose. GUSTAFSON *et al.* (1955) reported that in hamsters under several different conditions sucrose and fructose caused more dental caries than glucose, lactose and maltose. Likewise, in a trial with rats, GRENBY (1963) reported that a 56 per cent reduction in caries scores occurred when sucrose was replaced by glucose, and KRASSE (1965a) in a study with albino hamsters observed very low caries

scores with glucose in contrast to high scores with sucrose. KRASSE (1965b) in a study on the implantation of caries-inducing streptomycin-resistant streptococci into hamsters' oral cavities reported a low recovery of such organisms when a commercial animal food or a glucose-containing diet was used in contrast to a high recovery rate when a sucrose diet was used instead. In a study with dental students, CARLSSON and EGELBERG (1965a) observed little plaque formation when a relatively low carbohydrate diet was fed and when the same diet was supplemented by 50 g of glucose daily but larger amounts of plaque if sucrose was consumed instead of glucose. However, in contrast, CARLSSON and EGELBERG (1965b) found no difference in the fairly rapid accumulation of plaque in dogs whether a soft diet composed of protein and fat was used with or without added sucrose.

Since our diet 2700, which contained 67 per cent sucrose, has been used as the breeding diet for numerous generations in our colonies of white rats and as the standard assay diet, the possibility existed that our selection of breeders for each generation on a phenotypic basis unwittingly resulted in a circumstance whereby sucrose would promote experimental caries in these populations at greater rates than other mono- or disaccharides. Likewise, the control diet in all our assays for the periodontal syndrome in the rice rat has been diet 700, which also contained sucrose. However, in the latter case, unlike the white rat, the breeding diet has been Purina Laboratory Chow (Ralston Purina Company, St. Louis, Missouri). In addition, since this diet did not promote sufficient progression of the periodontal syndrome to permit selection of breeders on the basis of phenotypic evidence, matings were made on the basis of the performance of the parents' siblings.

Therefore, experiments were undertaken in which sucrose was replaced by other mono- and disaccharides to determine whether sucrose was uniquely appropriate for the production of experimental dental caries in the occlusal sulci and on the smooth surfaces and also for the formation of plaque and its sequelae, the periodontal syndrome, in rice rats. Two experiments were conducted with rice rats in which all sucrose was replaced by glucose, maltose, confectioner's sugar, whole wheat flour or white flour, or one-half of the sucrose by lactose. Three experiments were conducted with white rats in which all sucrose was replaced by either maltose or glucose or one-half of the sucrose was replaced by lactose.

MATERIALS AND METHODS

The first two experiments were conducted with rice rats of our strain which has been prone to develop the periodontal syndrome. The composition of the diets is shown in Table 1. The first experiment consisted of three groups. The rice rats in Group 1 were fed diet 700, which is the routinely used assay diet for the production of the periodontal syndrome and contains 67 per cent of the major nutrient-bearing components as sucrose. The littermates in Group 2 were provided diet 717 in which all sucrose had been replaced by glucose. The subjects in Group 3 were given diet 727 where one-half of the sucrose had been replaced by lactose. The second experiment was composed of 5 groups with Group 1 serving as controls fed diet 700. The

TABLE 1. COMPOSITION OF DIETS IN GRAMMES

Diet No.	700	717	727	737	738	739	740	2700	2717	2727	2737	2000	2005	2006	2007
Sucrose	670	—	335	—	—	—	—	670	—	335	—	—	—	—	560
Glucose	—	670	—	—	—	—	—	—	670	—	—	—	560	—	—
Lactose	—	—	335	—	—	—	—	—	—	335	—	—	—	—	—
Maltose	—	—	—	670	—	—	—	—	—	—	670	—	—	560	—
Confectioner's sugar	—	—	—	—	—	—	670	—	—	—	—	560	—	—	—
Whole wheat flour	—	—	—	—	670	—	—	—	—	—	—	60	60	60	60
White flour	—	—	—	—	—	670	—	—	—	—	—	—	—	—	—
Vitaminized casein*	240	240	240	240	240	240	240	240	240	240	240	—	—	—	—
Skim milk powder	—	—	—	—	—	—	—	—	—	—	—	280	280	280	280
Vitaminized corn oil*	50	50	50	50	50	50	50	50	50	50	50	—	—	—	—
Salt mixture*	40	40	40	40	40	40	40	40	40	40	40	—	—	—	—
Whole liver substance†	40	40	40	40	40	40	40	40	40	40	40	10	10	10	10
Cellulose‡	—	—	—	—	—	—	—	150	150	150	150	—	—	—	—
Alfalfa herb powder§	—	—	—	—	—	—	—	—	—	—	—	30	30	30	30
Sodium chloride	—	—	—	—	—	—	—	—	—	—	—	20	20	20	20
Brewer's yeast‖	—	—	—	—	—	—	—	—	—	—	—	40	40	40	40
Totals	1040	1040	1040	1040	1040	1040	1040	1190	1190	1190	1190	1000	1000	1000	1000

*J. dent. Res. 26, 47 (1947).

†Wilson Laboratories, Chicago, Illinois.

‡Cellu flour®, The Chicago Dietetic Supply House, Inc., Chicago, Illinois.

§S. B. Penick Co., 258 Brunswick St., Jersey City, New Jersey.

‖Nutritional Biochemicals Corp., Cleveland, Ohio.

littermate subjects in Groups 2 through 5 received diets 737, 738, 739 and 740, respectively, in which all sucrose had been replaced completely by maltose, whole wheat flour, white flour or confectioner's sugar. These experiments were begun at weaning and continued for 84 days. All diets and tap water were provided *ad libitum*.

The third and fourth experiments were conducted with rats from our colonies of mutant albino and Harvard caries-susceptible rats, respectively. Both experiments were composed of four groups of littermates. The control rats in each Group 1 were provided with diet 2700 where the sole carbohydrate was sucrose. The littermates in the two Group 2's were fed diet 2717 in which all sucrose had been replaced by glucose. The animals in both Group 3's were provided with diet 2727 in which one-half of the sucrose had been replaced by lactose. The rats in Group 4 were given diet 2737 in which all sucrose had been replaced by maltose. The composition of these diets is presented in Table 1. All diets and tap water were provided *ad libitum* for the 60 days after weaning.

The fifth experiment was conducted with weanling rats of the Harvard susceptible strain and was composed of four groups. Group 1 was provided with diet 2000 (KEYES and JORDAN, 1964) which contains 56 per cent confectioner's sugar as well as the lactose present in the 28 per cent skim milk powder. Groups 2, 3 and 4 were provided with modifications of diet 2000 in which the confectioner's sugar was replaced in diets 2005, 2006 and 2007 with either glucose, maltose or granulated sucrose, respectively. The composition of these diets is shown in Table 1. This experiment was terminated after 60 days.

The five experiments were terminated by sacrifice of the subjects with excess ether inhalation and the heads stored in 95% ethanol. The periodontal syndrome in the rice rat was scored by the method of GUPTA and SHAW (1956). Carious lesions in the occlusal sulci were scored by the procedure of SHAW *et al.* (1944), while those on the smooth surfaces were scored by the method of SHAW (1966).

At the time the rats in Experiment 3 were sacrificed, samples of smooth surface plaque were taken from two rats in each of Groups 1, 2 and 4 for bacteriological studies. For comparative purposes, plaque samples obtained from five humans and oral swabbings from five hamsters were collected. The mixed microbial flora derived from these samples was studied for its ability to synthesize extracellular slimes presumed to be polysaccharides from glucose, maltose, and sucrose in the following manner. Plaque samples or swabbings were inoculated into media consisting of trypticase (BBL), 2%; NaCl, 0·2%; K_2HPO_4, 0·4%, KH_2PO_4, 0·2%; and supplemented with 5 per cent of either glucose, maltose or sucrose. The mixed cultures were incubated in Brewer jars filled with 95 per cent H_2 and 5 per cent CO_2 at 35°C for 3 days. After incubation, the cultures were blended in a Servall Omniminer and centrifuged to remove bacterial cells. Extracellular bacterial slime present in the culture supernatant liquors was precipitated by adding 1·5 vol. of ethanol. The turbidity which developed was estimated on a 0 to 4+ scale. Alcohol treated uninoculated culture medium and mixed cultures grown in sugar-free broth were used as controls.

To determine if the extracellular slime consisted in part of a dextran-like polysaccharide, the precipitated material was washed twice with ethanol and dissolved in

saline. The saline solutions were tested for precipitation with antidextran antiserum in gel diffusion plates as previously described (GIBBONS and BANGHART, 1967). Controls consisting of normal rabbit serum and dextran absorbed antiserum were included.

RESULTS

The data for Experiments 1 and 2 are presented in Table 2. The weight gains permitted by the three diets in Experiment 1 with different carbohydrate sources were very similar, both among males and females. Likewise, the periodontal syndrome as

TABLE 2. INFLUENCE OF TYPE OF CARBOHYDRATE ON DEVELOPMENT OF PERIODONTAL SYNDROME IN THE RICE RAT

Group	Diet	No. of rats		Wt. gain (g)		Soft tissue lesions		Alveolar bone resorption	
		♂	♀	♂	♀	No. of areas	Ext. of areas	No. of areas	Ext. of areas
Experiment 1									
1	700	8	10	54·3 (4·2)	29·2 (2·4)	26·2 (0·9)	50·8+ (5·3+)	27·8 (0·1)	66·2+ (4·7+)
2	717	9	9	49·6 (2·8)	29·7 (2·0)	27·7 (0·2)	61·3+ (2·9+)	28·0 (0)	74·2+ (2·7+)
3	727	7	6	50·0 (4·9)	31·6 (3·6)	27·8 (0·2)	43·7+ (3·5+)	28·0 (0)	59·0+ (2·6+)
Experiment 2									
1	700	12	15	35·4 (2·7)	18·9 (2·0)	27·6 (0·3)	46·7+ (3·0+)	28·0 (0)	62·3+ (2·6+)
2	737	10	7	32·0 (3·2)	25·0† (2·0)	24·9† (1·1)	23·3+* (0·7+)	27·6† (0·2)	38·9+* (1·6+)
3	738	10	9	46·4† (3·2)	17·2 (1·3)	17·4* (2·1)	9·2+* (1·1+)	24·6* (0·7)	18·6+* (1·2+)
4	739	9	8	41·2 (3·4)	19·4 (1·8)	23·3* (1·0)	12·0+* (0·5+)	27·2† (0·3)	21·8+* (1·1+)
5	740	9	9	30·6 (2·8)	18·4 (2·7)	28·0 (0)	41·3+ (4·1+)	28·0 (0)	56·5+ (4·3+)

Values in parentheses are standard errors of means.
*1% level of significance.
†5% level of significance.

measured for lesions of the soft tissues or in terms of alveolar bone resorption were very similar for the three groups. A slight tendency was noted for a more rapid progression of the syndrome in Group 2 with glucose than in Group 1 with sucrose and for a slower progression of the syndrome in Group 3 with one-half of the sucrose replaced by lactose than in Group 1. However, these differences were not statistically significant at the 5 per cent level. Within the limits of the conditions in this experiment, glucose and a mixture of one-half lactose and one-half sucrose were statistically indistinguishable from sucrose in their ability to promote plaque formation, breakdown of the soft tissues, and alveolar bone resorption. However, the increase permitted by glucose and the decrease caused by one-half sucrose and one-half lactose

resulted in differences between Groups 2 and 3 that were statistically highly significant ($p < 0{\cdot}01$).

The weight gains supported by the five diets in Experiment 2 were in general not as great as usual, for which we have no explanation. The females in Group 2 fed diet 737 containing maltose and the males in Group 3 fed diet 738 containing whole wheat flour attained better and more nearly normal body weights than any other groups.

The substitution of maltose for sucrose in Group 2 resulted in a highly significant reduction in the periodontal syndrome as measured for soft tissues and for alveolar bone resorption. This reduction was of a percentage somewhat greater than for the substitution of one-half of the sucrose by lactose in Experiment 1. The substitution of whole wheat flour or of white flour for sucrose in Groups 3 and 4 resulted in even greater reductions in the periodontal syndrome than substitution of sucrose by maltose. In Group 5 the replacement of sucrose in the form of granulated sugar by finely ground confectioner's sugar had no influence on the periodontal syndrome. No bacterial evaluations were made in these two experiments to determine whether different distributions occurred within the microflora or if any of the micro-organisms had different metabolic properties as a result of the provision of the different carbohydrates.

The results of Experiment 3 with the mutant albino strain of rats that have been prone to develop carious lesions in the occlusal sulci and on the smooth surfaces are presented in Table 3. The weight gains for all four groups were similar among both males and females. In Group 3 where one-half of the sucrose had been replaced by lactose, while most of the rats grew normally, some of the rats grew very poorly, which depressed the average weight gain and increased the variability within the males and females of this group. This observation suggests that the level of lactose provided was on the borderline of toxicity at the 5 per cent level of fat provided in these diets. In these 4 groups, none of the rats was free of carious lesions in the occlusal sulci. The initiation and progression of this type of lesion were virtually identical for the rats in Groups 1, 2 and 4, irrespective of whether sucrose, glucose or maltose was the carbohydrate source. However, in Group 3 where one-half of the carbohydrate was lactose, a tendency toward a lower number of carious lesions ($p < 0{\cdot}05$) and a lower extent of carious lesions (insignificant) in comparison to Group 1 were observed.

With respect to carious lesions on the smooth surfaces of the molars, some rats in each group were caries-free. In particular, in Group 3, 42·1 per cent had no evidence of this type of lesion. In Groups 1, 2 and 4, the initiation and progression of lesions on the smooth surfaces were very similar to each other. However, highly significant reductions in the average number of carious molars and the average number of carious lesions and a significant reduction in the extent of carious lesions were observed in Group 3 where partial sucrose replacement by lactose had been initiated.

Clearly both for carious lesions in the occlusal sulci and on the smooth surfaces, sucrose, glucose and maltose were indistinguishable in their ability to promote experimental dental caries in this experiment. However, one-half replacement of sucrose by lactose definitely decreased the initiation and progression of lesions on the smooth surfaces of the molars; while the results were not as definitive with regard to the lesions in the occlusal sulci, the data suggested a similar but lesser effect.

TABLE 5. INFLUENCE OF TYPE OF SUGAR ON DEVELOPMENT OF CARIOUS LESIONS IN OCCLUSAL SULCI AND ON SMOOTH SURFACES IN THE MUTANT ALBINO RAT

Group	Diet	No. of rats		Wt. gain (g)		Carious lesions in occlusal sulci				Carious lesions on smooth surfaces			
		♂	♀	♂	♀	Caries-free rats (%)	No. of carious molars	No. of carious lesions	Ext. of carious lesions	Caries-free rats (%)	No. of carious molars	No. of carious lesions	Ext. of carious lesions
1	2700	12	12	181 (6)	118 (3)	0	6·7 (0·3)	14·0 (0·8)	50·2+ (3·1+)	4·2	4·4 (0·4)	4·5 (0·4)	11·5+ (0·9+)
2	2719	10	11	167 (8)	114 (3)	0	6·0 (0·6)	13·4 (0·9)	48·2+ (3·2+)	9·5	3·8 (0·4)	4·0 (0·5)	10·0+ (1·3+)
3	2727	10	9	159 (12)	107 (5)	0	5·8 (0·5)	10·6† (1·4)	39·0+ (6·1+)	42·1	2·2* (0·5)	2·2* (0·5)	5·8+† (1·5+)
4	2737	10	13	186 (6)	113 (3)	0	7·3 (0·3)	14·8 (0·7)	52·7+ (3·5+)	17·4	4·0 (0·5)	4·1 (0·5)	10·4+ (1·4+)

Values in parentheses are standard errors of means.
*1% level of significance.
†5% level of significance.

Mixed plaque cultures derived from rats in Groups 1, 2 and 4 were found to synthesize significant quantities of extracellular slime when grown in either glucose, maltose, or sucrose broth (Table 4). The relative quantity of material synthesized from

TABLE 4. SYNTHESIS OF EXTRACELLULAR SLIME BY MIXED ORAL CULTURES DERIVED FROM RATS, HUMANS AND HAMSTERS

	Sample number	Sucrose broth		Glucose broth		Maltose broth	
		Slime production*	Dextran†	Slime production	Dextran	Slime production	Dextran
Rats							
Group 1	1	4+	4+	3+	1+	4+	1+
(sucrose diet 2700)	2	4+	4+	3+	1+	4+	1+
Group 2	1	2+	4+	3+	2+	4+	1+
(glucose diet 2717)	2	4+	4+	3+	1+	4+	1+
Group 4	1	4+	4+	3+	1+	4+	2+
(maltose diet 2737)	2	4+	4+	4+	1+	4+	2+
Humans	1	4+	4+	0	—	0	—
	2	4+	4+	0	—	0	—
	3	4+	4+	0	—	0	—
	4	3+	4+	0	—	0	—
	5	2+	4+	0	—	0	—
Hamsters (laboratory chow)	1	4+	4+	0	—	0	—
	2	4+	4+	0	—	0	—
	3	4+	4+	0	—	0	—
	4	2+	4+	0	—	0	—
	5	1+	4+	0	—	0	—

*Extracellular slime present in culture liquor judged by ethanol precipitation.
†Intensity of precipitin band formed with antidextran antiserum.

each sugar did not differ markedly between plaque cultures derived from rats maintained on either a sucrose, maltose, or glucose diet. In contrast, samples of human dental plaque and oral swabbings from hamsters formed detectable amounts of extracellular slime from sucrose alone. None of the cultures from the rat, the hamster, or human subjects produced extracellular material in sugar-free broth.

Saline solutions of all slime samples reacted with antidextran serum, but not with normal rabbit serum or dextran absorbed antiserum, indicating that the slimes contained material serologically similar to dextran. The precipitin bands which developed with extracellular material synthesized in sucrose broth by plaque cultures from the albino rats were more intense than those which formed with the slimes synthesized in glucose or maltose broth. This evidence suggests that the material formed from sucrose contained a higher proportion of dextranlike polysaccharide than that synthesized from other sugars.

The results of Experiment 4 are tabulated in Table 5. The weight gains for the females were very similar for all four groups. Among the males, slightly but statistically insignificantly lower rates of growth were observed among the males in Groups 2 and

TABLE 5. INFLUENCE OF TYPE OF SUGAR ON DEVELOPMENT OF CARIOUS LESIONS IN OCCLUSAL SULCI OF HARVARD CARIES-SUSCEPTIBLE RATS FED DIETS OF THE 2700 SERIES

Group	Diet	No. of rats ♂	No. of rats ♀	Wt. gain (g) ♂	Wt. gain (g) ♀	Caries-free rats	No. of carious molars	No. of carious lesions	Ext. of carious lesions
1	2700	10	11	196 (5)	121 (5)	0	6·4 (0·3)	10·8 (0·8)	38·8+ (3·6+)
2	2717	12	12	186 (6)	129 (4)	0	4·9* (0·4)	8·8 (1·0)	27·0+† (3·7+)
3	2727	11	13	183 (4)	120 (4)	0	6·8 (0·3)	11·8 (0·9)	42·0+ (3·0+)
4	2737	12	12	196 (6)	122 (3)	0	7·4† (0·3)	13·0† (0·7)	42·3+ (2·8+)

Values in parentheses are standard errors of means.

*1% level of significance.

†5% level of significance.

3 than 1 and 4. The incidence of dental caries in the occlusal sulci varied somewhat in dependence upon the type of carbohydrate. In Group 2 where glucose replaced all sucrose, the number of carious molars was decreased by a highly significant degree ($p<0{\cdot}01$), the number of carious lesions was decreased but not at a statistically significant level, while the extent of carious lesions was reduced by a statistically significant amount ($p<0{\cdot}05$). The replacement of one-half of the sucrose in Group 3 by lactose did not cause any discernible change in any of the three criteria. In Group 4 the complete replacement of sucrose by maltose resulted in modest increases in all three criteria which were statistically significant at the 5 per cent level for the number of carious molars and the number of carious lesions. Lesions on the smooth surfaces were not present in this experiment except for small lesions in three rats in Group 3.

The results of Experiment 5 are tabulated in Table 6. In all four groups, the males and females grew more slowly than comparable rats of the same strain in Experiment 4 maintained on diets of the 2700 series. Slow rates of growth on diets of the 2000 series have been consistently observed in our laboratories in comparison wtih diets of the 2700 series. Female rats in all four groups grew at almost identical average rates. However, among the males, growth was more rapid on diets 2005 and 2007 where glucose and sucrose, respectively, had been used in place of confectioner's sugar. Both increases were statistically significant at the 5 per cent level.

Among the four groups, one rat in Group 2 was free of carious lesions in the occlusal sulci. The average values for carious lesions in the occlusal sulci among the rats in Group 2 where glucose was used in place of confectioner's sugar were less than the values for Group 1 by amounts which were highly significant. For example, the average extent of carious lesions for Group 2 was 37·8 per cent less than for Group 1. However, even with this reduction, the carious process could still be considered to be progressing rapidly among the rats in Group 2. In Group 3 where maltose was used instead of confectioner's sugar, modest reductions in the average number of

TABLE 6. INFLUENCE OF TYPE OF SUGAR ON DEVELOPMENT OF CARIOUS LESIONS IN OCCLUSAL SULCI AND ON SMOOTH SURFACES OF HARVARD CARIES-SUSCEPTIBLE RATS FED DIETS OF THE 2000 SERIES

						Carious lesions in occlusal sulci				Carious lesions on smooth surfaces			
Group	Diet	No. of rats		Wt. gain (g)		Caries-free rats (%)	No. of carious molars	No. of carious lesions	Ext. of carious lesions	Caries-free rats (%)	No. of carious molars	No. of carious lesions	Ext. of carious lesions
1	2000	11	12	120 (5)	92 (4)	0	8·1 (0·2)	16·2 (0·7)	57·1+ (2·8+)	17·4	4·1 (0·6)	5·5 (1·0)	11·4+ (2·2+)
2	2005	13	8	133† (5)	92 (6)	4·8	6·5* (0·5)	11·1* (1·2)	35·5+* (3·9+)	57·1	2·1† (0·7)	2·7† (0·9)	5·6+† (2·0+)
3	2006	10	12	115 (3)	88 (3)	0	8·2 (0·3)	15·3 (0·7)	49·1+ (3·7+)	27·3	2·6† (0·5)	3·2† (0·6)	6·5+† (1·4+)
4	2007	12	11	137† (6)	88 (4)	0	7·6 (0·3)	13·7† (0·8)	48·9+† (2·6+)	43·5	2·3† (0·5)	3·0† (0·8)	7·0+ (2·1+)

Values in parentheses are standard errors of means.

*1% level of significance.

†5% level of significance.

carious lesions and in the average extent of carious lesions were observed which were not statistically significant. In Group 4 where sucrose as granulated sugar was used instead of the finely powdered form of sucrose, confectioner's sugar, reductions were observed in all three caries scoring criteria which were statistically significant at the 5 per cent level for the average number of carious lesions and for the average extent of carious lesions.

With respect to carious lesions on the smooth surfaces, some rats in each group did not develop detectable lesions. The percentage of rats caries-free with respect to the smooth surfaces increased from 17·4 per cent in Group 1 to 27·3 per cent in Group 3, to 43·5 per cent in Group 4, to 57·1 per cent in Group 2. The average prevalences of carious lesions on the smooth surfaces were lower in Groups 2, 3 and 4 than in Group 1 by amounts which were of statistical significance at the 5 per cent level for all comparisons except the average extent of carious lesions for Group 4.

DISCUSSION

Under our experimental conditions, the initiation and progression of the periodontal syndrome in the rice rat was not altered significantly by substitution of sucrose by the same level of glucose, or confectioner's sugar, or by one-half replacement of sucrose by lactose. Tendencies for glucose to increase and lactose to decrease the occurrence of the syndrome were observed. However, replacement of sucrose completely by maltose caused highly significant reductions and replacement by whole wheat flour or white flour resulted in even greater reductions in the periodontal syndrome. This same type of substitution is not known to have been tested by other investigators. Earlier studies have shown that replacement of sucrose by dextrin resulted in moderate reductions in the periodontal syndrome and that replacement of sucrose by ground rice resulted in highly significant reductions (SHAW and GRIFFITHS, 1961).

The three experiments on dental caries initiation and progression in white rats indicated that under the currently prevailing conditions in our laboratories the replacement of sucrose by glucose had much less influence than had been reported by GRENBY (1963) for rats or by SHAFER (1949), GUSTAFSON *et al.* (1955) and KRASSE (1965a) for hamsters. Particularly when diet 2700 and its modifications were fed to rats of the mutant albino strain, sucrose, glucose or maltose appeared to be indistinguishable in their ability to support the carious process. When a mixture of one-half sucrose and one-half lactose was used in this dietary series, some reduction in occlusal lesions was observed as well as a significant reduction in lesions on the smooth surfaces. However, when diets of the 2000 series were used, some additional variations in the ability of the different carbohydrates to support the carious process were observed. Sucrose, as granulated sugar, and maltose were very similar to each other. Glucose tended to result in less caries involvement in the occlusal sulci but had no different effect than sucrose or maltose for lesions on the smooth surfaces. However, confectioner's sugar, which is finely ground sucrose with 3 per cent starch added, proved to be better able to support the carious process in the occlusal sulci and on the

smooth surfaces than granulated sugar, maltose or glucose. The difference between confectioner's sugar and granulated sugar had not been expected but may be explained on the basis of the extremely fine particle size of the former product which may be able to pass more easily into the sulci and be more readily adherent to the dental plaque on the smooth surfaces. However, the same substitution of confectioner's sugar for granulated sugar in diet 2700 did not cause any demonstrable difference in the initiation and progression of carious lesions on the smooth surfaces or in the occlusal sulci (unpublished data).

Again in Experiment 4 with the rats of the Harvard caries-susceptible strain and diets of the 2700 series, reductions in dental caries were observed when glucose was used in replacement of sucrose. This result was comparable to that seen with rats of the Harvard susceptible strain fed diets of the 2000 series and different from the result with rats of the mutant albino strain fed diets of the 2700 series. These results suggest the interplay among the strain of rats, the type of diets and the oral flora which enters into every experiment on the initiation and progression of dental caries.

Recently data have been accumulating which suggest that extra-cellular bacterial slimes are essential for plaque and caries formation (CARLSSON, 1965; GIBBONS and BANGHART, 1967). Rodent and human cariogenic streptococci studied by FITZGERALD and KEYES (1960), KRASSE (1965a, 1965b) and GIBBONS *et al.* (1966) have been found to synthesize large quantities of extracellular dextran primarily from sucrose, and this polysaccharide has been found to be a component of the matrix of human dental plaque (GIBBONS and BANGHART, 1967). KRASSE (1965a, 1965b) reported that hamster cariogenic organisms of this type became established, and induced plaque and caries formation in hamsters maintained on a sucrose-containing diet, but not in animals maintained on diets containing certain other carbohydrates.

