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SUCROSE

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SUCROSE

Summary

A number of papers of general interest on the subject of sucrose are available, but one in particular is most informative - Fewkes, Parker and Vlitos (514). Sections in this paper cover the chemistry of sucrose, the biological aspects (plant physiology, biosynthesis, translocation and accumulation) and sucrose as a food. More than 100 references are listed. During the process of reviewing papers for inclusion in this monograph, there emerged a controversial aspect to sucrose, exemplified by a comparison of Fewkes et al. and a popular paperback by John Yudkin, entitled "Sweet and Dangerous". (Bantam edition published May, 1973. Not included in Documents, but available at local bookstores.) The former treatment of sucrose presents the favorable aspects of the substance; Yudkin is known internationally for his insistence that sucrose is harmful and should be sharply restricted in its use. He calls sugar "The Quiet Killer", associating sugar consumption with heart disease and other often fatal diseases. Fewkes et al., in response to Yudkin state "However, direct evidence that sucrose, by virtue of its fructose content, is a specific contributory factor in the aetiology of these diseases has not been obtained". Or, further, on the subject of the implication of sucrose as a causative factor in dental caries, Fewkes et al. state: "Nevertheless, this problem is not necessarily a consequence of sucrose intake, but rather of inadequate dental hygiene and poor dietary control", and, "there is not corroborated evidence of any long-term deleterious effect on health, specfically attributable to sucrose." An element of bias, "for or against" sugar in the diet, may be observed in many reports of general nature; a most glowing example, favoring the use of sugar, is that of Hockett (748, 749).

Several papers are briefly noted here for their historical interest; both were published in 1942, during World War II. Harding (703) in America, in a paper "The Facts About Sugar", is preparing the public for rationing of sugar to about 2 pounds per week. Following a discussion of American profligacy in sugar consumption and an observation that "high sugar consumption leads to increased incidence of and death from catarrhal troubles, bronchitis and pneumonia", Harding concludes with the following paragraph:

> "Rationing has been invoked not because of scarcity of sugar but in part because of panic buying and largely because we lack shipping facilities to bring luxury supplies of sugar into this country. We must ration to effect equitable distribution. We shall in any case have more than enough sugar for our health's sake this year. For we normally waste and eat too much of it. We shall no doubt be far better off physically

if our ration finally drops to the German or even to the British level, assuming we have an otherwise well-balanced diet sufficiently rich in energy foods."

A few months later, the Council on Foods and Nutrition published a report in the Journal of the American Medical Association (1963) which was prefaced by the following:

Recent restrictions on the sale of sugar in the United States have focused attention on some of the nutritional problems associated with this commodity. Some restrictions of the consumption of sugar may be desirable from the standpoint of public health. The consumption of sugar and of other relatively pure carbohydrates has become so great during recent years that it presents a serious obstacle to the improved nutrition of the general public. The Council decided to summarize its views on this subject and has authorized publication of the following report.

Franklin C. Bing, Secretary

SOME NUTRITIONAL ASPECTS OF SUGAR, CANDY AND SWEETENED CARBONATED BEVERAGES

Here the objection (indirect) to sucrose is that it may replace sources of minerals and vitamins essential to good nutrition. Various suggestions have been made for enriching sugar as is done with flour, or for making sugar more nutritious by combining it with minerals and vitamins. In any event, too much sugar is consumed for the nation's nutritional welfare. The present (1942) restrictions in the use of sugar will help improve the nutritive quality of American diets.

Food and Cosmetics Toxicology (1979) reviews briefly the evidence relating sucrose as "a liability to myocardial infarction" in an article entitled "Sweet Mystery of Life" which appeared in 1971. An introductory comment labels such a relationship "a vexed question", and after descriptions of numerous experiments on animals and man, presents arguments by Yudkin that the relationship of smoking, sugar intake and ischaemic heart disease does not necessarily impute to smoking the real cause of ischaemic heart disease. "The basis for such a conclusion certainly seems, at present, too confusing to be convincing."

Yet another viewpoint is presented by MacDonald (1063) in a paper of 1969; "Sucrose: What Else Besides Caries?" "In terms of disease in man the only charge that can be laid against sucrose with any expectation of a verdict of guilty is its special ability to facilitate the development of dental caries".

John Yudkin of Queen Elizabeth College, University of London, has

for over a decade proclaimed sugar to be "deadly dangerous" and has insisted that it be banned from the diet. His missionary zeal has, however, failed to convince many researchers that sugar is "all that bad"; the world consumption of some 72,000,000 tons in 1971 (160,000,000,000 lbs!) would suggest that Dr. Yudkin is a "Voice Crying in the Wilderness". Much of the evidence that Yudkin presents in support of the "anti-sugar" hypothesis is based upon epidemiological studies of world consumption of sugar and other dietaries from which he has concluded that sugar and not fats is most closely correlated to the incidence of ischaemic disease (1934). The association between diabetes and myocardial infarction and atherosclerosis is underlined by Yudkin. Research has indicated that the dietary factor most closely paralleling the prevalence of diabetes is the consumption of fat; but fat has no deleterious influence on sugar tolerance and actually reduces the susceptibility to diabetogenic agents. Yudkin believes that the association of diabetes with fat consumption is purely fortuitous; the dietary intakes of fats and sugars are closely allied. Fat intake is a coincidental index to sugar intake; the aetiology of myocardial infarction is most likely multifactoral, with sugar consumption the most important dietary factor.

A summary of Yudkin's arguments for his position is presented in a paper introduced by Joan Mayer of Harvard University, in Postgraduate Medicine, 1968 (1936). The most important point, perhaps - is the observation that there are considerable metabolic differences in the form of carbohydrate in the diet such as starch versus sugar and the subsequent effects. Sucrose is more rapidly hydrolyzed and absorbed than is starch and the products, glucose and fructose may have different effects than the glucose alone derived from starch. Yudkin does state that "much still remains to be done in order to identify the mechanisms by which sucrose can produce the disease processes leading to coronary thrombosis". His final paragraph from the report:

> "Not everyone will necessarily agree with our opinion that the evidence incriminating sugar in coronary thrombosis is not more convincing than that incriminating fat. But most will agree that the tremendous research effort over a dozen years or more, by thousands of workers in dozens of countries, has failed to establish conclusively that the major cause of coronary thrombosis is a high intake of fat. If for no other reason than this, the hypothesis that a high intake of sugar is a major cause deserves to be tested by equally intensive research. The prize is the possibility of substantially reducing the toll of a disease that kills over 100,000 people each year in Great Britain alone."

The editors of Nutrition Reviews commented in 1970 (1974) under the title Sugar and Coronary Heart Disease: "Cigarette smokers use more sugared hot beverages than nonsmokers, an observation which confounds a superficial association between sugar and coronary heart disease". A number of studies are cited in which sugar consumption was seen to be higher among cigarette smokers than nonsmokers. Speculations are raised:

Are smoking and tea or coffee drinking related to an underlying psychological need or are they merely socializing habits shared by chance? Does the greater use of sugar in hot beverages among smokers suggest an altered taste threshold for sweetness? Does oral-pharyngeal dryness in smokers lead to greater consumption of beverages? It was concluded" that the apparent association between coronary heart disease and sugar is neither as straightforward nor as clear as originally hypothesized."

A comprehensive review of the literature on dietary factors and coronary heart disease is presented by R. Masironi (1105) in the Bulletin of the World Health Organization, 1970. The abstract is presented here:

> "Mortality data from arteriosclerotic and degenerative heart disease (AHD) and per capita consumption of total fat, saturated fat, sucrose, simple sugars, complex carbohydrates, and protein, and calorie intake for 37 countries were statistically evaluated to investigate possible relationships between dietary factors and incidence of AHD. On a geographical basis, consumption of total and saturated fats is strongly and positively correlated with the death rates, while consumption of complex carbohydrates is negatively correlated. No correlations were found with temporal changes in death rates or with differences within one country. These findings are discussed in the light of the works of many other investigators. It is concluded that the relation of diet to AHD is still controversial, and that the development and severity of the disease cannot be confidently attributed to any single dietary factor nor to blood cholesterol. The contributing effects of other factors, such as physical activity, mental stress, and affluence, are also discussed."

An epidemiological presentation correlating death rates and AHD throughout the world is followed by discussion of dietary factors related to AHD; total calorie intake, proteins, fats, carbohydrates and minerals and non-dietary factors such as physical activity, mental stress, emotional stress, etc. More than 100 references are cited.

Schiweck (1533) in 1970 delivered an address to a group of dentists; the published report, entitled "Is Sugar Harmful For The Human Organism", consists chiefly of a refutation of Yudkin's claim that sugar is harmful.

The problems of sugar consumption in modern industrial societies as represented by Czechoslovakia and the German Federal Republic are presented in considerable detail by Hruby and Wirths (771) in 1971. The authors view sugar as "empty calories" and generally accept the hypothesis of Yudkin and others who support the negative evaluation of sugar.

A recent "Letter to the Editor" in the Washington Post indicates the continuing nature of the controversy over sugar:

On the Controversy Surrounding Sugar and Nutrition

The Washington Post reported on Sept. 15 that Dr. Michael Jacobson and 180 supporters had called upon General Mills to abandon its new presweetened cereal, "Mr. Wonderfull's Surprise." This appeal seems impressive, but some important background information concerning it might lend perspective to the controversial nature of the facts surrounding this subject.

Dr. Jacobson solicited support from the Society for Nutrition Education for an appeal to breakfast cereal companies in general not to produce high-sugar products and to General Mills in particular to stop production and marketing of this product. When the request was offered as a resolution to be adopted by the society at its annual meeting in August, it was tabled on the grounds that the medical literature regarding the effects of sugar is controversial and should be further evaluated prior to endorsement of any resolution which advocated a decreased consumption of sugar.

It is indeed important to draw public attention to nutrition problems. However, even experts do not always agree on the interpretation of studies linking diet and health. It is a disservice to mislead the public with oversimplifications. Although there is no doubt that bad dietary practices contribute to many health problems, existing evidence does not support the unqualified assertion that "sugar and saturated fat can raise diabetes and heart attacks."

Educators and media must assume a more responsible attitude toward interpreting information about the effects of diet on health. For example, the evidence that obesity contributes to heart disease and diabetes is much more conclusive than is evidence of any correlation between intake of sugar and these disorders. Obesity is caused by excessive consumption of calories from any food regardless of whether the sources of these calories are sugar, cereal, bread, meat or potatoes—or simply from not exercising enough to burn up these calories. Following good nutritional practices based on medical facts about obesity may require difficult personal adjustments but would be a beneficial approach to reducing the risk of heart attacks and diabetes.

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Boyd et al. (184) determined the oral LD_{50} of sucrose in Wistar albino rat to be 35.4 plus or minus 7 g/kg body weight. Adult males of 300-500 g weight were given doses ranging from 5 to 80 g/kg intragastrically as 60 ml/kg solutions in water. All animals given 5-24 g/kg survived; those given 60-80 g/kg died within 5 hours. Female rats treated similarly showed an LD_{50} of 29.7 plus or minus 3.7 g/kg, which though lower than that for males, was not significantly so. Death from the higher doses was apparently due to marked capillary congestion of the brain and meninges; and from possibly a fulminating gastroenteritis. Death from lower doses was marked by renal lesions and vacuolar degeneration of the convoluted tubules. Survivors showed polydipsia, polyuria, alkalinuria and some hyperthermia between 3 and 6 days following treatment.

Constantopoulos and Boyd (335) studied the influence of a number of factors on the toxicity of sucrose in Wistar albino rats. The LD_{50} for doses given to fasting animals was not significantly different from that of sucrose given immediately after eating. A single dose of 38 g/kg (the LD_{50}) given in water to volumes of from 35 to 60 ml/kg gave death rates proportional to volume of dose. The mortality rate for a dose of 37.5 g/kg in 50 ml/kg solution was higher for female rats than

for males, and the premortem symptoms were also more pronounced in the female. The mortality rate for older, heavier rats was 40% versus 9.8% for younger, lighter animals. Successive dosing with sublethal doses of sucrose builds a tolerance to otherwise lethal doses. Dosing 5 days a week versus 7 days a week for 5 weeks produced no significant differences in mortality rates; seasonal differences in dosing (winter or spring) were also not significant.

Repeated doses of variable strengths given over long periods of time (a few months to about 10 months) to mice and rats subcutaneously produced no tumors: reported in HEW, PHS publication No. 149.

Harper and Worden (708) gave rats diets containing about 80% carbohydrate (glucose, fructose or sucrose) for 26 weeks; comparative toxicities were found to rank fructose most toxic, glucose least toxic, and sucrose of intermediate toxicity.

The LD_{50} (100 days) for sucrose was determined by Constantopoulos and Boyd (336) who gave several hundred young male albino rats doses varying from 20 g/kg to 45 g/kg daily, intragastrically, for up to 100 days or until 60% of the animals had expired. The maximum dose of sucrose with no deaths (LD_0 (100 days)) was about 20 g/kg/day; the smallest dose resulting in 100% deaths (LD_{100} (100 days)) was about 35 g/kg/day. The LD_{50} (100 days), plus or minus one standard error, was determined to be 28.5 plus or minus 1.3 g/kg/day. The authors have proposed an expression for ranking LD_{50} doses for drugs by a 100-day LD_{50} index (LD_{50} (100 days)/LD_{50} (1 day)); a tabular presentation for a number of drugs and sucrose index values is given.

The carcinogenic and tumor-promoting potentials of sucrose, cyclamate and saccharin given in the diet of female Swiss mice for 18 months by Roe et al. (1452) were shown to have no significant influence.

A 2-year study by Hueper (776) of a reported carcinogenicity of various sugars injected subcutaneously in mice and rats produced no tumors in the mice, and the dozen tumors seen in the rats were also observed in controls. The sucrose was given as a 25% solution twice weekly. It was suggested that reported carcinogenicity in other studies may have been due to impurities in the sugar used, e.g., polycyclic hydrocarbons or dibenzanthracene chemicals from the charcoal used in filtering and decolorizing processes of sugar manufacture.

Whitnah and Bogart (1876) produced reproductive abnormalities in female rats kept on a diet of which the carbohydrate portion was 30% starch and 20% sucrose; two other groups had diets with 30% starch and 20% lactose, or 50% starch, and showed normal reproductive capacity. After being kept on these diets for several years, females on each diet were changed to regular stock diet, placed with fertile males for 40 days or until pregnant. One female on sucrose produced a live litter but was afterwards sterile. The sucrose-fed animals matured earlier but showed few oestrous cycles; ovarian abnormalities were cytologically evident. The authors speculated that the action of the sucrose was on the pituitary function, but definite conclusions were not possible.

Sugar in the diet has been condemned not only because of a pre-

sumed relationship to ischaemic heart disease or atherosclerosis but also because of its cariogenic property. Stewart et al. (1652) conducted experiments with caries-resistant and caries-susceptible strains of rats to demonstrate the role of heredity in development of dental caries. Sucrose or finely ground rice comprised the carbohydrate in the diet and note was taken of the time elapsed between beginning the diet and the appearance of positive evidence of caries. The caries-susceptible animals showed caries much sooner than the resistant strain. A powdered sugar was substituted for the granulated sucrose in another experiment, and proved even more effective in shortening the time for caries to appear, contrary to other research findings that hard, coarse-particles increase caries incidence. In general, the hereditary influence on caries production in these experiments was significantly apparent.

A similar study by Haldi et al. (692), testing the relative cariogenicity of sucrose in solution versus sucrose as solid, using a caries-susceptible strain of Wistar rat, demonstrated that the aqueous sugar solution was significantly less cariogenic than was the dry granulated sucrose added to the diet. It was further observed that the cariogenicity of sugar depended upon direct oral contact with the teeth; a highly cariogenic diet given intragastrically by cannula produced no caries.

Food and Drug Research Laboratories, Inc. (1980) reported negative results in teratogenic studies of sucrose given to mice and rats. Dosages of 1600 mg/kg for 10 consecutive days in pregnant females produced no harmful effects on either dam or embryos.

Podolsky (1357) reported a quarter of a century ago on sugar as a medicine and its uses for curing or relieving the symptoms of a wide variety of illnesses ranging from seasickness, sleeping sickness, delirium, etc. to the pain of angina pectoris and varicose veins. The enthusiasm of the report must cause some distress among those who find sucrose a dangerous substance which should be removed from present-day diets.

A recent (1971) novel application of sugar in the therapy of hiccups was reported by Engleman and Lanktons (473) who found that a spoonful of ordinary, granulated white sugar, swallowed "dry" was very effective in stopping hiccups in 19 of 20 cases, some of whom had suffered from hiccups for up to 6 weeks. The authors suggested that it was the local irritation by the dry sugar on the pharynx and the subsequent interference with the afferent vagal impulses associated with hiccups, by impulses induced by way of the pharyngeal plexus, that makes sugar effective.

Four cases of allergic toxemia attributable to sucrose of cane origin were described by Randolph and Rollins (1409); similar cases by the same authors of beet sugar sensitivity were also reported in a second paper (1410). The "fatigue syndrome" of allergic origin included myalgia, mental confusion, depression, extreme fatigue, nervousness, tenseness and so-called "jitteriness". The exclusion of sucrose from the diet resulted in either complete relief from the symptoms or nearly so. Sucrose has been shown to be immunologically active and shows a

positive precipitin reaction when tested against Type 2 pneumococcus anti-serum. Invert sugar of cane origin elicits allergic responses in many cane-sensitive patients; monosodium glutamate of beet origin also produces reactions in beet sugar sensitive cases. The authors suggest that labelling the type of sugar employed in food processing should be federally regulated.

A congenital incapacity to eat sweet foods or sugar in any form was reported by Davidenkov (382) in 1940. Several series of brothers and sisters suffered from aglycophagia or the inability to eat sugar, an anomaly that appeared in early childhood and persisted during the lifetime of the individuals. Nonsweet polysaccharides were not rejected. The author stated that the aglycophagic syndrome was not like an allergic response to sugar, but most like myoplegia. Burgess et al. (231) described in 1964, 7 cases in which a congenital absence of sucrase resulted in a sucrose intolerance much like that observed by Davidonkov. A discussion of carbohydrate tolerance, enzyme defect and the genetic implications of sucrose intolerance is presented.

Dietary excesses of carbohydrates or fats have been frequently associated with adolescent acne and seborrheic dermatitis. Bett et al. (139), using dietary questionnaires given to 16 hospital patients with acne and a like number with dermatitis, found that the patients with seborrheic dermatitis had a significantly higher sugar consumption than in control subjects but those with acne vulgaris differed little or not at all.

A method for selecting daily dose-levels of drugs was described by Boyd (183) in 1971. From among several methods available, Boyd selected one based on decreasing fractions of the acute LD_{50} from data obtained by oral administration of the substance by intragastric cannula for a period of 100 days. Following a determination of the acute oral, single-dose LD_{50}, decreasing factors of that dose, beginning with about 90% are given daily for 100 days or until death, and the LD_{50} (100 day) established. The ratio between the LD_{50}'s (LD_{50} (100 day)/ LD_{50} (1 dose) is called the 100-day LD_{50} index. A tabular presentation of several index values includes that for sucrose, or 80.6 plus or minus 3.7. The LD_{50} (100 days) for sucrose was 28.5 g/kg/day, and an earlier determination of the LD_{50} (1 dose) was given as 35.4 g/kg by Boyd et al. (184).

A review of laboratory evidence implicating sucrose as the "arch criminal" in the genesis of dental caries was presented by Newbrun (1252) in 1967. An hypothesis is presented which suggests that only sucrose provides the proper substrate for the extracellular polysaccharides, the synthesis of which by certain oral microorganisms, provides the structural matrix of dental plaque. It has been shown that sucrose-containing diets are markedly more cariogenic than diets with glucose, lactose, maltose, maize or wheat flour. Individuals with hereditary fructose intolerance, a metabolic disorder - avoid all forms of sweets containing sucrose or fructose and have shown a marked freedom from dental caries.

As early as 1957, Yudkin (1932) proposed that sugar in the diet was more closely related to the incidence of coronary thrombosis, world wide - than were hydrogenated fats. The evidence was epidemiological, suggestive of a relationship between high standards of living and coronary thrombosis; increased sugar use was a measure of higher living standards throughout the world. Yudkin also suggested that multiple causes were responsible for the disease and that more information regarding dietary influences and the effects of exercise, smoking, etc. would be required for determining the relative importance of factors contributing to coronary thrombosis.

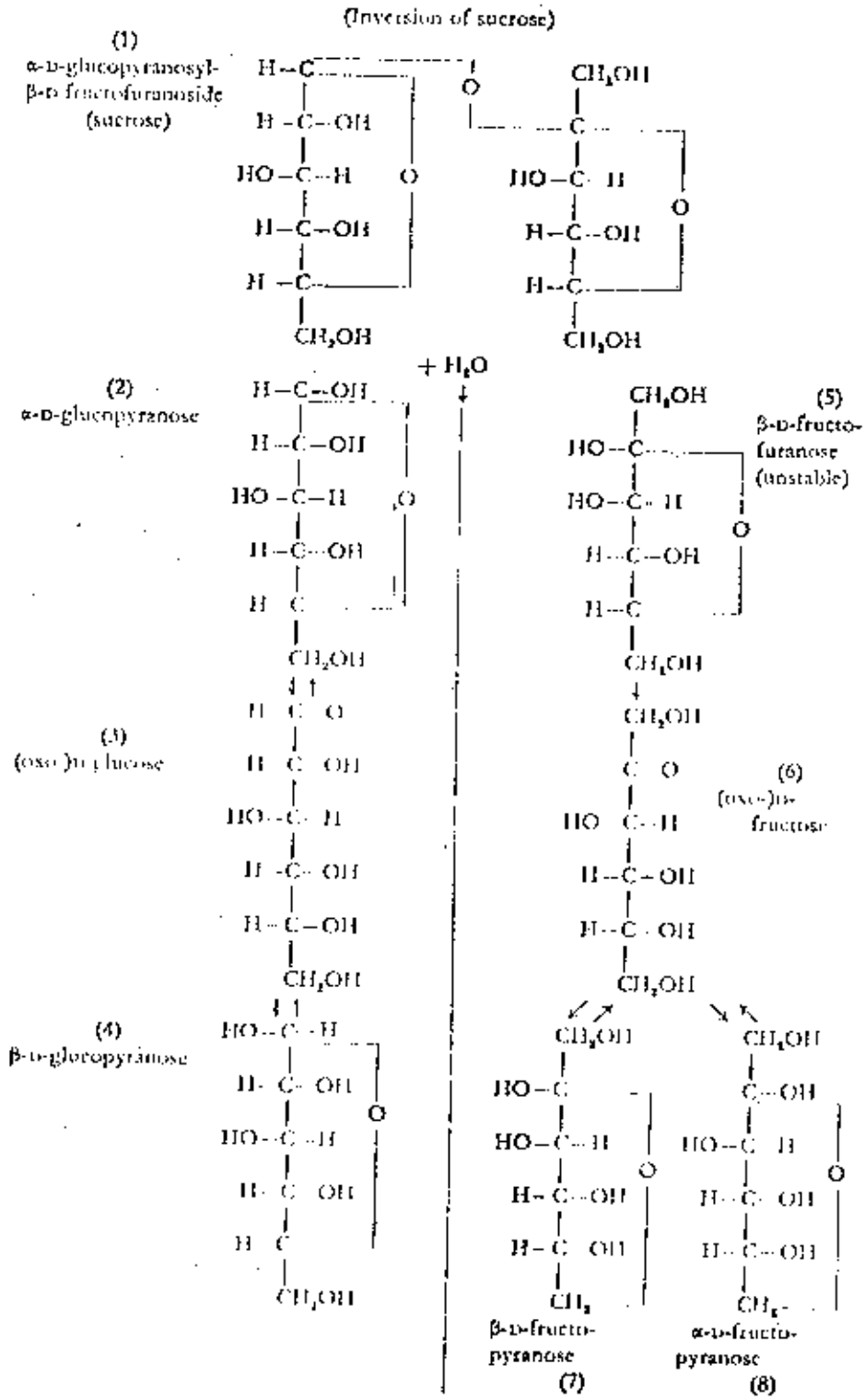
Burns-Cox et al. (232), in 1969 repeated earlier studies by Yudkin and others with a view to reproducing the positive results correlating sugar consumption and myocardial infarction. A questionnaire was used to collect data on 80 cases of infarction and 160 control patients; age, social class, marital status and sugar consumption were noted. Smoking habits related to sugar use were also noted. A stronger correlation between the incidence of myocardial infarction and smoking was found than a relationship to sugar consumption, as Yudkin and others had reported. Burns-Cox et al. suggested that consumption of refined sugar was unlikely to prove a major factor in the incidence of myocardial infarction.

Russian interest and research in the atherogenicity of sucrose is presented in a paper by Markelova and Lyapkov (1089). Cholesterol absorption and transport in male rats of Wistar strain were studied by means of C^{14}-tagged cholesterol and sodium acetate. One hundred and fifty male rats weighing 110-130 g were fed diets containing either cornstarch or sucrose as the carbohydrate portion for 30 days. At the end of the test period the animals were subdivided into two groups. One group was injected with C^{14}-tagged cholesterol (25 microcuries/rat), the other group received C^{14}-tagged sodium acetate (150 microcuries/rat). The cholesterol-treated were sacrificed at 24 and 72 hours after injection; the acetate treated were sacrificed at 2 hours. Cholesterol and fatty acid radioactivities were determined; lipoprotein lipase activity, lipoproteins, free fatty acids and blood sugar were also determined. The serum cholesterol levels were somewhat increased and fatty acid synthesis was accelerated. An increase in B-lipoproteins was observed, which resulted in prolonged retention of serum cholesterol. The high rate of sucrose absorption may account for the changes in lipid exchange; fructose may exert some influence on the biosynthesis of fatty acids. Hyperinsulinism is also considered as a consequence of sucrose ingestion with subsequent accelerated synthesis of fatty acids. The lipid exchanges observed in the experiments may be considered factors contributing to atherogenesis.

Earl and John Benditt (127) have recently (1973) reported the results of an investigation concerning the nature of atheroschlerotic plaque origin in human aorta and iliac artery walls. Identification of genetically X-chromosome-linked glucose-6-phosphate dehydrogenase in heterozygotic females indicated that plaques are monoclonal in origin. The most satisfactory explanation for this finding lies in genetic

mutation, with likely causes, chemical or viral mutagens.

The hydrolysis of sucrose after ingestion is shown in the following diagram from NAS/NRC information; the final effect of the digestive process is the total oxidation into carbon dioxide and water with the liberation of the same amount of energy utilized by the plant in manufacturing sucrose:



It has been suggested that the presence of hiatus hernia, mental defects and sucrosuria (sucrose in the urine) in the same individual comprises a clinical syndrome. Freier (564) made a study of this "triad", giving hospital patients and volunteers 20 g of sucrose daily in the diet of patients with mental defect and hiatus hernia, mentally normal with hiatus hernia, relatives of both groups, and normal controls. Urine was collected and analyzed for sugar. Sucrosuria was not uncommon in normal people; sucrosuria among children with lower oesophageal abnormalities was, however, more than twice as frequent as that found in normal individuals. The results of the study were insufficient to demonstrate a specific syndrome of the three factors.

A similar, earlier report by Moncrieff and Wilkinson (1178) described three cases of early childhood sucrosuria involving digestive abnormalities. In one case, the infant died and autopsy showed a congenital brain defect, eliminating a causal relationship between sucrosuria and mental retardation. The authors also concluded that insufficient evidence was available to establish a syndrome, particularly of sucrosuria and mental retardation.

Metais (1144) in 1967 reviewed the problems associated with anomalies of sugar metabolism, including digestion, absorption, etc., but more particularly, he discussed the genetic problem dealing with congenital sugar intolerances and the status of knowledge at that time.

Dahlqvist (365) in 1967 reviewed research in the metabolism of disaccharides, pointing out the failure of textbook treatments to present the modern view which presents an intracellular digestion of disaccharides rather than an earlier concept in which disaccharidases were secreted with the "intestinal juice" into the lumen of the small intestine. Three principal methods employed in studies of disaccharide digestion are described and the results which have lead to the intracellular concept are presented.

MacDonald and Turner (1063) reported a sex-related difference in serum fructose levels in healthy men and women given either 2 g/kg sucrose or mixture of glucose and fructose; the serum fructose levels were significantly higher in both sexes after sucrose intake, but significantly lower in women than in men. The authors explained the difference as due to sex hormones. The mixture of glucose and fructose produced slight differences in serum fructose. Insignificant changes in serum glucose in either instance were observed.

Intraperitoneal injection of sucrose in rats produces liver vacuoles resembling lysosomes according to a report by Brewer and Heath (198). Six to eight milliliters of 50% sucrose were injected into rats weighing 250-300 g; at intervals the animals were sacrificed and liver specimens prepared for light and electron microscopy. Cytoplasmic vacuoles showed acid phosphatase activity normally seen in the lysosomes; it was suggested that they had their origin in lysosomes, which they resembled both cytochemically and morphologically.

The malnutritional diseases of kwashiorkor and marasmus are both

due to protein deficiencies, but excess calories from carbohydrates usually lead to depot fat and fatty liver in kwashiorkor, - characteristics which are absent in marasmus, due to calorie deficiencies as well as to lack of protein in the diet. MacDonald studied the nutritional relationship of dietary carbohydrate and lipid metabolism using rabbits. The diets contained a constant protein component but the carbohydrates were varied. The lipid deposits in the liver varied directly with the amount of carbohydrate in the diet; sucrose produced more liver lipid than an equal weight of starch in the diet. A similar experiment with adult male humans, given diets with either maize starch or sucrose, produced similar results; sucrose increased the serum lipid levels and starch produced a fall in serum lipid levels. The traditional dermatological association of acne and high carbohydrate intake would appear justified according to MacDonald, in view of changes in fatty acid composition of the sebum in skins of men tested in the foregoing experiment.

The assumption that "all carbohydrates are alike", with similar metabolic effects, was refuted by Al-Nagdy et al. (30) (including Yudkin), who experimented with rats fed diets in which the carbohydrate portions were of 70% corn starch or sucrose. Difference in growth of weanling rats over a six-month period was apparent; sucrose diets were less efficiently utilized than starch. Respiratory quotients were lower for sucrose and serum cholesterol levels higher. The results of experiments by others in varying the fat in the diets showed no similar changes in growth, body fat or liver function. Bender and Thadani (124) have recently investigated the metabolic differences between sucrose and starch in an elaborate series of experiments. Some of the findings were: glucose oxidation and lipogenesis in liver of rats given a sucrose diet were severely depressed; the metabolic pathways differed for sucrose and starch; there were no sex differences observed in the tissues of animals with different carbohydrates; liver lipogenesis differed in three strains of rats used, but adipose tissue lipogenesis was depressed in all three strains; sucrose generally depressed metabolism, possibly through the effect on the function of glucose-6-phosphatase and dehydrogenases.

A number of investigators of the differences in sucrose versus starch in the diet have demonstrated an influence of sucrose on the insulin system in rats and in man. Cohen and Teitelbaum (313) used rats to test the carbohydrate effect on the "insulin reserve"; oral glucose tolerance tests performed at intervals on rats fed either sucrose or starch showed a marked impairment of glucose tolerance on sucrose diets. The effect was reversible and renewable when the animals were changed to a starch diet and again returned to sucrose. Liver fat content was increased on sucrose, with diminished insulin-like serum activity, possibly due to the rise in blood glucose. Yudkin and Szanto (1943) recently studied male patients with peripheral vascular disease and observed that a hyperinsulinism existed on sucrose diets, with increased blood platelet adhesiveness and insulin levels. Subjects showing hyperinsulinism on sucrose diets gave electrophoretic platelet mobility tests characteristic of atherosclerosis. Haldi and Wynn (688) in an earlier study, attempted to demonstrate in human subjects whether

or not sucrose would induce hypoglycemic reactions similar to those reported for glucose. Both glucose and sucrose produced a rise in blood sugar followed by a gradual return to normal levels; the authors concluded that hypoglycemia was not a usual response to large amounts of sugar. It was also suggested that the results indicated a rapid inversion of sucrose in the gastrointestinal tract. Szanto and Yudkin (1686) in another recent study have shown that a high sucrose diet produces no change in levels of blood cholesterol or phospholipids, but did increase significantly the serum triglycerides and immunoreactive insulin. The authors suggest that the role of sucrose in ischaemic heart disease may lie in the hyperinsulinism resulting as shown in a number of subjects studied. Factors known to be associated with both ischaemic heart disease and hyperinsulinism are peripheral vascular disease, maturity-onset diabetes, obesity, smoking and hypertension. It is suggested that habitual high consumption of sucrose by some individuals may make them susceptible to development of occlusive atherosclerotic disease.

Alternate carbohydrates, starch or sucrose, in the diets of young male volunteers were studied with regards to the effect on serum cholesterol, phospholipids, fatty acids and triglycerides, by Akinyanju et al. (18). Platelet stickiness and urinary sugar were also measured. Both starch and sucrose raised the cholesterol and phospholipid concentrations. Sucrose increased serum triglycerides; starch produced a fall. Both carbohydrates increased platelet stickiness. Sucrose was found in the urine after the sucrose diet; none was observed during the starch diet. Contrary to general belief, sucrose is not completely hydrolyzed before absorption, according to Akinyanji et al.

Intravenous sucrose solutions in therapy of a number of diseases ranging from alcoholism to asthma were widely used in the 1930's. Rigdon and Cardwell (1445) in 1942 reported a study of terminal clinical hospital cases in which hypertonic sucrose solutions were employed during therapy. Autopsies showed in some cases renal lesions, possibly due to the sucrose; rabbit experiments produced similar kidney damage, especially where dehydration precedes the injection of sucrose. Anderson (44) in 1941 had described sucrose nephrosis distinguishable by its hydropic or vacuolar degeneration. Caution was recommended in the use of sucrose therapy in cases involving pre-existing renal lesions, as in cases with diarrhea or chronic dysentery.

SUCROSE

Chemical Information

I. Nomenclature

A. Common Names

1. Sugar
2. Saccharose
3. Cane Sugar
4. Beet Sugar

B. Chemical Names

1. Sucrose
2. beta-D-fructofuranosyl-alpha-D-glucopyranoside
3. alpha-D-glucopyranosyl-beta-D-fructofuranoside

C. Trade Names

None

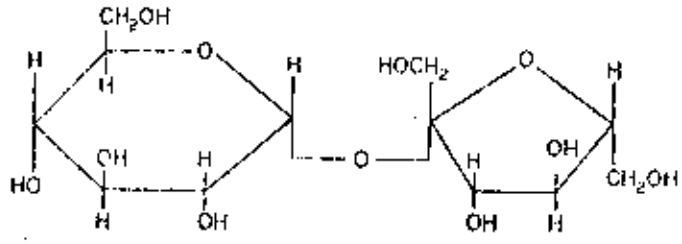
D. Chemical Abstracts Registry Number

000057-50-1

II. Empirical Formula

C12H22O11

III. Structural Formula



1. Sucrose, α-D-glucopyranosyl-β-D-fructofuranoside

IV. Molecular Weight

342.30

V. Specifications

From: Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, CAC/RS 4 (1968).

RECOMMENDED INTERNATIONAL STANDARD FOR WHITE SUGAR

1. SCOPE

This standard applies to white sugar except that paragraph 3.1.4 (Loss on drying) does not apply to white sugar in lump or cube form or to crystal candy sugar (crystal korizato) or to rock sugar (korizato).

2. DESCRIPTION

White sugar is purified and crystallized sucrose (saccharose).

3. ESSENTIAL COMPOSITION AND QUALITY FACTORS

3.1 Specification A

3.1.1	Polarization	not less than 99.7°
3.1.2	Invert sugar content	not more than 0.04% m/n
3.1.3	Conductivity ash	not more than 0.04% m/m
3.1.4	Loss on drying (3 hours at 105°C)	not more than 0.2% m/m
3.1.5	Colour	not more than 60 ICUMSA units

3.2 Specification B

3.2.1	Polarization	not less than 99.5°
3.2.2	Invert sugar content	not more than 0.1% m/m
3.2.3	Conductivity ash	not more than 0.1% m/m
3.2.4	Loss on drying (3 hours at 105°C)	not more than 0.1% m/m
3.2.5	Colour	not more than 150 ICUMSA units

4. FOOD ADDITIVES

4.1 Sulphur dioxide

Specification A	not more than 20 mg/kg
Specification B	not more than 70 mg/kg

5. CONTAMINANTS

Specifications A and B

5.1 Arsenic (As)	not more than 1 mg/kg
5.2 Copper (Cu)	not more than 2 mg/kg
5.3 Lead (Pb)	not more than 2 mg/kg (temporarily endorsed)

6. HYGIENE

It is recommended that the products covered by the provisions of this Standard be prepared in accordance with the appropriate sections of the General Principles of Food Hygiene recommended by the Codex Alimentarius Commission (Ref. No. CAC/RCF 1-1969).

From: NAS/NRC

DESCRIPTION: A white, free-running, easily soluble granular powder having a good sweet flavor, free from burnt off-flavors or odors.

Moisture	0.05% maximum	
Sucrose Assay	99.90% minimum	
Sieve Analysis (5 minute Rotap)	5 to 20% thru US #80 screen	
Extraneous Matter		
Macro	None	
Micro	None	
Microbiological Examination		
Standard Plate Count	1000/g maximum	
Yeast-Mold Count	100/g maximum	
E. coli	Negative	
Thermophilic Spore Count, NCA Method		
Aerobic, maximum	5 Spls/Lot	Average
Total Thermophiles	150/10 g	125/10 g
Flat Sour Type	75/10 g	50/10 g
Anaerobic, maximum		
Sulfide Producers	2 Spls +	10/10 g
Gas Producing	3 Spls +	4 of 6 tubes

DESCRIPTION: An off-white, clear, sweet tasting, viscous liquid produced from No. 2 grade raw cane sugar.

Solids	67.0 ± 0.2° Brix
Invert Sugar	0.4% maximum
Ash	0.04% maximum
pH (10% solution)	6.8 ± 0.2
Color	0.20 to 0.35 Horne Color
Clarity	Clear
Flavor and Odor	Characteristic sweet flavor; free from any objectionable foreign flavors and odors.
Extraneous Matter	none
Microbiological Examination:	
Standard Plate Count	1000/g maximum
Yeast-Mold Count	100/g maximum
E. coli	Negative

VI. Description

A. General Characteristics

Monoclinic sphenoidal crystals, crystalline masses, blocks or powder; sweet taste; stable in air; hygroscopic when finely divided, absorbing up to 1% moisture which is given off when heated to 90 degrees. Decomposes 160-186 degrees, chars or caramelizes.

B. Physical Properties

Soluble in H_2O, 1 gram/0.5 ml or in about 0.2 ml boiling H_2O; soluble, 1 g/170 ml alcohol or 100 ml methanol. Moderately soluble in glycerol or pyridine. Does not reduce Fehling's solution, form an osazone, or show mutarotation. Dilute acids and invertase hydrolyze sucrose to glucose plus fructose, or "invert sugar". Sucrose can be fermented, but resists bacterial decomposition as concentrated solutions.

Further elaboration of the chemistry of sucrose is provided in a paper by Fewkes et al. (514) which may be found in the documents section of this monograph. The formation of sucrates and complexes as a consequence of the weak polybasic acid behavior of sucrose is noted as are the derivatives resulting from the polyhydric alcohol nature of sucrose, e.g., esters, ethers and urethanes.

VII. Analytical Methods

Polarimetric and chemical analyses may be found in Methods of Analysis, AOAC, Sections 29.025, 29.026, 29.031 and 29.032.

As isotope dilution analytical method, modified to use a liquid scintillation counting technique, is described in a paper by McGagin and Eis (1127) and is included in this monograph (see for details).

VIII. Occurrence

The following paragraphs are taken from Sucrose Chemicals published by the International Sugar Research Foundation, 1970; (792) the introduction is included in this monograph:

Sucrose, commonly known as table sugar, is by far the most abundant carbohydrate found in the sap of land plants. It is one of the few organic compounds available in a state of unexcelled purity, in highly crystalline form, on a very large scale, and at low cost. It has been produced since 2000 BC from the juice of the sugar cane and since the early 1800's from the sugar beet.

The combined annual world production of sucrose from sugar cane and beet was 77 million tons in 1968. Of this total the United States (including Puerto Rico) produces six million tons and consumes ten million tons. Other major producing countries (in approximate order of size) are the USSR, Cuba, Brazil, India, France, Germany, Australia, Mexico and Taiwan.

Beets provide much of the sugar for Europe and central and western United States, and cane the rest. Other limited sources include sorghum and maple.

Sucrose is used almost exclusively by the food and other consumer goods industries for sweetening. Of the ten million tons consumed in the United States each year, 27% is sold directly to home and institutional users. The remainder goes to the baking, confectionery, ice cream, beverage, and other food industries. Pharmaceuticals, cosmetics, and other non-food users consume a hundred thousand or so tons annually. The commercial utilization of sucrose chemicals is increasing. Only a handful of compounds are presently used by the chemical and allied industries or other nonfood groups. Sucrose octaacetate and sucrose acetateisobutyrate are manufactured in substantial volume. Both esters are additives for specialty polymers, chiefly in adhesives. The octaacetate is a bitter chemical also used as a denaturant for alcohol. Cyanoethyl sucrose is manufactured on a limited basis for modification of specialty adhesives in the electronics industry. Mannitol is an important product in the food industry.

The Sugar Research Foundation has sponsored work on applications of sucrose chemicals. The most promising results have been obtained with sucrose ester detergents and surface coatings. Sucrose surfactants have been produced by a number of companies in the United States, Germany, Japan, and elsewhere. Although still under test, they show excellent promise as nontoxic, nonionic, highly detersive agents. Sucrose-based surface coatings also exhibit good properties, particularly those made from sucrose linseedate. Various other polymers and plastics have been prepared or modified with sucrose.

Sucrose is synthesized by a number of bacterial enzymes from glucose-1-phosphate and fructose or from free glucose. The chemical

synthesis was accomplished 20 years ago, and at best has yielded about 6% (514). A recent paper by Ness and Fletcher (1246) describes one technique for the synthesis of sucrose through the use of 1,3,4,6-tetra-O-benzyl-D-fructofuranose condensed with 2,3,4,6-tetra-O-benzyl-alpha-D-glucopyranosyl chloride, the preparation of which form a mixture of anomers of 2,3,4,6-tetra-O-benzyl-1-O-(p-nitrobenzoyl)-D-glucopyranose is described. The details of chemistry and chromatographical techniques may be seen by reference to the document section of this monograph.

Biological Data

I. Acute Toxicity

Rat

During the course of experimentation on the toxicities of drugs given to rats, Boyd et al. (184) raised the question of how much influence the high sugar content of the vitamin-deficient synthetic diets given the animals might have upon the experimental data. Drugs being tested were also frequently administered as syrups, and the total sugar ingested approached estimates of the LD_{50} of sucrose. A literature review of sucrose experimentation proved unsatisfactory in relationship to the problem expressed by Boyd et al; the present report describes their studies on sucrose.

Adult male Wistar albino rats, weighing 300-500 g, were fed a commercial, Purina diet and water ad libitum. Sixteen hours before administration of sucrose, the animals were placed in separate metabolism cages and given only water. The sucrose solutions in water were given intragastrically as 60 ml/kg of dosages ranging from 5 to 80 g/kg. Nineteen different dosages in all were given; the lowest four and highest three doses were given to 4-8 rats per dose, the remaining doses were given to 16-20 animals per dose. Forty-eight controls received distilled water only.

Various clinical measurements were made daily, 6 days a week, for 2 weeks, followed by a one-month casual observation period. Urinalyses of 24-hour specimens were performed. Autopsies of those animals not surviving the sucrose were made within 30 minutes of death; survivors were sacrificed and autopsied at 2 weeks or 1 month. Gross and cytological data were collected from various organs.

All animals receiving doses of 5-24 g/kg sucrose survived; those given 60, 70 or 80 g/kg died within 5 hours. The frequency distribution of deaths vs. time for doses intermediate peaked at 4 hours, was depressed at 9 hours and leveled off at 12-48 hours. The LD_{50} for all deaths from sucrose was 35.4 ± 7 g/kg; 30 female albino rats similarly treated showed an LD_{50} of 29.7 ± 3.7 g/kg, not significantly different from that of males.

The early clinical signs following the administration of the sucrose were hypokinesia, prostration, cyanosis, abdominal bloating and diarrhea; except for cyanosis, all these signs also appeared in the controls given only distilled water, but less markedly during the first 6 hours and not persisting for 24-48 hours as in those animals given sucrose. Recovery in those animals which survived appeared complete. Doses higher than 30-35 g/kg mean lethal dose produced sudden death in less than 10 hours, apparently from marked capillary congestion of the brain and meninges. A fulminating gastroenteritis may have contributed to early death, particularly in the light of reports that the intravenous lethal dose is higher than the oral lethal dose reported here.

Doses of sucrose lower than the LD_{50} dose result in a delayed death of between 10 and 48 hours; renal lesions are marked in these circumstances, with swelling and vacuolar degeneration of the convoluted tubules. The reported effects of a high sucrose diet on the liver were not observed in the present experiments; the liver was observed to recover rapidly from toxic oral doses of sucrose and appeared histologically normal at 24 hours, the weight, however, was significantly depressed at 1 month. Polydipsia, polyuria, alkalinuria and some hyperthermia were observed between 3 and 6 days in the survivors of sucrose doses lethal to some, but these symptoms had largely disappeared at 1 month after treatment.

Constantopoulos and Boyd (335) studied a number of factors which influence the toxicity of sucrose in albino rats of a Wistar strain. The sucrose was given dissolved in distilled water in various doses and volumes by intragastric cannula. Seven factors were included in the studies, five of which were tested by single, acute doses, and two involved short-term, multiple doses.

Factor 1: Food in the stomach. Five groups of 20 male rats each, weighing about 180 g, were allowed to eat before treatment; the LD_{50} acute oral dose of sucrose was found to be 37.24 ± 7.3 g/kg. Male rats in previous experiments given a single dose of 60 ml/kg following a 16-hour fast showed an LD_{50} of 35.4 ± 7 g/kg. The difference between the results of dosage on an empty stomach and one filled with food was not significant.

Factor 2: Volume of distilled water vehicle. Six groups of 15 rats each (males), weighing about 176 g were given a single dose of 38 g/kg sucrose in 35 to 60 ml/kg distilled water. The death rate was proportional to the volume of the dose; the larger the volume, the higher the mortality rate. Most animals succumbed between 3 and 7 hours after treatment; symptoms included hypokinesia, cyanosis, abdominal bloating, diarrhea and hypothemia. The animals which survived several days showed no observable signs of toxicity.

Factor 3: Sex of the animals. Sixty unstarved male rats weighing about 181 g, and 16 females of about 176 g weight were given 37.5 g/kg sucrose in 50 ml/kg solution. The mortality rate was higher for females than for males; 81% females died within 24 hours versus 38% males. The premortem symptoms were similar in both sexes, but more pronounced in females.

Factor 4: Body weight and age of animals. Eighty-two unstarved males weighing about 118 g, and 20 weighing about 182 g were given 37.5 g/kg sucrose in 50 ml/kg dose. The mortality rate for the lighter, younger animals was 9.8%; the rate for the heavier rats was 40%.