It is interesting that mixed cultures derived from oral swabbings of hamsters synthesized extracellular slime from sucrose, but not from glucose or maltose. Cultures of human dental plaque responded to these sugars in a similar manner to those from hamsters. CARLSSON and EGELBERG (1965a) have reported that sucrose, but not glucose, promoted plaque formation in man. In contrast to these findings, the albino rats studied in the present investigation developed masses of bacterial plaque and dental caries when fed diets containing glucose or maltose, as well as sucrose. In addition, mixed plaque cultures derived from rats on each of these dietary regimes were capable of synthesizing extracellular material from each of these sugars. These data indicate that the bacterial flora resident in these albino rats differed from that inhabiting the hamsters and humans studied in this investigation. Such microbial differences would seem to account for the divergent findings concerning the abilities of various sugars to promote caries in different species. Further studies are needed to explain the different results observed with different combinations of diets and strains of rats.

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Résumé—Le remplacement de saccharose dans le régime, provoquant des lesions de parodontolyses, par du glucose ou du sucre de pâtissier ou par un remplacement par moitié de lactose n'a pas d'effet significatif sur la fréquence et la gravité de la parodontolyse chez le rat du riz. Le remplacement de saccharose par du maltose provoque, par contre, une réduction significative: le remplacement par de la farine de blé complète donne même une réduction plus importante.

Trois expériences combinant diverses espèces de rats et de régimes sont réalisées pour tester la potentialité cariogène des sucres. Le remplacement total du saccharose par du glucose dans le régime 2700 n'a pas d'effet sur la fréquence de la carie dentaire chez le rat de l'espèce albinos; il produit une réduction modérée chez les rats de l'espèce Harvard, susceptible à la carie. Le remplacement total du sucre de pâtissier par du glucose dans le régime 2000 donne une réduction hautement significative dans le déclanchement et la gravité de la carie dentaire, à la fois dans les fissures occlusales et les surfaces lisses. Cependant, le remplacement de sucres en poudre et de saccharose par du sucre de pâtissier donne aussi des réductions significatives des deux types de lésions. De même que le saccharose, le maltose provoque le même développement rapide de lésions carieuses dans les trois expériences, mais moins de caries des surfaces lisses ont été observées dans l'une des trois investigations. Le remplacement de la moitié du saccharose par du lactose a donné, dans un essai, une fréquence moins élevée de caries des fissures et des surfaces lisses, dans un autre essai, mais non dans le suivant.

Des études de cultures mixtes de rats, soumis à des régimes contenant du saccharose, glucose ou maltose indiquant que le maltose et le saccharose semblent synthétiser plus de matériel extra-cellulaire que le glucose. Un peu de dextrane est synthétise à partir de chaque hydrate de carbone. Des études comparatives menées avec des echantillons de plaques de 5 patients et des frottis buccaux de 5 hamsters montrent que du matériel extra-cellulaire n'est synthétise que par le saccharose.

Zusammenfassung—Der vollständige Ersatz von Rohrsucker in der das Periodontalsyndrom verursachenden Diät durch Glukose oder durch Puderzucker, oder der hälftige Ersatz durch Laktose zeigte keinerlei signifikante Wirkung auf Prävalenz und Schweregrad des Periodontalsyndroms bei der Reisratte. Der Ersatz des Rohrzuckers durch Maltose verursachte eine signifikante Reduktion des Periodontalsyndroms; Ersatz durch Vollweizenmehl oder Weißmehl führte zu noch größeren Reduktionen des Syndroms.

Drei Versuche mit verschieden kombinierten Rattenstämmen und Diäten wurden ausgeführt, um die kariesproduzierende Fähigkeit der Zucker zu vergleichen. Der vollständige Ersatz des Rohrzuckers durch Glukose bei der Diät 2700 ergab keinerlei Wirkung auf das Auftreten von Karies bei Ratten des mutanten Albinostammes, er verursachte jedoch mittlere Reduktionen bei Ratten des kariesanfälligen Harvard-Stammes. Der vollständige Ersatz von Puderzucker durch Glukose bei der Diät 2000 verursachte sowohl in den okklusalen Fissuren als auch an den Glattflächen hochsignifikante Reduktionen hinsichtlich Beginn und Schweregrad kariöser Läsionen. Der Ersatz körnigen Rohrzuckers durch Puderzucker verursachte jedoch auch signifikante Reduktionen bei beiden Arten von Läsionen; Maltose unterstützte wie Rohrzucker die schnelle Entwicklung kariöser Läsionen in allen 3 Versuchen; in einem von 2 Experimenten war jedoch die Anzahl der Läsionen an den Glattflächen geringer. Der Laktoseersatz für eine Hälfte des Rohrzuckers führte in einem Experiment zur verminderten Entstehung kariöser Läsionen in den Fissuren und an den Glattflächen, jedoch nicht im zweiten Experiment.

Untersuchungen an gemischten Kulturen von Ratten, die mit Rohrzucker, Glukose oder Maltose enthaltenden Diäten gefüttert worden waren, führten in einem Experiment zu der Beobachtung, daß Maltose und Rohrzucker die Synthese extrazellulären Materials etwas mehr zu förden schien als Glukose. Aus jedem der Kohlenhydrate wurde etwas Dextran synthetisiert. Vergleichs-studien mit Plaquesproben von 5 Menschen und Mundabstrichen von 5 Hamstern zeigten, daß extrazellulärer Schleim allein aus Rohrzucker synthetisiert wurde.

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THE FERMENTATION OF SORBITOL BY CERTAIN ACIDOGENIC ORAL MICROORGANISMS

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SORBITOL possesses certain properties such as sweetness and humectant action that would make it a desirable constituent of oral preparations. The question of whether it would be fermented by oral microorganisms and hence contribute to caries, however, arises. Grubb[1] conducted studies along this line and, in general, arrived at the conclusion that it would be favorable, compared with glucose and sucrose, since it was fermented more slowly than these sugars when it was fermented at all. Our studies have been designed to obtain a more definite answer to the question of sorbitol fermentation by oral microorganisms both by pure culture and the mixed flora of saliva and to determine certain factors in this fermentation that would be pertinent to the situation in the oral cavity.

MATERIALS

The pure cultures employed in this investigation were freshly isolated from saliva of caries-susceptible individuals, unless otherwise noted. Since the saliva was obtained by paraffin stimulation, the isolated organisms should also include some from the dental plaque. The lactobacilli were isolated on tomato juice agar at pH 5 and designated "rough" and "smooth" according to their colonial morphology. These designations probably correspond generally to *Lactobacillus acidophilus* and *L. casei*, respectively, as described by Rogosa, Wiseman, Mitchell, and Disraeley.[2]

The chemicals employed in this investigation have all been C.P. grade. "Sorbo," commercial grade sorbitol (manufactured by The Atlas Powder Company), was also tested and the results with this product are entirely comparable to those with C.P. sorbitol.

The methods employed in this investigation are standard and need not be described in detail. In the experimental section will be found brief descriptions of how these methods were employed in the several aspects of the study.

EXPERIMENTAL

Pure Culture Studies.—One of the first problems encountered in this study was finding a carbohydrate-free medium which would support the growth of the oral bacteria under investigation. In an attempt to find such a medium,

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a preliminary study of 7 lactobacillus, 6 streptococcus, and 3 staphylococcus cultures was conducted. The cultures were maintained in brain-heart infusion broth. Three loops of each culture were inoculated into 5 ml. of 2 per cent heart infusion broth and into 2 per cent trypticase broth containing 0.1 per cent yeast extract with and without 1 per cent glucose, glycerol, and sorbitol. Bromcresol purple at a concentration of 0.2 per cent was used as an indicator for acid production. Examinations of the cultures were made at 24 and 48 hours.

Of the 7 lactobacilli, 2 were described as "rough" forms on the basis of their colonial morphology when grown on tomato juice agar at pH 5.0. The other 5 were described as "smooth" strains which presented a smooth, glistening type of growth. It had been previously observed that the so-called "rough" strains grew with difficulty in tomato juice broth.

On comparing the 2 test media for support of growth and acid production, trypticase yeast extract broth appeared to be superior. In addition, it was observed that maximal growth and acid production were obtained in broth containing glucose. Growth in media containing sorbitol or glycerol or no added carbohydrate was considerably slower. The results for acid production in the presence of the various substrates are shown in Table I. In the 2 cases where sorbitol was fermented by the lactobacilli, it was not indicative of strong acid production. The 2 "rough" strains of lactobacilli did not grow, and hence no production of acid was observed.

TABLE I
FERMENTATION REACTIONS OF ORAL MICROORGANISMS

TYPE OF ORGANISMS	NUMBER TESTED	NUMBER FERMENTING GLUCOSE	NUMBER FERMENTING GLYCEROL	NUMBER FERMENTING SORBITOL
Streptococci	6	6	3	2
Lactobacilli (smooth)	5	5	0	2
Lactobacilli (rough)	2	0*	0*	0*
Staphylococci	3	3	2	0

*No growth.

Inasmuch as unmodified tomato juice broth (TJB) contained a considerable amount of fermentable carbohydrate, it was not usable as a test medium. It was necessary to remove most of the carbohydrate from the tomato juice (TJ) by inoculating it with yeast cells and incubating for 24 hours. Media containing 40 per cent fermented tomato juice, 2 per cent trypticase, 0.1 per cent yeast extract, 1 per cent peptone, and 5 mg. of malate, citrate, alpha-ketoglutarate, and the sodium salt of ethyl oxalacetate were added per tube. Glycerol, glucose, and sorbitol were the test carbohydrates.

Fermentation of the glucose and sorbitol by the "smooth" strains was more rapid than in previous observations. "Rough" strains of the lactobacilli grew very little if any. Glycerol was attacked by only 2 streptococci and none of the lactobacilli. Further attempts to develop a medium which would give consistent growth and acid production of both "rough" and "smooth" forms of the lactobacilli were made by separately adding various

organic compounds. Among these were adenosine triphosphate, adenylic acid, magnesium hexose diphosphate, lactose, maltose, and sucrose. None of these compounds stimulated the growth of the "rough" strains.

It was finally found that by adding Tween 80 at a concentration of 0.05 per cent to the medium containing 40 per cent fermented tomato juice, 2 per cent trypticase, 0.1 per cent yeast extract, 1 per cent peptone, and the acids of the tricarboxyllic acid cycle previously used, we could obtain good growth of the "rough" strains. Rogosa and Mitchell[3] had previously reported on the beneficial effect of Tween 80.

With the development of such a medium, we were able to run fermentation tests on all the lactobacilli. Thirty lactobacillus cultures were used in these tests. Of the 30 cultures, 11 were of the "rough" variety. Six of the "smooth" organisms were from caries-immune individuals.* Only one "rough" organism from caries-immune individuals was studied. All the rest of the organisms studied were from susceptible individuals. The "smooth" cultures were maintained in untreated TJB while the "rough" strains were maintained in untreated TJB with 0.05 per cent Tween 80. These cultures were tested for their ability to ferment glucose, glycerol, and sorbitol at a concentration of 1 per cent. The media were distributed in tubes containing 5 ml. each. The media were inoculated with approximately 0.1 ml. of the culture (24 to 48 hours old). The results of the fermentation test are shown in Table II for glucose and sorbitol as substrates. Glycerol is not included in the results, since none of the lactobacilli attacked it.

TABLE II

FERMENTATION OF GLUCOSE AND SORBITOL IN 48 HOURS BY "SMOOTH" AND "ROUGH" STRAINS OF LACTOBACILLI FROM CARIES-SUSCEPTIBLE AND CARIES-IMMUNE INDIVIDUALS

SOURCE	TYPE OF CULTURE	NUMBER TESTED	NUMBER FERMENTING GLUCOSE	NUMBER FERMENTING SORBITOL
Cares susceptible	Smooth	13	13	10
Caries susceptible	Rough	10	10	0
Caries immune	Smooth	6	6	3
Caries immune	Rough	1	1	0

It was found that none of the rough strains fermented sorbitol, and that they fermented glucose only very weakly.

Similar results were later obtained employing the medium described by Rogosa, Wiseman, Mitchell, and Disraeley,[2] but with the potassium dihydrogen phosphate and sodium acetate omitted.

Studies of pH were carried out in an attempt to measure possible differences between strains in rates and amounts of acid production with the various substrates. Tubes containing 10 ml. of each type of media previously used in the fermentation tests were set up so that pH measurements could be taken at intervals of 3, 6, 12, 24, 36, and 48 hours. The media were adjusted

*In this study an adult free of caries and fillings and having no missing teeth was considered as caries-immune.

to an initial pH of 7.0. The inoculum consisted of 1 ml. of 24 to 36 hour culture of "smooth" organisms grown in TJB or "rough" grown in TJB with Tween 80 added. A Beckman pH meter was used for readings.

Preliminary pH studies carried out with "smooth" strains of lactobacilli and streptococci in broth containing 0.2 per cent trypticase and 0.1 per cent yeast extract had shown that the pH of glucose reached 5.0 with the lactobacilli in 12 to 24 hours and with the streptococci in 6 hours. Sorbitol was fermented much slower than glucose. Streptococci fermenting sorbitol produced a pH of 5.0 in 12 hours and the lactobacilli gave evidence of very little change during this time period (see Table III).

TABLE III

CHANGES IN pH WITH REPRESENTATIVE STREPTOCOCCI AND LACTOBACILLI (SMOOTH TYPE

	ADDITIVE TO BASAL MEDIUM	TIME IN HOURS						
		0	3	6	12	24	36	[illegible]
Lactobacillus No. 2	———	7.0	7.0	7.0	7.0	6.8	6.9	7.
	glucose	6.9	6.9	6.8	6.6	4.4	4.3	4
	glycerol	7.0	7.1	7.0	7.1	6.8	7.0	7.
	sorbitol	7.0	7.1	7.0	6.7	6.3	5.4	4.9
Lactobacillus No. 3	———	7.0	7.0	6.8	7.0	7.4	7.5	7.5
	glucose	6.9	6.8	6.6	5.9	5.1	5.2	5.2
	glycerol	7.1	7.0	6.9	7.0	7.4	7.4	7.5
	sorbitol	7.0	7.0	6.8	7.0	7.2	7.5	7.5
Lactobacillus No. 17	———	7.0	7.1	7.1	7.0	6.9	7.0	7.3
	glucose	6.9	6.9	6.8	6.4	4.8	4.6	4.7
	glycerol	7.0	6.9	7.1	7.0	6.9	7.1	7.3
	sorbitol	7.0	6.9	7.0	7.0	7.0	7.0	6.7
Streptococcus No. 1	———	7.0	6.8	6.7	6.8	6.8	7.2	7.4
	glucose	6.9	6.5	5.4	5.0	4.6	4.6	4.8
	glycerol	7.0	6.8	6.4	5.7	5.1	5.2	5.5
	sorbitol	7.0	6.7	6.5	5.6	5.0	5.0	5.0
Streptococcus No. 14	———	7.0	6.9	6.9	6.8	6.8	6.9	7.3
	glucose	6.9	6.9	6.7	6.0	4.9	5.0	5.1
	glycerol	7.0	7.0	6.9	6.7	6.4	6.3	6.4
	sorbitol	7.0	6.9	6.9	6.8	7.0	7.0	7.3
Streptococcus No. 19	———	7.4	7.3	7.5	7.5	7.5	—	—
	glucose	7.1	6.5	4.9	4.6	4.5	—	—
	glycerol	7.4	7.3	7.2	7.0	7.1	—	—
	sorbitol	7.3	7.0	5.8	5.0	4.9	—	—

Since the results of other studies by Smith[3] suggested that fructose might be readily metabolized by the "rough" strains, we included it as a test substrate. Table IV shows the pH produced by the fermentation of glucose and sorbitol by "smooth" and "rough" strains and of fructose by the "rough strains." It was observed that the fermentation of glucose appeared to be somewhat slower in this medium than in the medium containing trypticase and yeast extract. The difference was probably due to the greater buffering power of the medium.

Serial transfers of cultures of lactobacilli in the presence of sorbitol failed to yield organisms capable of greater acid production from sorbitol. Those not fermenting sorbitol still failed to do so after 8 transfers. These results,

together with similar negative results by Thunberg and manometric techniques, demonstrated that those lactobacilli failing to ferment sorbitol apparently were genetically incapable of such action and did not readily acquire such ability.

TABLE IV. DISTRIBUTION OF "ROUGH" AND "SMOOTH" STRAINS OF LACTOBACILLI BY pH PRODUCED WITH GLUCOSE, SORBITOL, AND FRUCTOSE IN 48 HOURS

TYPE OF CULTURE	NUMBER OF STRAINS TESTED	GLUCOSE		SORBITOL		FRUCTOSE	
		pH 5.0 OR BELOW	ABOVE pH 5.0	pH 5.0 OR BELOW	ABOVE pH 5.0	pH 5.0 OR BELOW	pH 5.1 TO 5.7
Rough	11	4	7*	0	11‡	4	7
Smooth	19	17	2†	5	14	§	§

*pH values stopped at 6.7 or higher.
†Lowest pH, 5.1.
‡No appreciable activity.
§No test.

Saliva Studies.—In order to obtain some information on the influences of sorbitol on the mixed flora of the mouth, sorbitol and other materials were added to fresh saliva. Changes in pH and in flora were followed; in addition, transfers to similar saliva media were made and the same changes observed. Very similar results were noted in the 14 saliva samples employed so the results of the pH studies are recorded as averages in Table V.

TABLE V

AVERAGE pH CHANGES IN SALIVA WITH ADDED FERMENTABLE MATERIALS

HOURS INCUBATION	GLUCOSE 1%	SORBITOL 1%	GLYCEROL 1%
	Initial Samples		
12	4.8	6.9	7.1
24	4.6	7.0	7.6
	First Transfer		
12	4.2	6.2	7.0
24	4.2	6.3	7.6
36	3.9	5.7	7.9
	Second Transfer		
6	5.5	6.4	7.6
24	4.2	5.4	7.6
48	3.6	5.7	7.7*

*At this time 2 samples demonstrated glycerol fermentation; one sample showed a pH of 5.1 and another 6.2, indicating the development of a glycerol-fermenting population in these salivas.

Microscopic examination of the saliva samples showed the following selective actions of the added compounds: (1) Glucose plus saliva favored the growth of streptococci early in the incubation period followed, after 24 hours, by growth of lactobacilli. Streptococci rapidly disappear after 24 hours. (2) Glycerol favored the development of gram-negative rods. The odor of each tube as well as the pH changes were indicative of proteolysis. (3) Microscopic examination of the 14 samples containing sorbitol after 48 hours' incubation of the second transfer showed large numbers of gram-negative rods, gram-negative cocci and, in most samples, gram-positive rods and cocci.

Results of a series of 3 successive transfers in sorbitol-saliva in another experiment also showed a predominance of gram-negative rods. When a 1:1,000 dilution was streaked on plates containing sorbitol, only gram-negative rods could be isolated. They were sorbitol fermenters. Further incubation of the plates resulted in a basic reaction demonstrating that these organisms are also proteolytic.

In view of the beneficial effects of certain surface-active agents on the growth of some oral lactobacilli, saliva samples containing the above materials plus Tween 80 or Triton A-20 were compared with those not containing these agents. No significant differences were noted.

Metabolic Studies.—In addition to the more or less direct tests reported above, detailed studies on the metabolism of sorbitol by both cells and cell-free extracts of a "smooth" strain of lactobacillus yielded certain relevant information. While a complete report on these experiments will be presented, we should note here certain of the results obtained.

Sorbitol was shown to be attacked by an adaptive enzyme or enzymes. This was demonstrated by growing cells in the presence of either sorbitol or glucose and then testing washed suspensions of these cells for acid production. In the results of such an experiment reported in Table VI, acid production was followed by potentiometric pH determinations with heavy suspensions of cells, standardized turbidimetrically with a Beckman Model B spectrophotometer.

TABLE VI

ACID PRODUCTION BY GLUCOSE AND SORBITOL GROWN CELLS

SUGAR OR ALCOHOL IN GROWTH MEDIUM	SUGAR OR ALCOHOL TESTED WITH CELL SUSPENSION	pH AT TIMES (MIN.) INDICATED						
		0	5	10	15	20	25	30
Glucose	Glucose	7.0	6.6	5.8	4.8	4.4	4.2	4.1
	Sorbitol	7.0	6.9	6.9	6.8	6.7	6.65	6.6
		7.0	7.0	7.0	7.0	7.0	7.0	7.0
Sorbitol	Glucose	7.2	6.6	5.5	4.7	4.2	–	4.1
	Sorbitol	7.2	7.0	6.8	6.6	6.3	–	5.9
		7.2	7.2	7.2	7.2	7.2	–	7.1

These results, in addition to demonstrating the adaptive nature of the enzyme or enzymes acting on sorbitol, further demonstrate the slower rate of acid production from sorbitol. It should also be pointed out that the pH changes described here with cell suspensions are not dependent upon growth as they were in previous tests of fermentability. Thus, the slow rate of acid production reported above in growing cultures was not due to the inability of the cells to grow rapidly.

Demonstration of the adaptive nature of the enzyme or enzymes attacking sorbitol has certain consequences that are associated with the phenomenon of adaptive enzyme production. One of these involves the competition that is commonly directed against the production of an adaptive enzyme by other substrates. In this case, one might expect that glucose would inhibit the production of the enzyme or enzymes active on sorbitol. The results in Table VII demonstrate this competitive effect of glucose, by showing that in the

presence of small amounts of glucose, the glucose is first utilized, then adaptation, and growth begins with the sorbitol present. The experiment consisted of following acid production during growth in media containing 1 per cent sorbitol plus small amounts of glucose.

TABLE VII

ACID PRODUCTION WITH SORBITOL AND GLUCOSE BOTH PRESENT

	pH AT TIMES (HOURS) INDICATED											
	0	3	4.5	6	7	8.5	9.5	10.5	12	14	16	18
1% Sorbitol*	6.9	6.7	6.6	6.5	6.45	6.5	6.5	6.4	6.3	6.15	5.8	5.55
1% Sorbitol + 0.05% glucose	6.9	6.7	6.6	6.4	6.0	6.0	6.0	5.95	5.9	5.75	5.6	5.3
1% Sorbitol + 0.01% glucose	6.9	6.7	6.55	6.3	5.75	5.6	5.6	5.6	5.55	5.45	5.3	5.15

*A small amount of glucose, probably 0.01 per cent, was introduced with the inoculum.

It will be seen that after an initial rapid drop in the pH proportional to the amount of glucose present, a period of 3 to 4 hours followed in which no further acid production was detected. This inactive period demonstrates that the cells had not adapted to sorbitol while glucose was present. After this period further decreases in pH demonstrate adaptation to the sorbitol.

In examining acid production from sorbitol and glucose manometrically, by release of CO_2 from bicarbonate buffer,[4] it was shown that only about 1 micromole of acid (21 μl CO_2 observed; 22.4 μl theoretical value) was produced per micromole of sorbitol while approximately 2 micromoles (40 μl CO_2 observed; 44.8 μl theoretical value) were produced per mole of glucose or fructose. This denotes the production of 1 micromole of a nonacid product and is consistent with hydrogen balance requirements since sorbitol is more reduced than the hexoses. This nonacid product is undoubtedly glycerol, and its production can be responsible for the significantly lower rate of acid production from sorbitol. These results also indicate that this strain of lactobacillus has a homolactic type of fermentation, as would be anticipated.

Aerobically, it has been demonstrated manometrically (O_2 uptake) that the optimum pH for sorbitol oxidation lies between pH 7 and 8, with a rapid decrease in activity on either side of this optimum.

Although we have not investigated the metabolic activities of the streptococci as we have the lactobacilli, it would be expected that they behave in very much the same manner as the lactobacilli because of their general physiologic similarities.

SUMMARY AND CONCLUSIONS

On the basis of the results outlined above, coupled with knowledge of bacterial physiology and the caries problem, it is our conclusion that the use of sorbitol would not contribute significantly to acid production and, thereby, to dental caries. It is even possible that benefits might arise from its use.

This conclusion is based on the following: (1) Only some strains of the various species studied ferment sorbitol. None of the rough strains of lacto-

bacilli nor staphylococci tested fermented sorbitol. (2) With strains fermenting sorbitol, acid production was slow, the quantity small, and conditions must be optimal. These conditions include a near neutral pH, continued presence of sorbitol, and virtual absence of glucose. (3) In the mixed flora of saliva, sorbitol leads to selection of gram-negative organisms, not the acidogenic cocci and gram-positive rods, and the pH believed necessary for decalcification is not reached. (4) Ability to ferment sorbitol is not readily acquired and there appear to be certain theoretical limits to the amount of acid that can be produced from sorbitol.

The authors wish to thank Dr. Gordon Green for the isolation of some of the organisms employed in this study.

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CARIES-CONDUCIVE EFFECT OF A PURIFIED DIET WHEN FED TO RODENTS DURING TOOTH DEVELOPMENT

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THE USE of refined carbohydrates, especially sugar, in modern diets has been for many years one of the most discussed factors relative to dental caries. Half a century has elapsed since the above relation first was investigated extensively.[1] But it is still disputable whether the caries-producing effect of a high sugar intake is caused primarily by an unfavorable effect on the oral environment or on the character of the teeth themselves. An inverse relation has been shown to exist between children's consumption of protective foods and of refined carbohydrates.[2] The relative importance attached to a decrease in the former, versus the simultaneous increase in the latter food source, consequently is a matter of controversy among research workers.

The following work deals first with an experimental approach, Part I, which makes provision for the desired separation of the "external" and the "internal" effect of a purified high carbohydrate ration on the production of dental caries. Part II consists of a reinterpretation of earlier observations in animals in the light of the results of this experimental approach.

PART I. EXPERIMENTAL APPROACH

The caries susceptibility of three species of rodents has been compared under three major experimental variables:

1. Animals were born to mothers subsisting on a stock diet of natural food constituents and continued on the same diet throughout and after completion of tooth development.

2. Animals were born to mothers subsisting on the stock diet, but at weaning were transferred to a purified ration high in sucrose and complete in known nutritional essentials.

3. Animals were born to mothers subsisting on the purified ration and continued on the same ration throughout the experimental period.

The composition and nature of the rations are shown in Tables 1 and 2. The purified ration was prepared from adequate quantities of all those nutriments which are known to assure general health, growth and reproduction in the rodent. When supplemented with ascorbic acid, this ration is essentially

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2. Sognnaes, R. F., and White, R. L., Oral Conditions of Children in Relation to State of General Health and Habits of Life, *Am. J. Dis. Children* 60: 284, August, 1940.