Factor 5: Tolerance to sucrose. Twenty rats were given 37.5 g/kg sucrose daily, 5 days a week for 34 days; the 10 survivors were then given 45 g/kg, presumably an LD_{100}. None died. The same dose,

45 g/kg sucrose given to 13 controls, not previously treated, produced an 85% mortality. The 10 survivors of the "primed" groups were given repeated doses of 45 g/kg on four successive occasions and all but 2 animals survived. A repetition of the experiments indicated, again - that a tolerance can be produced by successive dosing with sublethal doses of sucrose.

Factor 6: Dosing 5 versus 7 days per week. Two groups of 20 male rats each were given daily sucrose, either 26.8 g/kg daily for 7 days a week or 37.5 g/kg 5 days a week over a period of 5 weeks (the total dose was the same for each group). The mortality rates were not significantly different.

Factor 7: Influence of season. Four groups of 20 rats each, weighing 150-175 g, were given a single dose of 37.5 g/kg sucrose; three groups treated in winter (January, February and March) and one group in the spring (June). No significant seasonal difference was observed.

The concluding discussion by the authors is of considerable general interest; the reader may wish to refer to the paper, a reprint of which is included in this monograph in the documents section.

II. Short-Term Studies

Rat

Constantopoulos and Boyd (336) determined the LD_{50} (100 days) dose for sucrose given to rats. The experiment used 280 young male albino rats weighing 150-175 g initially and fed a standard Purina chow and water ad lib. Sucrose (Certified Sucrose, Fisher Scientific Co.) of daily doses ranging from 20 to 45 g/kg were given intragastrically to at least 20 rats each dose and matched by 8 controls receiving distilled water. Each dose was administered daily for 100 days or until 60% of the animals expired. Clinical data were gathered before dosing and at weekly intervals during sucrose administration: body weight; food and water intake; calorie consumption; caloric temperature; urinary volume, pH, glucose and protein. Gross and microscopic pathology were observed within 1 hour of death either by the treatment or upon sacrifice of those animals surviving to 100 days.

The LD_{50} was calculated at weekly intervals and results were analyzed statistically. The LD_{50} (100 days) plus or minus one standard error was determined to be 28.5 ± 1.3 g/kg/day. The maximum dose of sucrose with no deaths, or LD_0 (100 days) was 19.8 ± 1.9 g/kg/day; the smallest dose which resulted in 100% deaths, or the LD_{100} (100 days) was 35.5 ± 0.5 g/kg/day.

The clinical signs observed included drowsiness, pallor, prostration, hyporeflexia, ataxia, diarrhea and soiling of fur. Deaths from smaller doses followed the appearance of hypokinetic hypothermic cachexia; larger doses resulted in respiratory failure followed by

convulsions. A summary of autopsy, histopathological findings, is shown in the following table:

Table 1. *Summary of histopathological findings at autopsy*

Organ	Deaths after treatment for	
	Less than 1 wk	9–100 days
Adrenal gland	Lipoid vacuoles prominent	Cortical hypertrophy; sinusoidal congestion and occasional haemorrhage
Aorta (thoracic)	Minor capillary-venous congestion of tunica adventitia	Occasional vacuolation of tunica media; occasional small aneurism
Arterioles (splenic)	Normal appearance	Occasional vacuolation of tunica media
Brain	Capillary-venous congestion of brain and meninges	Capillary-venous congestion and occasional haemorrhage of brain and meninges; infiltration of meninges with granulocytes; occasional brain abscess
Gastro-intestinal tract:		
Cardiac stomach	Capillary-venous congestion of lamina propria and submucosa	Hypertrophy of stratified squamous epithelium, muscularis mucosae and muscularis externa; hyperaemia of lamina propria and submucosa
Pyloric stomach	Capillary-venous congestion of lamina propria and submucosa, occasionally with haemorrhage, lysis of gastric glands and granulocyte infiltration	Mostly normal appearance; occasional capillary haemorrhage and granulocyte infiltration
Small bowel	Capillary-venous congestion of lamina propria and submucosa	Hypertrophy of villi, muscularis mucosae and muscularis externa; hyperaemia
Cecum	Capillary-venous congestion of lamina propria and submucosa	Hypertrophy of Lieberkuhn glands, muscularis mucosae and muscularis externa; hyperemia
Colon	Capillary-venous congestion of lamina propria and submucosa	Normal appearance to slight hypertrophy
Heart	Minor capillary congestion of myocardium	Occasionally capillary congestion with haemorrhage and thrombosis
Kidneys	Glomeruli shrunken; capillary-venous congestion occasionally with haemorrhage; fine vacuolation of tubules	Capillary-venous congestion, occasional haemorrhage and thrombosis; tubular cloudy swelling, vacuolation and necrosis; occasional shrinkage of glomerular tuft
Liver	Areas of granulation and vacuolation of hepatic cells	Normal appearance or sinusoidal congestion with areas of centrilobular vacuolation and necrosis
Lungs	Capillary-venous congestion and areas of venous thrombosis	Areas of capillary-venous congestion and venous thrombosis
Muscle (ventral abdominal wall)	Normal appearance	Occasional weak cross-striation
Pancreas	Acinar glands shrunken with loss of zymogenic granules and areas of necrosis	Acinar glands shrunken with loss of zymogenic granules and areas of necrosis
Salivary (submaxillary) glands	Normal appearance	Occasional atrophy of serous glands and loss of zymogenic granules
Skin	Normal appearance	Normal appearance
Spleen	Normal appearance	Contracted red pulp
Testes	Normal appearance	Varying degrees of deficiency of sperm and inhibition of spermatogenesis
Thymus gland	Minor loss of thymocytes	Varying degrees of loss of thymocytes and of capillary-venous congestion

The authors have proposed an expression for ranking LD_{50} doses of various drugs by 100-day LD_{50} index, which gives a ratio of LD_{50} (100 day) over LD_{50} (1 day) or acute toxic dose. For sucrose, the authors had previously determined the LD_{50} (1 dose) to be 35.4 ± 7.0 g/kg. The following table shows the 100 day LD_{50} index values for a number of drugs relative to that of sucrose:

Table 4. *LD_{50} (100 days) of several drugs expressed as the 100 Day LD_{50} Index*

Drug	LD_{50} (100 days) ± SE* (g/kg)	LD_{50} (1 dose) ± SE* (g/kg)	100 Day LD_{50} Index
Atropine sulphate	0·078 ± 0·005 (1)	0·588 ± 0·055 (2)	13·2 ± 0·8
Acetylsalicylic acid	0·243 ± 0·056 (3)	1·48 ± 0·04 (4)	16·4 ± 0·5
Pilocarpine nitrate	0·156 ± 0·020 (5)	0·911 ± 0·111 (6)	17·1 ± 2·2
Paracetamol	0·77 ± 0·02 (7)	3·71 ± 0·83 (8)	20·6 ± 0·5
Phenacetin	1·12 ± 0·02 (9)	4·14 ± 0·71 (9)	27·1 ± 0·5
Benzylpenicillin	4·14 ± 0·20 (10)	6·7 ± 0·1 (10)	61·8 ± 3·0
Sodium chloride	2·69 ± 0·12 (11)	3·75 ± 0·43 (11)	71·7 ± 3·2
Caffeine	0·150 ± 0·003 (12)	0·192 ± 0·018 (12)	78·2 ± 1·6
Sucrose	28·5 ± 1·3	35·4 ± 7·0 (13)	80·6 ± 3·7

* All the drugs were given intragastrically to albino rats except atropine which was given intramuscularly to rabbits.

Numbers in parentheses refer to the following references: (1) Boyd & Boyd, 1962; (2) Boyd & Boyd, 1961; (3) Boyd, 1968b; (4) Coldwell & Boyd, 1966; (5) Boyd & Jarzylo, 1968; (6) Covert & Boyd, 1962; (7) Boyd & Hogan, 1968; (8) Boyd & Berezsky, 1966; (9) Boyd & Hornsroth, 1968; (10) Boyd & Selby, 1962; (11) Boyd, Axel & Knight, 1966; (12) Boyd, Dolman, Knight & Sheppard, 1965; (13) Boyd *et al.* 1965.

Harper and Worden (708) reported, in abstract form, comparative toxicity studies on glucose, fructose and sucrose when comprising about 80% of the diet in rats over a period of 26 weeks. Body weight gains were significantly lower than in controls only in the glucose-fed animals; fructose increased heart, liver and kidney weights, liver water and protein. Sucrose produced less marked effects, presumably due to its fructose component. The toxicities of the three carbohydrates ranked fructose most toxic, glucose least toxic, and sucrose of intermediate toxicity.

III. Long-Term Studies

None

IV. Special Studies

Carcinogenicity

The following data are taken directly from Survey of Compounds Which Have Been Tested For Carcinogenic Activity", HEW Public Health Service Publication No. 149, 1969: 1968-1969 Volume and Supplements 1 and 2:

Reference	Animal	Strain or type	Sex	Preparation and dose	Site and route	Animals with tumors	Survival	Duration of Experiment
Nonaka, 938	Rats	White	---	35% aq. soln. 2 ml/100 g body wt. daily	s.c.	0	---	289 days
Takizawa, 1938	Mice	---	---	25% aqueous soln.	s.c.	0[2]	---	---
Takizawa, 1939	--do--	---	---	Conc. aq. soln., repeated inj.	inj.	0	---	200 days
Suntzeff, 1940	9 mice	A, D, C57 and AKA	M	Sat. soln. in water 6 times weekly	s.c.	0	---	mo.
Zarshin, 1940	20 rats	Albino	---	50% aq. soln. 2-3 cc. daily for 120 days	s.c.	0	---	6 mo.

2. A slightly fibrous growth noted

Roe et al. (1452) fed groups of 50 female Swiss mice diets containing sucrose (10%), sodium cyclamate (5%) or saccharin (5%) to test the carcinogenic and tumour-promoting potentials of these substances. The experiment lasted for 18 months, and the results were negative with regard to 4 main observations: there was no difference in the survival times on the three diets; the sucrose diet produced a slightly greater rate of gain in weight; neoplasm and hyperplastic changes in the forestomach showed no influence of the sweeteners; and tumour-incidence in general showed no significant influence on the part of the three substances.

Hueper (776) tested a number of sugars that other investigators had reported to have carcinogenic action when subcutaneously injected in rats and mice. Twice weekly injections of 25% solutions were given to rats and mice into the region of the nape of the neck. Sixty rats or mice (30 males, 30 female) were tested for each of 8 sugars; Bethesda black rats 3 months old to begin, and C57BL mice 2 months old were used, as well as 60 rats and mice serving as controls. The injections were continued for 2 years after which survivors were sacrificed; autopsies were performed and histological examinations made.

The results on sucrose are as follows: of the 60 rats treated with sucrose injections, 12 tumors were observed during an interval of from

10 to 24 months: 6 sarcomas of ileocecal lymph nodes; 1 mesothelioma of the pleura; 2 carcinomas of the uterus; 1 lymph angiosarcoma of the subcutis; 1 cholangioma; and 1 adenofibroma of the breast. No tumors were seen in the mice injected with sucrose over the 2-year period. None of the tumors seen in the rats were found at the site of injection. The controls displayed a similar variety of tumors and it was concluded that the sugar was not carcinogenic; the tumors were described as "environmental background tumors, unrelated to the sugar injections". The author suggested that the apparent carcinogenicity of sugars reported by various investigators may be due to carcinogenic impurities present in the sugars used, e.g., polycyclic hydrocarbons from improperly prepared charcoal used in the filtering, decolorizing process, or dibenzanthracene chemicals eluted from charcoal by concentrated sugar solutions.

Reproduction

The relationship of carbohydrates in the diet of rats and abnormalities in the reproductive capacity of female rats was the subject of studies reported by Whitnah and Bogart (1876). Three experimental diets, differing only in the carbohydrate portion, were fed to many animals over a 2-year period: In two diets, starch comprised 30% of the carbohydrate, and 20% either sucrose or lactose; the third diet was 50% starch. Detailed studies were made of the reproductive systems of the female rats during this period.

Seven females from the group fed lactose, five from the sucrose-fed, and two of the starch-fed were changed from the experimental diets to a regular stock diet and kept with fertile males for 40 days or until pregnancy was detected. The results are shown in the following table:

TABLE 2.—*The effect of feeding various carbohydrates on the reproductive ability of rats*

Animal no.[1]	Carbohydrate fed	Age of animal: When placed with male	Age of animal: When litter was born	Size of litter	Condition of young
		Days	*Days*	*Number*	
70a	Lactose	79	107	10	Normal.
78a	do	79	103	6	All died within 3 days.
84b	do	79	102	7	Normal.
90b	do	79	105	5	Do.
121c	do	56	80	4	1 died, 3 normal.
125c	do	56	93	8	Normal.
125d	do	48	85	8	Do.
1	Sucrose	80		0	
2	do	70		0	
37	do	70	111	3	2 died, 1 weakly.
67b	do	79		0	
119c	do	56	80		Abortion.
124c	Starch	56	91	10	Normal.
127c	do	48	87	7	Do.

[1] The animals having like letters were litter mates.
[2] After producing 1 litter, this female was kept with a fertile male for 40 days without producing another litter.

The data indicate that reproduction among the lactose- and starch-fed animals was normal except possibly one litter born to a lactose-fed female; the single female that produced a five litter among the sucrose-fed animals was afterwards sterile. Studies of the sexual activity of females on the various diets indicated that the sucrose-fed females matured earliest, but showed only a few oestrous cycles. Cytological evidence indicated ovarian abnormalities among females feo the sucrose diets. The authors speculated regarding the action of sucrose in the production of sexual abnormalities in these studies, together with other evidence and pointed to a possible action of sucrose on the pituitary function. No definite conclusions were possible, however.

Teratogenicity

A study similar to that of Roe et al. but with a different emphasis was reported by Klotzsche (916) in 1969. Cyclamate, saccharin and sucrose were added to the diet of pregnant White New Zealand rabbits in a variety of doses during the sixth to eighteenth day of gestation (the period of organogenesis). Administration of the dose was intra-gastric by throat tube: saccharose (sucrose) was given in doses of 2, 4 or 10 g/kg/day; sodium cyclamate, 50, 100 or 250 mg/kg/day; and saccharin, 5, 10 or 25 mg/kg/day. Some controls received 20 ml water/kg/day, others were untreated.

On the 29th day, the animals were sacrificed and foetuses removed. Implantations, absorptions and corpora lutea were noted; full term foetuses were killed and prepared for study. Anomalies and deformities appeared equally in test animals and controls. None of the three substances showed a teratogenic or embryotoxic effect. The author points out the purity of the cyclamate used in the experiment (the pure sodium cyclamate was free from contaminants even when tested by thin layer chromatogram) and suggests that the positive effects found by some experimenters were due to contaminants or the very high doses of cyclamate employed. Of some interest is the notation that only in England had purity requirements for cyclamates been established.

The Food and Drug Research Laboratories, Inc. (1980) reported in 1973 on two teratogenic studies, using pregnant mice and rats fed up to 1600 mg/kg of sucrose for 10 consecutive days. No clearly discernible effects on nidation or maternal or fetal survival were observed. Postmortem abnormalities distinguishing treated from control animals were not seen.

Cariogenicity

The role of heredity and diet in the development of dental caries was investigated by Stewart et al. (1652) using caries - resistant and susceptible strains of rats. Four different experimental diets were given to animals at 25 days of age: caries-susceptible, sucrose diet (Ss); susceptible, finely ground rice (Sr); caries-resistant, sucrose (Rs); and resistant (Rr), finely ground rice. Observations for

cavities in the lower molars were made at two-week intervals; the animals were sacrificed at the appearance of positive evidence of caries, and time noted in days elapsed between beginning of diet and appearance of caries. Mean caries times and the number of rats in the experimental groups were as follows: Ss, 44 rats, 92 days; Sr, 17 rats, 58 days; Rs, 50 rats, 368 days; and Rr, 8 rats, 372 days. The difference between the susceptible (S) and resistant (R) rats on each diet and the time required for caries appearance was significant. An additional experiment, substituting powdered sugar for the granulated sucrose in the diet of 11 susceptible rats, was performed. The mean caries time for this group was only 65 days compared to 92 days for the granulated sugar diets in the first test. This result conflicts with other research indicating that hard, coarse particles increase caries incidence, but too few animals were tested with the powdered sugar to be significant. Hereditary influences on caries production were, however, manifest for the several diets.

A study performed by Haldi et al. (692) to determine the relative cariogenicities of sucrose as a solid and in solution used albino rats, twenty sets of three weanling litter mates in all, from a colony of Wistar strain previously observed to be caries-susceptible. The 60 animals were distributed into three groups, each group receiving one of the trio of weanlings. During the 90 days of the experiment, group I (control) was fed a complete sugar-containing diet by intragastric cannula; groups II and III were fed the same diet by stomach tube but with sucrose omitted. Group II received in addition weighed amounts of granulated sugar in food cups; Group III similarly received a 40% sugar solution available from a delivery tube. All three groups received the same amount of sugar, whether by stomach tube or orally as solid or solution.

After 30 days of experimental feeding, all animals were desalivated surgically to accelerate the production of caries. At the conclusion of the experiment, the animals were decapitated and upper and lower jawbones were removed and prepared for microscope examination of the teeth. The average number and extent of carious lesions for the three experimental groups are shown in the following table:

TABLE 1

Dental caries incidence in albino rats when sucrose was fed by various methods

GROUP	METHOD OF FEEDING	NO. OF RATS	AVERAGE WEIGHT INCREASE Male	AVERAGE WEIGHT INCREASE Female	NO. OF CARIES-FREE RATS	AVE. NO. OF CARIOUS MOLARS	AVE. NO. OF CARIOUS LESIONS	AVE. CARIES SCORE
			gm	gm				
I	Entire ration by stomach tube	8	231	196	8	0	0	0
II	Granular sucrose ingested orally (remainder of ration by stomach tube)	13	232	182	2	3.8	4.9	7.5+
III	Sucrose in solution ingested orally (remainder of ration by stomach tube)	13	252	208	7	0.8	0.8	1.2+

Critical ratios[1]

GROUPS	AVE. NO. OF CARIOUS MOLARS	AVE. NO. OF CARIOUS LESIONS	AVE. CARIES SCORE
I vs. II	5.4	5.4	4.2
I vs. III	2.7	2.7	2.4
II vs. III	3.9	4.7	3.4

[1] *Critical ratio:* The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Whenever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; whenever the critical ratio is between 2.0 and 2.9, the difference is of borderline significance; when the critical ratio is 3.0 or higher, the difference is highly significant.

From the experimental data, the authors concluded that sugar in solution was much less cariogenic than the dry, granular form. It also appeared that the cariogenicity of sugar is dependent upon direct oral contact with the teeth; a highly cariogenic diet fed by stomach tube produces no caries. The authors disclaimed any effort to relate the experimental effects on rats to a comparable influence of sugar upon human teeth, except in a general fashion; that the cariogenicity of sugar in the rat experiments was greatly reduced by dissolving the sugar before ingestion was, however, obvious to the authors.

Newbrun (1252) reviewed recent laboratory evidence implicating sucrose as the "arch criminal" of dental caries. An hypothesis is advanced that presents sucrose as the only substrate for extracellular polysaccharide synthesis by certain oral microorganisms. Extracellular polysaccharides serve as the structural matrix of dental plaque and as

a reservoir of substrate for the plaque organisms in between meals, thus promoting dental caries. Certain organisms synthesize dextrans or glucose polymers, and others form levans or fructose polymers, by virtue of extracellular enzymes showing high specificity for sucrose. The mechanism involved was not known.

Certain strains of oral streptococci have been shown to be cariogenic when innoculated into gnotobiotic or conventional rodents and are able to form extracellular polysaccharide - more from sucrose than from comparable amounts of glucose, maltose, lactose or fructose. In animal and in human studies, a sucrose-containing diet is markedly more cariogenic than in similar diets with glucose, lactose, maltose, maize or wheat flour substituted for sucrose.

A metabolic disorder, hereditary fructose intolerance, characterized by symptoms of nausea, vomiting, malaise, sweating, tremor, coma and convulsions following the ingestion of fructose leads to an avoidance of all forms of sweets, cookies, cakes and candy, etc. which contain sucrose or fructose by the individual suffering the disease. Studies of individuals so affected show a marked freedom from caries. The author concludes: "Of all the cast of carbohydrate characters sucrose stands indicted as the arch criminal of dental caries".

Heart Disease

In 1957, Yudkin (1932), Prof. of Nutrition at the Univ. of London, presented an analysis of the relationships of diet and coronary thrombosis, "hypothesis and fact". The report is of interest for several reasons: it deals with the still controversial relationship of hydrogenated fats in the diet and the incidence of coronary thrombosis; and presages the insistence by Yudkin to the present day* that sugar in the diet shows a more significant correlation to coronary thrombosis than is found in fats. Yudkin considers the epidemiological evidence presented by those who attempt to support the dietary fat - coronary thrombosis hypothesis is inconclusive and less satisfactory than the evidence which correlates higher living standards in general with the incidence of coronary thrombosis; there is, Yudkin contends - a "better relationship with intake of sugar than with any other nutrient examined". He suggests that multiple causes, "predisposing" and "precipitating", are responsible for the disease and that more information regarding the influence of diet, exercise, smoking, etc. is required to determine the relative importance of the factors related to coronary thrombosis.

Conflicting reports of presumptive correlation between sugar intake and arteriosclerotic heart disease led Burns-Cox et al. (232) to repeat earlier studies by Yudkin and others in comparing dietary histories of hospital patients with myocardial infarction with those

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*Yudkin lists 30 papers related to this subject and similar diseases in the bibliography of his book "Sweet and Dangerous" of 1972, mentioned in the Summary of this monograph.

of other patients or healthy subjects. Medical histories and diagnosis of myocardial infarction, dietary histories and the use of a standard questionary were employed in a study of 80 infarct and 160 control patients. Age distribution, social class, marital status and sugar consumption were noted (the latter data were derived by tabular conversion factors). The relationship between smoking habits and sugar consumption was also noted.

The object of this study was to reproduce the positive findings of Yudkin correlating sugar consumption and myocardial infarction; the object was not attained. Burns-Cox et al. found a stronger association between infarction and smoking than between infarction and sugar consumption, and concluded that data from this and other studies suggested that consumption of refined sugar was not likely to be a major and specific factor in the production of myocardial infarction.

A recent (1973) paper by Earl and John Benditt from the University of Washington School of Medicine (127) is unrelated in a direct sense to a study of sucrose, but it reports on a genetic basis for atherosclerotic plaques in man. Sucrose has been proposed as an agent contributing to atherosclerosis and degenerative heart disease in man, and for that reason, the paper by the Benditts is of some relevance. The controversy over sugar versus cholesterol as dietary villains in contributing to arteriosclerosis and ischaemic heart disease generally neglects explanations or hypotheses concerning the mechanism involved. Epidemiological evidence lacks the credibility that the research efforts reported by the Benditts carries. The abstract of the report is presented as follows:

ABSTRACT The main cellular elements of atherosclerotic plaques are smooth muscle cells. Because these plaques differ from their precursors in the underlying artery wall in several ways, we have asked the question: Are human atherosclerotic plaques polyclonal or monoclonal in their origin? The X-linked glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in heterozygotic females has been used to obtain an answer. 30 plaques of different degrees of complexity and 59 samples of normal aorta and iliac artery walls from four females, 25–79 years old, were investigated. The data show that fibrous caps even of relatively large atheromatous plaques, 0.5 mm or greater in diameter, are composed of cells that produce solely or predominantly one enzyme type, whereas samples of artery wall media and intima as small as 0.1 mm² are regularly composed of a mixture of cell types. If plaques were a response to injury akin to a healing wound, a reaction to a growth stimulant, or formed due to an organization of a mural thrombus, they would be expected to be polyclonal. Hence, the results imply that atherosclerotic plaques in human beings arise by another mechanism. The mechanism compatible with the monoclonal nature of atherosclerotic plaques is mutation, and the likely causes are chemical mutagens or viruses.