Table I.—Components of the purified and the natural ration

Components	Purified ration (experimental)	Purina laboratory chow [illegible]ers (stock diet)
Carbohydrates	67% (granulated cane sugar)*	48.16%
Protein	24% (casein)	26.18%
Fat	5% (corn oil)	5.35%
Fiber and moisture	0	13.52%
Ash	4% (salt mixture)†	6.49%
Calcium	0.53%	1.17%
Phosphorus	0.55%	0.87%
Magnesium	0.033%	0.196%
Iron	150 p.p.m.	327 p.p.m.
Manganese	50 p.p.m.	106.0 p.p.m.
Copper	15 p.p.m.	16.8 p.p.m.
Cobalt	0.65 p.p.m.	0.14 p.p.m.
Potassium	0.45%	0.90%
Sodium	0.44%	(?)
Chlorine	0.76%	0.57%
Iodine	20 p.p.m.	4.5 p.p.m.
Zinc	30 p.p.m.	(?)
Sulphur	480 p.p.m.	(?)
Fluorine	2.5 p.p.m.	21.0 p.p.m.
Vitamins		
Thiamin	3.5 p.p.m.	12.85 p.p.m.
Riboflavin	3.5 p.p.m.	7.49 p.p.m.
Nicotinic acid	25.0 p.p.m.	65.6 p.p.m.
Vitamin D	1.5 USP U/Gm.	0.45 USP U/Gm.
Vitamin A	30.0 USP U/Gm.	9.0 USP U/Gm.
p-Amino-benzoic acid	300 p.p.m.	(?)
Pantothenic acid	20 p.p.m.	50.6 p.p.m.
Pyridoxine	3.5 p.p.m.	(?)
Inositol	1,000 p.p.m.	(?)
Choline	1,000 p.p.m.	(?)
Liver concentrate	4% (supplement)	(?)
Other factors included	None	Unrecognized kinds and amounts

*In experiments where the sucrose fraction has been exchanged by dextrin or whole wheat flour, the ration has been named accordingly in the text and tables.
†Modification of Phillips' and Hart's salt mixture (*J. Biol. Chem.* 109:657, 1935) by Shaw, J. H., Carious Lesions in Cotton Rat Molars. *J. D. Res.* 26:47 (February) 1947.

the same as that which has been shown to give excellent growth in the primate.[3] The Purina laboratory ration is made from natural food constituents: Its known components have been recorded from analysis made by the Purina Company (*Care and Feeding of Laboratory Animals*, B-2362). A stock diet especially made for feeding of hamsters, rats and mice, this product is kept as uniform as possible with a low content of alfalfa which assures a constantly lower fluorine content than in similar products.[4] The trace amount of fluorine in the purified ration is not added for general nutritional

3. Waisman, H. A., and others, Studies on Nutritional Requirements of Rhesus Monkeys. *J. Nutrition* 26:205 (August) 1943.

4. Schaefer, H. C., personal communication, Purina Company.

Table 2.—Comparison of particle size of purified ration and coarse caries-producing ration

Ingredients	Amount (%)	Percentage distribution of particles (Grams per 100 Gm. of sample)			
		Over 20 mesh	20–40 mesh	40–60 mesh	Below 60 mesh
Purified ration					
Cane sugar	67	0.3	39.2	42.4	18.1
Casein (and B-vitamins)	24	0	26.0	42.2	31.8
Salt mixture	4	2.2	18.1	12.5	67.2
Corn oil (and fat-sol. vit.)	5	0	0	0	(5)
Sum of ingredients	100	0.3	33	39	27
Subject to mastication*	24	0	6	10	8
Coarse ration†					
Coarse corn‡	60	100.0	0	0	0
Linseed meal	6	19.6	31.7	19.2	29.5
Alfalfa meal	3	0.2	37.9	21.4	40.5
Sodium chloride	1	0	24.5	64.4	11.1
Powdered milk	30	0	0	0	100.0
Sum of ingredients	100	61	3	2	34
Subject to mastication*	69	61	3	2	3

*Portion which is not dissolved readily in saliva.
†Hoppert-Webber-Caniff diet. Analysis taken from Sognnaes, R. F., Studies in Masticatory Efficiency of Rats. *Am. J. Orthodont. & Oral Surg.* 27: (Oral Surg. Sec.) 383 (July) 1941.
‡Unlike whole grain or larger food checkers (such as our "stock diet") the 20 mesh particles are too small to be manipulated well by the rats' foreleg paws and gnawed off by the incisors. As a result the molars tend to fracture and become carious after masticating the 20 mesh particles (Sognnaes, 1941).

purposes; it arises from minor impurities in the carbohydrate fraction and the salt mixture.[5]

The experimental program was carried out in two series. In Series A, 53 Syrian hamsters (Table 3), 43 Long-Evans strain rats (Table 4) and 36 white mice (Table 5) were grouped as indicated in the tables.

The main experimental variable studied in this series was the effect of the ration used before eruption of the teeth. In Series B the observations were extended to a larger variety of rations and a greater number of animals of one species, in all 110 rats, grouped as shown in Tables 6, 7 and 8.

After sacrifice of the animals the jaws were examined under binocular microscope (x 30). Representative jaws were photographed, roentgenographed and finally prepared for histologic examination.

The scoring of the molar lesions (based on the binocular examination) was done by a modification of the method previously used in hamsters[6] and adapted to the rats and mice to make the records of the three species comparable. Because the purified, caries-producing ration was powdered finely, the lesions were not complicated by the artificial, mechanical fractures encountered in earlier work, when rats were fed coarse diets[7] (Table 2).

The carious lesions to be described were more nearly comparable to human caries than any experimental lesions seen

5. Shaw, J. H., and others. Dental Caries in the Cotton Rat. IV. Inhibiting Effects of Fluorine Additions to the Ration. *Proc. Soc. Exp. Biol. & Med.* 59:89 (May) 1945.

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7. Sognnaes, R. F., Studies on Masticatory Efficiency. IV. Mastication and Experimental Rat Caries. *Am. J. Orthodont. & Oral Surg.* 27: (Oral Surg. Sec.) [illegible] (October) [illegible]

Table 3.—Pre-eruptive influence of purified ration on the caries susceptibility of the teeth of Syrian hamsters

Group & no. of hamsters	Ration during dental development		Oral environment after eruption of the teeth					
	Prenatal life	Lactation (infancy)	Ration	Age period exposed* (months)	Carious molars	Carious cavities	Caries score	Score per months of oral exposure
1a 13	Stock	Stock	Stock	1st-5th (4)	0.8	0.8	0.8	0.2
1b 15	Stock	Stock	Stock	1st-8th (7)	1.0	1.2	1.2	0.2
1 28	(Average)			(4-7)	0.9	1.0	1.0	0.2
2a	Stock	Stock	Sucrose	4-8 (4)	2	2	2	0.5†
	Stock	Stock	Sucrose	4-8	2	3	3	0.8
	Stock	Stock	Sucrose	4-8	3	4	4	1.0
	Stock	Stock	Sucrose	3-8 (5)	5	5	5	1.3
	Stock	Stock	Sucrose	3-8	5	6	6	1.6
	Stock	Stock	Sucrose	3-8	6	6	7	1.8
	Stock	Stock	Sucrose	3-8	4	7	8	2.1
					3.8	4.7	5.0	1.3
2b	Stock	Stock	Sucrose	1-5 (4)	2	2	2	0.5
	Stock	Stock	Sucrose	1-5	4	6	6	1.6
	Stock	Stock	Sucrose	1-5	5	4	6	1.6
	Stock	Stock	Sucrose	1-5	5	5	7	1.8
	Stock	Stock	Sucrose	1-5	5	6	7	1.8
	Stock	Stock	Sucrose	1-5	7	9	9	2.4
					4.6	5.3	6.1	1.6
2c	Stock	Stock	Sucrose	1-8 (7)	10	14	18	2.4
	Stock	Stock	Sucrose	1-8	10	19	25	3.2‡
	Stock	Stock	Sucrose	1-8	10	19	31	4.0
	Stock	Stock	Sucrose	1-8	8	17	34	4.4
					9.5	17.3	27.0	3.5
2 17	(Average)			(4-7)	4.5	7.9	10.6	1.9
3a	Stock	Sucrose	Sucrose	1-3 (2)	4	5	9	4.5
	Stock	Sucrose	Sucrose	1-3	5	9	14	7.0
	Stock	Sucrose	Sucrose	1-3	7	13	17	8.5
	Stock	Sucrose	Sucrose	1-3	7	14	27	13.5
	Stock	Sucrose	Sucrose	1-3	8	23	35	17.5
					6.2	12.8	20.4	10.2
3b	Sucrose	Sucrose	Sucrose	1-3 (2)	8	27	40	20.0
	Sucrose	Sucrose	Sucrose	1-3	9	33	51	25.0
	Sucrose	Sucrose	Sucrose	1-3	10	35	53	26.5
					9	31.6	48.0	23.8
3 8	(Average)			(2)	7.3	20.0	31.0	15.3

*Figures in parentheses indicate the actual number of months during which the teeth were exposed to the [illegible] after-eruption into the oral cavity.

or reported in previous publications by the author. Indeed, no significant differences from human caries can be pointed out at present.

Experiments in Hamsters, Rats and Mice

Series A.—The findings in hamsters, rats and mice have been recorded in Tables 3, 4 and 5, respectively; the latter permits a direct comparison of the three species. All tables contain the number of animals in each group, the breeding diet, the caries-producing experimental diet and the period during which the animals were exposed to the latter. The number of carious molars, affected areas (cavities) and carious score (that is, areas times first, second or third degree penetration) are listed. The tabulated lesions are those seen by binocular microscope.

Table 4.—Pre-eruptive influence on the caries susceptibility of rats' molars

No. of rats	Ration during dental development		Oral environment after eruption of teeth					
					Dental effects			Score per months of exposure
	Prenatal life	Lactation	Ration	Length of exposure (months)	Carious molars	Carious cavities	Caries score	
15	Stock	Stock	Stock	7–9	0	0	0	0
2	Stock	Stock	Sucrose	3	0	0	0	
	Stock	Stock	Sucrose	3	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	5	0	0	0	
	Stock	Stock	Sucrose	7	0	0	0	
	Stock	Stock	Sucrose	7	0	0	0*	
12				3–7	0	0	0	0
3	Stock	Sucrose	Sucrose	4	1	1	1	
	Stock	Sucrose	Sucrose	4	1	1	1	
	Stock	Sucrose	Sucrose	4	2	2	2	
	Stock	Sucrose	Sucrose	4	3	3	3	
	Stock	Sucrose	Sucrose	4	3	3	4	
6				4	2.0	2.0	2.2	0.6
4	Sucrose	Sucrose	Sucrose	2	0	0	0	
	Sucrose	Sucrose	Sucrose	2	1	1	1	
	Sucrose	Sucrose	Sucrose	2	1	1	1	
	Sucrose	Sucrose	Sucrose	2	2	2	2	
	Sucrose	Sucrose	Sucrose	2	2	2	2	
	Sucrose	Sucrose	Sucrose	2	3	3	3	
	Sucrose	Sucrose	Sucrose	2	3	4	4	
	Sucrose	Sucrose	Sucrose	2	4	4	4	
	Sucrose	Sucrose	Sucrose	2	4	5	5	
	Sucrose	Sucrose	Sucrose	2	4	5	5	
10				2	2.4	2.7	2.7	1.4

Table 5.—Comparative increase in caries susceptibility of hamsters, rats and mice when "bred" on a high sucrose diet (Series A)

Species	Number of animals	Breeding diet	Posteruptive (experimental)		Average caries incidence		
			Diet	Period (months)	Molars affected	Areas affected	Caries score
Hamsters.....	6	Stock	Sucrose	4	4.6	5.3	6.1
Rats.....	12	Stock	Sucrose	4	0	0	0
Mice.....	15	Stock	Sucrose	4	0	0	0
Hamsters.....	3	Sucrose	Sucrose	2	9.0	31.6	48.0
Rats.....	10	Sucrose	Sucrose	2	2.4	2.7	2.7
Mice.....	21	Sucrose	Sucrose	2	0.4	0.5	0.5

A comparison of the history of the three groups of animals shows that the hamsters (Table 3) were the only ones developing caries when bred and fed on the stock diet. But the incidence was very low with an average of only one affected molar per animal after seven months. When bred on the stock diet and then, from weaning time, fed the sucrose diet (Group 2), the number of carious teeth, after only four months, was four times higher, after seven months, nine times higher than the above control.

When the sucrose diet was begun during lactation (Group 3a), caries became several times as severe as in Group 2 in less than half the experimental period.

In the next experimental group (3b), offspring of parents bred on the sucrose diet, the extent of caries again doubled over that in Group 3a. Males and females were represented equally, but within each group the females have a tendency to take their place in the lower caries score, which accounts for the range. In animals placed on the sucrose diet before, during and after tooth development, respectively, there was a ratio of 20:10:2 in carious destruction per month of posteruptive oral exposure to the diet. Figure 1 illustrates this ratio; some of the carious lesions are shown in Figures 2 and 3. Figure 2 illustrates the development of a lesion found only in teeth which developed on the sucrose diet. (P. H. Keyes, who has inspected these lesions, has not observed any in the hundreds of hamsters studied by him.)

This location of caries in the smooth lingual surfaces of the maxillary second molars has not been reported previously in animals and may be of significance as a possible counterpart for this type of caries in man.

Figure 3 shows the pathogenesis of the more common lesions of the hamsters, namely, those of the maxillary, occlusal and mandibular buccal surfaces of the first molars. It is interesting to note that the secondary dentin formation is

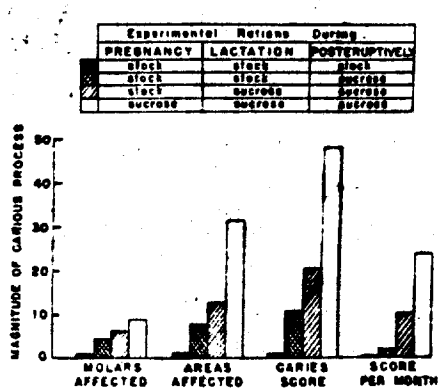

Fig. 1.—Pre-eruptive influence of the purified (sucrose) ration on the caries susceptibility of hamsters

Table 4.—Influence of prenatal and postnatal diet on experimental rat caries* (Series B)

Group	No. animals	Prenatal diet	Postnatal experimental			Dental findings at end of experiment			
			Diet	Start (days)	Duration (months)	Car. mol.	Car. cav.	Car. score	Score per month
Parents	Father	Stock	Sucrose	150	6‡	0	0	0	0
	Mother #1	Stock	Sucrose	90	8	0	0	0	0
	Mother #2	Stock	Sucrose	90	8‡	0	0	0	0
1st litter of #1	4 rats	Stock	Whole wheat	10	6	0	0	0	0
		Stock	Sucrose	10	6	0	0	0	
		Stock	Sucrose	10	6	4	4	7	
		Stock	Sucrose	10	6	4	5	8	
		Stock	Sucrose	10	6	4	8	11	
	4 rats	Stock	Sucrose	10	6	3.0	4.2	6.5	1.1
2nd litter of #1		Sucrose	Dextrin	21	3	0	0	0	
		Sucrose	Dextrin	21	3	0	0	0	
		Sucrose	Dextrin	21	3	1	1	1	
		Sucrose	Dextrin	21	3	1	2	2	
	4 rats†	Sucrose	Dextrin	21	3	0.5	0.7	0.7	0.2
		Sucrose	Sucrose	10	3	4	7	10	
		Sucrose	Sucrose	10	3	4	8	13	
		Sucrose	Sucrose	10	3	5	8	13	
		Sucrose	Sucrose	10	3	6	9	13	
	4 rats†	Sucrose	Sucrose	10	3	4.7	8.0	12.2	4.1
1st litter of #2		Stock	Sucrose	10	4	1	1	1	
		Stock	Sucrose	10	4	1	1	1	
		Stock	Sucrose	10	4	2	2	2	
		Stock	Sucrose	10	4‡	3	3	3	
		Stock	Sucrose	10	4	3	3	4	
		Stock	Sucrose	10	5	1	1	1	
		Stock	Sucrose	10	5	4	4	6	
		Stock	Sucrose	10	5	4	5	6	
		Stock	Sucrose	10	8	5	10	16	
	9 rats	Stock	Sucrose	10	4-8	2.6	3.3	4.4	0.9
2nd litter of #2	4 rats	Sucrose	Whole wheat	22	3	0	0	0	0
		Sucrose	Sucrose	10	3	4	8	9	
		Sucrose	Sucrose	10	3	6	9	12	
		Sucrose	Sucrose	10	3‡	5	7	20	
		Sucrose	Sucrose	10	3	7	13	20	
	4 rats	Sucrose	Sucrose	10	3	5.5	9.2	15.2	5.1

*Brothers and sisters from succeeding pregnancies.

‡Mother on sucrose diet for four months preceding birth of litter.

[illegible] the illustration in Figure [illegible] for [illegible]

marked from beginning to end. Structural faults have not been observed in the enamel sections studied so far. No chemical or physical properties have been tested as yet.

No caries developed in rats (Table 4) exposed to the sucrose diet for seven months, provided formation of the teeth had been completed on the stock diet. But when the rats were conceived as well as raised on the sucrose diet, caries developed after a period of only a few months of oral exposure of the teeth. Under similar experimental conditions even the mice showed some initial lesions, as seen from Table 5, after two months posteruptive feeding. In the latter table where comparable experimental periods are recorded, the species-difference becomes apparent as well as the comparative effect of the breeding diet on the final caries score.

It is interesting to compare the caries incidence in these groups because of the differences in the anatomy of their teeth. The depth and narrowness of the occlusal fissures are related inversely to the caries susceptibility. The hamster molars with their wide shallow grooves were found to be most susceptible, the rat and mouse molars least so. There must be other and more fundamental reasons for this species difference.

The differences in the final carious destruction within each species, on the other hand, seems to be influenced greatly by the dietary variable introduced before eruption of the teeth, more so, indeed, than by the oral environment after their eruption.

Table 7.—Influence of the prematernal diet on experimental rat caries*

Experimental conditions before tooth eruption			Experimental conditions after tooth eruption					
Pre-conceptive		Pregnancy lactation	Diet	Period (months)	Molars affected	Areas affected	Caries score	Score per month
Diet	Period (months)							
Sucrose	2	Sucrose	Sucrose	3	2	2	2	
					2	2	2	
					3	4	4	
					3	6	9	
					3	8	9	
					4	5	7	
					4	6	8	
					4	7	9	
					4	8	8	
					4	8	12	
Average	for 10 rats				3.3	5.6	7.0	2.3
Sucrose	4	Sucrose	Sucrose	3	4	8	9	
					4	7	10	
					4	8	13	
					5	8	13	
					5	7	20	
					6	9	12	
					6	9	13	
					7	13	20	
Average	for 8 rats				5.1	8.6	13.7	4.5

Table 8.—Summary of effects of different carbohydrates on experimental rat caries (Series B)

Group	No. rats	Ration during dental development		Posteruptive experimental conditions and findings						
		Pregnancy	Lactation	Ration	Age at start (months)	Duration (months)	Carious molars	Carious cavities	Caries score	Score per month
A	5	Stock	Stock	Stock	1	7	0	0	0	0
B	7	Stock	Stock	Whole wheat	1	3	0	0	0	0
C	6	Stock	Stock	Dextrin	1	6	0	0	0	0
D	12	Stock	Stock	Sucrose	1–3	3–8	0	0	0	0
E	17	Stock	½ part sucrose	Sucrose	½	4–8	2.7	3.9	5.7	0.9
F	4	Sucrose*	Sucrose	Whole wheat	1	3	0	0	0	0
G	19	Sucrose*	Sucrose	Dextrin	1	3	0.7	0.8	0.8	0.3
H	8	Dextrin*	Dextrin	Dextrin	0	3	0.7	0.7	0.7	0.2
I	14	Dextrin*	Dextrin	Sucrose	1	3	3.0	5.0	7.0	2.3
J	10	Sucrose†	Sucrose	Sucrose	0	3	3.3	5.6	7.0	2.3
K	8	Sucrose*	Sucrose	Sucrose	0	3	5.1	8.6	13.7	4.5

*Mothers started on given ration four months preceding pregnancy.
†Mothers started on given ration two months preceding pregnancy.

During the past year the work has been limited mainly to the rats (Series B); first, because caries of a very satisfactory nature could be produced at will in this animal and, second, because the rat seemed to have some additional experimental advantages over other caries-susceptible rodents: they reproduce well and have been studied extensively.

The experiments of Series B were designed to re-examine and extend the initial observations under best possible control.

Series B.—When one is studying prenatal effects on the development of the offspring, it is impossible to investigate the effect of different diets with true litter of the animals of a given litter can be compared with that of their parents, that of preceding and succeeding litters (their siblings) and that of the next closest related litters (their cousins). Furthermore, it is necessary to be sure that the start of the posteruptive exposure to the sucrose diet is identical in compared groups. These requirements are fulfilled in the animals compared in Table 6. The results fully confirm the findings of the experiments in Series A.

It is seen from Table 6 that the caries score per month is four to five times greater in those animals whose teeth were formed on the sucrose diet than in animals bred on the stock diet. It is particularly important to note that the oral

was identical in the compared groups. Little or no caries developed except where the sucrose ration was fed both before and after the eruption of the teeth. In the first place the teeth of the parents (bred on the stock diet) were unharmed by a period of oral exposure to the sucrose diet twice as long as that of their litters. Second, there was a very low caries incidence in the litter mates which were changed from the sucrose to a dextrin or to a whole wheat ration at weaning time. Caries developed in a few months in the litter mates and siblings which were fed continuously the sucrose diet. (The animals selected for illustration in Figure 4 are marked ‡ in Table 6.)

Animals whose teeth were formed completely on the sucrose diet developed severe caries in three months (see rat 223 in Figure 4). Animals placed on the sucrose diet at the stage of final calcification and maturation of their teeth developed caries in four months (rat 96). But the parents, placed on the sucrose ration when mature, developed no caries in six to eight months.

The first series of experiments (Series A) had given rise to the suspicion that not only the prenatal diet but also the "pre-maternal" diet was of importance, that is, the diet of the mother preceding conception of the litter. Following up this possibility, litter mate mothers were fed the sucrose diet for two and four months, respectively, preceding mating as well as during pregnancy. The caries scores of their litters are compared in Table 7 and show a caries-score ratio of about 1:2. This interesting suggestion should be investigated by a long term experiment on succeeding litters from the same mothers. The influence of the pre-maternal diet on the health of the offspring is not known to have received much experimental study before. If confirmed, it would make efforts toward mass prevention of caries a long term project.

In Table 8 is a comparison of all groups of rats of Series B. Primarily, it serves to confirm the previous findings on a larger scale. The most important additional finding recorded in this table is also the most recent one, namely,

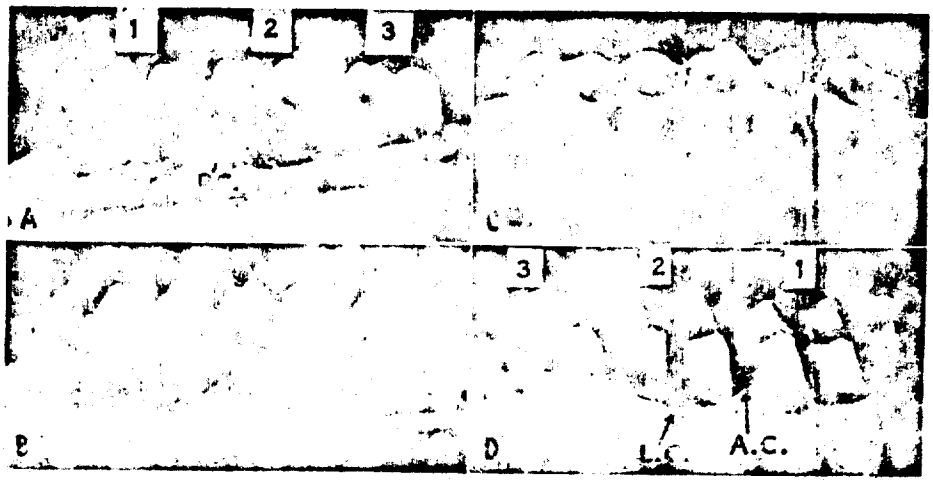

Fig. 2.—Lingual aspect of the maxillary molars of hamsters showing the development of smooth surface caries. The animals were placed on the purified diet after the first molar had erupted, while the second and third molars were still inside the jaw. The latter teeth had most caries, but least exposure to the sucrose ration in the oral cavity. P. C.: Precaries. A. C.: Approximal

that observed in Groups H and I. These groups consist of three litters born to mothers subsisting on the purified ration, except that dextrin instead of sucrose served as the carbohydrate fraction. At weaning time litter mates were divided in two groups, one of which (1) was transferred to the sucrose ration.

It is noted that this group developed a caries frequency comparable to rats bred on the sucrose diet. Their litter mates, which were continued on the dextrin ration (Group H), barely developed caries. This throws further light on the caries-producing property of this purified ration. Soluble carbohydrates seem to be potent caries-producing factors after the eruption of the teeth, as already shown in the cotton rat.[8] The maternal and prenatal influence, on the other hand, does not seem to be related to sugar in itself but may be related to the degree of refinement of the carbohydrate fraction. As a refined carbohydrate, sugar, rather than dextrin, usually is referred to as the more important factor to be considered in human caries.

Nevertheless, the animal findings do suggest that a purified ration, complete in all known nutritional essentials but containing a large proportion (67 per cent) of refined carbohydrates, is extremely conducive to caries, provided its ingestion is begun before and continued during tooth development. This appears to hold true whether sucrose or dextrin is the carbohydrate component of the ration fed before eruption of the teeth. Eruption age seems to be one of the critical periods determining the future dental health.

Group D of Table 8 again confirms the fact that the purified ration is not caries-producing for rats whose teeth were formed on the nonpurified diet. The same observation recently has been made in a personal communication by Greep of the Harvard School of Dental Medicine. He fed this ration to rats which, in addition, were exposed to endocrine imbalance and deficient mineral intake after eruption of the teeth. In contrast to this, Figure 5 shows the pathogenesis of the unquestionable lesions produced in our animals which have been fed the purified ration both before and two to six months after eruption of the teeth.

PART II. REINTERPRETATION OF OBSERVATIONS IN OTHER ANIMALS

Research on dental caries has adhered almost exclusively to the traditions of Miller's chemico-parasitic hypothesis.[1] Yet this hypothesis does not explain satisfactorily a number of important experimental observations. It is the purpose of the following section to analyze some of the most notable of these discrepancies with a view to examining their compatibility with the results of the experimental findings just reported.

It was thought to be a simple matter to reproduce caries in animals through carbohydrate fermentation and bacteriologic action in the oral cavity—if that be the all-important prerequisite to caries production. Therefore several attempts have been made to imitate Miller's etiologic requirements in experimental animals.

Guinea Pigs.—Howe[9] fed a group of 18 guinea pigs a diet of milk, rolled oats, carrots and green leaves in which were incorporated excessive amounts of sugar and starches. The teeth of the animals were covered continuously with adhesive masses of these refined carbohydrates and

8. Shaw, J. H., and others, Dental Caries in the Cotton Rat, II. Production and Description of the [illegible]

9. Howe, P. R., Dental Caries [illegible]

appeared very dirty. In no animal did any lesions of the teeth develop after nine to twelve months' exposure. Neither could Howe[10] induce caries by the feeding of Bacillus acidophilus and other micro-organisms isolated from carious lesions in man[11] although the oral flora constantly showed their aciduric reaction.

Monkeys.—Extending the work to monkeys,[12] it was shown that the same held true in the primate. They were fed a diet containing considerable amounts of sugar and white flour plus a sticky candy made by boiling cane sugar in water. The presence of aciduric organisms could be demonstrated constantly in the mouth of the animals. The teeth were covered with a mass of adhesive carbohydrates, which could be removed only with difficulty, but cleaning disclosed no effect on the underlying tooth substance.

No caries developed after nine months' feeding except in one animal that suffered from gastro-intestinal disturbance. While the experimental period may have been started too late to affect the teeth pre-eruptively, this negative finding presents strong evidence against the purely environmental effect of a high sugar intake on the teeth. Howe then concentrated on his famous pioneer work on nutritional factors affecting the dental conditions of the primate.

Howe's negative evidence on the importance of the oral environment alone was supported by contemporary and later observations. McIntosh, James and Lazarus-Barlow[13] described an organism isolated from human caries, constantly present in the mouth and capable of producing acids through fermentation of carbohydrate material. But when they tested this organism experimentally by introduction into the mouths of monkeys and rabbits, these investigators failed to produce lesions in the teeth.[14]

The extensive examinations of almost 3,000 monkeys by Schultz[15] showed that caries, if present, affected especially those animals which had been captured when young. This may suggest that the change from a natural diet had occurred early enough to permit a pre-eruptive influence upon the teeth.

Knap[16] tried to simulate human food habits in the monkey but could not induce caries by feeding cooked food containing sugar. In his experiments the teeth could not have been influenced by the "civilized" food before eruption.

Recently, Shaw and others[17] reported a higher than usual incidence of caries in a group of 138 rhesus monkeys which had been fed a high sucrose diet, essentially the one used in the present study with the addition of vitamin C. If the change in the oral environment, associated with the high sucrose diet, was the deciding cause of the carious destruction, then it seems strange that only a few of their monkeys developed visible (obvious) caries before a year or more of exposure to their new oral environment. They observed a remarkable resistance of the enamel to caries as compared to the dentin, once that was reached. It is noted that all the deciduous teeth were erupted before the animals were placed on the sucrose diet and that these teeth remained healthy all through the exposure. Only the permanent teeth, recently erupted, matured

10. Howe, P. R., Food Accessory Factors in Relation to the Teeth. *J. D. Res.* 3:7 (March) 1921, and *J.A.D.A.* 8:692 (August) 1921.

11. Howe, P. R., and Hatch, R. E., A Study of the Micro-organisms of Dental Caries. *J. M. Res.* 36:481, 1917.

12. Howe, P. R., Further Studies of the Effect of Diet Upon the Teeth and Jaws. *J.A.D.A.* 9:55 (January) 1922.

13. McIntosh, J., James, W. W., and Lazarus-[illegible]

14. McIntosh, J., James, W. W., and Lazarus-Barlow, P., Investigation into the Aetiology of Dental Caries. *Brit. J. Exper. Path.* 5:175 (June) 1924.

15. Schultz, A. H., Eruption and Decay of Permanent Teeth in Primates. *Am. J. Phys. Anthropol.* 19:489 (January-March) 1935.

16. Knap, M., Caries bij apen en dietproeven met het cog op caries bij den macacus. *Ned.-Ind. Tand. Tijdschr.* 9:71 and 103 (March) 1941.

17. Shaw, J. H., Elvehjem, C. A., and Phillips, P. H., A Survey of the Incidence of Dental Caries [illegible]

Fig. 3.—Pathogenesis of occlusal and buccal caries of the mandibular molars of hamsters. A heavy layer of secondary dentin is noted both under the initial lesion (above) and the advanced lesion (center). Periodontal destruction also is seen (center and below)

or developed during the experimental period, can have been affected. This assumption has been confirmed in a personal communication. Indeed, 80 per cent of the animals finally developed caries in these teeth after three years. Our findings may suggest that the sucrose diet in this particular case was acting on the development and final maturation of the teeth as well as on the [illegible]

Dogs and Rats.—Mellanby[18] failed to produce caries in dogs on soft sticky foods, excessive use of carbohydrates, daily introduction of Lactobacillus acidophilus and suppression of salivary flow. Nearly as negative were her findings in rats. The difficulty of producing caries in the latter animal by high sugar diets and oral micro-organisms has been demonstrated by several authors.

Rosebury and Karshan[19] failed to produce a single lesion in rats fed a synthetic diet containing excessive amounts of fermentable carbohydrates and introduced in the form of an adhesive paste containing L. acidophilus isolated from the human mouth. Indeed, similar aciduric organisms were found as natural inhabitants in the rats' mouths. Furthermore, the animals were exposed to deficiency in the calcifying property of the diet and to a large sugar intake as well as pulpal injury. Yet no carious lesions developed in rats kept under such experimental conditions from weaning time to 3 months of age. This corresponds well with the duration of our posteruptive feeding but differs in so far as the Rosebury-Karshan experiment was started too late to affect the formation of the teeth.

Klein and Harris[20] found organisms resembling the L. acidophilus described by the Bunting group in connection with human caries in the mouth of normal stock rats. The caries freedom of these rats adds further doubt to the causative sufficiency of these micro-organisms in producing caries.

Lilly[21] extended Rosebury and Karsh-

18. Mellanby, May, Diet and the Teeth. An Experimental Study. Part II. Medical Research Council Report 153. London: His Majesty's Stationery Office, 1930.

19. Rosebury, Theodor, and Karshan, Maxwell, Studies in the Rat of Susceptibility to Dental Caries. I. Bacteriological and Nutritional Factors. *J. D. Res.* 11:121 (February) 1931.

20. Klein, Henry, and Harris, M. M., A Note on the Presence of Aciduric Bacteria in the Mouths of Rats. *J. D. Res.* 11:149 (February) 1931.

21. Lilly, C. A., Failures to Produce Experimental Dental Caries in the White Rat with High Carbohydrate Diets and Bacillus Acidophilus [illegible]

an's experimental period to twelve months with the same negative result. No caries developed on a synthetic diet containing 63 per cent sucrose plus tri-weekly oral inoculations of a pure culture of L. acidophilus. As in the previous case, his approach differed from ours in so far as his rats were too old to permit an influence on the formation of their molars by the sucrose ration.

Jackson[22] has reported an experiment which serves as a very good control to our experiments. Like ours, his rats were of the Long-Evans strain of the Mus norvegicus, and he fed them a similar purified diet except that it contained even higher amounts of sucrose (up to 80 per cent). He placed the animals on this diet at weaning time and kept them on it for as long as seven months. The rats remained normal in health, vigor and appearance, and no difference was found between the teeth of the experimental group and the control group which was fed a sugar-free diet. Both groups maintained good health. Under these conditions (after normal tooth formation) it is evident that "high sugar diets for long periods are not necessarily injurious to the teeth."

There are two interesting rodents the use of which has opened new possibilities in experimental caries production lately, namely the Syrian hamster (Cricetus auratus)[23] and the cotton rat (Sigmondon hispidus hispidus).[8] Both are extremely susceptible to caries on various types of diets, particularly high carbohydrate diets, and would seem particularly valuable in the study of environmental factors involved in the caries process.

Aside from the two latter species,[24] which are not known as well as the others, caries is not produced easily in experimental animals by adhesive sugar pastes alone or in combination with oral inoculation of L. acidophilus isolated from human caries, by the constant existence of an aciduric oral reaction or the excessive feeding of fermentable refined carbohydrates for long periods after the eruption of the teeth. The chemico-

Fig. 4.—Comparison of caries susceptibility of parents (bred on stock diet), first litter (placed on stock diet during lactation) and second litter (bred on purified ration). After eruption the teeth were exposed to a high sucrose ration for a period of eight months (mother), six months (father), four months (first son) and three months (second son), respectively

22. Jackson, C. M., The Effects of High Sugar Diets on the Growth and Structure of the Rat. *J. Nutrition* 3:61 (July) 1930.

23. Arnold, F. A., Jr., The Production of Carious Lesions in the Molar Teeth of Hamsters (C. auratus). *Pub. Health Rep.* 57:1599 (October 23) 1942.

24. Hamsters and cotton rats are slower in dental development than the white rat. Consequently, their molar development and maturation, at least in the third molar, may be affected [illegible]

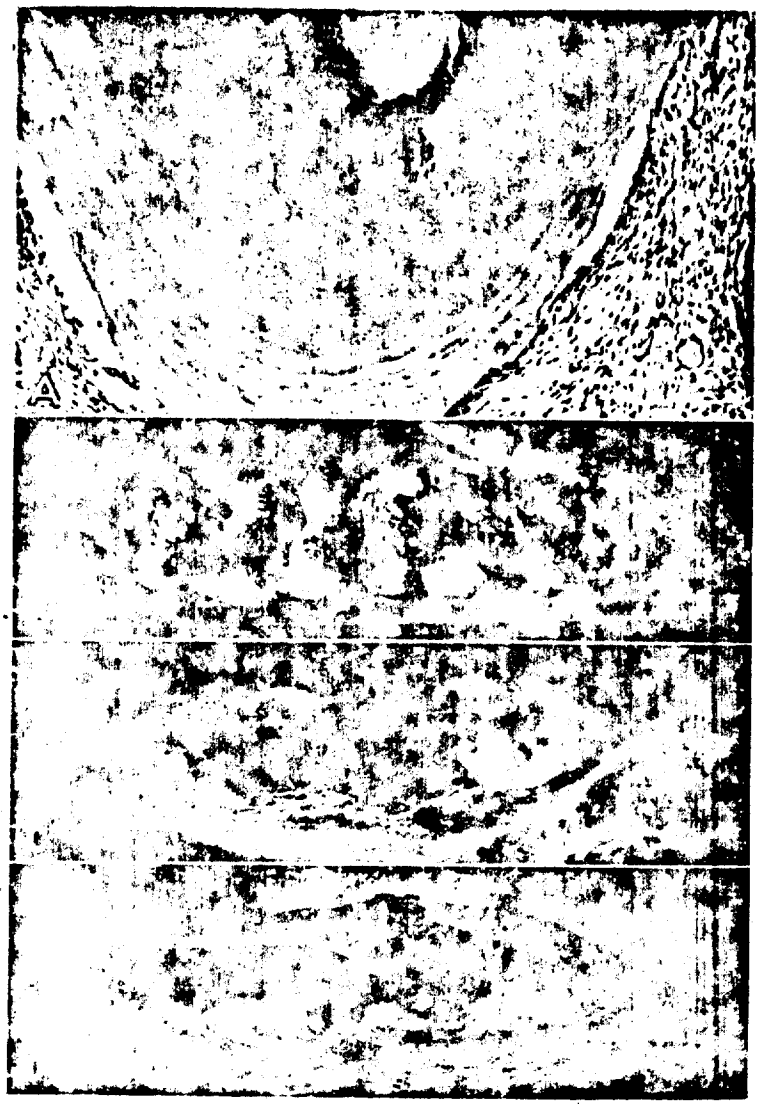

Fig. 5.—Pathogenesis of caries in rats placed on the purified, finely powdered (sucrose) ration before tooth eruption and kept on that diet for a period of two (A) to six (D) months after eruption of their molars. Invasion of the dentinal tubules and secondary dentin formation can be seen in the initial stage (A). Fractures are not in evidence even after considerable undermining of the enamel (B)

fice. It is suggested that previous failures to produce caries in various animals, including the monkey, by high sucrose diets may have been due to the fact that the did not extend to the second generation or covered too short periods within one generation to overcome the caries resistance of the species, strain or teeth in

COMMENTS AND CONCLUSIONS

We know of no earlier experiments which have shown that prolonged intake of refined carbohydrate diets over more than one generation, which would be a fitting parallel to the experience of civilized man today, is conducive to the production of dental caries. To some extent, such a parallel seems to have been attained by the experimental approach of the present investigation. The results do not appear inconsistent with the previous failures to produce experimental caries by high sugar diets when these earlier studies (Part II) are reinterpreted in the light of a developmental as well as environmental effect of such diets.

Purified Ration.—The mechanism of this developmental influence is not known. In the experiments reported here, the purified caries-producing ration was complete in all known nutritional essentials. It is particularly remarkable that it contained twice as high content of vitamin A and D as the stock diet. The general health of the animals was excellent. The growth of animals fed the sucrose diet was at least as good as that of the animals on the stock diet.

The reproduction was very satisfactory in the rats and mice. The hamsters were less satisfactory in so far as they have a tendency to eat their litters. It has been reported that rats fed highly purified diets have not cared for their young, have not had enough milk or have been unable to raise their litters satisfactorily.[25, 26] The large sucrose fraction of such diets has been suspected as the cause of these difficulties. Nevertheless it has been possible to breed rats for five generations on diets containing 66 per cent refined carbohydrate, 33 per cent as sugar.[27] Indeed, rats have grown and reproduced well on purified rations with as high as 80 per cent sucrose content.[22] The above differences may have been related to the adequacy of the diet.

Prenatal Influence.—It does not seem likely that the prenatal influence on the teeth of the animals can have been related to the relatively large intake of sugar in itself, to the physical property of the diet or to the direct shortage of any known nutritional essentials.

Unrecognized Factors.—The possibility may be suggested that some unrecognized mechanism or factors, possibly in the nature of trace elements, have played a part in deciding the caries resistance of the teeth. As far as fluorine is concerned, no evidence of dental fluorosis was observed in any groups of our animals such as reported by Dale and others[28] in hamsters fed a rabbit chow diet contaminated with fluorine.

The literature suggests that the inhibitory effect of fluorine on experimental caries is most marked if given *after* tooth eruption in amounts of 100 to 250 p.p.m. and that the effect appears relatively limited if given only during tooth development[29] as in this study. More work is needed on this phase. In the meantime one cannot disregard completely the possible operation of this element or others, the importance of which is still unknown.

After reviewing earlier investigations on the nutritional importance of various

25. Whitnah, C. H., and Bogart, R. J., Reproductive Capacity of Female Rats as Affected by Kinds of Carbohydrates in the Ration. *J. Agric. Res.* 53: 327, 1936.

26. Minnich, P. S., and Smith, A. H., Studies on

27. Fisher, D. P., The Effect of High Sugar Diets. *M. Rev. of Rev.* 38:693 (December) 1932.

28. Dale, P. P., Lazansky, J. P., and Keyes, P. H., Production and Inhibition of Dental Caries in Syrian Hamsters. *J. D. Res.* 23:445 (December) 1944.

29. Hodge, H. C., and Sognnaes, R. F., Experimental Caries and a Discussion of the Mechanism of Caries Inhibition by Fluorine. In *Dental Caries*

trace elements, the author is impressed by the fact that a total purification of synthetic diets has not been possible without great limitation to its general adequacy and digestibility. As a result animals have not lived long enough to make possible a study of effects of a prolonged depletion into second generations. Yet it has been concluded that several specific trace elements are unessential to the well-being and general health of animals. This does not prove the unimportance of such elements, singly or combined, to the prolonged health of all tissues over several generations. As far as the tooth enamel is concerned, it has indeed lower ability than most tissues to recover later whatever was missing during its development. The hypothesis of a nutritional depletion resulting from food purification deserves further experimentation. In surveys[30-31] of human caries, it is desirable to consider the part played by an excessive ingestion of refined carbohydrates *before* tooth eruption.

1. The findings indicate that hamsters, rats and mice are susceptible to experimental caries when fed a purified, finely powdered ration, complete in known nutritional essentials and high in refined carbohydrates, particularly if its ingestion is begun during tooth formation.

2. The caries susceptibility appears to be influenced by an unrecognized mechanism, a nutritional factor or combination of factors operating before eruption of the teeth from conception to maturation of the offspring and their individual teeth.

3. It is possible that the long suspected relation between dental caries and the excessive consumption of refined carbohydrates may be partly in the nature of an unrecognized indirect influence on the quality of the developing teeth and that the effect may be accentuated through generations.

30. Sognnaes, R. F., A Possible Role of Food Purification in the Etiology of Dental Caries. *Science* 106: 447 (November 7) 1947.

31. Sognnaes, R. F., Analysis of Wartime Reduction of Dental Caries in European Children. *Am. J. Dis. Children:* In press.

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J. Dental. Res. 45: 1551-1561, 1966.

Effects of Different Types of Human Foods on Dental Health in Experimental Animals

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SYNOPSIS IN INTERLINGUA

LE EFFECTOS DE DIFFERENTE TYPOS DE ALIMENTATION HUMAN SUPER LE SALUTE DENTAL DE ANIMALES EXPERIMENTAL.—Studios clinic in patientes con marcatemente active carie dental monstrava que numerose differente alimentos e bibitas habeva esite consumite, in multe casos inter le repastos regular. Con le objectivo de testar experimentalmente le cariogenicitate relative de ille alimentos e bibitas, 53 de typo representative esneva offerite ad libitum durante 28 dies a recentemente dislactate rattos de linea Osborne-Mendel. Le resultatos de iste experimentos animal ha clarificate le datos dietari de previe studios clinic. Illes indica que multe communemente consumite alimentos e bibitas es possibilemente cariogene.

In a comprehensive clinical study of over 80 patients with rampant dental caries who were referred by their dentists and physicians to the National Institute of Dental Research, an attempt was made to find definite factors responsible for the etiology of caries.* In a small percentage of the patients, the development of rampant caries appeared to be related to the development of an abnormal physical condition such as xerostomia following X-ray radiation treatment involving the salivary glands, Sjögren's syndrome, or possibly to the effects of prolonged medication and malnutrition. However, in most cases, the history of past illnesses, the physical examination, and medical tests offered little or no explanation for the development of rampant caries. The most common findings of factors associated with the development of rampant caries were a history of frequent or excessive eating of sweets (sugar-containing foods) at and between meals, the growth of extensive bacterial plaques adhering to tooth surfaces (which was sometimes associated with a marginal gingivitis), and lower pH levels in bacterial plaques and food material retained in carious areas than the pH of the mucous membranes and saliva. From these and earlier pH studies,[1-4] it appears that the last of these three factors is largely dependent on the interaction of the first two factors. However, the extent to which the first two factors are related is not yet well understood.

It was clear from the dietary records that a great variety of different foods and beverages were consumed by almost all persons studied. The question of how much each of these might have affected the development of caries was not easy to answer. Whereas the excessive consumption of one particular food material might make it highly suspect of inducing rampant caries in one patient, the excessive consumption of a different food material would be similarly suspect in another patient. In neither case could one rule out the contributory effects of many other food materials which were consumed in lesser amounts but which, when added together, might have played a major role in the production of rampant caries. Furthermore, it was difficult to know whether any of the food materials consumed might actually have acted to retard or inhibit the development of caries through some possible anticariogenic properties. Thus, as suggestive as these clinical studies were, there was an obvious need for adequately controlled experiments to evaluate the cariogenicity

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* This study included physical, dental and medical examinations, hematological and salivary tests, dental and chest X-rays, salivary flow and lactobacillus counts, direct stereomicroscopic examination of the oral environment at 6X to 60X, magnification using the Zeiss Ortoskop, phase microscopic and microchemical examinations of dental plaque material removed from fissure carious tooth surfaces and gingival pockets, intraoral pH measurements, fluorescent antibody studies to identify specific strains of oral streptococci, and detailed records of diets, beverage and eating habits, and oral hygiene. In order to secure some kind of "control" for this clinical study, comparisons were made with other members of the patients' families, most of whom did not have rampant caries. This study will be published separately.

and anticariogenicity of the different foods and beverages that people like and commonly consume.

There have been a great many observations, discussions, and controversies published in the literature concerning the role of different foods and particularly sweets in the etiology of caries. However, with the possible exception of the Vipeholm study,[4] in which an increase in rate of development of caries was observed in long-term hospital patients who were fed known diets containing certain sweets, there seems to be little controlled experimental proof to show which foods are cariogenic and which noncariogenic in humans. The great difficulty of establishing and enforcing scientifically adequate experimental controls for food consumption over several years' time, as well as the legal and moral problems involved, have generally limited attempts to study experimentally the effect of food materials on the development of caries in human populations.*

In view of the long time required to carry out experimental caries studies in humans and the extreme difficulties in securing scientifically adequate dietary controls for any given food material, it seems important to try to evaluate the cariogenic and anticariogenic properties of these individual foods, beverages, and refreshments in laboratory animal experiments, where conditions can be much more adequately controlled and where carious lesions which appear similar to carious lesions in humans can be produced in a relatively short period of time. While the results of animal experiments may not necessarily be directly applicable to humans, and while there are always some uncontrolled variable factors even in the most carefully controlled animal experiments, nevertheless, there is no scientific evidence to indicate that the dental caries process in laboratory animals is essentially different from the dental caries process in humans. Such animal experiments can serve to test the validity of the empirical recommendations and conflicting theories on which we have had to base food selection methods for the control of caries. Animal experiments can also furnish a valuable background on which to base future human studies. Of course, studies with human populations are necessary to establish food practices for the control of caries on an incontestable foundation.

Since, in addition to caries, other dental conditions such as erosion, calculus formation, periodontal disease, and malocclusion were observed in patients or members of their families during the rampant caries study, an attempt was made to study similar conditions where practical in these animal experiments. In addition, observations were made on the density (porosity) of maxillary and mandibular bones in these animals.

Materials and Methods

This study was based on a long series of laboratory animal experiments in which carious lesions comparable to the various kinds of carious lesions found in the molar teeth of humans (occlusal fissure, proximal, buccal, and lingual surface carious lesions) as well as caries in incisors have been produced in the teeth of rats when they were fed certain cariogenic diets. In these experiments many different factors had been studied for their effects on the development of caries, including different strains of rats, diets, feeding conditions, cage conditions, social conditions, chemicals and antibiotics added to the diet or drinking water, and inoculation of animals with micro-organisms isolated from animals with caries or from carious lesions in humans. It was clear from these previous experiments that almost all of these factors may exert some effect on the development of caries and that the etiology of caries is necessarily multifactorial in nature. Furthermore, we almost always found some experimentally uncontrolled variation in the caries susceptibility of different animals in the same experimental group which may have resulted from individual differences in heredity, gestation, birth, infection, and feeding before the animals were weaned and placed on the experiment. Therefore, even though the diet was obviously one of the major factors in determining the development of caries, it was important to try to reduce the experimental variability among animals in order to compare the cariogenic properties of a large number of different food materials

* This difficulty in controlling food consumption is in striking contrast to the relative ease in controlling fluoride consumption in experimental studies in which 1 ppm of fluoride has been added to the drinking water, a procedure which has reduced dental caries about 50 per cent in cities where it has been used over long periods of time.

Since many of the food materials which people eat are by themselves nutritionally inadequate, it was also important to be able to keep the animals alive throughout the experimental period.

A number of preliminary experiments were done in which animals were fed various foods for various times to find suitable experimental conditions to meet these problems. To a large extent this was accomplished by the following procedures.

The NIH Animal Production Unit was requested to furnish 20- to 21-day-old newly weaned Osborne-Mendel stock strain rats of 30–40 Gm. in weight and in as many groups of 60 of the same sex as were available.

Osborne-Mendel strain rats were selected because in several previous experiments comparing NIH strains of rats they have been most uniformly susceptible to caries and also have grown the best.[5] Animals 30–40 Gm. in weight were used because in previous experiments animals below 30 Gm. weight often did not grow well, and weanling animals above 40 Gm. weight had frequently not been as susceptible to caries as those within the 30–40 Gm. weight range. Multiple groups of 60 were used because each cage rack held 60 small animal cages, and the use of this number produced maximum convenience and efficiency for duplication of experiments as well as an adequate number for representation of many different foods in a single experiment.

After the animals were received they were conditioned for 3 days before an experiment was started by putting all of one sex together in a large animal cage $2\frac{1}{2} \times 2\frac{1}{2}$ ft. square with a $\frac{1}{4}$-in.-mesh wire screen bottom. The NIH Animal Production Unit had regularly fed mother rats a standard laboratory diet* and water with daily supplements of white bread and milk, carrots and kale added from birth to weaning. During the conditioning period, the laboratory diet was replaced by the basic diet of the experiment (Stephan diets 580 or 581), because the former was known to contain crude fiber material which sometimes impacted in the molar fissures and interfered with experimental caries production. This 3-day conditioning period enabled the newly weaned animals to adjust better to the necessity to secure food in the absence of their mothers and tended to reduce the mortality of newly weaned animals. It also mixed the animals and permitted them to interchange the parasitic micro-organisms they harbored, which tended to give a more uniform caries susceptibility. At the end of the conditioning period, animals from the large cage were distributed among the different experimental groups in small-animal cages at random. The small-animal cages were of uniform size with $\frac{3}{8}$-in.-mesh stainless steel wire construction. The animal room was air conditioned and kept at a temperature of 80° F. (27° C.). Distilled water for drinking was furnished to all animals at all times.

The nutrients required for growth and survival in our society are generally met by eating a combination of foods three times a day at regular mealtimes. However, in the clinical study it was found that people with rampant caries sometimes had the habit of eating only one or two complete meals a day, often skipping breakfast and/or lunch. They generally made up for this by frequent between-meal consumption of sweets and soft drinks. It seemed obvious that if these between-meal sweets were the only food given to the animals in experiments, some of them would not survive very long. Therefore in addition to the particular beverage or food material to be tested, the animals were also given a basic diet which would furnish a minimum amount of nutrients essential for growth and survival. The basic diet could be considered to be the equivalent of the meals eaten by people. Because we sought to learn whether the different foods to be tested might have any anticariogenic effects as well as any cariogenic effects, two types of basic diets were used in parallel experiments. The first, Stephan diet 580, was developed in 1950 as a simple low-fat, high-sugar, fine-particle diet which produced smooth-surface as well as fissure caries in rats. The second, diet 581, is like diet 580 without the sucrose and was found to be practically noncariogenic by itself. The composition of these basic diets is given in Table 1.

In a preliminary experiment in which the basic diets were given as a single meal 9–11 A.M. daily, the time allowed for eating did not prove adequate in all instances since

*Purina Lab Chow, Ralston Purina Co., St. Louis, Mo.

TABLE 1

BASIC DIETS

Diet	Constituents	
Diet 580 (cariogenic)	Dried skim milk powder	32%
	Sucrose (granulated cane sugar XXX fine)	66%
	Whole dried liver substance (Wilson)	2%
Diet 581 (noncariogenic) .	Dried skim milk powder	32 parts
	Whole dried liver substance (Wilson)	2 parts

vegetables, fresh fruits, dried fruits, and snacks. They were made available to the animals continously, 24 hours a day, so that in effect they could be consumed ad lib., at and between meals.