Hereditary Sugar Intolerance

The inherited absence of intestinal sucrase was reported by Burgess et al. (231) in 7 cases in which jejunal mucosa biopsies showed an enzymatic deficiency in sucrase which was distinguishable from an acquired carbohydrate intolerance resulting from disease involving the small intestine. The absence of intestinal sucrase results in severe diarrhea following ingestion of sucrose and an inability to metabolize sugar as shown by the presence of the carbohydrate in the urine and feces after oral ingestion of sucrose. The authors suggest that cases of hereditary sucrose intolerance are not uncommon.

The 7 case histories, described in some detail, were all of newborn infants who immediately demonstrated diarrhea and symptoms of digestive upset which were relieved by removal of sugar (sucrose) from the diet. Testing for sucrose intolerance in all cases clearly indicated the absence of sucrose and an inability to metabolize sucrose. Maltase and lactase activities were also determined in all 7 cases, palantinase activity measured in 5 cases, and cellobiase estimated in one case. The results, compared with findings of normal children are shown in the following two tables:

TABLE 1

DISACCHARIDASE LEVELS IN JEJUNAL MUCOSA FROM PATIENTS WITH CONGENITAL SUCRASE DEFICIENCY

Case No. and Sex	Age (yr.)	Enzyme Levels (units)			
		Lactase	Maltase	Palatinase	Sucrase
1 M	51	18·2	6·2	0·21	0·45
2 F	13	19·8	46·4	1·1	0·24
3 F	2	27·9	2·0	1·2	0·14
4 M	6	15·5	13·2		0·39
5 M	31	18·1	7·6	1·7	0·14
6 F	2	8·8	1·4		0·29
7 M	1 month	20·0	27·6	2·4	0·28
Means		18·1	15·3	1·4	0·27

Units: 1 unit = 1 mmole substrate split/g. mucosa/min.

TABLE 2

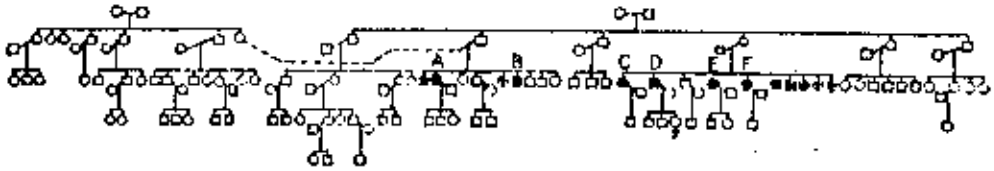
DISACCHARIDASE LEVELS IN JEJUNAL MUCOSA FROM NORMAL CHILDREN

Sex	Age (yr.)	Enzyme Levels (units)			
		Lactase	Maltase	Palatinase	Sucrase
F	6	8·5	63·3	—	20·2
M	21	6·6	88·9	—	12·4
M	2	12·4	43·2	6·9	19·0
F	1	8·5	53·0	—	18·0
M	12	7·5	64·5	6·5	17·4
Means		8·7	62·6	6·7	17·4
Ranges		6·6-12·4	43·2-88·9		12·4-20·2

Units: 1 mmole of substrate split/g. mucosa/min.

The authors present a discussion of carbohydrate tolerance, enzyme defect and genetics. The site of disaccharide hydrolysis during absorption by the intestine has been shown to reside intracellularly and not in the intestinal lumen. The hereditary nature of sucrose intolerance is suggested by the occurrence of affected sibs in a number of recorded cases, as well as in the fact that the symptoms date from the first few days of life when sucrose had been given. In one case, a history of transient diarrhea in the father of two affected sibs and his intolerance to the enzyme palatinase, suggested that the condition was inherited as an autosomal dominant (the sexes are equally affected); other investigators believe that is is an autosomal recessive. The authors support the latter hypothesis, in the light of their own experimental data.

An anomaly consisting of a congenial incapacity to eat any kind of sweet food was reported in a paper by Davidenkov (382) in the Journal of Heredity, 1940. Two series of brothers and sisters contained members unable to eat sugar in any form: one series of 7 children had 2 cases of aglycophagia; a second series of 5 children had 4 affected with the anomaly, which in all cases appeared in early childhood and persisted throughout the lifetime of the affected individuals. Efforts of the aglycophagic individuals to swallow sweet foods resulted in nausea and unpleasant physical weakness. Clinical studies of one affected male revealed no abnormalities of nervous system or viscera. The degree of aglycophagia was not total in all cases but appeared somewhat milder in several instances. Non-sweet polysaccharides were not rejected, suggesting that the abnormality was not due to disturbed carbohydrate metabolism. The aglycophagic syndrome did not appear to be a kind of allergy but highly peculiar and most like myoplegia. The pedigree of the families involved was presented by the author as follows:



FAMILIAL INHERITANCE OF AN INABILITY TO EAT SUGAR

Figure 1

Pedigree showing the two groups of cousins who were unable to eat sugar in any form. The one individual (B) who underwent extensive clinical tests became flushed, feverish, and complained of pain in his stomach when given sugar at the clinic. His conception of the perfect meal was tea without sugar, rye bread and brine pickles. The affected individuals lived mostly on rye bread and dairy products. The crossed symbols indicate death in infancy.

Allergenicity

Four cases of allergic symptoms attributable to sucrose were described by Randolph and Rollins (1409). A variety of clinical allergic responses were observed which are referred to as the fatigue syndrome of allergic origin, or allergic toxemia: myalgia, mental confusion, depression, extreme fatigue, nervousness, tenseness, and so-called "jitteriness". In each of the cases described, the patient was unaware of the role of dietary sucrose in the allergic symptoms due to typical masked type of food allergy. With the avoidance of sucrose, allergic symptoms were either absent of greatly relieved. The presence of dextran as a contaminant of sucrose has been suggested as responsible for sugar allergy, but this has not been clearly demonstrated. Immunological studies were suggested by the authors for clarification of the nature of sugar allergy: sucrose has been shown to be immunologically active and shows a positive precipitin reaction when tested against Type 2 pneumococcus antiserum. Invert sugar of cane origin when injected intravenously causes allergic responses in many cane-sensitive patients.

A report by the same authors (1410) describes cases of beet sugar sensitivity and a sensitivity to monosodium glutamate derived from beets. A point is made by Randolph and Rollins regarding the failure of federal labeling regulations to differentiate the types of sugar used in processed foods, which may be of some consequence to individuals sensitive to one or more of the different sugars employed (this was written in 1950). The allergic manifestations of beet sensitivity are similar to those noted in the preceding report: myalgia; fatigue; various mental symptoms; and asthma, rhinitis, urticaria, dermatitis, headache and gastrointestinal symptoms.

Therapy

A letter to the editor of the New England Journal of Medicine in 1971 by Engelman and Lonklons (473) presented an unusual application of sugar in the therapy of hiccups. The authors stated that one teaspoonful of ordinary white granulated sugar, swallowed "dry", stopped immediately the hiccups of 19 out of 20 patients, some of whom had suffered from persistent and intractable hiccups for up to 6 weeks. Several cases had recurrent hiccups within 10 to 24 hours after the initial treatment, but responded favorably to a second or third treatment. The mechanism for the action of the sugar was not known, but was possibly due to local irritation of the pharynx and subsequent interference in the afferent vagal impulses associated with hiccups by impulses in the vagus nerve by way of the pharyngeal plexus.

A quarter of a century ago, Podolsky (1357) reported in the journal, Sugar - a remarkable recitation of the uses of sugar as a medicine; unfortunately, though numerous investigators, medical practitioners - are cited by name, there is not a bibliography of references. In brief, sugar is a veritable "miracle drug" - a panacea! It has cured or

relieved seasickness, vomiting of pregnancy, sleeping sickness, delirium, convulsion, headache, pain, liver damage, jaundice, irritation in stomach ulcers, shock, the wasting effects of pneumonia, weakened heart action, the pain of angina pectoris, irregular heart rhythm, heart poisoning by pneumonia toxins and uremic poisoning, glaucoma, low blood pressure, varicose veins and mercury poisoning.

The final paragraph of Podolsky's report follows:

> Sugar is the fuel which fires life's engines. It is the means by which the body is run. It does not require much conversion to reduce it to a form readily available for use by the cells of the body. It is the most quickly utilized of all foods. It is the only food which can be injected directly into the veins without setting up a train of unpleasant reactions. It spares cell destruction in starved conditions, frees the tissues of undesirable excesses of water and other fluids, and bolsters up bodily energy quickly and without much effort.

Toxicometry

Boyd (183) reported in Clinical Toxicology, 1971, a method for selecting the daily dose in multiposal predictive toxicometrics. Three methods are noted: multiples of estimated daily therapeutic cose; increasing fractions of the acute LD_{50}; and decreasing fractions of the acute LD_{50}. The latter was selected as producing the most information in the least time. Oral administration by intragastric cannula for a period of 100 days was chosen as a standard technique in the studies. The acute oral LD_{50} (single dose) is first determined and the associated clinicopathological syndrome of toxicity established. Decreasing fractions, from about 90% of the LD_{50} are given daily for 100 days or until death, and the daily dose that kills 50% of the test animals is calculated, and the LD_{50} (100 days) established. From this information, a 100-day LD_{50} index is expressed as the quotient of the LD_{50} (100 days)/LD_{50} (1 dose). The index values for a number of agents including sucrose are shown in the following table: the LD_{50} (100 days) for sucrose was 28.5 g/kg/day):

Table I
Values for the 100-Day LD_{50} Index

Agent	100-Day LD_{50} Index ± S.E.	Reference
Phenacetin[a]	8.6 ± 3.1	[6]
Atropine[b]	13.2 ± 0.8	[1]
Aspirin[c]	16.4 ± 0.5	[2]
Pilocarpine[c]	17.1 ± 2.2	[11]
Dicophane (DDT)[c]	17.6 ± 0.4	[12]
Paracetamol[c]	20.6 ± 0.5	[9]
Phenacetin[c]	27.1 ± 0.5	[10]
Benzylpenicillin[c]	61.8 ± 3.0	[13]
Sodium chloride[c]	71.7 ± 3.2	[4]
Caffeine[c]	78.2 ± 1.6	[8]
Sucrose[c]	80.6 ± 3.7	[14]

[a] Drug given orally to guinea pigs.
[b] Drug given intramuscularly to rabbits.
[c] Drug given orally to albino rats.

Variable results were obtained when different time intervals or durations of doses of different drugs were employed, but in general, a 100-day interval proved useful in the determination of chronic toxicity of drugs.

Acne and Seborrhea

The scourage of the adolescent, acne vulgaris, and seborrheic dermatitis are diseases frequently associated with dietary excesses as an aetiological agent, particularly the fat or carbohydrate, or of both; changes in diet are often recommended as part of the treatment of these diseases. Bett et al. (139) presented the results of studies using questionnaires given to 16 hospital patients with diagnoses of seborrheic dermatitis and a like number with acne. The dietary questionnaire and the results with regards the sugar intake of test and control subjects are shown as follows:

DIETARY QUESTIONARY

NAME ..... AGE .....

N.B.—IF THERE IS AN ALTERNATIVE MARKED* PLEASE CROSS OUT THE ONE WHICH DOES NOT APPLY

If your weight has increased in the last few years, are you making a serious effort to check or decrease it? Yes/No*

If "Yes," are you restricting sweet or sugary foods, or sugar? .....

.....

.....

.....

If you are on a special diet *now* why are you on this diet? .....

.....

How long have you been on this diet? .....

Have you *ever* been on any other special diet? Yes/No*

If "Yes"

Why were you on this diet? .....

Approximately when did you start this diet? .....

How long did it last? .....

If your eating habits have permanently changed as a result of being on the diet, in what way? .....

.....

Go through an average day in your mind, and write down how many cups of tea and coffee you consume:

	Tea	Coffee	Other hot beverages (Cocoa, chocolate, etc.)
Before breakfast	..... cups	..... cups	..... cups
At breakfast	..... cups	..... cups	..... cups
Mid-morning break	..... cups	..... cups	..... cups
Midday meal	..... cups	..... cups	..... cups
Teatime	..... cups	..... cups	..... cups
Evening meal	..... cups	..... cups	..... cups
Bedtime	..... cups	..... cups	..... cups
Other	..... cups	..... cups	..... cups

How much sugar do you take in tea? .....teaspoons

Are the spoons level or heaped? .....

How much sugar do you take in coffee? .....teaspoons

Are the spoons level or heaped? .....

Have you always taken the same amount of sugar in these beverages? Yes/No*

If "No," how much did you take before? .....

When did you change? .....

Do you *regularly* use artificial sweeteners, e.g. saccharine, saxine, etc. Yes/No*

How long have you used them? .....

How much of the following do you eat or drink *per week*?

Sweets, toffees, and fancy chocolates .....lbs. .....oz.

No. of 2-oz. chocolate bars .....

Fizzy drinks, non-alcoholic .....glasses

(inc. tonic water, ginger-beer, etc.) .....small bottles

Fruit squash .....glasses

Fruit juice (tinned or bottled) .....glasses

How many a *week* do you have of the following:

Porridge .....portions

Plain breakfast cereals .....portions

Sugar-coated breakfast cereals .....portions

Jam or marmalade .....teaspoons

Sweet biscuits .....number

Pudding or sweet at midday (including tinned fruit) .....portions

Pudding or sweet for tea or evening meal (including tinned fruit) .....portions

Individual cakes and/or slices of cake .....number

How many spoons of sugar do you take on breakfast cereals? .....

Are they teaspoons or dessertspoons? .....

Are they level or heaped? .....

How many spoons of sugar do you take on porridge? .....

Are they teaspoons or dessertspoons? .....

Are they level or heaped? .....

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—— and Morland, J. (1967). *Amer. J. clin. Nutr.*, 20, 503.

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TABLE I.—*Sugar Intake of Subjects With Acne and of Control Subjects (Control 1: Patients With Warts; Control 2: Healthy Factory and Office Workers)*

Group	No.	Age (Mean, Years)	Sugar Intake (g./day)			
			Total		Beverage	
			Mean	Median	Mean	Median
Acne	16	19·5	121	103	65	70
Control 1	16	20·0	111	97	47	48
" 2	16	19·7	120	114	63	55

No significant differences in sugar intake between groups ($P > 0·05$).

TABLE II.—*Sugar Intake of Subjects With Seborrhoeic Dermatitis and of Control Subjects (Control 1: Patients With Warts; Control 2: Healthy Factory and Office Workers)*

Group	No.	Age (Mean, Years)	Sugar Intake (g./day)			
			Total		Beverage	
			Mean	Median	Mean	Median
Dermatitis	16	38·2	170	185	101	109
Control 1	13	36·5	108	109	65	56
" 2	16	38·1	110	110	61	57

Significant differences.—Total sugar intake: dermatitis and control 1—$P < 0·01$. Dermatitis and control 2—$P < 0·02$. Beverage sugar.—Dermatitis and control 1—$P < 0·02$. Dermatitis and control 2—$P < 0·05$.

Only in the cases of seborrheic dermatitis was there found a significantly higher consumption of sugar than in the control subjects; in the patients with acne vulgaris there was no difference. The authors could not from these data conclude that the sugar intake was a cause for the disease of seborrhea, or that diet was not an important element in acne, but the data were indicative of the value of controlled experimentation in the relationship of dietary sugar on the course of the disease seborrhea dermatitis and acne vulgaris.

Biochemical Aspects

I. Breakdown

Sucrose in solution undergoes hydrolytic decomposition in the presence of hydrogen ions. The transformation of dextrorotary sucrose to a levorotary mixture of monosaccharides, D-glucose and D-fructose, is called inversion, and the equivalent mixture of monosaccharides is known as invert sugar. In living organisms the hydrolysis of sucrose is catalyzed by glycosidases, and the invert sugar may be fermented to alcohol, lactic, butyric and acetic acids, etc. In addition to hydrolysis, other biochemical reactions are known: alcoholytic, glycerolytic, phosphorylytic and arsenolytic decomposition of sucrose. Certain bacteria, such as species of Leuconostoc, may form polysaccharides from sucrose, of which dextran is important; dextran is composed of D-glucose units and forms jelly-like masses during beet sugar production, clogging filters and retarding crystallization. The final result or breakdown of sucrose in the digestive process is the total oxidation into carbon dioxide and water, liberating the energy utilized in the plant photosynthesis of sucrose; nutritively, this energy is expressed as 3.95 k cal/g.

The inversion of sucrose is shown by the diagram on the following page:

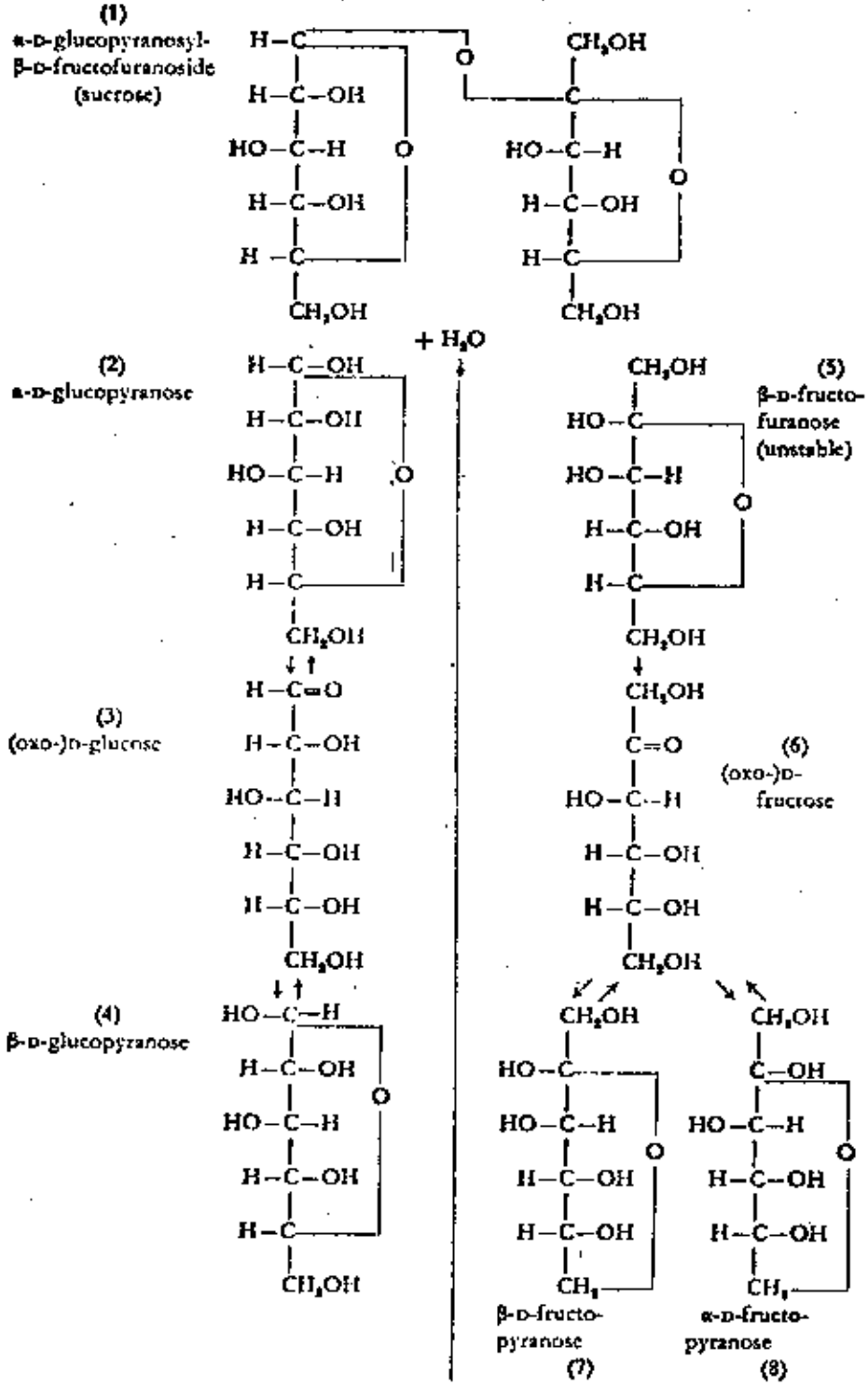
II. Absorption - Distribution

A report by Akinyanju et al. (18) of a comparative study of starch and sucrose diets in young male volunteers noted sucrose in the urine during intakes of 500 mg/day sucrose but not when starch was ingested. The implication was that, contrary to popular belief, sucrose is not entirely hydrolyzed before absorption.

The textbook version of the metabolism of disaccharides which portrays the disaccharidases as being secreted with the intestinal juice into the lumen of the small intestine is not true, according to Dahlqvist (364) in a review paper. The digestion of disaccharides occurs intracellularly in the wall of the small intestine. Dahlqvist describes a number of studies which have led to the intracellular concept of disaccharide digestion. Three principal methods have been employed: tissue fractionation methods; in vitro incubation with intestine; and histochemical staining of enzyme activity.

Tissue fractionation has yielded isolated brush border membrane preparations showing a high yield of disaccharidase activities; the preparations are, however, "contaminated" with cytoplasmic fragments and the results are not clear-cut. Incubation studies with slices of everted intestine in vitro have generally used sucrose as substrate; the glucose fragments accumulate in the tissue in large amounts, but most of the fructose liberated remains in the incubation medium.

(Inversion of sucrose)



Sucrose does not appear in the tissue, and the indications are that it is split close to the surface of the mucosal cells; the intestinal invertase appears to be separated from the intestinal lumen by some kind of membrane. The following schematic drawing shows the relationship of invertase and lumen in the mucosal cell:

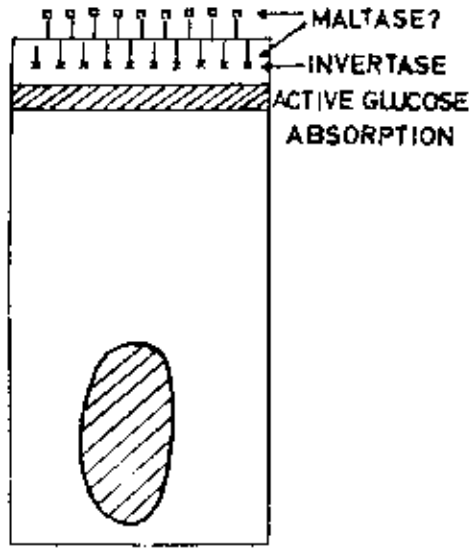


FIG. 4. Schematic drawing of the localization of the invertase and maltase activities in the epithelial cells of the small-intestinal mucosa, as indicated by in vitro incubation experiments.

Histochemical staining techniques have been elaborated that give results which fit those of the fractionation and incubation studies. The distribution of the disaccharidase activity is shown by staining to be largely concentrated in the distal parts of the villi. The epithelial cells regenerate at the bottom of the crypts and migrate up the sides of the villi, during which time the enzymes are developed, reaching maximum at the distal ends of the villi. The general picture as described by Dahlqvist has been demonstrated for many years, but ignored in textbook treatments.