In a preliminary experiment, 240 rats were used, 1 per cage, but in the main experiments described here 960 rats were used, with 4 animals per cage. Experiments were run in duplicate, so that there were 8 animals per group, or 16 animals for each food tested. Control groups were run in duplicate for each experiment, so that there were 16 control animals for each basic diet.

TABLE 2

EXPERIMENTS 6350–98: LIST OF FOODS TESTED

Carbohydrates	*Candies*	*Cookies*	*Biscuits*
Sucrose	Caramels	Chocolate sandwich	Teething biscuit
Dextrose	Chocolate	Vanilla wafers	Melba toast
Starch	Marshmallows	Baby cookies	Soda crackers
Confectioners' sugar	Mints	Chocolate graham	Graham crackers
Sorbitol†	Chewing gum	Honey grahams	Dog biscuits*

Breads	*White Bread with Spreads*	*Soft Drinks*	*Vegetables*
White bread	Bread+raspberry jam	Cola	Carrots
Whole wheat	Bread+grape jelly	Orange drink	Lettuce
Rye	Bread+peanut butter	10% sucrose water	Cabbage
Raisin	Bread+peanut butter+grape jelly	10% dextrose water	Potato
Cracked wheat	Bread+butter	Whole milk	Spinach

Fresh Fruits	*Dried Fruits*	*Snacks*	*Experimental Diets* (24 hours)
Apples	Figs	Peanuts	580
Bananas	Apricots	Popcorn	581
Oranges	Dates	Potato chips	585
Lemons	Raisins	Corn chips	593
Grapes			1503

No supplements (Control I and Control II)

* Diet records showed that occasionally some children who have dogs will try to eat dog biscuits.

† Sorbitol is actually a hexitol or "sugar alcohol."

several control animals on diet 581 lost weight and died. Therefore, in the following experiments the timing for meals was changed to two meals daily, given 9–10 A.M. and 3:30–4:30 P.M., and this proved adequate for survival of animals during the experimental periods.

Fifty-three representative foods and refreshments to be tested for cariogenic and anticariogenic properties were selected from diet sheets, which had been kept by patients with rampant caries. These test foods are listed in Table 2 and included several different carbohydrates, candies, cookies, biscuits, breads, bread with spreads, soft drinks,

The 2 control groups received 1 of the basic diets for 2 hours a day but no test food. In addition, 2 other groups received the basic diets (580 or 581) as experimental diets for 24 hours a day, and 3 groups received Stephan diet 585[6] or 593[6] or Keyes diet 1503[7] for purposes of comparison. Together these made a total of 60 groups. Thus, the 53 groups of animals which were given the test foods for 24 hours also had the opportunity to consume 1 of the 2 basic diets during two 1-hour periods per day (equivalent to mealtimes). The groups on the 5 comparison experimental diets (which included diets 580 or 581 as experimental diets rather

that as basic diets) had the diet available 24 hours daily, but the animals in these 5 groups did not get any additional diet or supplement. Distilled water was furnished to all animals, ad lib. The animals were weighed at the start of the experiment, at weekly intervals, and at the end of the experiment.

The basic diets (580 or 581) were fed in cups with nonscatter lids, which were placed in the cage and removed according to schedule. These cups were also used for the experimental diets. The solid test foods were fed in separate glass cups and were replaced with fresh cups daily. The liquid test foods and also drinking water were given from wide-mouth, rubber-stoppered bottles with stainless steel drinking tubes which extended into the cage. They were replaced with fresh bottles of liquids daily.

The experiments were concluded after the test foods had been fed for 28 days, when the animals were anesthetized and sacrificed. The heads were defleshed after autoclaving, the teeth sectioned with a rotary saw and scored for caries according to the method of Keyes.[8] Observations were made at 15× magnification under a stereomicroscope, and the location and extent of each carious lesion, the number of carious teeth, and the caries scores were recorded for each animal. In addition to this detailed evaluation for dental caries, an effort was made to evaluate quantitatively the effects of foods on total dental health. Stereomicroscopic observations were made for dental erosion, periodontal disease, dental calculus formation, adherent dental plaque formation, attrition, incisor pigmentation, alveolar bone density (porosity), and growth, and scored on a 0–4 scale. Most of these data are too extensive to include in a publication of this size. A comparison and statistical analysis of the data on caries scores and some data on the foods producing dental erosion are given in this paper, and only a brief analysis of the other conditions studied is included.

Results

A comparison of the effects of the 53 different foods on dental caries development is given in Table 3. This table lists for each food the mean caries score and the standard error of the mean for both the experimental groups fed basic diet 581 and those fed basic diet 580. At the top of Table 3 are listed the foods which were noncariogenic and possibly caries inhibitory. The foods are listed in order of increasing caries scores for the experimental groups with basic diet 581. Where the caries scores were the same, the foods are listed in order of increasing caries scores with basic diet 580. Thus, only those scores with diet 581 are necessarily in increasing order as listed. It should be noted that the control groups have not been placed at the top of the list even though caries was not produced with the diet 581 controls, since a mean caries score of 11.3 was produced with the diet 580 controls.

To get an over-all view of the experimental data, the list may conveniently be divided into five parts on the basis of statistical significance. Part *A* of Table 3, which includes the first five food materials (dog biscuit, popcorn, sorbitol, peanuts, and whole milk) produced no caries with diet 581 and also produced significantly less caries ($p < .01$ calculated with Students' t test) when fed with diet 580 than the diet 580 control; i.e., these test foods probably had some anticariogenic properties under the conditions of these experiments. Thus, foods like these may possibly have some potential beneficial properties from the standpoint of decreasing dental caries when an otherwise cariogenic diet is consumed. Obviously, this finding is suggestive for further studies that try to isolate any anticariogenic substances from such foods or to define more clearly the mechanisms by which they reduce caries with diet 580.

Part *B* of Table 3, like Part *A*, includes foods which produced no caries with basic diet 581, but unlike Part *A* the caries scores of foods with diet 580 in Part *B* were not significantly different at the $p < .01$ level from the control group, which had a mean caries score 11.3. Thus, foods such as these might be considered to be probably neither harmful nor beneficial from the standpoint of dental caries. It should be pointed out, however, that in Part *B* the lemons, dried apricots and oranges produced dental erosion in all cases even though they produced no caries with diet 581. This dental erosion will be described later.

Part *C* of Table 3 lists foods which produced a little caries, with a caries score less than 2.0 in the group fed diet 581. These foods could be considered to be slightly

TABLE 3

Comparison of Caries Scores Produced by Different Foods

Food Material Tested	Experiments with Noncariogenic Supplement (Basic Diet 501)		Experiments with Cariogenic Supplement (Basic Diet 500)	
	Mean	S.E.	Mean	S.E.
A:				
Dog biscuits	0.0	0.00	0.0	0.00
Popcorn	0.0	0.00	1.8	0.53
Sorbitol	0.0	0.00	1.0	1.00
Peanuts	0.0	0.00	2.3	1.61
Whole milk†	0.0	0.00	4.2	0.79
B:				
Lemons*	0.0	0.00	4.3	1.42
Corn chips	0.0	0.00	7.0	1.98
Cabbage	0.0	0.00	7.0	1.69
Lettuce	0.0	0.00	9.7	3.33
Control (basic diet only 2-hour supplement)	0.0	0.00	11.3	2.03
Dried apricots*	0.0	0.00	11.3	3.53
Oranges*	0.0	0.00	16.8	3.17
C:				
Soda crackers	0.3	0.24	11.6	2.81
Spinach	0.6	0.49	4.6	2.99
Cracked wheat bread	1.3	0.91	13.7	2.80
Potato chips	1.6	0.68	6.7	2.93
D:				
Whole wheat bread	2.0	0.13	17.7	2.01
Carrots†	2.1	0.05	21.1	1.42
Cornstarch†	3.3	0.49	10.2	2.28
White bread and butter	4.9	1.00	22.1	5.80
White bread and peanut butter	5.2	1.88	18.0	4.43
Graham crackers	8.7	1.81	22.7	3.98
Raisin bread	9.0	4.11	17.2	4.55
Melba toast	9.0	3.17	19.6	2.73
White bread	9.2	1.31	15.1	2.92
E:				
White bread and raspberry jam	10.2	1.63	33.0	4.81
Figs	10.3	1.92	29.0	6.06
Rye bread	12.7	2.98	29.0	6.30
Chewing gum	14.0	3.67	27.2	7.19
Caramels	16.0	6.21	50.4	9.42
Chocolate graham crackers	18.0	3.77	39.1	8.76
Baby cookies	18.5	1.25	22.0	6.27
Honey graham crackers	19.2	2.92	21.6	4.44
Apples*	19.4	8.01	81.7	7.17
Vanilla wafers	19.7	3.56	30.2	5.52
Bananas	21.0	6.06	73.7	7.60
Chocolate sandwich cookies	23.8	1.60	92.2	7.93
Grapes*	24.1	3.92	42.4	10.55
Candy mints	24.7	8.40	42.12	4.95
Teething biscuits	29.0	5.27	29.2	5.43
Cola*	29.6	8.46	40.2	9.59
Marshmallows	30.1	3.28	42.2	5.63
Dextrose	30.6	3.56	43.5	8.66
Raisins	30.9	5.32	61.6	7.78
10% sucrose water	32.3	12.20	20.6	5.00
Dates	32.7	3.39	38.5	6.28
Milk chocolate	34.1	8.90	41.2	11.30
White bread and jelly	36.7	7.22	36.2	9.44
Confectioners' sugar	38.4	4.68	60.2	8.12
10% dextrose water	41.3	9.88	45.5	8.66
Orange drink*	43.5	8.96	26.1	10.27
Sucrose	62.1	8.57	51.9	7.10

* Produced dental erosion

† Produced a higher than average caries score > 1.5

cariogenic, although the caries effects were not statistically significant at the $p < .01$ level with either basic diet.

Part *D* of Table 3 lists foods which produced caries scores from 2.0 to 10.0 with diet 581. These foods could be considered to be moderately cariogenic. With one exception (cornstarch) the foods gave increased caries scores with diet 580, but only in the case of carrots was the increase statistically significant at the $p < .01$ level in the groups fed basic diet 580.

Part *E* of Table 3 lists 27 foods which produced caries scores greater than 10.0 with diet 581. Practically all these foods were significantly cariogenic with diet 581 ($p < .01$), and most of them significantly increased caries with diet 580. The foods in Part *E* can be considered to be from moderately to highly cariogenic under the conditions of these experiments. All of them contained some fermentable sugar, and sucrose was the most cariogenic of all.

Table 4 lists the caries scores produced in the groups of rats fed the experimental diets 24 hours a day, giving a comparison of cariogenicity of these diets with the foods consumed by humans. It can be seen that diet 581 remained essentially noncariogenic when it was fed 24 hours a day and only one animal developed a minimal carious lesion in this group. However, in the group fed diet 580 24 hours a day, the mean caries score was 63.0, which was much higher than the control group fed diet 580 only 2 hours a day (mean caries score: 11.3) and was practically the same as the group fed sucrose with diet 581 (caries score: 62.1). Keyes diet 1503,[7] which Keyes designed to induce caries in hamsters, gave a mean caries score of 23.5 when fed to rats in these experiments. Stephan diet 585,[6] which is a coarse corn particle diet patterned after the Hoppert, Webber, and Canniff type but specifically designed to stimulate the growth of oral lactobacilli, gave a mean caries score of 38.8. Stephan diet 593,[5] which is a synthetic cariogenic diet patterned after diet 580, gave a caries score of 12.0 in these experiments. Thus, many human foods give caries scores comparable to these cariogenic experimental animal diets. The rats fed the standard laboratory diet did not develop any caries.

As was stated before, at the same time that microscopic examination of the rat jaws were made for carious lesions, observations were also made for dental erosion, dental calculus, attrition, periodontal destruction, visible plaque formation, incisor pigmentation, and porosity of alveolar bone. An arbitrary scoring system, 0–4, was used for all conditions observed to estimate the extent of differences, with 0 equal to none and amounts increasing through 1, 2, 3, and 4, with 4 representing the most extreme form of the condition observed.

A summary of the data for dental erosion is given in Table 5. Since only seven of the foods tested produced dental erosion, these foods are listed individually. The 46 other foods tested, which did not produce erosion, are given as a single group as are also the experimental diets. The pH of all foods was measured at the start of the experiments with a pH meter* and glass electrode. A little

TABLE 4

COMPARISON OF CARIES SCORES PRODUCED BY EXPERIMENTAL ANIMAL DIETS FED 24 HOURS A DAY FOR 28 DAYS

Diet	Mean Caries Score	Standard Error
580 (Stephan)	63.0	±12.37
581 (Stephan)	0.1	± 0.01
1503 (Keyes)	23.5	± 4.14
585 (Stephan)	38.8	± 3.99
593 (Stephan)	12.0	± 1.41
Lab chow	0.0	± 0.00

TABLE 5

DENTAL EROSION VS. FOOD pH

Food	pH	Erosion Scores (581)	Erosion Scores (580)
Cola	2.5	2.9	2.1
Apples	4.3	2.7	1.4
Orange slices	3.4	2.4	1.1
Lemon slices	2.5	1.9*	2.0
Grapes	3.4	1.9	1.4
Dried apricots	3.5	2.4	2.0
Orange drink	2.9	1.5	1.0
All (46) other supplements	4.6–8.2	0.0	0.0
Six experimental diets	5.8–6.4	0.0	0.0

* Also included marked erosion of incisor teeth.

In general, erosion scores were slightly higher with diet 581 than 580, except for lemon slices. Since diet 581 did not contain sugar (sucrose) as did diet 580, the animals ate more sweet supplements with diet 581 than diet 580.

* Beckman pH meter, Beckman Instruments, Fullerton, Calif.

distilled water was added to foods where necessary in order to get a fluid which could be measured. It can be seen in Table 5 that all the foods which produced dental erosion were all quite acid in reaction (below pH 4.5), whereas all of the foods which did not produce dental erosion were only moderately acid or alkaline in reaction (above pH 4.5). These results are in agreement with other experiments in which experimental dental erosion has been produced in rats with acids and acid salts in the drinking water or diets. It was interesting to observe that the beverages with an acid reaction produced erosion chiefly on the lingual surfaces of the molar teeth where the animal's tongue contacted them, whereas the more solid foods produced erosion chiefly on the occlusal surfaces. Erosion of the incisor teeth was also seen in some cases.

The observations on periodontal disease as scored by estimating the extent of alveolar bone destruction showed that relatively little alveolar bone destruction occurred during the 28 days of the experiment. In most cases this seemed associated with the impaction of hair in the interproximal spaces and periodontal membrane between the teeth, and no mean scores above 2 were recorded for any of the foods. Mean periodontal scores above 1.0 were produced only with sucrose, dextrose, confectioners' sugar, and cola beverage; but none of these was significantly different from the controls at the $p < .01$ level.

Dental calculus formation, scored on the method of Baer, Stephan, and White,[9] was also not very extensive. The lowest calculus scores occurred when supplements of fresh fruit were given and the highest scores with whole milk and starch. Again, none of the differences was significant at the $p < .01$ level when compared with the control groups. The calculus was all of the supragingival or salivary type, although in a few cases transparent and pearly calculus was formed as well as the rough or chalky type. In no case did this calculus formation appear to be associated with alveolar bone destruction.

Bacterial plaque material could be seen on the teeth of live animals, but most of this did not adhere to the teeth after the rat heads were prepared for microscopic observation. In many cases where caries was extensive, relatively thick deposits of dried plaque material remained around carious tooth surfaces, and the bacterial nature of this material could be demonstrated readily by stained smears and phase-microscopical examination at 1,000× magnification. However, due to the possible loss of plaque material in the preparation of rat heads for microscopical examination, no attempt has been made to correlate plaque material with caries here.

Differences in the porosity of alveolar bone were seen in the sawed sections of the jaws, and it was obvious that the foods which were deficient in minerals or other essential nutrients tended to produce porous alveolar bone formation in some of the animals in these experiments. As might be expected, the dog biscuit produced very dense alveolar bone formation, whereas in the animals fed sucrose or the other carbohydrates with diet 580, the alveolar bones were relatively spongy or porous.

During the 28 days of the experiment, the animals were in a very active growth period, and an animal would normally be expected to gain over 100 Gm. on an optimal diet. Most of the supplements tested were nutritionally deficient in several respects and did not support maximum growth rates when fed with either diet 580 or diet 581. Besides the standard laboratory diet, the only food tested which gave optimal growth was the dog biscuit. Near-optimal growth was given by supplements of peanuts, baby cookies, and the different breads and bread with spreads; but most other supplements gave definitely suboptimal growth. Particularly poor growth was produced by 10 per cent cerelose solution and the cola drink. Since the animals seemed to develop a great fondness for these sweet beverages, it is not surprising that growth was affected unfavorably. While the observations on the effects of the foods tested on growth and development of bone structure are significant, the individual data on these observations are too extensive to give here.

Discussion

The results of these experiments indicate that many of the types of foods commonly eaten by people with rampant caries may be highly cariogenic when they are eaten without restriction by rats. Certain other

foods may be relatively noncariogenic or possibly even exert a protective effect against caries under similar conditions.

In general, foods which were most cariogenic had a relatively highly fermentable sugar content but this relationship was not necessarily quantitative. It did not seem to matter whether the sugar content was produced by the addition of a refined sugar (sucrose or dextrose) or whether the sugar content was naturally present in food material, such as fructose, sucrose, or dextrose, which may be present in fruits such as bananas, apples, grapes, or raisins. From a biochemical standpoint, this result is not surprising in view of the fact that apples normally contain about 11 per cent sugar, fresh bananas may contain 19.2 per cent sugar, grapes 14.9 per cent sugar, and raisins 63 per cent sugar.[10] The finding that some fruits may induce caries in rats is very important, since it furnishes evidence from controlled experiments that these foods need to be re-evaluated from the dental caries standpoint. This finding fails to support the frequently voiced opinion that foods with naturally occurring sugars (sucrose, dextrose, fructose, or maltose), such as fruits, are noncariogenic or that fruits in general contain some unknown protective substances which presumably render them noncariogenic. Also, there seems to be no valid reason to believe that the sucrose naturally present in sugar beets or sugar cane is chemically changed during the sugar refining process or is any different from the sucrose present in many fruits. Thus, from the results of these considerations and experiments, there appears to be a need for a reappraisal of the scientific evidence regarding current programs which advocate the feeding of fruit such as apples to children in schools as a preventive measure for the control of dental caries. Of course, it should also be recognized that the physical properties of foods such as apples may exert a beneficial cleaning effect on the teeth when the food is eaten. At the same time the chemical properties of the food may exert a harmful acid or acid-producing effect, which may tend to produce dental erosion or dental caries. The effects of a given food on the teeth might depend upon a balance of several factors and particularly how or how often it was eaten. In these experiments, the animals seemed to like to eat most of the foods tested, and the only fruit which they did not consume in a relatively large amount was lemon. The animals which were given lemon slices developed dental erosion, but not dental caries. It was surprising that rye bread produced a significant caries score, but the formula of the particular brand of rye bread which had been used showed that sugar had been included in it.

It would seem important to recognize that starch alone may have some cariogenic properties. The less cariogenic intermediate group of foods generally contained more starch than sugar. Thus, cornstarch proved to be somewhat cariogenic in the diet 581 experiments (caries score 3.0), since its difference from the control was significant at the $p < .01$ level. However, the contrast between cornstarch and sucrose was striking, and sucrose proved to be highly cariogenic (caries score 62.0). The differences of the sucrose group from either the starch group or control group were significant at the $p < .01$ level.

The comparison of the control group with the whole milk group, whole wheat bread group, or peanut group, showed no difference, i.e., none was cariogenic. Thus, these experiments suggest that not all foods which are consumed between meals are by themselves necessarily cariogenic, and a food such as whole milk alone may possibly serve as a noncariogenic beneficial between-meal snack. The fact that whole milk proved noncariogenic as well as diet 581 (which contains approximately 50 per cent lactose), indicates that lactose (milk sugar) is a relatively noncariogenic sugar. It is known that many oral micro-organisms may readily ferment dextrose, fructose, and sucrose but not readily ferment lactose or sorbitol. Also, the author has found that lactose did not lower the pH of dental plaques as did sucrose and some other sugars.[11] Since lactose is apparently solely of mammalian origin,[12] one may raise the question as to why mammals have lactose secreted by the mammary glands rather than dextrose (glucose), which is the sugar normally present in the blood. Among the reasons, one could speculate that from an evolutionary standpoint this might be a naturally selected biological protective mechanism against the potential destructive fermentation action of glucose by bacteria on the teeth.

Potential anticariogenic effects of foods as

well as the caries-increasing effects are suggested in the experiments in which diet 580 was the basic diet. In these experimental groups, even though the basic diet was fed for only 2 hours daily, some caries were produced in the control group without any test food, as well as in the test-food groups. Here some test foods, such as peanuts, gave a significant caries-reducing, or cariostatic, effect; and some test foods, such as sucrose, gave a significant caries-increasing, or additional cariogenic, effect. Of course, a few may exert a cariostatic effect by reducing the animals' appetite for the cariogenic diet. These differences require a careful analysis and suggest that further studies be made for anticariogenic properties in different foods.

In general, the effects of the test foods given with basic diet 580 were consistent with the effects when basic diet 581 was given, but it was clear that since diet 580 contained 66 per cent sucrose, the animals were not as hungry for more sugar or sugar-containing foods as when diet 581 was the basic diet. For example, it was observed during the experiments that when a supplement of 10 per cent sucrose solution in H_2O was given, the animals on diet 581 drank more of it and less plain water than when diet 580 was given. Thus, with diet 580, where more than one source of sugar was given, the animals could satisfy their taste for sweets from either of the sources during the meal period and thus during the in-between-meal periods the desire for sweets was lessened. This may offer an explanation as to why caries was increased in a relatively greater extent by three of the four drinking fluids containing sugar when no sucrose was present in diet 581, than when sucrose was present in diet 580.

It is important to recognize that the physical properties of foods may greatly affect their retention on tooth surfaces, and that retention of foods on tooth surfaces after eating also affects the development of caries.

In the previous study with humans, several severe cases of dental erosion were seen in addition to caries. The histories of three of these cases indicated a prolonged and excessive consumption of acid foods, including grapes in one case, of apples in another case, and of citrus fruits in the third. In the present animal experiment, of the 53 foods tested there were just seven which produced erosion in the teeth. None of the comparison diets or controls produced any cases of erosion. It was very interesting that the pH levels of these seven foods were all below the pH levels of any of the other foods or diets and that all animals that consumed these foods had some degree of erosion.

While it should always be recognized that animal experiments are not necessarily directly applicable to humans, the experiments described in this paper have been based on previous clinical studies which have indicated the important role of between-meal consumption of various types of foods in the development of rampant caries and also of dental erosion. Thus, these animal experiments may lend support and help to clarify some of the conclusions indicated from clinical observations. At the present state of knowledge, we need to integrate the results of clinical and epidemiological studies in humans and controlled laboratory and animal experiments to arrive at the most intelligent ways with which to solve the problems in the prevention of dental diseases.

Summary

The experiments described in this paper are generally consistant with our clinical studies of rampant dental caries, which indicate that there are many foods which may contribute to the development of caries when consumed in excess between meals. They also indicate that there are some very important differences in the effects on the teeth of different types of foods and beverages commonly consumed by people between meals. They help in clarifying some of the confusion that has existed regarding which foods may or may not be cariogenic and do not lend support to the contention that sugar (sucrose) present in foods naturally is different in cariogenicity from the same sugar when it has been refined and added to foods. The experiments support those dentists who have taught that the habitual and excessive between-meal consumption of candy, cookies, and soft drinks may lead to the development of rampant caries in susceptible individuals. Since scientific evidence for this conclusion has been accumulating for a long time, the problem of applying this knowledge seems

largely to have been limited to trying to educate people to avoid eating candy and sweets.

The results of these experiments indicate that this advice may not be sufficient to control rampant caries since many other foods which people like contain readily fermentable carbohydrates and are potentially cariogenic. Unfortunately, prohibition of eating sweets like any other prohibition is apt to be resented by people, consciously or unconsciously, and must be considered as a negative approach. However, the results of these experiments also suggest a more positive approach to the dental health problem. A small number of the foods tested, such as cows' milk, proved noncariogenic. Since most people in our society tend to consume more and more snacks and prepared convenience foods, the recognition, selection, or development of a greater variety of noncariogenic foods of this type would seem to offer a more positive answer to the problem. Further experiments along this line are being conducted.

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A Preliminary Report:

Caries Experience and Between-Meal Eating Habits of Pre-School Children

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INTRODUCTION

Early in 1955 the dental staff of the West Tennessee Regional Office of the Tennessee Department of Public Health became interested in a study investigating the relationship of dental caries experience and between-meal-eating-habits of five-year-old children.

Five-year-old children were selected for the study, since the dental staff routinely sees more than 1,000 five-year-olds and their parents at pre-school conferences each spring. At these pre-school conferences it is possible for the dental officer or dental hygienist to interview each child and one or both of his parents.

PILOT STUDY

A pilot study was planned for March, April and May of 1955 and data for the study were collected at 53 pre-school conferences in eight West Tennessee Counties.

A total of 1,373 dental inspections and child parent interviews were conducted by members of the dental staff. Members of the dental staff participating in the study included two dental officers, a dental extern and a dental hygienist.

Information collected at these pre-school conferences included d. e. f. teeth for each child inspected and a dietary history of items each child usually ate between meals.

For the pilot study, a simple check type questionnaire was used to record individual d. e. f. caries experience and the different types of confections usually eaten between meals by the child.

The d. e. f. caries experience was obtained by counting d. e. f. teeth. The kind of confections usually eaten between meals was obtained by questioning the child and parents. Although personnel conducting the interviews were inexperienced in interviewing techniques, an effort was made to be as objective as possible by permitting the child and parents to do most of the talking after the initial question of: "What do you usually eat between meals?"

For purposes of tabulation, confections were grouped as follows: candies, chewing gum, carbonated drinks, pastries, peanut butter, jellies, jam or syrups, chocolate milk, and frozen products. No attempt was made to record non-cariogenic foods such as fresh fruits or vegetables.

FINDINGS

The findings of the study are shown in Table I where the percentage of children regularly eating various between meal confections is shown. "Regularly" was arbitrarily defined as almost every day.

Table I. Per cent of 1,373 West Tennessee pre-school children regularly eating various between meal confections.

Confections	*Per cent of children regularly eating confections*
Candies	38%
Chewing gum	35%
Carbonated Drinks	32%
Pastries	30%
Peanut Butter	18%
Jelly, Jam or Syrup	15%
Chocolate Milk	12%
Frozen Products*	10%

* Probably too low, since frozen products are a seasonal confection and these interviews were done in early spring.

There appeared to be a direct relationship between the number of different types of confections routinely eaten between meals and the number of d. e. f. teeth observed. The relationship was suspected and is confirmed in Table II.

Table II. Comparison of d. e. f. teeth and number of different kinds of confections regularly eaten between meals.

No. of Confections eaten regularly	*d. e. f. teeth*
0	2.3
1-2	4.5
3+	8.0

SUMMARY AND CONCLUSION

The study indicated that approximately one-third of these 1,373 pre-school children consumed candy, chewing gum, or soft drinks, between meals almost every day. The study also indicated a direct relationship between the number of these confections and d. e. f. caries experience.

1956 STUDY

In the spring of 1956 the West Tennessee Dental Staff decided to repeat the study, and revised the objective of the study to investigate the relationship between d. e. f. teeth and the actual number of times confections were etaen between meals for the day previous to the child health conference. With the help of the Nutrition and Statistical Services of the Tennessee Department of Public Health, the questionnaire was revised to record this more specific information.

The methodolgy of collecting the data was much the same as the previous year except the children and parents were questioned specifically about what the child ate between meals the previ-

ous day and also how many times he may have had various cariogenic items.

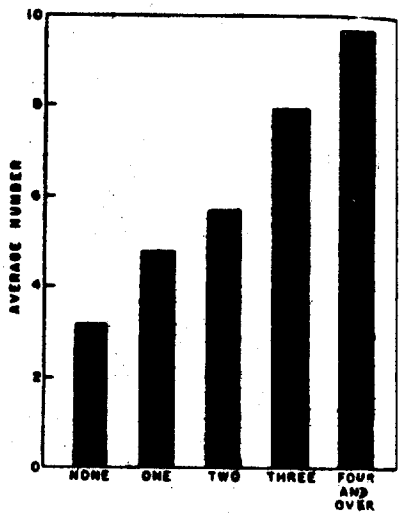
Data for the 1956 study were collected at 36 pre-school conferences in 13 counties and included a sample of 1,124 five-year-old children. One dental officer, two dental externs and one dental hygienist collected the data during March, April and May of 1956. These data were tabulated and summarized by the Statistical Service of the Tennessee Department of Public Health.

FINDINGS

As was suspected there appeared to be a direct and consistent relationship between the number of d. e. f. teeth and the number of times confections were eaten between meals on the day previous to the pre-school conference. The number of between-meal sweets eaten during the one day previous to the conference is not significant in itself. However, in a sample as large as 1,124 children it appears that a one day diet history can be used as an index to measure in between meal eating habit patterns.

This relationship between d. e. f. teeth and number of between meal sweets is shown in Figure 1.

Figure 1. AVERAGE NUMBER OF def TEETH PER CHILD ACCORDING TO BETWEEN-MEAL SNACKS, PRESCHOOL CHILDREN, WEST TENNESSEE, 1956.



SUMMARY AND CONCLUSION

The positive relationship between d. e. f. teeth and number of between-meal confections was confirmed as suspected. However, the almost consistent progression of one additional d. e. f. tooth for each additional between-meal confection was not anticipated.

It appears from this study that in a large enough sample of children a fairly accurate prediction of d. e. f. caries experience can be made by even a moderately accurate between-meal diet history for only one day.

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Relation of oral biochemistry of sugars to the development of caries

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Although scientists' knowledge of the etiology of dental caries is fragmentary, there is general agreement that the minimal oral conditions that must be fulfilled before the initial carious lesion may be anticipated are twofold; namely, the presence of microorganisms and fermentable carbohydrates. Accordingly, it is important to review the knowledge concerning the oral physiology and biochemistry of sugars and allied substances.

The work herein reported is limited to that of the author and his associates.[1-3] It is not to be inferred that the observations and conclusions are original with us; rather, they are in agreement with and supplementary to those of many other investigators. It is only by dovetailing data from a variety of sources that the pertinent body of knowledge is established. A further limitation of this presentation is that it concerns itself exclusively with *in vitro* and *in vivo* observations with human subjects and materials. This does not mean that the vast amount of information accumulated from animal investigations is unimportant, but rather that it is beyond the scope of this paper. Parallel animal studies from these laboratories have been reported in detail elsewhere.[4]

The presence of sugars in and about the teeth is explainable on the basis either of their adhering to the teeth during the act of digestion or their being secreted into the oral cavity as a normal constituent of the saliva. It is, therefore, important to ascertain whether the salivary glands normally secrete sugar in significant quantities. It is not unusual in surveying the older literature to find statements supporting the view that saliva normally contains appreciable quantities of sugar and that these values are further increased during certain pathologic states as, for example, diabetes. In all probability, these beliefs may be explained by the nonspecific nature of biochemical methods available to early research workers for the detection of sugars. Virtually all of the present investigators active in the field are in agreement that the quantity of sugar secreted in the saliva is negligible.

Presented as part of a panel discussion "Effects of sugars and other carbohydrates on the teeth" before the Sections on Research, Pedodontics and Public Health Dentistry, ninety-fifth annual session, American Dental Association, Miami, Fla., November 9, 1954.

Dean, University of Alabama School of Dentistry.

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Table 1 • Daily glucose* variation

Subject	1st day†	2nd day	3rd day	4th day	5th day	Av.
1	15	11	14	32	23	19
2	20	16	14	8	15	14
3	20	23	19	32	10	21
4	17	22	23	21	15	19
5	17	15	16	40	11	20
6	20	9	21	16	16	16
7	21	11	11	15	26	17
8	23	8	32	25	23	22
9	19	21	23	16	13	18
10	18	22	28	18	13	20
11	16	20	25	33	18	22
12	19	12	26	18	14	18

*Reducing substances calculated as glucose.
†All values are mg. %.

GLUCOSE LEVELS IN SALIVA

The author has studied the normal salivary glucose level of 12 subjects over a period of six days. When samples were taken two hours after eating, values which ranged from 14 to 22 mg. per cent were found. He concurs with other workers that most, if not all, of this value is attributable to reducing substances other than glucose. These data may be seen in Table 1.

In addition to the observations on normal persons, the possibility of a divergent observation in diabetic children has been considered. From a consideration of the data accumulated in many laboratories, including those at the University of Alabama, it may be concluded that there is no appreciable elevation of salivary sugar in persons afflicted with this condition. It might, therefore, be said that analyses of saliva, two hours after eating, reveal negligible quantities of glucose and that there is evidence that the normal value is profoundly influenced by physiologic or pathologic factors. In making this statement [illegible] it [illegible] remembered that glucoprotein[illegible] are [illegible] constituents of saliva and that hydrolysis of these materials could influence the salivary sugar [illegible] possibility is in [illegible]

It is probable that some of the conflicting reports found in the early literature regarding the presence of sugar in the saliva are attributable to a lack of understanding of the normal oral clearance mechanism of sugars. Those investigators who took samples immediately after the ingestion of meals found high sugar values. Those making analyses several hours after eating found none. These divergent conclusions may be reconciled in the light of more recent studies wherein salivary glucose levels have been determined before eating and at stated intervals after the ingestion of food and confections. In the laboratory at the University of Alabama School of Dentistry, the glucose concentration of saliva before and after the chewing of gum and the eating of hard and soft mints, chewy candies, cookies and bread and jam has been observed. These observations are reported in Table 2. It will be noted that although the pre-eating sample has minimal glucose, it is not unusual for concentrations in excess of 500 mg. per cent to occur in the mouth after the eating of typical snacks.

Subsequently, these studies were extended to include the salivary clearance of glucose after the ingestion of an Army field ration. This was done because we were interested in observing the variations in salivary sugar levels with something more complex than a snack. The Army field ration offered the opportunity of accumulating data on a standardized meal containing sugars and allied substances in the form of hard biscuit, flavored drink and candy. The data for three test subjects are presented in Table 3. From these observations it may be concluded that it is possible to maintain high salivary glucose levels for an hour or [illegible] by ingesting a meal containing sugars and allied substances in a variety of physical forms.

[illegible]

Table 2 • Glucose* concentration of human saliva after use of food and confections

Substance	No. subjects	Time of eating in min.	Av. glucose concentration in mg. %			
			0 time†	5 min.‡	15 min.‡	30 min.‡
Chewing gum, Dentyne, 3.2 Gm.	28	30 (chewing)	19	484	147	26
Soft mint, Birch Bit, 2.5 Gm.	12	4	16	662	131	23
Hard mint, Toronto Mint, 1.9 Gm.	12	9 (av.)	21	377	259	24
Chewy candy, Choclette, 5.9 Gm.	11	7	24	1,909	269	26
Cookie, vanilla wafer, 8.2 Gm.	12	2.5	16	889	117	18
Bread and jam, 76.0 Gm.	12	3	25	1,475	149	22

*Reducing substances in saliva calculated as glucose.

†Zero time value is control value taken immediately before beginning the ingestion of the test substances.

‡Time intervening since the beginning of ingestion of the test substances.

ingesti n of the hard candy taken at the end o the field ration meal. In order that w might study this further, a group of four subjects volunteered to suck lollypops. T e data accumulated in this experimer t may be seen in Table 4. It is appare that the ingestion of certain confections as exemplified by the lollypop makes for a considerable and prolonged elevation in the salivary glucose level. It is of great importance that the oral clearance of representative sugar-containing food continues to be studied, and it is most fortunate that this problem has received the continuing interest of competent researchers, including Bibby[5] and Lundqvist[6] and their associates.

The variations in the preliminary studies pointed to the need for expanding the knowledge of the factors influencing the clearance of oral glucose. Although the importance of the rate of salivary flow, the morphology of the dental arches and the action of the muscles of mastication was recognized, an investigation of these factors has been deferred until after the role of the physical nature of the sugar supplement and the manner in which it is consumed has been studied in more detail.

Six young adults were utilized as test subjects, and salivary samples were taken before and at stated intervals after the ingestion by eating, sucking, drinking and chewing of 500 mg. quantities of glucose. These observations are presented in Table 5. It may be concluded that the clearance of the sugar is obviously dependent on

5. Bibby, B. G.; Goldberg, H. J. V., and Chen, E. Evaluation of caries-producing potentialities of various foodstuffs. J.A.D.A. 42:491, May 1951.

6. Lundqvist, C. E. Oral sugar clearance; its influence on dental caries activity. Odont. Revy Suppl. 1, 1952.

3 • Clearance from oral cavity of glucose* contained in Army field ration

	Beginning of ingestion period	Halfway through biscuits and drink	10 min. after biscuits and drink	Halfway through candy	10 min. after candy
	47 (0)	1,920 (13.5)	300 (41)	2,480 (58)	255 (90)
B	28 (0)	700 (10.5)	145 (33.5)	1,480 (55)	325 (83)
	30 (0)	1,100 (8)	365 (26)	3,480 (53)	380 (82)

[illegible] are recorded as mg.

Table 4 • Clearance from the oral cavity of glucose* (mg. %) contained in lollypops

Subject	0 time	15 min.	30 min.	1 hr.	2 hr.
A†	27	6,160	3,840	245	72
B	23	700	1,290	870	47
C	22	1,290	5,160	2,440	54
D	21	4,320	6,160	1,060	64

*Reducing substances in saliva calculated as glucose.
†Subject consumed candy in approximately one hour. Others needed between one and one-quarter hours.

the physical form in which it is fed and that the quantity of sugar is of secondary importance.

CONVERSION OF SUCROSE TO GLUCOSE

All the data reported in the foregoing experiments are based on analyses of saliva for glucose. This has been done because the methods available for detecting this sugar are well established and because it is assumed with reservations that other cariogenic substances, particularly the polysaccharide starch and the disaccharide sucrose, must be hydrolyzed to six-carbon reducing sugars before they are of importance in caries etiology. Obviously, many of the experimental snacks and meals contained starch and sucrose, and it seems logical to expect that they contribute in part to the glucose salivary levels. The oral hydrolysis of starch to glucose has been studied in considerable detail, but little is known of the ability of sucrose to be hydrolyzed by oral enzymes. In an effort to extend our knowledge of this phenomenon, we have studied the *in vitro* and *in vivo* abilities of saliva to convert sucrose to glucose. It has been found that if 1 per cent sucrose is added to 10 cc. of saliva, the glucose concentration of the mixture increases from a control value of 15 mg. per cent to 30 mg. per cent within two minutes. If the sucrose concentration of the saliva is increased to 10 per cent, the two minutes [illegible] concentration is increased to 200 mg. per cent or more. These data are shown in Figure 1.

The *in vitro* experiments have been supplemented by limited *in vivo* observations. A small group of human subjects volunteered to ingest 1 Gm. portions of a ration previously shown to be cariogenic in the Syrian hamster. This ration had 5 per cent sucrose but no glucose. Beginning at two minutes after the ingestion of the diet and continuing through 16 minutes at two-minute intervals, determination of the salivary concentration of actual and potential glucose was made. (The term potential glucose represents sucrose present in the saliva that could be converted to reducing substances.) The results of the *in vivo* experiments were found to parallel the results of the *in vitro* experiments; namely, that within two minutes there appeared in the saliva amounts of glucose in excess of 100 mg. per cent. These data may be seen in Figure 2.

After a consideration of the results, it has been concluded that appreciable quantities of dietary sucrose are converted to reducing sugars almost immediately on contact with certain enzymes normally present in the saliva or salivary microorganisms. It has been concluded further that the salivary glucose deter-

Table 5 • Amount (mg.%) of glucose* in saliva before, during and after use of 500 mg. glucose

How used	Time in minutes				
	0	2	9	16	30
Eaten (cube)†	18	1,425	68	20	14
Sucked (wafer)	18	3,304	1,860	1,125	229‡
Rinsed (solution)§	21	832	105	25	17
Chewed (gum base)‖	23	725	204	144	27

*Reducing substances in saliva calculated as glucose.
†The glucose cube (cube) was eaten within 1 minute.
‡Three of the six subjects had not completely dissolved their wafer. The 30 minute average value for those dissolving the wafer was 91 mg. per cent.
§The glucose solution (1 cc.) was rinsed in the mouth for one minute prior to swallowing.
‖The glucose in the gum base was chewed for the experimental period.

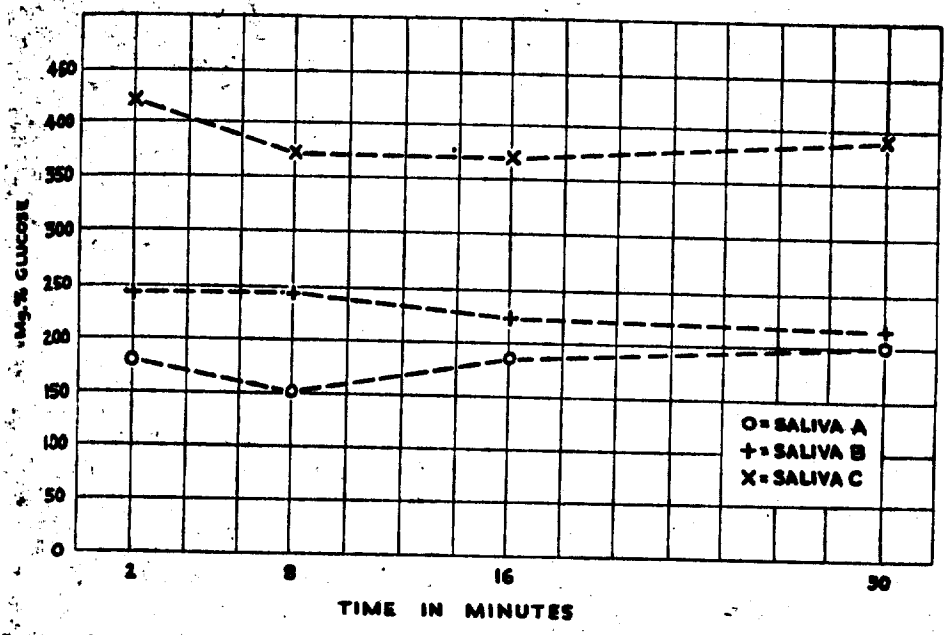

Fig. 1 • Glucose formation in saliva-sucrose mixtures (10 cc. saliva with 10 per cent sucrose). Term glucose represents reducing substances in saliva calculated as glucose. There is a possibility that some of the glucose formed could not be detected because it was broken down by microbial fermentation

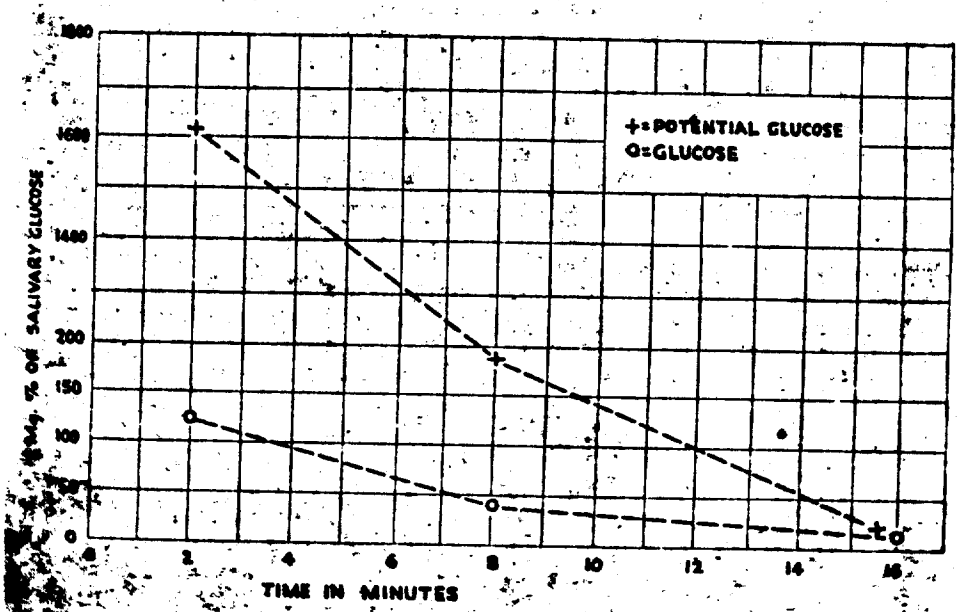

Fig. 2 • In vivo salivary sucrose to glucose conversion after ingestion of 1 Gm. 5 per cent sucrose ration. Values for glucose and potential glucose include fructose whose reducing equivalent has been calculated as glucose. There is a possibility that some of the glucose formed could not be detected because it was broken down by microbial fermentation

Table 6 • Acid formation in saliva-carbohydrate mixtures*

Substrate	pH of mixture at various time intervals						Terminal titratable acidity cc./0.025 N acid
	0 min.	30 min.	1 hr.	2 hr.	3 hr.	4 hr.	
Glucose	6.8	6.7	6.0	4.6	4.4	4.4	5.8
Sucrose	6.8	6.7	6.0	4.5	4.4	4.4	5.8
Starch	6.8	6.2	4.7	4.5	4.4	4.4	7.1
Fructose	6.9	6.6	4.9	4.4	4.4	4.3	4.0
White dextrin	6.8	6.1	5.0	4.5	4.3	4.3	5.3

*Mixtures contained 2.5 cc. of 10 per cent glucose, sucrose, fructose, and white dextrin and 5 cc. of 5 per cent starch plus 5 cc. of pooled saliva.

mined in our and in other oral carbohydrate clearance studies represents, in addition to the dietary glucose and the glucose formed by the hydrolysis of starch, a variable percentage of glucose derived from sucrose.

The data presented in the previous tables and illustrations throw considerable light on the normal glucose content of saliva as it is secreted by the glands, the extent and persistence of glucose in the saliva after test meals, the relation of the physical nature of the glucose-containing food to the glucose retention pattern, and the ability of enzymes in saliva to convert sucrose to glucose. Although all this is worthwhile information, its value is enhanced if it serves as a background for studying the ability of enzymes in the saliva to convert glucose and allied substances to organic acids, particularly if one concurs with the author in his belief that the latter are etiologic agents in the caries process. Such discontinuing investigation has been attempted by the author and his associates.

CONVERSION OF SUGARS TO ACIDS

The comparative [illegible] of representative monosaccharides and disaccharides to serve as a substrate for acid production by enzymes in saliva [illegible] been studied in some detail. To 5 cc. [illegible] aliquots of pooled saliva from caries-active patients [illegible] added 2.5 cc. of con[illegible] solution [illegible] the test carbohydrate [illegible] was followed by pH determinations of the mixtures at one-half, one, two, three and four hours and a terminal acidity recorded at the end of the four hour period. The data are reported in Table 6. It will be noted that the observations with fructose, sucrose, glucose, starch and white dextrin were comparable and that each was readily susceptible to the action of those enzymes in the saliva associated with acid production.

In subjecting these data to self-criticism, it was noted that each of the test materials could be designated as refined sugars and allied substances, and the immediate question that came to mind was whether or not their unrefined counterparts would be equally susceptible to the action of enzymes in the saliva. In an effort to clarify this point, the experiment was repeated and the susceptibility of honey, maple sugar, raw sugar and farina to acid formation was compared with that of refined glucose and sucrose. The data in Table 7 show that raw sugars are as prone to support acid production as are refined sugars.

As the studies progressed, we became increasingly aware that the conditions of our *in vitro* experiments were quite different from those occurring on the tooth surface. The principal differences were as follows: (1) whereas the sugar concentration in the mouth rarely [illegible] little [illegible] 15, [illegible] 10,000 [illegible]

Table 7.—Acid formation in saliva-carbohydrate mixtures*

Substrate	pH of mixture at various time intervals					Terminal titratable acidity cc./0.025 N acid
	0 min.	30 min.	1 hr.	2 hr.	3 hr.	
Glucose	6.8	6.1	5.3	4.6	4.4	4.3
Sucrose	6.9	6.3	5.6	4.7	4.4	4.0
Starch	6.9	6.3	5.5	4.6	4.4	5.9
Farina	6.8	6.0	5.5	4.7	4.4	6.3
Honey	7.0	6.5	5.9	4.8	4.6	4.0
Maple sugar	7.0	6.2	5.5	4.7	4.5	4.7

*Mixtures contained 2.5 cc. of 10 per cent glucose, sucrose, honey and maple sugar and 5 cc. of 5 per cent starch and farina plus 5 cc. of pooled saliva.

stant and (2) the concentration of oral enzymes in our saliva-sugar test tube mixtures was probably minimal when compared to conditions found in dental plaques. Accordingly, experiments were designed to reconcile partially the differences referred to.

To 5 cc. aqueous solutions containing 0 to 10,000 mg. per cent of glucose and sucrose were added 5 cc. aliquots of saliva from caries-active persons. Acid production of the mixture was followed by the recording of the pH every 30 minutes over a three hour period. After the last pH reading, the titratable acidity was measured. Typical observations are presented in Table 8. It is apparent that acid production is comparable in the 100, 500, 1,000 and 10,000 mg. per cent glucose solutions through the first hour and is comparable for the 500, 1,000 and 10,000 mg. per cent of glucose for the entire three hour period. Acid production in the 100 mg. per cent glucose solution ceased after one hour, and analyses of the saliva-glucose mixture indicated that all of the available glucose had been utilized within that period. Although the observations with sucrose are not recorded in the table, they are almost identical with those of glucose.

The probable influence of the concentration of salivary enzymes on sugar breakdown has been studied by comparing acid production of the supernatant and sedimentary fractions of centrifuged, pooled saliva from caries-active patients. Five cubic centimeters of 10 per cent glucose or sucrose has been added to the same volume of sediment or supernatant saliva, and the pH of the mixture has been determined at 5, 15, 30, 45 and 60 minutes of incubation. Acid production was demonstrable in the sediment within five minutes and in 60 minutes required 6 cc. of 0.025 normal sodium

Table 8.—Effect of carbohydrate concentration on acid production in glucose-saliva mixtures*

Initial conc. mg. % of glucose	pH of mixtures at various time intervals							Terminal titratable acidity cc./0.025 N acid
	0 min.	30 min.	60 min.	90 min.	120 min.	150 min.	180 min.	
0	6.8	6.3	6.2	6.2	6.2	6.2	6.2	2.0
100	6.8	5.8	5.3	5.1	5.1	5.1	5.0	3.1
500	6.8	5.7	5.3	4.7	4.6	4.4	4.4	5.9
1,000	6.8	5.8	5.0	4.6	4.5	4.5	4.4	5.7
10,000	6.8	5.9	5.1	4.7	4.5	4.8	4.3	5.9

*Mixtures contained 5 cc. of pooled saliva and 5 cc. of aqueous solution of glucose.

Table 9 • Acid production in glucose-saliva mixtures

Description of mixture	pH of mixture at various time intervals						Terminal titratable acidity cc./0.025 N acid
	0 min.	5 min.	15 min.	30 min.	45 min.	60 min.	
5 cc. supernatant saliva plus 5 cc. 10% glucose	7.1	7.0	7.0	6.9	6.9	6.8	2.0
5 cc. sediment saliva plus 5 cc. 10% glucose	7.2	6.5	5.5	4.3	4.0	4.0	6.0

hydroxide for neutralization. In contrast, no appreciable acid production was demonstrable in the supernatant sample. These data are recorded in Table 9.

Taken collectively, the data shown in Tables 8 and 9 fortify the belief that the concentrations of sugars and oral enzymes that are likely to be found on the tooth surfaces will support appreciable acid production. Whether or not this acid production is a direct etiologic factor in dental caries is beyond the scope of this paper but is the subject of continuing investigation in the laboratories of our institution.

COMMENT

In closing the author would like to make a strong plea for a more critical understanding of the suspected relation of oral sugar physiology and biochemistry to the etiology of dental caries. It is his personal opinion that generalizations are incompatible with the accumulated findings of responsible investigators. He would remind the many self-appointed armchair experts currently making pronouncements on the subject that professional opinion cannot establish scientific facts.

Method of Science • There are in science immense numbers of different methods, appropriate to different classes of problems; but over and above them all, there is something not easily definable, which may be called the method of science. It was formerly customary to identify this with the inductive method, and to associate it with the name of Bacon. But the true inductive method was not discovered by Bacon, and the true method of science is something which includes deduction as much as induction, logic and mathematics as much as botany and geology. *Bertrand Russell, Mysticism and Logic, 1918.*

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EFFECTS OF NATURAL AND REFINED SUGARS ON ORAL LACTOBACILLI AND CARIES AMONG PRIMITIVE ESKIMOS

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AND
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A field study was made of the Eskimos of the lower Kuskokwim River area, in the southwestern region of Alaska, the home of the most primitive of the American Eskimos. This region was chosen because of the opportunity afforded to observe both primitive and semicivilized natives. Many of the settlements are so isolated that contact with the white man and particularly with his foods has not been sufficient to effect any sign of dental caries. In a few settlements, on the other hand, where traders have brought in "store" foods, the natives show an exceedingly high incidence of caries. In 1935 we (L. W. and D. W.) showed that 85 per cent of the mouths free from caries contained no lactobacilli.[1] In 1936

This work was made possible by grants from the office of Indian Affairs, United States Department of the Interior, through the cooperation of Dr. J. G. Townsend, Director of Health.