The serum fructose levels in healthy men and women following sucrose intake were shown by MacDonald and Turner (1063) to be significantly higher than the serum levels after an intake of an

equivalent mixture of fructose and glucose. Ten male and eight female student volunteers were given either 2 g/kg sucrose or an equivalent mixture of glucose and fructose dissolved in water. Blood samples were obtained before and at 15, 30, 60, 90 and 120 minutes after the sugar intake; serum fructose and glucose levels were measured on each sample taken. Changes in glucose levels for sucrose or the glucose-fructose mixture were insignificant. The results are shown in the following tables:

TABLE I—MEAN (AND STANDARD ERROR) OF SERUM-GLUCOSE LEVELS AFTER SUCROSE AND AFTER FRUCTOSE AND GLUCOSE

	Mean (± S.E.M.) serum-glucose levels (mg. per 100 ml.) after (min.):											
	Sucrose						Fructose and glucose					
	0	15	30	60	90	120	0	15	30	60	90	120
Men	76 (2·9)	99 (5·0)	102 (5·3)	72 (3·9)	65 (5·0)	69 (3·9)	91 (4·5)	114 (4·6)	125 (10·5)	94 (9·1)	82 (4·0)	81 [illegible]
Women	92 (4·4)	124 (8·4)	124 (8·9)	108 (10·9)	85 (4·0)	88 (3·8)	84 (3·9)	112 (6·3)	112 (7·9)	97 (5·9)	93 (6·0)	85 [illegible]

TABLE II—MEAN (AND STANDARD ERROR) OF SERUM-FRUCTOSE LEVELS AFTER SUCROSE AND AFTER FRUCTOSE AND GLUCOSE

	Mean (± S.E.M.) serum-fructose levels (mg. per 100 ml.) after (min.):											
	Sucrose						Fructose and glucose					
	0	15	30	60	90	120	0	15	30	60	90	120
Men	0·2 (0·11)	3·1 (0·69)	5·8 (0·71)	8·2 (0·84)	6·1 (1·03)	9·8 (1·25)	0·0 (0·00)	1·2 (0·45)	3·7 (0·37)	5·0 (0·42)	5·8 (0·57)	6·3 (0·5[illegible]
Women	0·5 (0·38)	2·1 (0·61)	5·6 (1·12)	7·9 (0·90)	9·3 (0·80)	8·4 (0·62)	0·5 (0·38)	2·5 (0·63)	4·1 (0·69)	5·1 (0·52)	5·4 (0·37)	4·8 (0·62)

Some differences were observed between the serum fructose levels of men and women when given the fructose-glucose mixture; little difference was noted in the sucrose intake. The authors attributed the difference to the influence of the sex hormones rather than to differences in the absorption of dietary fructose which is significantly lower in women than in men. The following figures demonstrate the difference:

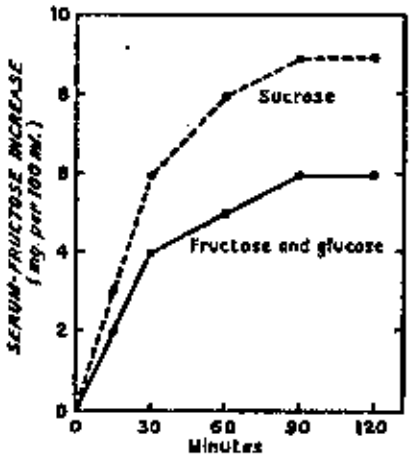


Fig. 1—Mean increase in serum-fructose level in men after sucrose and after fructose and glucose.

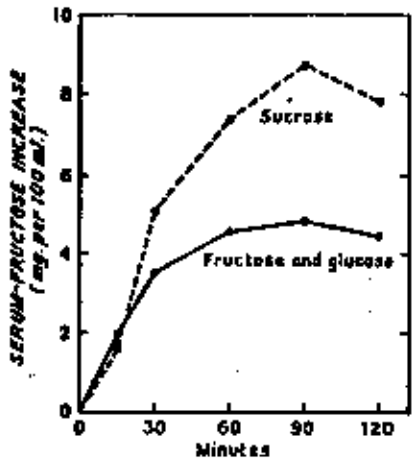


Fig. 2—Mean increase in serum-fructose level in young women after sucrose and after fructose and glucose.

One of the earliest recognized, possibly new - relationship of mental retardation, hiatus hernia and sucrosuria as a syndrome was reported by Moncrieff and Wilkinson (1178). Three cases of early childhood sucrosuria were described; various sugars were tested and urinalyses indicated that the digestive abnormalities resulted in a rapid absorption of sucrose and its elimination before the digestive process could be completely effective. The authors speculated at length regarding a possible genetic relationship of the three defects comprising the syndrome: metabolic error, esophageal anomaly and mental retardation. In the one case of the infant who died, autopsy revealed a congenital brain defect, ruling out an hypothesis that sucrosuria and mental retardation were associated in a causal relationship. The data from these three case-histories were insufficient to demonstrate a clear association of the elements of the "syndrome".

The presence of sucrose in the urine, or sucrosuria, has in the past been associated by investigators with hiatus hernia and mental defects; it was suggested that this triad of sucrosuria, hiatus hernia and mental defect comprises a clinical syndrome. Urine chromatography has become an important investigation of mentally retarded children due to the unusual constituents in the urine of the mentally afflicted, and sucrose can be chromatographically detected, whereas Benedict's test for urinary sugar does not reveal the nonreducing sucrose. The unusual syndrome or "triad" was the subject of a study by Freier (564) in 1961, who administered daily doses of 20 g sucrose to four different groups of patients and volunteers. Urine was collected over a 6 hour interval and the sugars identified by paper chromatography and concentrations determined. The four groups studied were: 3 patients with hiatus hernia and mental defect; 11 mentally normal patients with hiatus hernia; 41 relatives of the first 2 groups; and 38 normal controls. The results are summarized in the following table:

TABLE 2
ANALYSIS OF RESULTS

	Total	Sucrose Present	Per Cent With Sucrosuria
Group 1			
Patients with hiatus hernia and mental deficiency	3	3	100
Group 2			
Hiatus hernia, but mentally normal	11	9	82
Group 3			
Relatives of patients with hiatus hernia	41	18	44
Group 4			
Normal controls:			
Adults	20 } 38	6 }	34
Children	18	7	

Sucrosuria was shown to be not rare among normal people who have ingested no more sugar than may be found in a cup of tea. The incidence, however, of sucrosuria among children having lower oesophageal abnormalities was more than double that of normal individuals. An association between hiatus hernia and mental defect has been shown probable but the finding of clinical evidences of sucrosuria, hiatus hernia and mental defect together has not yet been sufficiently demonstrated to constitute a disease entity or specific syndrome.

The anomalies of sugar metabolism in general and disaccharide intolerance particularly are discussed in a paper by Metais (1148). The genetic problems associated with congenital sugar intolerances are described.

III. Metabolism and Excretion

As noted in the preceding section (Absorption-Distribution), sucrose is split into glucose and fructose at the surface of the intestinal mucosa and does not normally appear in the tissue as sucrose. The metabolism of sucrose, is - then, that of glucose and fructose. See Monograph on Corn Sugar for details.

IV. Effects on Enzymes and Other Biochemical Parameters

Brewer and Heath (198) injected intraperitoneally 6-8 ml 50% sucrose into albino rats weighing 250-300 g in studies of the formation of liver vacuoles. Three rats, two injected and one control, were treated, in each such group, and sacrificed at various time intervals. Liver specimens were prepared for light and electron microscopy. Mitochondria, endoplasmic reticulum, nucleus, microbodies, bile canaliculi and sinusoidal cell border appeared normal. Cytoplasmic vacuoles contained finely granular, structureless material, which after staining closely resembled that of lyosomes. Acid phosphatase concentrations were seen to be restricted to the lyosomes; after sucrose injection the phosphatase activity is seen in the vacuoles, suggesting that the origin of the vacuoles is the lyosome, which they resemble morphologically and cytochemically.

During the 1930's, sucrose solutions were widely used intravenously in the treatment of various conditions leading to tissue dehydration such as traumatic cerebral edoma, acute alcoholism, chronic hypertension, asthma, postoperative anemia and edema of nephrosis and nutritional inanition. Rigdon and Cardwell (1445) reported studies of 16 clinical hospital cases involving intravenous administration of hypertonic sucrose solutions during therapy. In some cases, autopsy showed renal lesions possibly related to the sucrose injection; epithelial cells in the convoluted portion of the renal tubules were swollen. It appeared that dehydration of the patient at the time of

administering sucrose was an important factor in the appearance of renal lesions, as was the quantity of sucrose given. Experiments with rabbits given various doses of sucrose after having fluids removed from the diet for from 1-4 days before injection showed considerable kidney damage similar to that observed in some of the human cases described. Smaller quantities of sucrose produce tubular lesions when fluids are restricted before and during administration intravenously; in the rabbit experiments, only large quantities of sucrose produced lesions when fluids are present in the diet.

Sucrose nephrosis was described by Anderson (44) as being readily recognizable from other types of nephroses by its hydropic or vacuolar degeneration. It was recommended that sucrose be added to the list of chemicals which produce tubular degenerative changes of nephrosis, and that caution be used in sucrose therapy where pre-existing renal lesions may enhance the effects of sucrose so that changes are produced from relatively small amounts. The lesions were described as similar to those seen in some cases of diarrhea or chronic dysentery.

The relationship of dietary carbohydrate and lipid metabolism was the subject of a study by MacDonald (1054) who was interested in the differences observed in the malnutritional diseases of kwashiorkor and marasmus as related to the influence of dietary carbohydrates. Kwashiorkor is characterized by not only the characteristics of protein deficiency, but of an excess of calories usually supplied by carbohydrates, leading to fatty liver and quantities of depot fat; marasmus shows deficient protein effects, without liver lipid or depot fat and is marked by deficient caloric intake as well. MacDonald experimented with rabbits; the diets were devised to maintain a constant protein intake but the carbohydrate, or caloric-intake was varied. The animals with excessive carbohydrate in the diet lost weight rapidly and the liver became fatty; total serum protein concentration also fell more rapidly. Observations of children with kwashiorkor showed similar characteristics, suggesting that excessive dietary carbohydrate together with low protein intake produces a derangement of the lipid metabolism.

Various carbohydrates in the diet produce different amounts and types of liver and depot fat; experiments with rabbits showed a direct relationship between liver lipid deposits and the mean daily carbohydrate intake. Weight for weight of carbohydrate, sucrose produced more liver lipid than starch. Seven adult males (human) were given diets of 500 g of either maize starch or sucrose added to a well balanced diet of protein, vegetables and fruit for 25 days after which the carbohydrate was changed. Weekly samples of serum lipid and depot fat were analyzed. The starch diet produced a fall in serum lipid levels; sucrose increased the lipid levels. Fatty acid pattern changes and serum lipid concentrations are shown in the following tables:

Table 1. *Changes in the concentration of serum lipid and its fractions found in adult males on a diet low in fat and high in starch or sucrose*

	Starch diet	Sucrose diet
Total serum lipid	Decreased	Increased
Sterol esters in serum	Decreased	Unchanged
Glycerides in serum	Unchanged	Increased
Phospholipid in serum	Decreased	Unchanged
Total cholesterol in serum	Decreased	Unchanged

Table 2. *Changes in the fatty acid pattern in the serum and depot lipids in adult males on a diet low in fat and high in starch or sucrose*

	Serum lipid		Depot fat	
Fatty acid	Starch diet	Sucrose diet	Starch diet	Sucrose diet
Myristic	—	—	Unchanged	Unchanged
Palmitic	Increased	Increased	Unchanged	Unchanged
Palmitoleic	Unchanged	Unchanged	Unchanged	Decreased
Stearic	Unchanged	Unchanged	Unchanged	Unchanged
Oleic	Increased	Unchanged	Increased	Increased
Linoleic	Decreased	Decreased	Decreased	Unchanged

MacDonald suggested that the differences found in the fatty acid proportions in the human experiments might offer some justification for the traditional dermatological association of acne and high carbohydrate intake. Sebum changes on the skins of the men in the foregoing experiments showed fatty acid composition changes related to the carbohydrate; the skin lesions seen in kwashiorkor may also be related. The author concluded that while dietary carbohydrates do influence lipid metabolism, the mechanism and metabolic pathways involved are largely unknown.

Bender and Thadani (124) investigated the metabolic differences between sucrose and starch in the rat and found that dietary sucrose depresses the rate of oxidation of glucose and of lipogenesis from glucose in both liver and adipose tissues. It was suggested that the effects observed may have been due to increased levels of various enzymes and consequent changes in the metabolic pathways.

Three different rat-strains were used in five experiments and sub-experiments. The diet was composed of 60 parts by weight of sucrose or cornstarch, 24 parts casein, 8 parts arachis oil, 5 parts salt mixture and 3 parts solka floc; in one experiment the oil was omitted and the carbohydrate increased proportionally. At the end of each experiment, the animals were sacrificed, liver and epididymal fat pads (adipose tissue) were removed and treated in vitro by a technique infusing radioactive C^{14}-glucose. After incubation, CO_2 was collected and measured for radioactivity by a liquid-scintillation spectrometer. Fats were chromatographically separated and the radio-

activity determined. Liver samples were analyzed for glycogen. Carcass water and fat were calculated.

The number, variety and details of experiments performed by Bender and Thadani preclude further treatment. An abstract by the authors, however, follows:

Abstract. (1) Male, weanling rats were fed on diets containing 60% Sucrose or 60% starch and the liver and adipose tissue were examined *in vitro* for their ability to oxidise glucose and to synthesise fat from glucose, using radioactive glucose as an indicator. (2) Compared with the livers of rats fed on starch those fed on sucrose showed a severe depression of both glucose oxidation and lipogenesis. When two forms of radioactive glucose were used, labelled in carbon-1 or universally labelled, the change in proportions of carbon dioxide liberated indicated that sucrose feeding had altered the metabolic pathways. (3) Adipose tissue also showed depressed lipogenesis after sucrose feeding and less carbon dioxide was formed from (1-^{14}C) glucose but that from (U-^{14}C) glucose was unchanged. (4) The effect of sucrose was apparent after 14 days feeding and persisted in experiments continued for 112 days. (5) The proportions of fat and glycogen in the liver and the fat content of the body were not altered by sucrose feeding. (6) Tissues from female rats behaved in the same way as those from male rats. (7) The effect of sucrose was compared in three strains of animals. The response of hepatic lipogenesis differed with the strain but lipogenesis in adipose tissue was depressed to a similar extent in all three strains. Glucose oxidation in both tissues varied in extent with strain; the strain that was not affected with respect to glucose oxidation in the liver was the most sensitive with respect to glucose oxidation in adipose tissue. (8) The sucrose effect was equally marked in fed and 24 hr-fasted rats. (9) Sucrose included in a fat-free diet had a depressant effect on glucose oxidation and lipogenesis in liver but not on glucose oxidation in adipose tissue. (10) The lipids formed from radioactive glucose by adipose tissue were separated by thin layer chromatography and examined for radioactivity. The results indicated that the depression of lipogenesis largely or wholly affected the triglycerides. (11) Rats were injected intraperitoneally with radioactive glucose after feeding on the respective diets. The extracted fat from kidney and adipose tissue, but not that from liver and blood, showed a lower radioactive count after sucrose feeding. (12). It is suggested that sucrose might exert its depressant effect on metabolism by (a) reducing the amount of glucose-6-phosphate available through stimulating the formation of glucose-6-phosphatase and (b) altering the proportion of glucose metabolised via the pentose shunt by stimulating the formation of glucose-6-phosphate dehydrogenase.

Akinyanju et al. (18) reported briefly in Nature a study of the comparative effects of diets containing starch or sucrose on the blood lipids of young male volunteers. Ten male students divided into 2 groups, 19 to 25 years of age were placed on a diet for one week (control) which provided 3310 calories of which 48% was from carbohydrate, 37% from fat, 11% from protein and 4% from alcohol (wine or beer). Blood pressure, electrocardiograms and blood lipids were measured and found to be within normal limits. During the second week the diet was changed to provide 3600 calories, of which 50% was from carbohydrate, either cooked wheat starch or sucrose, 30% from fat, 11% from protein and 9% from alcohol. Cholesterol was added as a supplement. After one week on this diet, the two groups were changed to the alternate carbohydrate diet for the third week of the experiment. Blood samples were taken each day before breakfast and analyzed for total serum cholesterol,

phospholipids, total esterified fatty acids and triglycerides. Platelet stickiness was also measured. Urinalyses for sugar were performed.

Serum cholesterol concentrations rose significantly in both diet groups without distinction between starch and sucrose; phospholipid concentrations behaved similarly. Serum triglyceride concentrations changed, rising during sucrose intake and falling during starch intake in both groups. All subjects showed an increase in platelet stickiness without distinction between starch and sucrose. Sucrose appeared in the urine of subjects during the sucrose intake up to 500 mg/day; no sucrose was observed in the urine during the starch diet, which implied that contrary to general belief, sucrose is not completely hydrolysed before absorption. The differences observed between diets of starch and of sucrose were not discussed as regards the significance relating the data to the aetiology of ischaemic heart disease.

A high sucrose diet given to nineteen healthy men during a study by Szanto and Yudkin (1686) produced no change in levels of blood cholesterol or phospholipids, but did increase significantly the triglycerides. In six of the test subjects, there was an increase in serum immunoreactive insulin during a glucose tolerance test, accompanied by increased platelet adhesiveness. The test volunteers were divided into two groups: group A, 10 men; group B, 9 men. Both groups for one week followed a normal diet; during the next two weeks, group A took diets high in sucrose, group B a diet of starch. Two weeks back on the normal diet were followed by two weeks of the alternate carbohydrate with group B receiving the high sucrose diet. Two more weeks on normal diets completed the experimental study. At the end of the first week and at two-week intervals thereafter, the subjects were weighed, their glucose tolerance determined and insulin response measured, plasma lipids assayed, platelet adhesiveness, plasma fibrinogen and clot lysis time determined. A summary of the test results is shown in the following table:

TABLE 3. Effect of dietary sucrose in nineteen male subjects (mean)

	Preliminary	Sucrose	Rest	Starch	Rest
kCal/day	3390	3550	—	3370	—
Weight (kg)	73·0	74·0*	73·4	73·6	73·5
Sucrose (g/day)	164	438	—	10	—
Lipids (mg/100 ml)					
Cholesterol	249	248	250	243	248
Phospholipid	230	231	231	220	230
Triglyceride	143	150*	142	149	142
Blood glucose (mg/100 ml)					
0 min	69	69	68	69	70
30 min	99	99	98	99	100
120 min	68	68	69	67	68
Serum insulin (μU/ml)					
0 min	22	23†	21	22	21
30 min	63	86†	67	64	61
120 min	34	49†	36	32	32
Platelet adhesiveness (%)	67	65	69	67	67
Fibrinogen (mg/100 ml)	255	256	259	257	255
Clot lysis time (hr)	5½	5	5	5½	5½

* or † Significant differences between these values and those for preceding and subsequent period.
*$P < 0.05$. †$P < 0.01$.

The only significant data appear in the changes produced in weight, triglycerides and serum insulin. The authors suggest that the effect of sucrose in a possible role in the development of ischaemic heart disease may lie in the hyperinsulinism produced as shown in 6 of the 19 subjects of this study. The factors known to be associated with ischaemic heart disease are peripheral vascular disease, maturity-onset diabetes, obesity, smoking and hypertension; all are associated with hyperinsulinism. Sucrose is now added to the list. An habitual high consumption of sucrose, in a proportion of the population, may result in susceptibility to the development of occlusive atherosclerotic disease, according to Szanto and Yudkin. They suggest that "sucrose-induced hyperinsulinism" may provide a predictive test for the identification of susceptible persons who will develop ischaemic heart disease if they continue to consume large quantities of sucrose.

Haldi and Wynn (688), following a report that ingestion of dextrose induced hypoglycemic reactions in human subjects, conducted an experiment to determine whether sucrose would induce a similar reaction. Forty-four medical students served as the test subjects; after fasting they were given a sucrose solution containing 1.5 g/kg body weight in 400 ml water. Arterial and venous blood samples were taken before administering the sucrose and at hourly intervals for the next four hours for analysis of sugar concentrations. Physical and behavioral reaction of the subjects were also observed. A similar experiment was performed with glucose; the results are shown below:

TABLE 1

*Blood sugar concentrations after ingestion of glucose and of sucrose (1.5 grams/kilo in 400 cc. water)**

SUGAR INGESTED	BASAL	MINUTES AFTER INGESTION				
		2	5	10	15	30
	Blood sugar—mgm.%					
Glucose	95	90	108	125	131	155
Sucrose	95	100	107	122	134	148

* Each value is an average of 26 experiments.

It was concluded from the data which showed a rise in blood sugar followed by a gradual decrease to normal levels only that hypoglycemia is not the usual response to the ingestion of large amounts of sucrose; physical and behavioral patterns of the test subjects were outwardly unchanged by the sugar intake. The similarity of the glucose and sucrose effects on the blood sugar suggests that sucrose is rapidly inverted in the gastrointestinal tract.

Yudkin and Szanto (1937) reported, in abstract form, a relationship between hyperinsulinism, sucrose-induced- and platelet adhesiveness in studies of 27 male patients with peripheral vascular disease versus 27 apparently healthy men. Platelet adhesiveness and insulin level were significantly correlated with sugar intake in the patients tested; no such correlation was observed in the healthy men tested. The experimental data are summarized in the following table:

Table 1. *Correlation between sugar intake, insulin level and platelet adhesiveness in twenty-seven men with PVD and twenty-seven 'healthy' men, before and during glucose tolerance test*

(Tau values, and *P* values for two-tailed test)

Time of insulin assay	Sugar intake and insulin		Sugar intake and platelet adhesiveness	
	Tau	*P*	Tau	*P*
	(A) Men with PVD			
Fasting	0·30	<0·05	0·28	<0·05
30 min	0·52	<0·002		
120 min	0·34	<0·02		
	(B) 'Healthy' men			
Fasting	−0·03	NS	0·18	NS
30 min	−0·19	NS		
120 min	−0·06	NS		

NS, not significant.

A second report by the same authors (Szanto and Yudkin (1686)) describes briefly an experiment in which young men were given a high-sucrose diet for 10 days during which before and after immuno-reactive insulin levels were determined. Six of the test subjects were selected, three showing "sucrose-induced hyperinsulinism" and three not showing the symptoms; all were then given a high-sucrose diet for 2 weeks. The electrophoretic platelet mobility was measured before the sucrose diet, after completion of the diet, and at 2 weeks after completion. The three subjects who had shown hyperinsulinism gave electrophoretic test results characteristics of atherosclerosis; the three subjects who had not shown the symptoms of hyperinsulinism gave electrophoretic results unchanged during the experiment. The atherosclerotic-like group pattern returned to normal two weeks after the experimental period.

Cohen and Teitelbaum (313) observed that the incidence of diabetes and atherosclerosis among recent Yeminite immigrants to Israel was much lower than among those who had resided there for more than 25 years, apparently due to the carbohydrate dietary differences rather than to the fat content which differed little in the two groups. The Yemenite carbohydrate intake was mostly starch, while the Israeli carbohydrate diet was about 40% sucrose.

A study was performed to demonstrate the effect of the kind of carbohydrate, sucrose or starch, fed to rats, on the "insulin reserve" of the animal, with protein and fat intake kept constant. Albino rats, weighing 100-300 g, were placed on a variety of diets: diet A was an animal-house stock diet; diets B, C, D, E were synthetic diets composed of 67% carbohydrate, 10% butter, 18% vitamin-free casein, and 5% salts and vitamins. In diet B, the carbohydrate was 67% starch; in diet C, 67% sucrose; in diet D, 27% starch and 40% sucrose; and in diet E, 34% starch and 33% sucrose.

Oral glucose tolerance tests were performed at intervals and the insulin-like activity was determined. No difference between diets A and diet B (starch) were observed regarding glucose tolerance. An impairment of glucose tolerance was observed in diet C (sucrose) after 21 days and becoming more pronounced at 40 days; the reduced sucrose percentages in diets D and E also resulted in glucose tolerance impairment, but required 40 to between 50 and 100 days to develop. The impairment was reversible when the animals were put back on starch diets, but only a few days on sucrose were required to renew impairment of glucose tolerance. The sucrose diet produced not only an impairment of glucose tolerance, but an increased fat content in the liver; the insulin-like activity of the serum was also diminished, which indicates a reduced insulin reserve. The intragastric load of sucrose compared to starch results in a rapid rise in blood glucose, stimulating the insulin system; repeated stimulation may lead to impairment of the insulin system in sucrose-fed animals.

A refutation of the criticism, directed at the advocates of sucrose as a dietary factor in the aetiology of ischaemic heart disease, wherein the major carbohydrate components of current human diets are assumed to be interchangeable and with identical metabolic effects is offered by Al-Nagdy et al. (30) including Yudkin. Experiments with rats fed diets containing about 70% carbohydrate, either as corn starch or as sucrose, showed that weanling rats grew more slowly on sucrose, and adult rats lost more weight, than those animals receiving starch. The amount of food intake was not a factor in the growth differences, but rather the sucrose was less efficiently utilized. After six months on the diets with either starch or sucrose, oxygen uptake and CO_2 output measurements showed an expected respiratory quotient of about 0.87 for rats on starch, but only about 0.75 for those on sucrose diets. Serum cholesterol levels were higher in rats on sucrose; liver fats were less when the animal was given the sugar diet after weaning, but were higher when the diets were not begun until age 3 months. The

authors concluded that there was no justification for the view that starch and sugar are metabolically identical. They further noted the contrast in altering carbohydrate in the diet with the consequent differences, and the reports of investigators who similarly altered the fat in the diet of rats with no differences in the growth, body fat, kidney and liver function and other measurements.

V. Drug Interaction

None

VI. Consumer Information

The widespread use of sucrose as a sweetener and preservative is subject to some controversy; the sugar has been charged with contributing to the high incidence of heart disease and dental caries, as well as encouraging poor dietary practices. Positive confirmation of these charges has not been established. Sucrose is cleared by MID to flavor sausage, ham, and miscellaneous meat products, in amounts sufficient for the purpose, and is GRAS for use in dietary supplements affirmed by FDA letter to trade association.

The following tables were compiled from data collected during a GRAS survey, NAS/NRC in 1972. Data from the Market Research Corporation of America (MRCA), on the frequency of eating the substance, produced the food consumption values, and the mean portion sizes in each food category were taken from USDA data.

Table 2 gives usage levels found in regular foods. Table 3 presents usage levels in infant formulas and baby food. Table 11 shows annual poundage data for the years 1960 and 1970. Table 13 reports the possible daily intakes of various food categories and the age groupings represented.

TABLE 2 — USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP I) USED IN REGULAR FOODS(R)

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
SUCROSE NAS 0328	01	BAKED GOODS(R)	51	11.41866	31.48817
	02	BREAK CEREALS(R)	6	26.70919	42.07918
	03	OTHER GRAINS(R)	13	1.42626	2.05451
	04	FATS OILS(R)	14	3.43124	13.50222
	05	MILK PRODS(R)	23	17.93889	21.80855
	06	CHEESE(R)	5	24.56225	25.20767
	07	FROZN DAIRY(R)	19	9.34587	14.87673
	08	PROCSD FRUT(R)	40	12.58393	17.16377
	09	FRUIT ICES(R)	8	12.37630	15.49706
	10	MEAT PRODS(R)	25	2.86743	3.44626
SUCROSE NAS 0328 (CONT.)	11	POULTRY(R)	10	1.50161	3.40499
	12	EGG PRODS(R)	*	1.90000	1.90000
	13	FISH PRODS(R)	*	3.41690	15.09497
	14	PROCSD VEGS(R)	26	13.25549	18.25246
	15	CONDM RELSH(R)	17	26.22036	38.02379
	16	SOFT CANDY(R)	23	44.74074	86.11353
	17	CONF FROST(R)	15	39.85787	49.69291
	18	JAM JELLY(R)	12	32.72478	40.26694
	19	SWEET SAUCE(R)	18	30.96578	36.83039
	20	GELATIN PUD(R)	17	19.10623	27.64013
	21	SOUPS(R)	10	.19596	.94269
	22	SNACK FOODS(R)	4	5.21489	6.39636
	23	BEV TYPE I(R)	22	13.03547	14.22625
	24	BEV TYPE II(R)	*	10.27431	18.27730
	25	NUT PRODS(R)	4	8.13720	9.79329
	27	GRAVIES(R)	19	5.66008	8.82468
	28	IMIT DAIRY(R)	8	16.23692	18.64297
	30	HARD CANDY(R)	8	48.97770	66.76674
	31	CHEWING GUM(R)	11	42.29549	63.07884
	32	GRAN SUGAR(R)	*	97.92500	98.47500
	34	INS COF TEA(R)	*	12.60000	13.30000
	46	SEAS. FLAVRS(R)	4	10.81475	15.42458

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TABLE 3 -- USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(B)

NAS SURVEY NO.	SUBSTANCE NAME	FOOD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0328	SUCROSE	82	CEREALS(B)	*	2.54630	3.70101
		83	FORMULAS(B)	5	4.75952	6.17033
		84	PROCSD FRUT(B)	*	12.24772	21.91103
		85	MEAT PRODS(B)	*	.44000	.44000
		86	POULTRY(B)	*	.56000	.56000
		89	PROCSD VEGS(B)	*	2.89298	5.66843
		90	PUDDINGS(B)	*	12.00001	21.74505
		91	SOUPS,MIXES(B)	*	.36000	.42000
		92	MEAT DINNER(B)	*	4.55361	4.62202
		93	COME DINNER(B)	*	1.36400	5.61910

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TABLE 11, PART A -- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1960/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1960	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FEMA	POUNDAGE REPORTED TO FEMA-- 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FEMA
SUCROSE NAS 0328	157/184	2,972,784,154	5,962,444,877	6,431,228,646			6,431,228,646

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & II), PER FOOD CATEGORY AND TOTAL DIETARY, BASED ON FOOD CONSUMPTION BY TOTAL SAMPLE - - - SEE EXPLANATORY NOTES IN EXHIBITS SECTION

SUBSTANCE NAME (SURVEY NO.)	FOOD CATEGORY NO. NAME	# OF FIRMS	(AGE)	POSSIBLE DAILY INTAKE, MG. AVERAGE	HIGH A	HIGH B
SUCROSE NAS 0328	01 BAKED GOODS(R)	51	0-5 MO.	388.214040	513.812700	1077.397780
			6-11 MO.	2900.187240	5914.555080	8048.795180
			12-23 MO.	6222.842700	10253.417880	17270.052650
			2-65+ YR.	15665.578320	23270.006260	42476.166240
SUCROSE NAS 0328	02 BREAK.CER(S)(R)	8	0-5 MO.	160.255140	454.456250	252.475060
			6-11 MO.	5856.149370	16972.095620	5361.697140
			12-23 MO.	6971.096590	13594.977310	10982.665980
			2-65+ YR.	5341.838000	13835.360420	6415.830000
SUCROSE NAS 0328	03 OTHER GRAIN(R)	13	0-5 MO.	7.131300	24.246420	10.224550
			6-11 MO.	138.347220	407.910360	198.356270
			12-23 MO.	233.906640	540.552540	335.365240
			2-65+ YR.	396.500250	835.723640	568.484680
SUCROSE NAS 0328	04 FATS OILS(R)	14	0-5 MO.	17.156200	17.156200	67.511100
			6-11 MO.	66.074720	257.343000	378.662160
			12-23 MO.	216.168120	431.748000	890.639860
			2-65+ YR.	600.467000	1084.271840	2362.888500
SUCROSE NAS 0328	05 MILK PRODS(R)	23	0-5 MO.	988.697500	717.554000	1166.861700
			6-11 MO.	11193.842400	53834.408850	13489.737200
			12-23 MO.	9770.673750	31285.354400	11776.659750
			2-65+ YR.	7005.845750	21634.253100	8535.377220
SUCROSE NAS 0328	06 CHEESE(R)	5	0-5 MO.	**********	24.582250	**********
			6-11 MO.	663.180250	2382.538250	890.607000
			12-23 MO.	1915.855500	5452.815500	1966.196250
			2-65+ YR.	2308.851500	5756.651000	2369.520900
SUCROSE NAS 0328	07 FROZN DAIRY(R)	19	0-5 MO.	93.458700	383.100670	146.767300
			6-11 MO.	887.857650	2467.309680	1412.789350
			12-23 MO.	1345.805280	3158.904060	2142.749120
			2-65+ YR.	2302.542720	5768.401790	3008.442250
SUCROSE NAS 0328	08 PROCSD FRUT(R)	40	0-5 MO.	591.444710	1585.575180	806.697190
			6-11 MO.	6518.435740	18233.265700	8890.832060
			12-23 MO.	12656.433580	25130.108210	17266.752620
			2-65+ YR.	14886.765190	31535.328580	20304.729910
SUCROSE NAS 0328	09 FRUIT ICES(R)	8	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	37.125900	99.010400	46.491160
			12-23 MO.	74.257800	259.902300	92.502360
			2-65+ YR.	66.634100	304.407500	108.479420

SUCROSE NAS 0328	10	MEAT PRODS(R)	25	0-5 MO.	31.541730	83.155470	40.108850
				6-11 MO.	593.550010	1600.025940	754.775820
				12-23 MO.	865.983650	1485.196170	1101.170520
				2-65+ YR.	2248.045120	3730.526430	2859.387940
SUCROSE NAS 0328	11	POULTRY(R)	10	0-5 MO.	7.508050	34.535030	17.024950
				6-11 MO.	52.562790	140.212520	132.754610
				12-23 MO.	99.106260	236.250340	224.729340
				2-65+ YR.	193.707650	457.528080	435.253710
SUCROSE NAS 0328	12	EGG PRODS(R)	*	0-5 MO.	**********	19.000000	**********
				6-11 MO.	9.600000	34.200000	9.500000
				12-23 MO.	17.100000	74.100000	17.100000
				2-65+ YR.	36.100000	131.100000	36.100000
SUCROSE NAS 0328	13	FISH PRODS(R)	*	0-5 MO.	3.415500	10.296750	15.094970
				6-11 MO.	44.445700	167.526100	146.229410
				12-23 MO.	184.620600	461.251500	815.106780
				2-65+ YR.	423.943600	1056.440100	1871.726680
SUCROSE NAS 0328	14	PRCCSD VEGS(R)	26	0-5 MO.	185.562860	556.688580	255.534440
				6-11 MO.	3181.077600	7422.514400	4320.594400
				12-23 MO.	5169.251100	8654.191970	7148.459400
				2-65+ YR.	11266.316500	18580.626680	15514.551000
SUCROSE NAS 0328	15	COOKH RELSH(R)	17	0-5 MO.	**********	26.020320	**********
				6-11 MO.	214.563040	550.048380	304.190320
				12-23 MO.	750.970640	2038.348980	1064.666120
				2-65+ YR.	2360.193440	5685.920560	3346.053520
SUCROSE NAS 0328	16	SOFT CANDY(R)	23	0-5 MO.	89.421480	694.814800	172.227060
				6-11 MO.	984.296280	3042.370320	1894.497660
				12-23 MO.	1565.925900	4160.088320	3013.473550
				2-65+ YR.	2594.962920	7874.370240	4994.554740
SUCROSE NAS 0328	17	CONF FROST(R)	19	0-5 MO.	**********	39.857820	**********
				6-11 MO.	39.657220	79.715640	49.692510
				12-23 MO.	79.715640	279.004740	99.385820
				2-65+ YR.	119.573460	318.862560	149.078730
SUCROSE NAS 0328	18	JAM JELLY(R)	12	0-5 MO.	**********	92.174340	**********
				6-11 MO.	2192.560250	7297.625940	2697.884980
				12-23 MO.	981.743400	3665.175360	1205.008200
				2-65+ YR.	1865.312460	5792.286060	2295.215580

SUCROSE NAS 0328	19	SWEET SAUCE(R)	18	0-5 MO.	92.857340	123.863120	110.491170
				6-11 MO.	278.692020	959.939180	331.473510
				12-23 MO.	505.110280	2353.399280	957.590140
				2-65+ YR.	2105.673040	5542.274620	2504.400[illegible]
SUCROSE NAS 0328	20	GELATIN PUD(R)	17	0-5 MO.	382.125600	515.859560	552.[illegible]
				6-11 MO.	2445.603640	7413.236640	3537.[illegible]
				12-23 MO.	2636.866640	6419.710080	3814.337940
				2-65+ YR.	3897.681120	10030.797000	5638.536520
SUCROSE NAS 0328	21	SOUPS(R)	10	0-5 MO.	.399920	2.[illegible]9400	1.[illegible]
				6-11 MO.	46.[illegible]	145.370920	219.646770
				12-23 MO.	69.566080	192.161[illegible]	328.055120
				2-65+ YR.	63.387320	160.966200	298.832730
SUCROSE NAS 0328	22	SNACK FOODS(R)	4	0-5 MO.	**********	5.214890	**********
				6-11 MO.	20.859960	57.363790	25.585440
				12-23 MO.	57.363790	161.601590	70.355960
				2-65+ YR.	67.793570	192.950930	83.152680
SUCROSE NAS 0328	23	BEV TYPE I(R)	22	0-5 MO.	312.851280	469.276920	342.870000
				6-11 MO.	2959.051690	10128.[illegible]190	3242.[illegible]790
				12-23 MO.	7065.224740	21152.638750	7743.147500
				2-65+ YR.	13556.[illegible]000	36159.500190	14857.700000
SUCROSE NAS 0328	24	BEV TYPE II(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	**********	10.274310	**********
				12-23 MO.	**********	20.548620	**********
				2-65+ YR.	3379.150750	9698.548640	9940.122900
SUCROSE NAS 0328	25	NUT PRODS(R)	4	0-5 MO.	**********	16.274400	**********
				6-11 MO.	301.076400	1090.384800	362.351750
				12-23 MO.	219.704400	732.348000	264.418830
				2-65+ YR.	423.134400	1261.266000	509.251080
SUCROSE NAS 0328	27	GRAVIES(R)	19	0-5 MO.	5.660080	16.980240	5.824680
				6-11 MO.	79.241120	220.743120	123.545520
				12-23 MO.	203.762880	577.328160	317.688480
				2-65+ YR.	469.786640	1205.597040	732.448440
SUCROSE NAS 0328	28	IMIT DAIRY(R)	8	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	227.316080	373.449160	261.001560
				12-23 MO.	129.895360	552.055280	149.143760
				2-65+ YR.	146.132280	243.553800	167.786730

SUCROSE NAS 0328	30 HARD CANDY(R)	8	0-5 MO.	.000000	.000000	.000000
			6-11 MO.	48.977700	146.933100	66.766740
			12-23 MO.	146.933100	440.799300	200.300220
			2-65+ YR.	293.866200	832.620900	407.600440
SUCROSE NAS 0328	31 CHEWING GUM(R)	11	0-5 MO.	**********	**********	**********
			6-11 MO.	42.295490	42.295490	63.076840
			12-23 MO.	42.295490	126.886470	63.076840
			2-65+ YR.	84.590980	169.181960	126.157680
SUCROSE NAS 0328	32 GRAN SUGAR(R)	*	0-5 MO.	783.400000	3917.000000	757.000000
			6-11 MO.	1762.650000	4994.175000	1772.550000
			12-23 MO.	2446.125000	6169.275000	2461.870000
			2-65+ YR.	8471.550000	22131.000000	8468.590000
SUCROSE NAS 0328	34 IRS COP TEA(R)	*	0-5 MO.	95.200000	415.[illegible]	95.000000
			6-11 MO.	667.600000	165[illegible].600000	704.900000
			12-23 MO.	781.200000	2622.400000	824.600000
			2-65+ YR.	15258.600000	32684.400000	16106.300000
SUCROSE NAS 0328	68 SEAS FLAVRS(R)	4	0-5 MO.	**********	**********	**********
			6-11 MO.	**********	1.021475	**********
			12-23 MO.	**********	2.1[illegible]	**********
			2-65+ YR.	1.021475	5.107375	1.542456
SUCROSE NAS 0328	82 CEREALS(B)	*	0-5 MO.	343.750500	593.287900	499.636350
			6-11 MO.	234.259600	613.658300	340.492920
			12-23 MO.	50.426000	229.167000	74.020200
SUCROSE NAS 0328	83 FORMULAS(R)	5	0-5 MO.	15977.709640	29271.[illegible]	20713.297410
			6-11 MO.	3259.511680	15511.275660	4220.[illegible]
			12-23 MO.	1047.094400	295.[illegible]	1357.472600
SUCROSE NAS 0328	84 PACCSE FRUT(B)	*	0-5 MO.	8003.495200	14253.415840	14461.279000
			6-11 MO.	8303.954160	26148.802200	14459.678340
			12-23 MO.	2057.616960	8610.147160	3681.693040
SUCROSE NAS 0328	85 MEAT PRODS(B)	*	0-5 MO.	35.200000	164.560000	35.200000
			6-11 MO.	24.640000	69.000000	24.640000
			12-23 MO.	3.960000	9.[illegible]	3.000000
SUCROSE NAS 0328	86 POULTRY(B)	4	0-5 MO.	26.100000	122.3[illegible]	26.100000
			6-11 MO.	24.940000	69.760000	24.940000
			12-23 MO.	3.480000	19.720000	3.480000

SUCROSE NAS 0328	89 PROCSD VEGS(B)	*	0-5 MO.	775.318640	1506.473820	1519.139740
			6-11 MO.	550.345140	1822.577400	1054.006990
			12-23 MO.	60.752580	101.254300	119.037030
SUCROSE NAS 0328	90 PUDDINGS(B)	*	0-5 MO.	955.110790	3494.012890	1678.355950
			6-11 MO.	1172.730970	4340.313590	2050.769850
			12-23 MO.	326.430270	374.790310	573.616350
SUCROSE NAS 0328	91 SOUPS,MIXES(B)	*	0-5 MO.	5.400000	21.600000	6.300000
			6-11 MO.	11.140000	29.920000	13.020000
			12-23 MO.	5.040000	16.380000	5.880000
SUCROSE NAS 0328	92 MEAT DINNER(B)	*	0-5 MO.	45.656160	182.544400	46.220210
			6-11 MO.	333.143550	1323.446900	337.407460
			12-23 MO.	27.301660	86.708590	27.732120
SUCROSE NAS 0328	93 COMB DINNER(B)	*	0-5 MO.	313.720000	1069.376000	1232.363000
			6-11 MO.	692.912000	1940.672000	2854.502800
			12-23 MO.	211.420000	804.760000	870.960000
SUCROSE NAS 0328	ALL CATEGORIES ********** ********** **********	182	0-5 MO.	30707.845160	67045.426150	46139.633960
			6-11 MO.	59201.417950	157164.073405	89481.762240
			12-23 MO.	67550.408490	163487.781720	101257.974200
			2-654 YS.	118002.538625	268537.022515	177251.038736

Appendix

A petition requesting GRAS status for an enzyme-treated beet sugar process has been recently submitted to the Food and Drug Administration. There is involved a new technique applied to the processing of beet sugar which utilizes an enzyme derived from the mold Mortierella vinacea var. The molasses from which sucrose is produced contains another sugar, raffinose - the presence of which prevents a maximum extraction by crystallization of the sucrose. The enzyme, known by common names Alpha-galactosidase or Melibiase, and a trade name, Melibia-D, hydrolyzes raffinose to sucrose and galactose which do not interfere with the crystallization of sucrose; the efficiency of the manufacturing process is thus improved.

The petitioner has submitted the required evidence demonstrating that the use of Melibia-D does not comprise a hazard to consumer or to the environment into which waste materials are discharged. Information relevant to consumer use of the sugar produced by the Melibia-D process is extracted from the petition and presented as an Appendix to the monograph on Sucrose. Included are: covering correspondence; the petitioner's monograph; diagrammatic representation of Melibiase production and application to beet sugar manufacture; and toxicity studies, summaries of these follow. Additional information and exhibits may be obtained by reference to F.D.A. Petition No. 3G0030.

Melibiase Toxicity Studies

I. Acute Toxicity

Duckling

One-day old Peking ducklings were orally intubated with various levels of Melibiase enzyme reacted molasses to screen for the presence of aflatoxins. No adverse effects were noted in behavior, bodyweight gain or in the livers examined microscopically.

Mouse

Acute toxicity of beet sugar produced by applying enzyme process was tested using mice. 1,000 and 2,000 mg of beet sugar per Kg of body weight per day were injected to the vein of mice for 14 days. Four mice were used for one group. It is concluded that LD_{50} is much more than 2.0 g per Kg, per day.

An acute oral toxicity study was conducted with Melibia-D (Melibiase Enzyme). The test material was administered at a dose level of 10.0 g/kg. Each dose was weighed individually, and the test material was flushed through the intubating needle with 10 to 12 cc of corn oil. Eight albino rats (four males and four females) were tested. The acute oral median lethal dose (LD_{50}) was found to be greater than 10.0 g/kg. General signs of intoxication exhibited by the rats following dosing included hypoactivity, ruffed fur, diarrhea, and diuresis. Necropsy revealed no gross pathologic alterations among any of the animals.

II. Short-Term Studies

Rat

One hundred and sixty albino rats were divided into four groups and treated as follows:

Group	No. Rats Male	No. Rats Female	% Reacted Molasses (Melibiase) in diet
1	20	20	0
2	20	20	12.5
3	20	20	25.0
4	20	20	50.0

No treatment-related changes were noted in the rate of growth of the animals or in the clinical chemistry, hematology or urinalysis of the rats examined during the 26-week feeding experiment.

Histopathological examination revealed no abnormalities attributable to the feeding of the reacted molasses.

Dog

Thirty-two dogs were divided into 4 groups, each consisting of 4 males and 4 females. They were treated as follows for 26 weeks:

Group	Male	Female	% Reacted Molasses (Melibiase) in diet
1	4	4	0
2	4	4	12.5
3	4	4	25.00
4	4	4	50.00

This report covers the feeding phase of the 26-week study.

Other than soft feces at the 25% and 50% test levels, no adverse effects were noted which could be attributed to the compound tested. No compound-related changes were noted in the blood chemistry, hematology or urinalysis of the dogs tested. Pathologic-anatomical evaluation of tissues revealed no compound-related changes.

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Effect of an "Atherogenic" Diet containing Starch or Sucrose on the Blood Lipids of Young Men

YUDKIN has suggested that the dietary change most likely to be an aetiological factor in ischaemic heart disease is the replacement of a large proportion of starch by sucrose[1]. It is thus relevant to investigate the differences that can be caused experimentally when starch or sugar is the principal dietary carbohydrate. We report here studies on human volunteers.

Ten male students between 19 and 25 yr old were studied for 3 weeks. During the first (control) week, each subject daily weighed and recorded his normal diet, and from this information the average daily intake was computed. It provided 3,310 kcalories, of which 48 per cent was from carbohydrate, 37 per cent from fat, 11 per cent from protein and 4 per cent from alcohol (Table 1). The subjects were also examined, and their blood pressure, electrocardiograms and blood lipids were measured and found to be within normal limits. They were then divided into two groups so that the average calorie intake during the preliminary week was similar in the two groups. During the 2 experimental weeks the volunteers were in residence, their feeding was supervised and their activity was kept as constant as possible.

The experimental diet provided 3,600 kcalories each day, of which 50 per cent was from carbohydrate, 30 per cent from fat, 11 per cent from protein and 9 per cent from alcohol (Table 1). One group received their carbohydrate—except for that in the wine, beer or milk—entirely as sucrose. This was weighed out and given daily. The subjects consumed it with their meals and throughout the day in beverages. The other group was given cooked wheat starch in the form of pancakes at the usual meal times. Various recipes were prepared from the ingredients shown in Table 2. The cholesterol supplement was dissolved in the fat by gentle warming and this mixture was used for all the cooking.