This paper is Unit 4, Section XIII, of "Dental Studies of the Eskimos of North America," directed by L. M. Waugh, D.D.S., Consultant Dentist, Office of Indian Affairs, and Dental Director (Reserve) of the United States Public Health Service.

T. J. Pyle, D.D.S., Supervisor of Dentistry for Alaska, was detailed by his office to aid the starting of the work and assisted in the original examinations of the natives and in the selection of subjects for the experiment; Mrs. Anna C. Schwalbe and Reverend F. Drebert, of the Moravian Mission, Bethel, Alaska, permitted the use of the Moravian church at Napakiak as a laboratory and clinical headquarters; Sammy Full Moon, Quinhagak, Alaska, served as native interpreter and assistant; Mr. Matfey Berezkin, native Alaskan priest of the Russian church, aided in soliciting the good will of the primitive Eskimos of his settlement; Philip Jay, D.D.S., University of Michigan, prepared the culture medium; William J. Gies, Ph.D., Professor Emeritus, Department of Biological Chemistry, Columbia University, gave counsel regarding phases of the research.

1. Waugh, L. M., and Waugh, D. B.: Dental Observations Among Eskimo: VI. Bacteriologic Findings in Most Primitive and Populous Native District of Alaska, J. Dent. Research 15:317 (Sept.) 1936.

Rosebury and L. M. Waugh,[2] in a similar series of experiments, found that 86.4 per cent of the mouths free from caries showed no lactobacilli and that 80.6 per cent of the mouths with caries had lactobacilli. Both these experiments were carried on in the same general region as was the present work.

Observations and data obtained by L. M. Waugh[3] among the Labrador Eskimos indicate that when the natives remain on their primitive diet, which is practically free from fermentable carbohydrates, caries is unknown, but that when "white man's" food is introduced, caries appears and is prevalent in proportion to the amount of "white man's" food indulged in. His observations and studies of native diets among the Eskimos of Arctic Alaska[4] bear out the same findings. He has also furthered these results by field studies among the primitive Eskimos of the lower Kuskokwim River region.[5]

Osborn, Noriskin and Staz,[6] working among the Bantu of South Africa, exposed extracted undecayed teeth in vitro to saliva mixtures of crude cane sugar and sucrose for from two to eight weeks and found that with the refined sugar the teeth showed a great amount of decalcification, while with the crude sugar they showed only slight decalcification. They noted that the acidity of the solutions in the different tubes was the same throughout the duration of the experiment and concluded that there must be some other factor present which would account for the difference in the decalcifications. Further study[7] led them to the belief that a protective agent is present in sugar in the natural state which is removed during the process of refining.

The present work was carried on in three native settlements on the lower Kuskokwim River area. While all three villages were fairly close to a large white settlement, little visiting was done to the trading stores, and, when done, could be ascertained. For this reason, no attempt was

2. Rosebury, T., and Waugh, L. M.: A Study of Dental Caries Among Eskimos of the Kuskokwim Area in Alaska: I. Clinical and Bacterial Findings, Am. J. Dis. Child. 57:871 (April) 1939.

3. Waugh, L. M.: Nutrition and Health of the Labrador Eskimos with Special Reference to the Mouth and Teeth, J. Dent. Research 8:428 (June) 1928.

4. Waugh, L. M.: A Study of the Nutrition and Teeth of the Eskimo of North Bering Sea and Arctic Alaska, J. Dent. Research 10:387 (June) 1930.

5. Waugh, L. M.: Dental Observations Among Eskimo: VII. Survey of Mouth Conditions, Nutritional Study, and Gnathodynamometer Data, in Most Primitive and Populous Native Villages in Alaska, J. Dent. Research 16:355 (Aug.) 1937.

6. Osborn, T. W. B.; Noriskin, J. N., and Staz, J.: Comparison of Crude and Refined Sugar and Cereals in Their Ability to Produce in Vitro Decalcification of Teeth, J. Dent. Research 16:165 (June) 1937.

7. Osborn, T. W. B.; Noriskin, J. N., and Staz, J.: Inhibition of in Vitro Decalcification in Teeth, J. Dent. Research 16:545 (Dec.) 1937.

made to assure isolation of the natives concerned in the experiment. If such an attempt had been made, it is probable that the work would have ended in failure, for the natives would not have tolerated any sort of confinement for so long a period. Two of the towns had one small trading store each, but with the cooperation of the white owners of these stores we were able to maintain a close check-up on the purchase of sweets during the period of study. The third town had no store of any kind. The first two towns mentioned are Oscarville and Napakiak; the third, Naposkiak.

During the survey, data as accurate as possible were collected regarding the normal sugar and flour intake of the people. To this end, the traders who sell these products to natives were questioned as well as the natives themselves. While the information thus gained was of necessity indefinite, owing to the migratory life of the natives, it did show that even the primitive Eskimos had been getting some small amounts of refined wheat flour and sugar for about thirty to thirty-five years. However, all attempts to link the amounts of flour and sugar purchased with the presence of caries were generally unrewarded. This failure was probably due to the fact that the amounts consumed by the individual Eskimo were small. Bread was made by mixing the flour with water and a little salt and baking the dough in a pan. The result was a brown-surfaced bread about ½ inch (1.3 cm.) in thickness. Sometimes a small amount of baking soda, if available, was added, causing the mixture to rise to a thickness of about 1 inch. The bread was eaten in very small amounts, one loaf lasting a family of four or five about a week. The Eskimos do not have bread all year, as only after a successful hunting and trapping season are they able to purchase the flour, and that purchase includes only as much as the success of their "catches" permits. Generally the average family has enough to last only through January or February, and they do not restock until late summer or early fall. The sugar is not used as the white man uses it. The Eskimos make a native candy by browning the sugar dry, then adding water and boiling it down. The result is similar to a hard maple sugar, yellowish brown and about ⅛ to ¼ inch (0.3 to 0.6 cm.) thick. Very few of the natives dissolve this in their tea, in the cup; most of them place a small piece in their mouth while drinking the tea and allow the tea to dissolve the surface as it passes over. One small piece may last an Eskimo for two or three cups of tea. An attempt was made to link this habit with the presence of caries by questioning individual natives closely as to how long they had been using sugar in this way, how often they had the candy, and how long it was retained in the mouth. However, no connection was shown between this use of sugar and the incidence of caries. The amounts of sugar purchased, as related by the traders, varied a great deal. One

native might purchase as much as 100 pounds (45 Kg.) at one time, but this was for his whole family, including "in-laws" and dependents, which usually meant about 15 persons. One hundred pounds was the highest amount recorded, the average being around 50 to 60 pounds (23 to 27 Kg.) per year for a family.

PROCEDURE

The routine procedure of the present work was to examine the mouth and teeth with a mouth mirror and a finely sharpened explorer, charting all caries and "catches" on an individual sheet. Caries was indicated by a soft, pulpy feeling when the explorer touched definitely decayed matter and when it was possible to see the grayish blue tinge of undermined enamel. A "catch" was indicated by definite entrance of the point of the explorer into a pit or a break in the surface of the enamel. In the original examination, natives with mouths free from caries were mostly sought, but many were taken whose teeth presented a slight amount of decay. Immediately after the examination, a saliva specimen was taken. This was treated according to the method of Bunting and Jay of the University of Michigan:

1. Paraffin-stimulated saliva, 1 cc., was mixed with 4 cc. of broth (meat infusion, 1 per cent dextrose, p_H 5).

2. Of this mixture, 0.1 cc. was streaked on a Petri dish medium of tomato juice agar.

3. Both broth and plate were incubated four days at 37.5 C.

4. Slides were made from both of these, stained according to the method of Gram, with carbolfuchsin as a counterstain, and examined microscopically.

5. Results were charted separately on specially mimeographed sheets, and no comparisons were made until after the whole work had been completed.

At the conclusion of the original examination and collection of saliva, each native was allowed to choose the sweet which he or she wished to eat. As the experiment was to continue over an extended period and the cooperation of the subjects was an essential factor to the continuance of the work, they were allowed their choice, but close observation was necessary to see that the foods were approximately equally distributed in each settlement. In only a few instances was it necessary to insist that some subjects take assigned foods. After the selection of the sweets had been made, the subjects were fed a definite quantity each day. The amount was not sufficient to interfere in any way with their native diet or food intake. Each feeding was organized and closely supervised by both or either of the authors and the technician, or by the technician, to see that each native ate only his own food and that he ate all of it.

The foods containing natural sugars were given in the following amounts daily: 100 Gm. of raisins, dried figs or dates; 70 Gm. of pure maple syrup or honey. The foods containing refined sugars were given in the following amounts daily: 3 figs with juice preserved in 65 per cent of cane sugar; a 5 cent size chocolate candy bar of a well known brand, sticky, soft and chewy; 1 small round lollypop, different flavors; 6 sugar cubes, chewed but not rinsed, or a sugar solution consisting of 6 cubes dissolved in a cup of water and rinsed immediately.

The feeding periods of the people varied from five to thirteen weeks, but practically all of the periods were of five and one-half weeks' duration.

The authors feel that a record of the work and the conclusions drawn therefrom are best shown by the tables which follow

TABLE 1.—*Incidence of Caries and Lactobacilli in the Mouths of Eskimos Fed Refined Sugar*

Food	Subject	Weeks of Feeding	Feedings	Caries at Start	Caries at End	Lactobacilli At Start	Lactobacilli At Two Weeks	Lactobacilli At End
Figs (preserved)...	A. E.	5	28	0	8	No	Yes	Yes
	N. S.	5	30	4	15	Yes	Yes	Yes
	N. M.	5	28	3	5	No	Yes	Yes
	O. W.	5½	24	0	0	No	Yes	Yes
	N. O.	5½	19	0	5	No	....	....
Candy bars.....	F. L.	5½	26	14	17	No	Yes	Yes
	M. A.	6	28	3	10	....	Yes	Yes
	J. M.	..	..	5	..	Yes	....	....
	E. J.	6	24	0	7	No	Yes	Yes
	W. M.	6	18	2	8	No	Yes	Yes
	S. M.* (see raisins)	5½	31	0	2	No	Yes	Yes
Lollypops.....	F. T.	5½	24	7	9	....	Yes	Yes
	S.	6	23	2 catches	7	No	Yes	Yes
	A. N.	5½	25	0	8	No	Yes	Yes
	S. S.	5½	26	0	4	No	Yes	Yes
	E. M.	5½	26	2	6	Yes	Yes	Yes
	A. J.	6	26	12 catches	20	No	Yes	Yes
	J. W.	10½	24	0	0	No	....	Yes
Sugar solution....	F. T.	5½ (see lollypops)		7	7	Yes	Yes	Yes
	Mr. N.	5½	28	0	0	Yes	Yes	Yes
	M. B.	5½	31	3	4	Yes	Yes	....
Sugar cubes.....	D. S.	5½	23	0	2	Yes	....	Yes
	M. S.	6	19	4 catches	4 catches	No	No	....
	S. T.	..	..	0	..	No	Yes	....

* This Eskimo was the interpreter. The first two weeks he was fed raisins, and the remainder of the summer, candy bars.

TABLE 2.—*Incidence of Caries and Lactobacilli in the Mouths of Eskimos Fed Natural Sugars*

Food	Subject	Weeks of Feeding	Feedings	Caries at Start	Caries at End	Lactobacilli At Start	Lactobacilli At Two Weeks	Lactobacilli At End
Raisins.....	E.	..	..	0	..	No	....	....
	J. N.	5½	27	2	6	....	Yes	Yes
	S. M.	2	14	0	0	No	No	....
	M. N.	5½	27	9	10	Yes	Yes	Yes
	A. K.	5½	23	0	0	No	....	No
	Mr. K.	5½	31	3	3	No	Yes	No
Dried figs.....	S. E.	13	86	0	0	No	No (5 wk.)	Yes
	V. E.	7	37	0	0	No	No	No
	D. W.	2½	14	0	0	No	No	....
	C. J.	5½	30	1 catch	1 catch	Yes	Yes	Yes
	E. F.	5½	30	5	8	Yes	Yes	Yes
	N. S.	5½	19	0	0	No	No	....
	W. E.	6	18	0	..	No	No	....
Maple syrup.....	Mrs. N.	5½	26	0	0	Yes	No	Yes
	N. A.	5½	27	0	0	No	No	No
	M. A.	5½	28	0	0	Yes	Yes	Yes
	H. J.*	13	88	0	11	No	No (6 wk.)	Yes
	A. W.	5½	25	1	4	Yes	....	Yes
Money.....	E. A.	5½	23	1	1	Yes	No	Yes
	A. A.	5½	23	0	0	No	No	No
	M. F.	13	51	0	0	No	No	No
Dates.....	M.	13	49	0	0	No	Yes	Yes

* This subject admitted that he ate "lots of candy" during the four weeks when he went to Bethel in the mornings to chop wood for the missionaries.

TABLE 3.—*Amount and Distribution of Foods Containing Natural Sugars*

Foods Given	Daily Amount, Gm.	Feeding Period	Average Number of Feedings	Subjects Given Each Food
Raisins	100	5½ weeks	24.4	6
Dried figs, prunes	100	5½ to 13 weeks	28.4	7
Pure maple syrup	70	5½ weeks (1 was 13 weeks)	28.8	5
Pure honey	70	5½ weeks (1 was 13 weeks)	32.0	2
Dates	100	13 weeks	40.0	1

TABLE 4.—*Amount and Distribution of Foods Containing Refined Sugars*

Foods Given	Daily Amount	Feeding Period	Average Number of Feedings	Subjects Given Each Food
Preserved figs (65% sugar)	3 figs with juice	5-5½ weeks	25.2	5
Candy bar	1 bar	5½-6 weeks	25.4	5
Lollypop	1	5½-6 weeks	26.2	5
Cube sugar	6 cubes	5½-6 weeks	21.0	2
Sugar solution	6 cubes in water	5½ weeks	29.5	3

TABLE 5.—*Summary of Observations on Growth of Lactobacilli in Mouths of Caries-Free Eskimos After Addition of Natural and Refined Sugars to Diet*

Sugar Given	Subjects Without Lactobacilli at Start	Subjects With Lactobacilli		
		At Start	After 2 Weeks' Feeding	After 5 Weeks' Feeding
Natural	86.68%*	13.32%	16.66%	11.11%
Refined	81.82%	18.13%	88.00%	100.00%

* This finding corroborates results reported by L. M. Waugh and D. B. Waugh in 1935 and by Rosebury and L. M. Waugh in 1936.

TABLE 6.—*Summary of Observations on Dental Caries in Eskimos After Addition of Natural and Refined Sugar to Diet*

Sugar Given	Caries-Free Subjects Who Remained Caries Free at End	Caries-Free Subjects Who Showed Caries at End	Subjects with Caries at Start Showing Caries Increase at End
Natural	92.31%*	7.69%	57.16% (average increase of 1.57 cavities)
Refined	27.27%	72.73% (average of 3.6 cavities)	100% (average increase of 4.9 cavities)

* The percentage would have been 100 except for H. J. (table 2) who reported eating "lots of candy" during a four week period when he visited Bethel daily.

SUMMARY AND CONCLUSIONS

The figures show the group fed various foods containing refined sugar to have been higher in dental caries at the beginning. This discrepancy may be accounted for in the dropping out of several Eskimos as well as in the fact that, in order to promote the necessary good will, the subjects were allowed to choose their own sweets. The foods were not divided by settlements but distributed as equally as possible among the people of all three villages. In many cases husbands and wives or other members of the same family selected different sweets. The percentage of those free from caries in the one group was compared with the percentage free from caries in the other, and the carious average of one group was compared with the carious average of the other. The results for the two groups are so drastically different that the deductions therefrom must be accepted as strongly indicative.

Practically 100 per cent of those free from caries in the group fed natural sugars remained free from caries, and showed a drop from the original percentage with lactobacilli. Of those with carious mouths in this group, 57.16 per cent showed an increase in cavities, with an average increase of 1.57 cavities, which might be considered a normal increase for actively carious mouths. From these figures one may conclude that with the Eskimo natural sugars do not initiate or cause an increase in the growth of oral lactobacilli; neither do natural sugars initiate or cause an increase in dental cavities.

Of those free from dental caries in the group fed refined sugars, 72.73 per cent showed inception of caries, with an average of 3.6 cavities. Of those with carious mouths in the group fed refined sugars, 100 per cent showed an increase in cavities, with an average increase of 4.9 cavities, which must be regarded as an increase far above normal for a five or six week period. In the group fed refined sugar 81.90 per cent showed initiation of the growth of oral lactobacilli, and these organisms were present in 100 per cent of the mouths at the end of the feeding period. From these figures one may conclude that with the Eskimo refined sugars initiate and cause an increase in the growth of oral lactobacilli and that they also initiate and cause an increase in dental cavities.

This is the first recorded field work on the effects of natural and refined sugars on oral lactobacilli and dental caries among the Eskimos and must be regarded as preliminary.

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DENTAL CARIES IN THE ALBINO RAT ON HIGH SUCROSE DIETS CONTAINING DIFFERENT AMOUNTS OF ALUMINUM

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It has been shown (Wynn, Haldi, Shaw and Sognnaes, '53) that two high-sucrose diets, which we have designated as the Emory and Harvard diets, differing from each other in some respects but containing identical amounts of sugar, show a pronounced difference in cariogenicity. In searching for a possible single factor which might account for this difference, it was noted that a very small amount of aluminum had been incorporated in the salt mixture of the Emory diet whereas none had been added to the Harvard diet. As the Emory diet was much less cariogenic than the Harvard diet, the present experiments were undertaken to determine whether additions of this trace element to the diet might serve somewhat like the fluoride ion in giving protection to the teeth against dental caries. In this connection it may be of interest to note that Pieruccini ('47, cited by McConnell, '52) has suggested that aluminum can substitute for calcium and phosphorus in the structure of apatite. This type of substitution, says McConnell, is not particularly surprising when considered in the light of the demonstration by Yoder and Keith ('51) of complete replacement of silicon by aluminum in synthetic yttrium garnetoid, although substitution of AlO_4 for SiO_4 was previously supposed to be very limited in orthosilicates.

It has been shown by Hove, Elvehjem and Hart ('38) that if aluminum is needed in the nutrition of the rat during its

most rapid period of growth, 1 μg per day will satisfy the requirement. Conversely, no deleterious action upon growth, reproduction or general well-being of animals has been observed when relatively large amounts of aluminum have been added to the diet. In the experiments of Myers and Mull ('28) 4 generations of rats were fed 2 mg of aluminum in the form of potassium aluminum sulfate per rat daily in addition to the stock diet. No abnormalities were noted on autopsy and all animals appeared healthy. Similar results were obtained by McCollum, Rask and Becker ('28). They found that aluminum compounds in the diet in concentrations as high as 600 p.p.m of the element aluminum exerted no noticeably deleterious action on growth, reproduction, or general well-being as judged by external appearance and by autopsy. In view of these observations on aluminum requirements of the rat and the effects of a relatively large intake, it was deemed unnecessary to extend our studies beyond the condition of the teeth. Notation was nevertheless made of the amount of food consumed and of the weekly change in body weight.

EXPERIMENTAL

Albino rats of the Wistar strain were selected at weaning in groups of 4 of the same sex from the same litter and placed on 4 rations that were identical in composition except for their aluminum content. The basic ration consisted of 64% sucrose, 20% casein, 8% fat, 4% yeast and liver extract, 4% salt mixture and all the known essential vitamins in adequate amounts. The composition of the salt and vitamin mixtures is given elsewhere (Haldi and Wynn, '52). The 4 rations will be referred to as rations A, B, C and D, respectively. No aluminum was added to ration A. To ration B there was added the same amount of aluminum (0.16 p.p.m.) as in the ration fed in our previous experiments (Haldi and Wynn, '52; Wynn, Haldi, Shaw and Sognnaes, '53), whereas 12.5 and 125 times this amount was added to rations C and D, respectively. These amounts of aluminum (2 p.p.m. and

.0 p.p.m.) may appear to be small, but it should be borne in mind that similarly small amounts of fluoride afford some protection to the teeth against dental caries.

There were two sets of experiments, one on non-desalivated and the other on desalivated animals. In the experiment on the non-desalivated animals, 14 rats were placed on each of the 4 rations. They were fed ad libitum for 180 days and at the end of this period were sacrificed and the teeth examined under a dissecting microscope for the evaluation of dental caries.

In the second experiment the major salivary glands were removed when the animals had been fed the rations 120 days. In this experiment there were 10 animals on each ration. They were continued on these rations 60 days longer and were then sacrificed and the teeth examined for caries. The method of desalivation has been described elsewhere (Haldi, Wynn, Shaw and Sognnaes, '53).

Carious lesions were graded in accordance with the criteria followed in our earlier study (Haldi and Wynn, '52). The total score for the animals on each ration was derived by adding the products that were obtained by multiplying the number of lesions of each grade by the grade itself; the average score by dividing the total score by the number of teeth. This latter value is particularly helpful in comparing the carious destruction of the teeth on the 4 rations.

RESULTS

The number of carious lesions and the caries score for the non-desalivated rats are presented in table 1 and for the desalivated rats in table 2. The males and females have been grouped together as we have found that there is no sex difference in caries susceptibility in our colony of albino rats.

Comparison of the data in tables 1 and 2 shows that desalivation resulted in both an increase in the average number of carious teeth per animal and in the severity of the lesions.

The differences in the total scores on the 4 rations were relatively small both in the non-desalivated and desalivated

animals. Statistically, these differences were not significan The critical ratio, for example, was less than one in the ca of the greatest difference which was between the non-desal vated animals on rations to which were added aluminum i

TABLE 1

Carious lesions and caries score of non-desalivated albino rats on rations contain various amounts of aluminum

ALUMINUM ADDED TO RATION	TOTAL NO. TEETH	TOTAL NO. CARIOUS TEETH	TOTAL NO. CARIOUS LESIONS	NO. OF LESIONS Grade 1	2	3	4	TOTAL SCORE	AV SCOR
				LOWER MOLARS					
None	84	32	38	21	15	2	0	57	0.68
0.16 p.p.m.	84	33	39	25	10	2	2	59	0.70
2.0 p.p.m.	84	31	39	24	10	3	2	61	0.73
20 p.p.m.	84	31	39	28	7	4	0	54	0.64
				UPPER MOLARS					
None	84	4	4	4	0	0	0	4	0.05
0.16 p.p.m.	84	5	6	6	0	0	0	6	0.07
2.0 p.p.m.	84	2	2	2	0	0	0	2	0.02
20 p.p.m.	84	3	5	5	0	0	0	5	0.06

TABLE 2

Carious lesions and caries score of desalivated albino rats on rations containi various amounts of aluminum

ALUMINUM ADDED TO RATION	TOTAL NO. TEETH	TOTAL NO. CARIOUS TEETH	TOTAL NO. CARIOUS LESIONS	NO. OF LESIONS Grade 1	2	3	4	TOTAL SCORE	AV SCOR
				LOWER MOLARS					
None	60	26	46	22	10	9	5	89	1.4[illegible]
0.16 p.p.m.	60	30	46	19	14	11	2	88	1.4[illegible]
2.0 p.p.m.	60	28	43	14	15	12	2	88	1.4[illegible]
20 p.p.m.	60	25	45	13	19	12	1	91	1.[illegible]
				UPPER MOLARS					
None	60	10	14	12	2	0	0	16	0.[illegible]
0.16 p.p.m.	60	12	15	11	3	1	0	17	0.[illegible]
2.0 p.p.m.	60	13	20	15	5	0	0	20	0.[illegible]
20 p.p.m.	60	12	16	10	4	2	0	24	0.[illegible]

amounts of 2 and 20 p.p.m. It may therefore be concluded t the addition of aluminum to the Emory diet was not the or responsible for making it less cariogenic than the rvard diet.

Incidental to these observations on the teeth it is of interest note that there was no perceptible difference in the general ysical condition of the animals on the 4 rations. The aver- weight of the 4 groups of males at the conclusion of the periment was 302, 304, 301 and 307 gm, respectively; and the females, 191, 194, 190 and 193 gm. The initial average eight of the groups at the beginning of the experiment did t vary by more than 1 or 2 gm. Although the animals were lowed to eat ad libitum the amount of food consumed by e different groups was remarkably uniform. The average ily consumption by the 4 groups of males was 11.2, 11.5, 1.2 and 11.3 gm, respectively, and of the females, 8.7, 8.9, 8 and 8.7 gm. Obviously, appetite and growth were not fected by the addition of aluminum to the rations in the mounts used in these experiments.

SUMMARY AND CONCLUSIONS

These experiments were undertaken to determine whether luminum, like fluoride, when added to the diet in small mounts may offer some protection against dental caries.

No significant differences were found in the number of arious molars or in the caries scores as a result of the addi- ion of aluminum in the amounts used in this study, either n non-desalivated or desalivated animals.

It may be concluded therefore that the difference in cario- enicity of two diets containing the same amount of sucrose ut with different amounts of aluminum, as reported previ- usly, is not due to the small amount of aluminum that was dded to the salt mixture of one of these diets. The explana- ion of the lower cariogenicity of one of the high sucrose diets must be sought elsewhere.

The addition of aluminum up to 20 p.p.m. had no effect on appetite or growth of the animals.

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Dental Caries in the Albino Rat Fed High Sucrose Diets of Relatively High and Low Pyridoxine Content[1]

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Pyridoxine as a dietary supplement is believed by Strean and his co-workers to have a suppressive effect on the initiation and spread of dental caries. This deduction has been drawn from experiments using hamsters, as well as children. Hamsters fed a cariogenic diet containing pyridoxine in a concentration of 10 ppm had fewer dental caries than a similar group fed the same diet with a concentration of 0.5 ppm (Strean, Gilfillan and Emerson, '56; Strean, Bell, Gilfillan, Emerson and Howe, '58). The later report included an experiment using 28 children divided into two groups of 14 each. One group received a lozenge containing 3 mg of pyridoxine three times a day after meals. At the end of a year this group, on the basis of their DMF (decayed, missing, filled) rating, had a lower caries score than the control group. In a preliminary report of a study scheduled to be conducted over a period of several years, Cohen and Rubin ('58) observed a slightly favorable trend in caries experience of a group of children receiving pyridoxine-containing lozenges as compared with another group receiving a placebo.

Strean ('57) has postulated that pyridoxine may effect a change in the oral flora whereby the lactobacilli requiring this vitamin as an essential nutrient, and not associated with dental caries, would multiply at the expense of *L. acidophilus*, *S. salivarius*, *S. mitis* and *S. mitior*, which some believe to be associated with tooth decay.

The interesting suggestion has been offered (Strean, Bell, Gilfillan, Emerson and Howe, '58) that the difference in cariogenicity of two high-sucrose diets containing the same amount of sugar which has been reported from our laboratories (Wynn, Haldi, Shaw and Sognnaes, '53) may have been due to a difference in the concentration of pyridoxine in the two diets. The experiments reported in this paper were undertaken to test the validity of this supposition.