Table 1. PROXIMATE COMPOSITION OF DIETS, MEAN DAILY VALUES (AND RANGE)

	Pre-experimental	Experimental
Kcalories	3,310 (2,500-3,940)	3,600
Fat (g)	149 (108-195)	120
Protein (g)	93 (74-116)	99
Alcohol (g)	18 (0-68)	46
Carbohydrate (g)	399 (298-537)	450
Sucrose (g)	160 (114-261)	

After the first experimental week, the diets of the groups were interchanged; thus each subject acted as his own control.

Blood samples were taken before breakfast and total serum cholesterol was estimated by the method of Zlatkis, Zak and Boyle[2], phospholipids by the method of King and Allott[3] and total esterified fatty acids by the method of Stern and Shapiro[4]. Triglycerides were calculated by the formula used by Albrink[5]. We also measured platelet stickiness in the same blood samples[6]. We examined the urine for the presence of sugars by taking 10 ml. of a 24 h sample of urine, passing it through a resin column, drying in a vacuum, taking it up in 0·5 ml. of water and spotting it on a Whatman No. 1 paper for descending chromatography.

The concentration of serum cholesterol before the experiment was 240 mg/100 ml. in each group. There was a significant rise which persisted throughout the 2 weeks (Table 3). There was no difference between the two groups or between the periods of the sucrose diet and the starch diet.

The effect on the concentration of serum phospholipids was somewhat similar. Again, there was no difference which could be attributed to the different carbohydrates.

The serum triglyceride behaved quite differently. In group 1, there was a small rise in the week during which the subjects took sucrose, and then a small fall when they changed to starch. In group 2, there were similar and rather larger changes with the different carbohydrates: a fall during the week with dietary starch, and a rise during the week with dietary sucrose. Although none of these separate changes reached the level of significance, there was a significant difference in triglyceride levels at 7 days between group 1 taking sucrose and group 2 taking starch, and also a significant rise in triglyceride level in group 2 when it changed from starch to sucrose.

Platelet stickiness increased in every subject between the first and second weeks, the average increase being some 35 per cent in both groups; there was no difference attributable to the type of carbohydrate.

Paper chromatography revealed sucrose in the urine of every subject taking sucrose, in amounts equivalent to about 500 mg per day. No sucrose was found in any of the specimens from subjects taking starch.

During the first week, the weight of the subjects in each group increased by an average of 2 lb.; there was no further change during the second week.

The experimental diets, containing cholesterol and saturated fat, led to a rise both in cholesterol and in phospholipids. This is similar to the finding of Beveridge, Connell, Mayer and Haust[7]. We found no difference in the cholesterol or phospholipid concentrations when starch or sucrose was in the diet; it may be that the effect of the other dietary components in producing a rise in serum cholesterol masked the effect of the different carbohydrates. It seems that the rise in serum cholesterol reaches a plateau after 1 week. This may be due to an increase in the rate of breakdown in cholesterol brought about by high dietary cholesterol, as suggested previously[7,8].

Our results with triglycerides differ in one or more respects from those of other workers. Hodges and Krehl[9] and Kuo and Bassett[10] also found an increase in triglycerides in subjects given dietary sucrose instead of dietary starch, but they did not use the "atherogenic diet" that we did. Macdonald and Braithwaite[11] used raw starch in their highly experimental diets, while we used cooked starch. Kuo, Feng, Cohen, Fitts and Miller[12] found it necessary to feed at least 85 per cent of the diet as sucrose in order to produce lipaemia, and Macdonald and Braithwaite[11] used diets with 77 per cent starch or sucrose; our subjects received 50 per cent of their diet as carbohydrate. Rifkind, Lawson and Gale[13] reported a fall in triglycerides in subjects in whom dietary sugar was restricted. It has been suggested[14] that this was due to a fall in weight of the subjects during the experiment, rather than to a change in the nature of the dietary carbohydrate. Our own subjects, however, also showed a fall in triglycerides during the first week when sugar was removed from their diets, in spite of the fact that they gained a small amount of weight.

Platelet adhesiveness increased with both diets, and was the same when the diet contained either starch or sugar. As with cholesterol and phospholipids, it is possible that any difference that may have been produced by the carbohydrate was masked by the increase caused by the basal diet with its cholesterol and saturated fat.

The appearance of sucrose in the urine raises some interesting questions. First, even though the amounts are small, it disposes of the general belief that sucrose is always completely hydrolysed before absorption. It is especially to be noted that this occurred even though the sucrose was taken in amounts not greater than those consumed by many people in their usual diets, and even though it was distributed throughout the day, again following the usual pattern of consumption. Second, there is the possibility that some of the effects of sucrose in producing metabolic change may be caused by small quantities of unhydrolysed sucrose acting on the tissues. This possibility has been considered by Aitken, Robinson and Yudkin[15] in experiments on rats; they were, however, able to demonstrate that the increase in a number of hepatic enzymes produced by dietary sucrose did not occur when the unhydrolysed sugar was introduced parenterally.

We thank the Leverhulme Trust Fund, the Max Rayne Foundation and General Foods Corporation for financial assistance, and the staff of St Stephen's Hospital for helping with the platelet counts.

Table 2. INGREDIENTS OF EXPERIMENTAL DIET. DAILY QUANTITIES

	(g or ml.)
Meat (beef, chicken, ham, bacon)	300
Egg	250
Starch or sucrose	400
Green vegetables	250–280
Milk	150
Beer (or wine)	1,200 (or 300)
Hydrogenated coconut oil	50
Cholesterol	4

Table 3. SERUM LIPIDS IN HUMAN VOLUNTEERS

Group 1

Period days	Initial 0	Sucrose week 7	Starch week 14
Cholesterol (mg/100 ml.)	240 (212–275)	331 (319–370)	346 (310–366)
Phospholipid (mg/100 ml.)	211 (205–253)	247 (220–267)	261 (241–259)
Triglyceride (mequiv./l.)	1·75 (0·52–4·66)	2·29 (1·20–2·89)	2·00 (0·59–3·05)

Group 2

Period days	Initial 0	Starch week 7	Sucrose week 14
Cholesterol (mg/100 ml.)	238 (205–268)	310 (287–387)	312 (285–351)
Phospholipid (mg/100 ml.)	229 (209–256)	246 (204–269)	247 (235–290)
Triglyceride (mequiv./l.)	1·58 (0·78–4·16)	0·75 (0·03–1·95)	1·89 (1·14–3·51)

Statistically Significant Differences ($P < 0·05$)

			p Value
Cholesterol	All subjects	Day 0 v. day 7	< 0·005
		Day 0 v. day 14	< 0·005
Phospholipid	All subjects	Day 0 v. day 7	< 0·005
		Day 0 v. day 14	< 0·005
Triglyceride Day 7		Group 1 v. group 2	0·028
"Switchover" test			0·024

These are median values of male students, five in each group, when given atherogenic diet with starch or sucrose.
For methods see refs. 16, 17 and 18.

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PHYSIOLOGY

Metabolic Differences between Starch and Sucrose

SINCE 1957, papers from this department have stressed that the dietary factor involved in the aetiology of ischaemic heart disease is likely to be sugar (sucrose) rather than fat[1]. The evidence incriminating dietary sugar—now quite strong—is chiefly epidemiological. Investigations of populations show that the rise in the incidence of the disease coincides with the rise in the consumption of sugar and not of fat[2-4]. Again, during investigations of individuals we have shown that the disease is most likely to occur in those that have been eating a great deal of sugar[5].

Our suggestion that dietary sugar is a cause of ischaemic heart disease has sometimes been criticized on the grounds of implausibility (for example, Kinsell[6]). Sugar, we are told, is rapidly hydrolysed and rapidly absorbed as glucose and fructose, and the fructose is rapidly converted into glucose; in what way then can sugar act differently from the other major dietary carbohydrate, starch, which also rapidly appears in the blood as glucose? Are not all digestible carbohydrates interchangeable, so that they have identical metabolic effects?

In seeking for a possible mechanism by which sugar may induce ischaemic heart disease, we have therefore taken as our first task the discovery of differences in the metabolic effects of dietary sucrose and dietary starch. Our second task will be to see whether we can demonstrate which of these differences may be responsible for the involvement of sugar in the aetiology of heart disease.

A great deal of evidence contradicts the common belief that all forms of dietary carbohydrates are interchangeable. Macdonald[7], and Antar and Ohlson[8], have summarized some of the differences induced in man and in experimental animals when sugar is substituted for starch in the diet. The effects include an increase in serum cholesterol, changes in depot fats and liver fats, and diminished growth. In addition, Cohen and Teitelbaum[9] have found that sugar-fed rats exhibit an impaired glucose tolerance.

During the past year, we too have found several differences between starch-fed and sucrose-fed rats. We are now reporting these briefly with the purpose of combating the view that all dietary carbohydrates are equal, and particularly that starch and sugar are interchangeable.

All experiments were carried out on rats given purified diets and water *ad libitum*. The diets contained about 70 per cent carbohydrate, either as corn starch or as sucrose; except where noted, they were adequate in all known nutrients.

Diets containing sucrose produced a lower growth-rate in weanling rats (Fig. 1), or a loss of weight in adult rats, compared with diets containing starch. The effect was more noticeable when the diets were low in protein. The lower rate of growth was not due to a lower food intake, nor was there any difference in absorption. The effect must therefore have been due to less efficient utilization of the food.

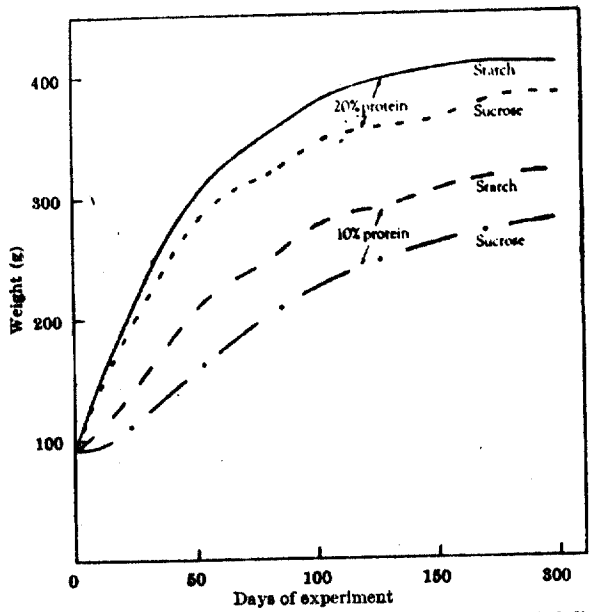

Fig. 1. Growth of male rats from the age of 28 days on purified diets containing two levels of protein, and 69 per cent carbohydrate in the form of starch or sugar. Each group consisted of four male rats

Measurements of oxygen uptake and carbon dioxide output were made in rats over periods of 24 h, about six months after they had been placed on the diets with starch or with sugar. The rats on the diet with starch gave the expected respiratory quotient of about 0·87. However, the rats on sucrose gave a respiratory quotient of about 0·75; this figure is far lower than that expected for a high carbohydrate diet, and similar to that expected (and, in fact, found) for animals on a high fat diet. We have at present no explanation for the low respiratory quotient of the rats on sucrose.

After feeding for six months, the level of serum cholesterol was higher in rats on the sugar-containing diet than in those on the starch-containing diet. The total body fat was significantly less in the sugar-fed rats. The liver fat was less in the sugar-fed rats when they were given the experimental diets from weaning; it was higher in the sugar-fed rats when they were given the diets from the age of three months (Table 1).

Table 1. BODY FAT AND LIVER FAT IN RATS FED DIETS CONTAINING TWO LEVELS OF PROTEIN, WITH 69 PER CENT CARBOHYDRATE AS STARCH OR AS SUGAR

Groups of four male rats fed on experimental diets for 6 months, from age 28 days (weaning) or age 3 months. Fat as percentage of dry weight of body or of liver

Young rats				Older rats			
Diet		Fat content		Diet		Fat content	
Carbohydrate	Protein	Body	Liver	Carbohydrate	Protein	Body	Liver
Starch	20%	35	12	Starch	10%	35	7
Sugar		30	9	Sugar		26	9
Starch	10%	32	9	Starch	5%	32	18
Sugar		32	7	Sugar		27	46

The major carbohydrate components of current human diets, especially in affluent countries, are starch and sugar (sucrose). Experiments have now shown conclusively that the substitution of one for the other in the diet of rats causes several metabolic changes. There is no justification for the view that starch and sugar are metabolically identical.

The effects of altering the carbohydrate in the diet stand in remarkable contrast to the effects of altering the fat in the diet. For example, Thomasson *et al.*[10] have given rats diets containing 54 per cent of the calories from fat, and compared natural soyabean oil with various hydrogenated soyabean and other oils. Some of these contained more than half of the fatty acids in the 'unnatural' *trans* form. They found no difference in body growth, body fat, kidney and liver function, and several other measurements.

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SUCROSE NEPHROSIS AND OTHER TYPES OF RENAL TUBULAR INJURIES*

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Sucrose has recently been added to the long list of chemical substances which may cause renal tubular damage.[1] Muller[2 3] is credited with suggesting the term "nephrosis" for the purely degenerative conditions of the kidney, meaning, presumably, the tubular nephropathies and not the degenerative vascular changes. Despite controversies over the use of this term, it seems established in medical nomenclature, and serves a useful purpose. One condition in which tubular degeneration is a prominent feature, the so-called "lipoid nephrosis," seems fairly well established as a distinctive disease entity.[4] The remainder of the nephroses is the result of poisons of metabolic, bacterial or chemical origin. The addition of sucrose to the list of chemical agents capable of producing nephrosis is of interest, not only because of the popularity of sucrose within recent years as a therapeutic agent, but also because the tubular change seems of peculiar and distinctive nature and appearance.[1] The tubular lesion, unless of mild degree, is easily recognizable, having an appearance quite different from the more familiar types of nephrosis, and may be termed a hydropic or vacuolar degeneration.

This effect of sucrose was first noted by Helmholz[5] in 1933. He described the effects upon the kidneys of rabbits which followed the injection of hypertonic solutions of sucrose, and also described the lesion in a human case. Since that time hypertonic sucrose has been used with increasing frequency as a therapeutic agent. It has been recommended, not only to lower the cerebrospinal fluid pressure[6 7 8] in cases of traumatic injury to the nervous system, but also to relieve cerebral symptoms and complications in arteriosclerotic hypertension[9 10] and as a diuretic.[9 11 12 13 14] If used in small amounts, and in properly selected cases with adequate renal function, there is no evidence that sucrose will have a harmful result.[1 15] However, the dele-

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terious effects of hypertonic sucrose when used in large amounts, or in the presence of inadequate renal functional reserve, has been confirmed by the experimental work and published cases of several authors.[1 5 12 15 16 17] It is intended here to report two more cases exhibiting this lesion, but with certain unusual features, and to make comparisons with some other types of tubular degeneration.

Case 1.—M. R, a negro girl 15 years of age, was brought to the hospital in the eighth month of pregnancy, in labor and comatose. She had had no medical attention during her pregnancy and planned to be delivered by a midwife. Edema of the feet and ankles had been present for four months, and for three weeks she had complained of severe headaches and periods of blindness and vertigo. The night previous to hospital admission she had repeated convulsions. The urine was at low specific gravity and contained 3+ albumin. The nonprotein nitrogen content of the blood was 26 mg. per 100 c. c. She delivered a stillborn baby shortly after admission, and following delivery the urinary output was very small. For its diuretic effect, and because of the cerebral symptoms, sucrose was administered on the two days following delivery, a total of 2,100 c. c. of a 25 per cent solution being given in seven periods of injection. The urinary output was somewhat increased, but a good diuretic effect was not ob-

Fig 2

Kidney, *Case 1.* High power view of convoluted tubules, showing the fine vacuolation and droplets in lumens. In some of the swollen epithelial cells no nucleus is visible.

Fig. 1

Kidney, *Case 1.* The diffuse, widespread nature of involvement of convoluted tubules is evident. The swelling, paleness, fine reticulation and vacuolation of the tubular cells are the characteristic features.

Fig. 3

Kidney, *Case 1.* Photomicrograph of convoluted tubules showing details of the fine reticulation and vacuolation.

tained. Infection developed, her condition deteriorated, and she died on the ninth day after delivery. Three hundred c. c. of 25 per cent sucrose were given the day before and the day of death. It is estimated that about 10 grams of sucrose per kilogram of body weight were given during the nine-day period.

Autopsy confirmed the presence of severe puerperal infection, including isolation of a non-hemolytic streptococcus and Staphylococcus aureus from the blood stream. Though the liver did not show lesions perfectly typical of eclampsia, some necrosis was found. The findings of particular interest were the renal lesions. The kidneys were large, each weighing 225 grams, and were pale and soft.

Sections of the kidneys showed an extreme degree of hydropic swelling and vacuolation involving all convoluted tubules of the cortex. These tubules, with their greatly swollen, pale staining cells, appeared in sharp contrast to the deeply staining glomeruli and medullary tubules. The swollen epithelial cells almost or completely occluded the lumen in many cases. The nuclei were not distended or swollen, but in many cells no nucleus was visible. The cytoplasm of these pale cells had a foamy, finely reticulated and vacuolated appearance, but was not water-clear, as in the cells of a hypernephroma. Small round globules frequently occupied the cytoplasm of the tubular cells, the lumen of the tubules, and the capsular spaces of the glomeruli. Neither fat nor glycogen could be demonstrated by special stains. Another and apparently independent change was seen in a few tubules in the form of hyaline droplets accumulated in the portions of the epithelial cells adjacent to the tubular lumens. Staining by azo-carmine resulted in clear differentiation of these droplets, and also indicated some thickening of the glomerular basement membrane. It seems likely that these changes are related to the eclamptic condition which was present.

Case 2.—M. J., a negro woman, aged 60 years, complained of a sudden sharp pain in the region of the right temple. Later, she was found in an unconscious condition and brought to the hospital. Her blood pressure was 200 systolic and 120 diastolic. The urine contained 4+ albumin. She was given intravenously a single injection, of 250 c. c. of a 25 per cent solution of sucrose. Death occurred eleven hours later. Necropsy showed generalized and renal arteriosclerosis with cardiac hypertrophy. Cerebral hemorrhage into the right internal capsule with secondary rupture into the ventricles had occurred.

The kidneys had some fine granularity of their outer surface and were pale and soft. Microscopic examination of the renal tissue revealed a fairly severe degree of sclerosis of arterioles and small arteries with a patchy fibrosis affecting some glomeruli and regions of interstitial tissue. Almost all of the convoluted tubules were involved by a variable but usually marked degree of swelling and vacuolation. The lining of some tubules seemed so swollen as to block the lumen. The epithelial cells showed fine reticulation of their cytoplasm, or small clear vacuoles. The inner margins of the cells were ragged and irregular. Many of the epithelial cells had no visible nucleus. Red blood cells were sickle-shaped.

COMMENT

It has already been shown by experimental studies and reports of several cases that this peculiar type of vacuolar nephrosis is the result of injection of sucrose.[1 5 12 15] The first of the cases presented here illustrates the severity of the change which may result from sucrose administration and the uniform, widespread nature of the involvement of the convoluted tubules. The second case shows the marked degree of change which occurs after a single injection into an adult of as little as 250 c. c. of a 25 per cent solution. It may be noted that in both cases the kidneys were the seat of the other pathologic changes. In one case, there were glomerular and tubular lesions apparently associated with pregnancy toxemia, and in the other case severe vascular lesions were present. In several other cases which have been previously described[1] sucrose nephrosis was found to be severe in kidneys involved by marked vascular disease. It may well be that interference with renal circulation by vascular change, or a similar effect produced by thickening of glomerular basement membrane, will markedly enhance the damaging effects of sucrose. Particularly if there was a reduction in filtration rate, it would operate to prolong the exposure of the epithelial cells to the sucrose in the filtrate. Further circulatory embarrassment may have been produced in the second case by the sickle-shaped red cells. These associated disturbances in the functional condi-

Fig. 4
Kidney, *Case 2.* Convoluted tubules showing marked degree of vacuolar change. A sclerotic arteriole is also evident.

tion of the kidney may explain the definite effects produced in *Case 2*, although the amount of sucrose administered was only about 1 gram per kg. of body weight.

There are several other points of interest and importance regarding this lesion. One of these is the nature of the change in the tubular cells. The swelling and vacuolation are apparently not associated with the presence of lipid or glycogen in the cytoplasm. Whether or not sucrose is present in the cells is not known, though the fact that there may be some slight tubular reabsorption of sucrose would suggest that this is not unlikely. The failure to stain the material in the vacuoles by any of the usual methods, and the resemblance of mild degrees of the change to cloudy swelling, in which there is known to be increased imbibition of water into the cell,[18] suggests that the change is probably a true hydropic degeneration. While some slight tubular reabsorption of sucrose may occur normally,[19] presumably by the dextrose reabsorption mechanism, Richards[20] has demonstrated that the effect of certain drugs injurious to the renal tubules is to cause greatly increased tubular reabsorption, to an extent that urinary output is greatly diminished. Experimentally, Helmholz[12 16] has been able to produce a great lowering of urinary output or definite anuria by injection of large amounts of sucrose. It may be that convolu[illegible] tubular cells are injured by contact with su[illegible] in glomerular filtrate to an extent that mar[illegible] absorption of sucrose and fluid may occur.

The effect of the tubular changes on re[illegible] function is of practical importance. Increa[illegible] imbibition of water is not necessarily very d[illegible] aging to cells, and may be a reversible cha[illegible] particularly if it is due to injury by exposure [illegible] anisotonic solutions.[21 22] That there is at le[illegible] a temporary effect on renal function seems pro[illegible] able from the extent of tubular damage whi[illegible] may occur. This is borne out by the exper[illegible] mental work of Helmholz and Bollman,[12] wh[illegible] were able to produce a lowering of phenolsulphon[illegible] phthalein excretion, and even definite anuria by injection of sucrose into rabbits. It seems equally plain, however, that little or no permanent damage results. But since temporary interference with renal functional efficiency may be of serious degree, the use of sucrose seems unwise where there is already renal damage and decrease of functional reserve.

The specificity of the hydropic degeneration resulting from sucrose also needs consideration. Klemperer[17] states that dextrose in very high

Fig. 5
Kidney from a case of poisoning by diethylene glycol. The hydropic degeneration is evident in the swollen tubular cells with the water-clear cytoplasm.

Fig. 6
Kidney from a case of enterocolitis with diarrhea in a child, the pale, swollen tubular cells showing a change similar to the damage caused by sucrose.

(50 per cent) concentration also produces vacuolation, though not so striking as the result of sucrose. Helmholz and Bollman[12] found that following injections of sodium sulfate, urea, dextrose and sorbital, similar changes were produced, but these were definitely less marked than those produced by sucrose. Hydropic degeneration has also been described as occurring in cases of chronic dysentery[23] and in poisoning by diethylene glycol.[24, 25]

As a comparative study, and in a search for lesions similar to sucrose nephrosis, three groups of cases were examined. In the first group were 380 cases showing tubular degenerative changes, picked from a series of 3,000 autopsies. Among these cases none was found which might be confused with the well-developed sucrose nephrosis. Some having the more severe degrees of cloudy swelling and fatty degeneration of the tubules resembled the milder changes in tubules after sucrose administration. Some of these cases had been given dextrose. Also in one case of diabetes mellitus an appearance suggestive of mild sucrose nephrosis was present. It may probably be attributed to some slight accumulation of glycogen in convoluted tubules.