PROCEDURE AND RESULTS

The Harvard high-sucrose diet, the composition of which is given elsewhere (Sognnaes, '48; Wynn, Haldi, Shaw and Sognnaes, '53) was used in all experiments. This diet contains 3.5 ppm of pyridoxine. Two sets of experiments were conducted: one on sialoadenectomized albino rats of our Emory-Wistar strain which has proved to be fairly resistant to dental caries, and another on intact animals of a more susceptible strain. In the first experiment 8 groups of triplicate litter mates were selected at weaning and sialoadenectomized by our usual procedure (Haldi, Wynn, Shaw and Sognnaes, '53). Whereas intact animals of this strain are fairly resistant to dental caries, when sialoadenectomized they develop caries on a cariogenic diet within a relatively short time. Beginning immediately after weaning, the three groups of litter mates were fed throughout the experiment the Harvard diet to which was added for each group, respectively, 1, 10 and 100 ppm of pyridoxine. At the end of a 70-day feeding period, the animals were killed and the teeth scored for dental caries by the method customarily followed in our laboratories (Haldi and Wynn, '52).

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TABLE 1

Carious lesions and caries scores of sialoadenectomized caries-resistant albino rats fed a cariogenic diet with different concentrations of pyridoxine

No. animals	Days on diet	Additional pyridoxine	Carious lesions[1]	Caries score[1]
		ppm		
8	70	1	24	59
8	70	10	24	61
8	70	100	22	55

[1] Average per animal.

The results of this experiment are presented in table 1.

From these data it is obvious that the addition of pyridoxine in a relatively large amount to a cariogenic diet did not affect its cariogenicity when fed to sialoadenectomized albino rats of the Wistar strain.

Strean, as stated previously, believes that pyridoxine may bring about a change in the oral flora. In this connection, it may be noted that the addition of pyridoxine to a synthetic medium increases the population of *L. casei* proportional to the quantity of this vitamin added to the medium (Bohonas, Hutchings and Peterson, '42) and that *L. casei* is the test organism for the biological assay of pyridoxine (Rabinowitz, Mondy and Snell, '48).

In view of the possibility that pyridoxine, once absorbed, may be returned to the mouth by way of the salivary glands, thereby having a more prolonged effect on the oral bacteria, we decided to conduct another experiment on intact rats of a strain more susceptible to dental caries. For this purpose we used albino rats from a colony developed from animals that had been supplied to us several years ago by the laboratories of the Naval Medical Research Institute. We had found that these animals, when fed a cariogenic diet with their salivary glands intact, develop caries in a relatively short time.

The animals were selected at weaning, using triplicate litter mates, with 20 animals placed in each group, and fed immediately the prescribed diets. The control group was fed the Harvard diet without supplementation and the other two groups, the same diet to which was added, respectively, 10 and 100 ppm of pyridoxine. When the animals had been fed the diets 120 days, they were killed and th teeth were given a score for dental cari in the same manner as in the precedin experiments. The results are given i table 2. In these experiments, as in tho on the sialoadenectomized rats of the Er ory-Wistar strain, the addition of pyridox ine to the diet did not reduce its carioge icity.

TABLE 2

Carious lesions and caries scores of intact cari susceptible albino rats fed a cariogenic diet with different concentrations of pyridoxine

No. animals	Days on diet	Additional pyridoxine	Carious lesions[1]	Caries score
		ppm		
20	120	0	17	40
20	120	10	18	35
20	120	100	18	38

[1] Average per animal.

DISCUSSION

In our previous studies, we found tha the high-sucrose diet referred to as th Harvard diet is much more cariogenic tha the Emory diet which contains the sam amount of sucrose. These two diets diff in their pyridoxine content with a co centration of 3.5 ppm in the Harvard an 10 ppm in the Emory diet. If Strean's sup position were correct that the difference i pyridoxine concentration may account fo the difference in cariogenicity of the tw diets, one would reasonably expect th the addition of pyridoxine to the Harvar diet would make it less cariogenic. Th proved not to be the case. The cariogeni ity of the Harvard diet was not diminish even though the pyridoxine content wa brought well above the level of that whic obtained in the Emory diet.

The results of our experiments are n in agreement with those of Strean and h co-workers ('56; '58). These investigat found that increasing the pyridoxine co centration in the diet from 0.5 to 10 pp resulted in a decrease in the cariogenici of the diet. It is possible that the respon of the hamster to the addition of pyrido ine to the diet may differ from that of t albino rat.

Another possibility should also be tak into account, namely, that the diet fed t hamsters, containing 0.5 ppm of pyrido

may have been deficient in this factor. If this were the case, the higher incidence and greater extent of caries observed when using this diet, as compared with the one containing 10 ppm, may have been related to a pyridoxine deficiency. It seems unlikely, however, that the hamsters suffered from a pyridoxine deficiency inasmuch as they survived over an experimental period of 7 to 10 months. Furthermore, the authors did not report acrodynia or other signs of pyridoxine deficiency. In our experiments, the basic Harvard diet provided 35 to 50 μg of pyridoxine in the normal daily food intake of 10 to 15 gm which doubtless satisfied the rat's pyridoxine requirements. Lepkvosky ('38) obtained optimal growth in rats with a daily dose of 10 μg. Sure ('40) reported later that 10 to 25 μg/day are adequate for continuous growth.

CONCLUSION

The addition of pyridoxine in relatively large amounts to a synthetic high-sucrose diet, originally adequate in its pyridoxine content, did not reduce the cariogenic properties of the diet when fed to sialoadenectomized albino rats of a fairly resistant strain nor to intact rats of a much more susceptible strain.

The difference in the cariogenicity of two high-sucrose diets containing the same amount of sugar, previously reported from our laboratories, cannot be attributed to the difference in pyridoxine concentration of the two diets.

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Cariogenicity of Acidic and Basic High-Sucrose Diets Fed Albino Rats

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Constant, Sievert, Phillips, and Elvehjem[1] have shown that basic minerals, fed through their diets to cotton rats, reduce the incidence and extent of caries in these animals. However, the addition of neutral or acid minerals was without any effect. These investigators suggested that the increase in the pH of the diets containing the basic minerals might have been responsible for the reduction in caries, but no direct evidence for this assumption was offered. Nizel, Harris, and Parker[2] have shown that the addition of metaphosphoric acid to a cariogenic diet fed to hamsters has a cariostatic effect. These investigators have postulated that the amount of soluble phosphate in the diet controls the degree of its cariogenicity. Although the pH of the diets used in this study were not given, one would expect the addition of metaphosphoric acid to decrease the pH of the diets fed these animals.

Since in one instance an increase in the pH of a cariogenic diet brought about a cariostatic effect and in another instance a decrease in the pH of a diet also brought about a cariostatic effect, it was deemed of interest to investigate the role of the pH level on the cariogenicity of a high-sucrose diet in our laboratories.

EXPERIMENTAL METHODS

Experiment 1.—Twenty littermate pairs of albino rats of the Emory-Wistar strain were selected at weaning and were sialoadenectomized by our usual procedure.[3] One of each pair was fed a highly cariogenic diet with a pH of 6.0, and the other was fed the same diet to which 1.5 per cent sodium carbonate (Na_2CO_3) had been added. The addition of the sodium carbonate elevated the pH of the diet to 6.9. The pH of the diets was determined by suspending 50 gm. of the diets in 50 ml. of distilled water and reading the pH of the slurries with the glass electrode. The composition of the diet (H-100), fed the animals in the experiments reported here, is given elsewhere.[4] All animals were individually housed in wire-mesh, screen-bottomed cages and were furnished the diets and distilled water ad libitum. At the end of an experimental period of 70 days, the animals were sacrificed. The teeth were removed, cleaned, and scored for the incidence and extent of caries by our usual methods.[5] The pH on the tooth surfaces was taken at 20-day intervals using an antimony electrode similar to the one described by Stephan.[6] The details of the construction and evaluation of these electrodes is given elsewhere.[7]

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Experiment 2.—Thirty triplicate littermate groups of albino rats of the Emory-Wistar strain were selected at weaning and sialoadenectomized as in Experiment 1. One member of each group was fed the highly cariogenic diet as in the preceding Experiment, the second the same diet to which had been added 3 per cent sodium carbonate, and the third the same diet to which had been added 1.4 per cent lactic acid (85 per cent). The pH of the three diets was 6.0, 8.9, and 4.7, respectively. The same procedures as were outlined in Experiment 1 were followed in this experiment.

Experiment 3.—Ten triplicate littermate groups of rats were selected at weaning and fed a non-cariogenic stock diet* until they had reached a weight of approximately 100 gm. The animals were then sialoadenectomized and fed the same cariogenic diet as was used in the first two experiments. Since the purpose of this experiment was to determine whether or not acidic and/or basic supplements were effective systemically, these materials were given by stomach tube in the following manner: 1 animal of each group was given 3 ml. of a suspension of diet in 20 ml. of water for each 10 gm. of food eaten; the second animal, the same quantity of a suspension made up with 15 per cent sodium carbonate; and the third animal, the same quantity of a suspension made up with 7 per cent lactic acid. This assured that the two groups of test animals in this experiment received the same amount of acid and base per gram of food eaten as those in Experiment 2. Otherwise, the same procedures outlined in the other experiments were followed in this one.

TABLE 1

CARIES SCORES AND pH READINGS ON TOOTH SURFACES OF RATS FED A CARIOGENIC DIET WITH AND WITHOUT ADDED Na_2CO_3*

No. of Rats	Diet	pH of Diet	pH on Teeth	Av. Caries Incidence	Av. Caries Score	C.R.†
20.....	H-100	6.0	6.9±0.11	24	46±2.7	1.5
20.....	H-100+ 1.5% Na_2CO_3	6.9	6.8±0.11	24	51±3.1	

* The ± values in the table are the standard errors of the means (S.E.M.).

† The critical ratio (C.R.) is the ratio of the difference between two means to the standard error of the difference between the means. When the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; 2.0–2.9, the difference is of borderline significance; 3.0 or more, the difference is highly significant (J. M. Dunning, *J. D. Res.*, 29:541, 1950).

RESULTS

The data obtained in Experiment 1 are given in Table 1. The addition of 1.5 per cent sodium carbonate to our cariogenic diet did not affect the caries incidence or the caries scores of animals fed these diets, although the pH of the test diet was increased from 6.0 to 6.9. No difference was found in the average pH on the tooth surfaces of the two groups of animals.

In Experiment 2, the addition of 3.0 per cent sodium carbonate to the cariogenic diet elevated the pH from 6.0 to 8.9 (Table 2) and also markedly reduced the cariogenicity of this diet. When, however, the pH of the diet was lowered to 4.7 by the addition of lactic acid to the diet, there was an increase in the average caries score, but this increase

* Purina Laboratory Chow, Ralston Purina Co., St. Louis, Missouri.

w s not statistically significant. It may also be seen in this table that the pH was ele v ed on the surfaces of the teeth of the animals fed the diet containing sodium carbon a . This difference was statistically significant, as was the decrease in pH on the to th surfaces of the animals fed the diet containing lactic acid.

The data given in Table 3 are the results of the third experiment, in which the ani-mals were given the basic and acidic supplements by stomach tube to determine whether or not these supplements would produce the same results systemically as they did when given in the food. The data obtained in this experiment indicate that neither

TABLE 2

CARIES SCORES AND pH READINGS ON TOOTH SURFACES OF RATS FED CARIOGENIC DIETS WITH ADDED Na_2CO_3 AND ADDED LACTIC ACID*

No. of Rats	Diet	pH of Diet	pH on Teeth	C.R.†	Av. Caries Incidence	Av. Caries Scores	C.R.†
30	H-100	6.0	6.7 ± 0.09	9.6	25	54 ± 3.9	5.1
30	H-100 + 3.0% Na_2CO_3	8.9	7.3 ± 0.09		21	31 ± 1.6	
30	H-100 + 1.4% lactic acid	4.7	6.3 ± 0.09	3.1	25	68 ± 4.2	2.2

* The ± values in the table are the standard errors of the means (S.E.M.).
† C.R. = critical ratio.

TABLE 3

CARIES INCIDENCE AND CARIES SCORES OF RATS FED A CARIOGENIC DIET AND INTUBATED WITH Na_2CO_3 AND LACTIC ACID IN SOLUTION*

No. of Rats	Diet	Intubated	Caries Incidence	Caries Scores
10	H-100	H_2O	25	46 ± 3.2
10	H-100	Na_2CO_3	25	47 ± 2.5
10	H-100	Lactic acid	24	43 ± 3.2

* The ± values in the table are the standard errors of the means (S.E.M.).

odium carbonate nor lactic acid, when intubated, affected the amount of caries that leveloped in this strain of rats when they were fed our cariogenic diets. The caries scores of the controls and of the two groups of test animals were practically identical.

DISCUSSION

The proponents of the acid decalcification theory postulate that acid, formed by bacterial enzymatic action on carbohydrate in the oral cavity, produces decalcification of the enamel that is the first step in the decay process. Those who have studied the effect of the various ammoniated compounds in dentifrices as cariostatic agents are of the opinion that the effectiveness of these agents is due to acid neutralization, together with a reduction in the aciduric bacteria present.[8, 9] However, in experimental animals[10-12] and in man,[13] excessive amounts of acids passing through the oral cavity lead to erosion and not to decay. It has also been shown that cariogenic diets of the same

pH may bring about quite different degrees in the caries attack.[14] These observations would lead one to conclude that the pH of a diet, alone, is not the only determining factor as to its cariogenicity.

Granados, Glavind, and Dam[15] have reported that dietary lactic acid did not significantly affect the caries scores of hamsters; however, there was a slight increase in the scores of the animals given lactic acid supplements. This is in agreement with the data presented in Table 2 of this report.

In the present study we also investigated the role of alkalinization of a cariogenic diet with sodium carbonate and found (Table 2) that the addition of this compound to the diet markedly reduced the caries conduciveness of the diet. The action of the Na_2CO_3 in making the experimental diet less cariogenic must be a local and not a systemic one, since this action disappeared when the experimental animals were intubated with Na_2CO_3 (Table 3). McClure and Muller[16] have reported that NaH_2PO_4 lost its cariostatic effect when intubated in their animals.

The question then arises as to the mechanism of the action of the Na_2CO_3 in reducing the caries conduciveness of the diet. Is it that the alkalinization elevated the pH in the mouth to a sufficiently high level to neutralize rapidly any acid that might have been formed, or is it that the Na_2CO_3 in the diet made the phosphate available to act as a cariostatic agent?

It is of interest to note (Table 2) that the elevation of the pH of the diet with the addition of the Na_2CO_3 was definitely reflected in an elevation of the pH on the tooth surfaces above the neutral point. However, this does not indicate that no decalcification had occurred because it is well known that decalcification of enamel by a chelating action may take place in alkaline media.

Evidence has been presented to show that phosphate added to a diet makes the diet less conducive to caries.[2, 11, 17] However, McClure and Muller[16] found that $CaHPO_4$ was not cariostatic when added to whole-wheat flour; yet, in another study,[18] $CaHPO_4$ added to bread did have a caries-preventive effect. McClure and Muller suggested, as a possible explanation for the positive effect in the bread diet, that NaCl present in the bread may have solubilized the $CaHPO_4$, thereby liberating phosphate. In the whole-wheat flour there was no NaCl present, and no cariostatic effect was found. In our laboratories we have found, in agreement with McClure and Muller's suggestion, that NaCl does have a solubilizing effect on $CaHPO_4$, but also we have found that Na_2CO_3 has even a greater solubilizing effect on this compound than NaCl in vitro.

It may be concluded from the results of these experiments that the presence of dietary lactic acid in our cariogenic diet did not appreciably increase the caries scores; on the other hand, alkalinization of our high-sucrose diet with Na_2CO_3 made this diet less cariogenic. It is suggested that there may be two possible mechanisms involved in its action: (1) maintenance of alkalinity on the tooth surface and (2) a solubilizing effect on the relatively insoluble phosphates in the diet. The solution of the problem must await further investigation.

SUMMARY

Albino rats fed a high-sucrose diet to which 1.5 per cent Na_2CO_3 had been added developed the same amount of caries as did their controls, fed the unsupplemented cariogenic diet. The pH of the two diets was 6.9 and 6.0, respectively.

The addition of 3.0 per cent Na_2CO_3 to the diet reduced the caries scores significantly, while the addition of 1.4 per cent lactic acid increased the caries score. This increase, however, was not statistically significant. The pH of the basic diet was elevated to 8.9 by the addition of the Na_2CO_3, and that of the acidic diet was decreased to 4.7. The pH on the tooth surfaces was 6.7, 7.3, and 6.3 in the rats fed the control, basic, and acidic diets, respectively.

Intubation of acid or base did not affect the caries scores, indicating that the cariostatic action of the basic diet was a local, rather than a systemic, one.

The cariostatic action of Na_2CO_3 in the diet may have two possible mechanisms: (1) maintenance of alkalinity on the tooth surface and (2) a solubilizing effect on the relatively insoluble phosphates in the diet.

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DIETARY HABITS AND THE DENTAL CARIES EXPERIENCE IN 200 CHILDREN

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MANY studies have been conducted to determine the relationship between dental caries activity and dietary carbohydrates in man. Surveys of the diet of primitive races[1-10] and the diet during wartime[11-13] tend to indicate that the accessibility of refined carbohydrates in the diet is related to the dental caries experience. When readily fermentable carbohydrates are not available, or when they are withdrawn from the diet, the dental caries experience decreases. Numerous studies[14-19] have supported the positive relationship between the readily fermentable carbohydrate in the diet and the dental caries experience. However, some workers have been unable to confirm this observation.[20-23]

The frequency of eating and the amount of carbohydrate consumed between meals also have been considered in previous studies but the results have been contradictory.[24-26] The value of milk as a "protective food" often has been considered, and previous studies have shown that a significant inverse relationship exists between the total milk intake and the dental caries experience.[18, 27] Excessive milk consumption, however, in one study has been associated with a higher dental caries incidence.[26]

The present study was undertaken to obtain additional information about the relationship of dietary habits and the dental caries experience in children.

MATERIAL AND METHOD

Two hundred cases were selected at random from the files of the Pedodontia Department. The survey group consisted of 106 boys and 94 girls, ranging in age from 5 to 13 years. Twenty-five children lived in rural areas, and 175 resided in Indianapolis, or nearby communities. A careful clinical examination was performed by (A. Z. or R. E. M.) with adequate light, mouth mirror, and sharp explorer, after the teeth had been dried with air. A complete roentgenographic survey including bite-wing films was available.

The records of each child included a 7-day diet survey. The parents previously had been requested to keep an accurate record of all foods and the amount consumed at both the mealtime and the between meal period. Only the dietary records that were complete, including the amounts of foods eaten, and those

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that had been prepared with apparent care and interest, were included in this study. Since the role of the diet in dental caries had not been discussed previously with the parents, it was hoped that the diet records were reasonably accurate.

One of the authors (A. Z.) critically reviewed the radiographs of each case to check the accuracy of the recording of carious lesions on the patient's record. In the case of the permanent teeth, the number of affected surfaces expressed as DMFS (decayed, missing, and filled surfaces) was determined. Missing posterior teeth, as a result of extraction, were counted as five surfaces, while a recording of three surfaces was made for each anterior tooth that had been lost as a result of dental caries.

With the aid of the compilations of Bowes and Church,[28] the diet of each child was reduced by calculations to equivalent teaspoons of sugar. One teaspoon of sugar was considered equivalent to 4 grams of refined carbohydrate. In calculating the sugar equivalent in the diet, only the refined foods or those to which sugar had been added were considered. Foods contained in the "Seven Basic Groups," with some exceptions, were not included in the calculation of the sugar equivalent unless free sugar was added during the preparation. One exception was dry cereal. The dried fruits, including raisins, prunes, peaches, apricots, and dates have a very high sugar content; therefore, their sugar equivalents were calculated and included in the total. These are foods commonly eaten by children, particularly between meals, and there is no reason to believe that they are any less cariogenic than refined foods. Although honey is considered as a natural sugar, its sugar equivalent was calculated as were the syrups and other spreads for bread.

The total amount of sugar was calculated for each child and expressed in teaspoons of sugar per week. A tabulation likewise was made for the sugar consumed between meals. Since the frequency of eating may play a role in the dental caries experience, this value was determined for each child. The data from the individual diet survey also were used as an indication of the child's milk consumption and this was recorded as glasses of milk per week.

PRESENTATION OF DATA AND DISCUSSION

The sex of the patient and susceptibility to dental caries was considered since there are conflicting data in the literature on this observation. Klein, Palmer, and Knutson[29] found that girls have a slightly higher DMFS rate than boys, while Potgieter and associates[26] reported no consistent sex difference. In the present study, the mean DMFS was found to be 7.04 for girls and 6.31 for the boys (Table I). This difference was not considered to be statistically significant, at the 65 per cent level of confidence using the critical ratio technic. When the dental status of the rural and city children was compared again, using the critical ratio technic, there was found to be a significant difference at the 4 per cent level of confidence. The mean DMFS for the rural children was 4.92, while that of the city children was 6.9 (Table II).

TABLE I

DIFFERENCE IN DENTAL CARIES EXPERIENCE BETWEEN BOYS AND GIRLS BASED ON DECAYED, MISSING, AND FILLED SURFACES (DMFS)

SEX	MEAN	STANDARD DEVIATION	STANDARD ERROR OF MEAN	NUMBER OF CASES
Boys	6.31	5.84	0.57	106
Girls	7.04	7.65	0.79	94

TABLE II

DIFFERENCE IN DENTAL CARIES EXPERIENCE BETWEEN RURAL AND CITY CHILDREN BASED ON DECAYED, MISSING, AND FILLED SURFACES (DMFS)

	MEAN	STANDARD DEVIATION	STANDARD ERROR OF MEAN	NUMBER OF CASES
Rural	4.92	3.89	0.78	25
City	6.9	7.04	0.53	175

The total weekly sugar intake equivalents were calculated for each of 200 children. The mean total intake was found to be 164 teaspoons, while the mean total between meal sugar was 54.5 teaspoons (Table III).

TABLE III

SUGAR INTAKE EQUIVALENTS OF 200 CHILDREN

	MEAN	STANDARD DEVIATION	RANGE FOR 95%
Total teaspoons of sugar per week	164	17.29	130-200
Total teaspoons of sugar between meals per week	54.51	6.38	42-67

Multiple correlations were made with the DMFS values (Table IV). An attempt was made to determine the relation between total weekly sugar consumption and the dental caries experience. There was found to be a low positive correlation of +0.10, indicating little relationship between these two values. There was found, however, a high positive correlation of +0.77 between the DMFS value and the amount of between meal sugar. A correlation between DMFS and the frequency of between meal eating showed a very low positive correlation of +0.18, which indicates a slight relationship. A correlation was computed to determine the relationship between the DMFS rate and total milk intake, and a very low negative relationship of -0.08 was obtained.

TABLE IV

MULTIPLE CORRELATIONS WITH DMFS VALUES

	CORRELATIONS
Total sugar intake	+0.10
Between meal sugar intake	+0.77
Frequency of between meal eating	+0.18
Milk intake	-0.08

In view of the high positive correlation between DMFS and the between meal sugar, a definitive type of study was done. The upper and lower quartiles

of the between meal sugar data were tested for significance, using the critical ratio technic. This procedure was employed to assist in determining whether the relationship of the two variables was causal or casual. The mean for the lower quartile was 7.79 and the upper quartile was 5.82, as shown in Table V. The resulting test of significance (C. R.) showed the difference not to be statistically significant at the 10 per cent level of confidence. This was interpreted to indicate that, while a high positive correlation exists between these variables, the definitive test on the upper and lower 25 per cent of the cases did not prove to be statistically significant. In view of the variables involved, it appears that there is need for further investigative work in this area.

TABLE V

CORRELATION OF LOWER QUARTILE AND UPPER QUARTILE OF BETWEEN MEAL SUGAR INTAKE AND DMFS

	NUMBER OF CASES	MEAN	STANDARD DEVIATION	STANDARD ERROR OF MEAN
Lower quartile	50	5.82	5.95	0.84
Upper quartile	52	7.79	6.99	0.97

DISCUSSION

It was observed in this study that approximately one third of the weekly total sugar intake, or an average of 55 teaspoon equivalents, was consumed in the form of between meal snacks. The total sugar intake equivalent was high in most children; however, there was little relationship between the total sugar consumed and the dental caries experience. This suggests that dental caries activity was not greatly influenced by a high total sugar intake as long as the sugar was taken at mealtime.

A positive correlation was noted between the DMFS incidence and the amount of sugar consumed between meals. This possibly could be explained by the fact that since one third of the total sugar intake was in the form of between meal snacks, there was a continuous concentration of fermentable carbohydrates in the mouth, and this continuous concentration of substrate favored the production of acids of sufficient potential to produce more carious lesions.

Observations made in previous studies have suggested that with an increased frequency of eating, particularly between meals, there would be an increase in the dental caries experience. In the present study, however, there was found to be very little relationship between the frequency of eating and DMFS. In a review of the dietary records, it was found, however, that the between meal snacks were not always of the cariogenic type. It may be further theorized that inaccuracies in the dietary records, if they do occur, are most likely to be in the recording of between meal snacks, particularly those consumed away from home. In view of this, perhaps relatively little importance should be placed on this observation.

Some investigators have shown that an increased milk intake beyond "reasonable amounts" is associated with an increase in dental caries. This association has been attributed to the fact that the appetite of the child drinking large

quantities of milk will become readily satisfied and there is a tendency to omit the basic portion of the meal and eat only the softer, "more attractive," high carbohydrate foods. This child gets hungry more readily and, as a consequence, there is a greater desire for between meal snacks. The results of the present study, however, did not support this observation. Further studies in this area are needed to clarify the conflicting results.

SUMMARY AND CONCLUSIONS

A study has been undertaken to accumulate additional evidence relative to the effect of dietary habits and the dental caries experience of 200 children.

1. The mean weekly total sugar intake equivalent was 164 teaspoons, while the mean between meal sugar intake equivalent was 55 teaspoons.

2. There was found to be very little relationship correlation between the total sugar consumed and DMFS (+0.10).

3. The total between meal sugar showed a high positive correlation with the DMFS (+0.77).

4. In the present study, there was found to be very little relationship correlation (+0.18) between the frequency of eating and DMFS.

5. There was a slight negative relationship correlation (−0.08) between the total milk intake and DMFS.

6. In equal numbers of children of the same ages, the mean DMFS of the girls (7.07) was higher than the mean DMFS of the boys (6.31). The difference, however, was not considered to be statistically significant using the critical ratio technic.

7. The children residing in the city had a higher DMFS (mean 6.9) than the rural children of comparable ages (mean 4.92). The resulting tests (C. R.) indicated a significant difference in the dental caries experience between the rural and city children at the 4 per cent level of confidence.

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