The hydropic degeneration in the kidney which results from poisoning by diethylene glycol was compared in four cases of death from administration of "elixir of sulphanilamide"! In one of these cases the tubular changes were quite mild, resembling cloudy swelling. In another the tubular damage was very severe, with necrosis and desquamation of lining epithelium. The remaining cases showed focal areas in which there was marked swelling and distortion of the lining cells of convoluted tubules. The cells had a water-clear cytoplasm, without the fine reticulation and formation of small droplets as seen in sucrose nephrosis. These differences, combined with the patchy distribution, produced a picture quite different from that of sucrose nephrosis.

The sections of kidney tissue from thirty-five cases of enterocolitis with diarrhea revealed an instance in which the renal changes appeared similar to those resulting from sucrose. The cells of the convoluted tubules were similarly swollen and had fine reticulation of their cytoplasm, but were not quite so pale staining as the cells affected by sucrose, and the vacuolation was not so marked. The change, however, was practically indistinguishable from that due to sucrose.

SUMMARY

(1) Sucrose may be added to the list of chemicals which produce tubular degenerative changes or nephrosis.

(2) Sucrose nephrosis is a peculiar type of hydropic or vacuolar degeneration affecting convoluted tubules.

(3) The probability is discussed that pre-existing renal lesions may enhance the effects of sucrose on the tubules so that changes are produced from relatively small amounts.

(4) The lesion produced by sucrose is compared with certain other types of renal tubular damage.

(5) The occurrence of a tubular lesion of similar appearance in some cases of diarrhea or chronic dysentery is confirmed.

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DISCUSSION (*Abstract*)

Dr. Robert A. Moore, St. Louis, Mo.—Last February, at the Barnes Hospital, we had an opportunity to observe at autopsy the kidneys of a lead worker who came to the Hospital with the signs and symptoms of uremia, and died some forty-eight hours after admission. At autopsy the appearance of the kidney was much as Dr. Anderson has shown. Some of the tubules in the cortex showed a peculiar hydropic degeneration.

I must confess that this slide was on my desk for some days, and finally we wrote as the diagnosis "hydropic degeneration of the renal tubules." Some time after that, Dr. Anderson's fir t excellent article on this subject appeared in the *Journal of the American Medical Association*. We immediately remembered this case, secured the history from the hospital, and found that the patient had been given sucrose intravenously. Since that time we have seen one additional case at the Barnes Hospital.

Even at the time that we originally studied this case we were impressed with the fact that the change occurred in some tubules and not in other tubules. The pathologic change, hydropic degeneration, was present in nodules throughout the renal substance. These nodules correspond to the nodules of tubular hypertrophy or dilatation seen in cases of chronic renal disease.

That raises the point Dr. Anderson has touched upon, which seems to me to be important. That is, what is the nature of the pathologic change? How much physiologic disturbance occurs as a result of the hydropic degeneration? Here in this kidney there is, I believe, some evidence that the change is severe and practically restricted to that part of the renal tubule which is morphologically the best preserved.

The second case showed only slight changes. In order to gain some additional information concerning the point of whether or not this lesion occurs only in damaged kidneys, I secured through the courtesy of Dr. Paul Wheeler, pathologist in charge at St. Louis City Hospital, the sections from five kidneys of individuals who had received intravenous sucrose. These kidneys in four instances were from patients who had had a traumatic injury to the cranial cavity or to the brain and were given the sucrose as a measure of dehydration. In one case the patient had rheumatic heart disease and was given sucrose purely as an experimental procedure.

The typical pathologic change in the renal tubules was present in only two of the five cases. In one case there was a severe degree of cloudy swelling. In two cases I would consider the renal tubules essentially normal, or, if you wish, there was a slight degree of cloudy swelling, depending upon the fixation and stain used. Thus, in two cases out of five examined, it h[illegible] curred in what, so far as we can determine, was a [illegible] viously normal kidney in young persons injured in [illegible] mobile accidents and given the sucrose as a treat[illegible] for edema of the brain. In the two cases at B[illegible] Hospital it occurred in previously damaged kidne[illegible]

However, on the basis of the observations o[illegible] first case at the Barnes Hospital, I am convinc[illegible] Dr. Anderson is, that there may be some severe d[illegible] to the kidney as a result of intravenous administr[illegible] of sucrose, and one should use it sparingly in case[illegible] renal damage. On the basis of the other observat[illegible] it would appear to be a safe therapeutic measure if t[illegible] is no previous renal damage, and if it is used in y[illegible] individuals. However, there should be additional stu[illegible] on this point.

I should like to ask Dr. Anderson if he has made [illegible] observation concerning renal functions in individ[illegible] who have received sucrose intravenously.

Dr. R. H. Rigdon, Memphis, Tenn.—I have been very much interested in this renal lesion for approximately a year and a half now. In my experience it has occurred only in those cases in which sucrose has been given.

In the first case under consideration here, we have a complicated problem. We have three separate factors to consider. One is the bacterial infection. Second, the patient had eclampsia. The correlation of clinical eclampsia with the pathologic changes, of course, presents a problem. A third factor is that the patient received sucrose.

This lesion which has been shown certainly is neither the lesion that we see in bacterial infections, nor that resulting from bacterial toxins. It is not the lesion that occurs in eclampsia. I am speaking now of the diffused tubular change. The focal areas that were called to your attention as colloidal degeneration in the epithelial cells of the tubules would suggest a closer relationship to the bacterial infection than to eclampsia. Although this case is complicated by a bacterial infection, it is obvious that the most diffuse lesion results from the sucrose. There are other cases reported in which there are no bacterial infection that show exactly the same renal change.

Dr. Cardwell and I, at the University of Tennessee, are studying the sucrose problem both in man and in experimental animals. We made a very serious mistake in our early work, and that was in bringing the data before our hospital staff about a year ago. They quit using sucrose. Since that time we have not been able to get any cases to study at autopsy. I learned my lesson at this time about accumulating pathologic data. We are now selecting cases and giving 250 c. c. of a 25 per cent sucrose solution and checking their nonprotein nitrogen.

We were unable to find any evidence of retention of nonprotein nitrogen in two of the cases we have studied. In one case we did get retention of nonprotein nitrogen. This brings up the question of the association of this lesion with other pathologic changes in the kidney, and also why do we get a retention of nonprotein nitrogen in some cases and not in others?

Our experimental observations have been few up to this time, but we have been impressed by one point, and that is the association of dehydration of the rabbit with the production of this renal lesion. Going back to

the eight or nine cases that Dr. Anderson has reported, we find that the majority of the cases were comatose at the time of admission. We have no indication as to whether or not they were dehydrated, but it is likely, I think, that if they were comatose their fluid intake may have been restricted for a period of time. There may be some correlation between dehydration at the time the sucrose was given and the occurrence of this renal lesion. Time only can tell whether this is true. Our experimental animals are being carried with this dehydration in mind.

The association of this tubular lesion with previous renal damage seems to be open to question. In some of these cases I cannot convince myself that there was a pathologic change present in the kidney before the patient was given sucrose. I am of the opinion that this lesion is not necessarily associated with previous renal damage. It may be, but more cases must be studied to determine the relation of this tubular lesion to previous renal damage.

We must always be on guard for new lesions developing as a result of therapeutic agents.

Dr. Wiley D. Forbus, Durham, N. C.—Last year during the meeting of the Southern Medical Association in Memphis, I had the opportunity of seeing the lesions which Dr. Anderson has shown us today. With that preliminary view of the lesion in mind, I have watched carefully for changes in the kidney in the routine autopsies of our department during the past year. In this material we have seen the lesion on two or three occasions. On one occasion we found that the entire tubular structure of the kidney was involved. This, of course, is in striking contrast to the observation of Dr. Moore, who found the lesion focally distributed. This variation in distribution of the lesion suggests to me that its development may be specifically determined by the functional state of the tubular epithelium in the respective functional units. Perhaps it is to be expected that only those functional units actually in a state of activity would be apt to undergo the peculiar alteration. The same line of thought might be applicable also to the occurrence of the lesion in the injured kidney in which, as Dr. Moore has described, the lesions developed only in the areas of tubular hypertrophy and were totally absent in the injured and at least presumably non-functioning tubules.

Dr. Lucien Y. Dyrenforth, Jacksonville, Fla.—It seems to me it would be very interesting to find out what is the fate of sucrose administered intravenously. I wonder if the author can tell us anything about that.

Dr. Anderson (closing).—Regarding the effects of sucrose on renal function in man, my own observations are few. In some of the first cases I described, the nonprotein nitrogen of the blood was determined at the time of entrance into the hospital, and following administration of sucrose was greatly increased. In some instances death was due to uremia. In experimental animals, sucrose has been shown to have a definite effect on renal function.

Dr. Rigdon's comments concerning dehydration are timely and interesting. In a dehydrated individual sucrose will be more concentrated in the fluid in contact with tissue cells, with the probability of more severe injury. Sucrose is obviously a bad thing to administer to a dehydrated patient, since it will markedly increase the dehydration.

The problem of focal and diffuse changes as a result of sucrose is pertinent, particularly because of the possible effect of previous renal lesions. In the first case discussed today, the changes were diffuse, and in the second case, there was a tendency to focal involvement. It is probable that previous renal damage is an important factor determining the site of the changes.

The fate of sucrose which has been injected into the blood stream is apparently to be completely excreted by the kidneys without being metabolized. In an individual with normal renal function, the sucrose is completely excreted within twenty-four hours, and almost all within ten hours. When there is failure of renal function, the excretion of sucrose is delayed and, depending upon the degree of renal failure, it may be as long as seventy or ninety hours before urinary excretion is complete. Clearance of sucrose from the blood is similar to that of urea or creatinine, and is comparably decreased in renal disease. Sucrose does not pass the barrier of the choroid plexus into the cerebrospinal fluid. I do not know what degree of diffusion occurs into other tissues of the body.

FOR ALBERT E. SACCO JR., M.D.

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Some Metabolic Effects of Dietary Sucrose

A. E. Bender and Pushpa V. Thadani

Abstract. (1) Male, weanling rats were fed on diets containing 60% sucrose or 60% starch and the liver and adipose tissue were examined *in vitro* for their ability to oxidise glucose and to synthesise fat from glucose, using radioactive glucose as an indicator. (2) Compared with the livers of rats fed on starch those fed on sucrose showed a severe depression of both glucose oxidation and lipogenesis. When two forms of radioactive glucose were used, labelled in carbon–1 or universally labelled, the change in proportions of carbon dioxide liberated indicated that sucrose feeding had altered the metabolic pathways. (3) Adipose tissue also showed depressed lipogenesis after sucrose feeding and less carbon dioxide was formed from (1–^{14}C) glucose but that from (U–^{14}C) glucose was unchanged. (4) The effect of sucrose was apparent after 14 days feeding and persisted in experiments continued for 112 days. (5) The proportions of fat and glycogen in the liver and the fat content of the body were not altered by sucrose feeding. (6) Tissues from female rats behaved in the same way as those from male rats. (7) The effect of sucrose was compared in three strains of animals. The response of hepatic lipogenesis differed with the strain but lipogenesis in adipose tissue was depressed to a similar extent in all three strains. Glucose oxidation in both tissues varied in extent with strain; the strain that was not affected with respect to glucose oxidation in the liver was the most sensitive with respect to glucose oxidation in adipose tissue. (8) The sucrose effect was equally marked in fed and 24 h-fasted rats. (9) Sucrose included in a fat-free diet had a depressant effect on glucose oxidation and lipogenesis in liver but not on glucose oxidation in adipose tissue. (10) The lipids formed from radioactive glucose by adipose tissue were separated by thin layer chromatography and examined for radioactivity. The results indicated that the depression of lipogenesis largely or wholly affected the triglycerides. (11) Rats were injected intraperitoneally with radioactive glucose after feeding on the respective diets. The extracted fat from kidney and adipose tissue, but not that from liver and blood, showed a lower radioactive count after sucrose feeding. (12). It is suggested that sucrose might exert its depressant effect on metabolism by (a) reducing the amount of glucose-6-phosphate available through stimulating the formation of glucose-6-phosphatase and (b) altering the proportion of glucose metabolised via the pentose shunt by stimulating the formation of glucose-6-phosphate dehydrogenase.

There are many reports in the literature dating from Kutz [1876], Frerichs [1876] and Lusk [1892], indicating that dietary carbohydrates differ in their physiological effects. More recently it has been

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shown that sucrose and starch differ in their effects. For example, rats show inferior growth when fed on sucrose [CHANG, 1962; AL-NAGDY, MILLER, QURESHI and YUDKIN, 1966], increased liver fat [MARSHALL and WOMAK, 1954], increased levels of various enzymes [FREEDLAND and HARPER, 1958; CARROLL, 1963; AITKEN, ROBINSON and YUDKIN, 1967; BARTLEY, DEAN, TAYLOR and BAILEY, 1967], rabbits exhibit growth failure and eventual death [MACDONALD and THOMAS, 1956], and human beings respond to sucrose feeding by an increase in serum triglycerides [MACDONALD and BRAITHWAITE, 1964]. Moreover, YUDKIN [1957, 1964] has indicated a relation between sucrose consumption in man and the incidence of ischaemic heart disease.

The present work was undertaken in an attempt to investigate metabolic differences between sucrose and starch, and the results show that dietary sucrose depresses the rate of oxidation of glucose and of lipogenesis from glucose in both liver and adipose tissue. It is suggested that these effects may be due to an increase in the level of the various enzymes and consequent changes in the metabolic pathways.

Experimental and Results

General Methods

Animals and diets. Rats of three different strains were used, the Lister hooded colony of Queen Elizabeth College (Lister), hooded rats from the Medical Research Council colony (MRC) and white rats purchased from Roebuck Farms (Ltd. (Roebuck). They were housed in groups of three or four to a cage on a wire-mesh grid at a temperature of 20–22° C.

Diets were composed of 60 parts by weight sucrose or corn starch, with 24 parts casein, 8 parts arachis oil, 5 parts salt mixture and 3 parts solka floc. In the fat-free diet used in experiment 3b the carbohydrate was proportionately increased. All the B vitamins were included in the diet and the fat-soluble vitamins were given separately once a week. Animals were allowed free access to food until the time of killing in all experiments except experiment 3a, which was designed to determine the effects of short periods of fasting.

Incubation of tissues. The rats were killed by a blow on the head and the liver and epididymal fat pads (as a source of adipose tissue) were removed immediately. The fat pads were cut in halves and, without cooling, transferred to 25 ml Warburg flasks containing 3 ml Krebs-Ringer bicarbonate at pH 7.3–7.4 previously gassed with O_2+CO_2 (95:5) together with 1 ml 0.1 M glucose solution containing 0.25 μc or 1.0 μc of (14 C) glucose. This yielded a final concentration of 450 mg glucose percent. The flasks were attached to manometers for ease of handling (no manometric readings were taken), gassed with the same mixture and the side arms closed with

'Subaseal' rubber caps. They were immersed in a water bath at 37° and the tissues were incubated for 3 h with continuous shaking. Each measurement made on adipose tissue was carried out in duplicate.

Livers were chilled in ice, cut into slices approximately 0.5 mm thick and treated in the same way as the adipose tissue. About 600 mg wet weight of liver, equivalent to about 100 mg dry weight, was used in each flask. Each measurement was carried out in triplicate.

Collection of CO_2 and measurement of radioactivity. At the end of the 3 hour-incubation period the flasks and manometers were removed from the bath and the reaction stopped by the addition of 0.5 ml 2 N H_2SO_4 injected by syringe through the rubber cap. The CO_2 was absorbed in 0.5 ml M Primene 81-R in methanol [OPPERMAN, NYSTROM, NELSON and BROWN, 1959] injected through the rubber cap into the side arm, and the flasks, still attached to the manometers, were left for 20 min at room temperature while the CO_2 was absorbed. (The injection of methanolic solution of Primene and the rapid expansion of the methanol were liable to cause a loss of CO_2 by driving the manometer fluid round the U-tube of the manometer. This was prevented by replacing the usual manometer fluid with mercury.)

The flasks were then detached from the manometers and the Primene-CO_2 solution was withdrawn from the sidearm and transferred with three rinsings, each of 0.5 ml methanol, into clear glass counting vials. One ml fresh Primene solution, 5 ml of scintillation fluid (0.06% 2,5-diphenyloxazol and 0.6% 1,4-bis-(5-phenyloxazol-2-yl)benzene in toluene) and 3 ml toluene were added and the radioactivity measured in an automatic liquid-scintillation spectrometer (Packard Tri-Carb model 3003).

Fat extraction. After incubation the contents of each Warburg flask were transferred to a centrifuge tube and the tissue rinsed free from adhering glucose solution by washing four times with Krebs-Ringer bicarbonate solution, centrifuging each time. The fourth washing was found to be virtually free from radioactivity.

The washed tissue was homogenised with 10 ml chloroform-methanol (2:1, v/v) in a glass Potter-Elvehjem homogeniser and a 4 ml sample of the homogenate was removed for a dry weight estimation. The remainder was filtered and washed according to the method of FOLCH, LEES and STANLEY [1957]. A 3 ml aliquot of the solution of fat was evaporated to dryness, weighed, then dissolved in 5 ml toluene and the radioactivity measured as above.

Separation of fats. This was effected by thin-layer chromatography by the method of VOGEL, DOIZAKI and ZIEVE [1962]. The spots were visualised by exposure to iodine vapour and transferred to counting vials and the radioactivity measured.

Glycogen estimation. Immediately the rat had been killed a sample of liver was removed as quickly as possible and frozen in liquid nitrogen. This was usually achieved within 5 sec of death. The frozen sample was weighed and the glycogen estimated by the phenol-sulphuric acid method of MONTGOMERY [1957].

Carcass water and fat. Carcass water was determined by drying the animals, including the gastrointestinal tract, for 48 h in an oven at 105°. The dry carcasses were crushed in a mortar and extracted in a Soxhlet extractor for 8 h with petroleum ether (60–80°). The total fat content was calculated from the difference between the weights of the dried carcass before and after extraction.

Calculation. The radioactivity of the CO_2 and the fat were expressed as counts/min/mg dry weight of liver or adipose tissue. Figures quoted are means of the duplicate measurements made on adipose tissue and the triplicate measurements made on liver.

The significance of the results was tested by the Mann-Whitney test [MANN and WHITNEY, 1947].

Experiments

Experiment 1. Comparison of dietary effects of sucrose and starch: male weanling rats of the MRC black and white strain, 4 weeks old, were fed on either the sucrose diet (5 rats) or the starch diet (4 rats) for a period of 14–36 days. They were killed at intervals throughout this period and the liver and epididymal fat pads were examined *in vitro* for their ability to oxidise glucose and for lipogenesis using both forms of radioactive glucose. Sixteen rats were used in a repeat experiment to confirm the results obtained with adipose tissue.

Table I shows the radioactive counts of lipids and CO_2 from liver and adipose tissue after incubation.

Liver: in the sucrose-fed animals compared with those starch-fed the oxidation of (U–^{14}C) glucose was reduced to 0.4 and that of (1–^{14}C) glucose to 0.6. This caused a change in the ratio of the CO_2 produced from the two forms of glucose (CU/C1) from 0.9 on starch diet to 0.6 on sucrose diet – indicative of a change in metabolic pathway. Lipogenesis from both (U–^{14}C) glucose and (1–^{14}C) glucose was reduced to 0.5.

Adipose tissue: the rate of oxidation of (U–^{14}C) glucose was not affected by sucrose feeding and the reduction of the rate of oxidation of (1–^{14}C) glucose to 0.8 was not significant. Lipogenesis from both forms of glucose was reduced to 0.7 by sucrose feeding but because of the spread of results the effect on (1–^{14}C) glucose was not significant. The findings were verified with an additional 16 rats and the results given in table I A show that the depression of lipogenesis from (1–^{14}C) glucose was significant (a larger amount of radioactivity was used in this experiment). They confirm the significant depression of lipogenesis from (U–^{14}C) glucose. Table I A also confirms that the oxidation of (U–^{14}C) glucose is not depressed by sucrose; the depression of oxidation of (1–^{14}C) glucose, previously found to be 0.8 and not significant, is now 0.7 and becomes significant.

Experiment 2a. Length of feeding period: six rats of the MRC colony were fed for 106–112 days, three on each diet, and the livers were incubated with (U–^{14}C) glucose. Six rats of the Lister colony were fed for 14 days and the livers incubated with (1–^{14}C) glucose.

Figure 1 shows the lipid and CO_2 counts together with the results of feeding for 14–36 days from exp. 1. The sucrose-depressant effect had been achieved after 14 days and persisted during the longer

Table I. Effect of dietary carbohydrate on lipogenesis and glucose oxidation. Weanling rats of MRC colony, fed 14–36 days

	Diet 60% starch (4 rats)	60% sucrose (5 rats)	Ratio activity on sucrose diet/ activity on starch diet	Significance of differences between sucrose and starch values[1]
Liver				
Lipid count/min/mg (with range)				
(U–^{14}C) glucose	49 (41–65)	23 (15–43)	0.5	0.016
(1 –^{14}C) glucose	40 (29–55)	18 (11–36)	0.5	0.032
Ratio CU/CI	1.3 (1.1–1.5)	1.3 (1.2–1.4)	1	NS
Carbon dioxide count/min/mg tissue				
(U–^{14}C) glucose	172 (156–204)	68 (63–82)	0.4	0.008
(I –^{14}C) glucose	207 (168–241)	133 (112–168)	0.6	0.008
Ratio CU/CI	0.9 (0.7–1.2)	0.6 (0.5–0.6)	0.7	<0.02>001
Adipose tissue Lipid count/min/mg tissue				
(U–^{14}C) glucose	27 (21–37)	19 (16–26)	0.7	0.029
(I –^{14}C) glucose	26 (15–35)	19 (16–23)	0.7	NS
Ratio CU/CI	1.1 (0.8–1.4)	1.0 (0.8–1.2)	0.9	NS
Carbon dioxide count/min/mg tissue				
(U–^{14}C) glucose	19 (13–27)	18 (14–32)	1.0	NS
(1 –^{14}C) glucose	23 (14–32)	18 (15–26)	0.8	NS
Ratio CU/CI	0.9 (0.4–1.8)	1.0 (0.8–1.4)	1.1	NS

[1] MANN-WHITNEY test

period of feeding. After this longer period the older rats had a lower rate of glucose oxidation than the younger rats on both diets but the sucrose-depression was proportionately similar to that in the younger animals.

Experiment 2b. Female rats: exp. 1 was repeated on female rats of the Lister colony and liver metabolism was found to be affected in the same way as that of the males. Glucose oxidation was reduced from a CO_2 count of 278 (range 258–310)/min/mg tissue on a starch diet to 184 (range 163–227) on sucrose diet, P = 0.008. Liver fat count was reduced from 60 (range 44–73) counts/min/mg dry wt liver on starch diet to 24 (range 20–31) on sucrose diet.

Table Ia. Confirmatory experiment

	Starch diet (4 rats)	Sucrose diet (4 rats)	Ratio activity on sucrose diet/ activity on starch diet	Significance of differences between sucrose and starch
Adipose tissue				
Lipid count/min/mg tissue				
(U-^{14}C) glucose	210 (113–333)	135 (80–217)	0.6	0.02
(I -^{14}C) glucose	241 (124–387)	154 (132–202)	0.6	0.036
Ratio CU/CI	0.92(0.54–1.45)	0.74(0.58–1.85)	1.2	NS
Carbon dioxide				
count/min/mg tissue				
(U-^{14}C) glucose CD	139 (100–195)	134 (75–327)	1.0	–
(I -^{14}C) glucose CI	264 (150–440)	196 (153–260)	0.7	0.047
Ratio CU/CI	0.56(0.38–0.73)	0.56(0.42–0.67)	1.0	–

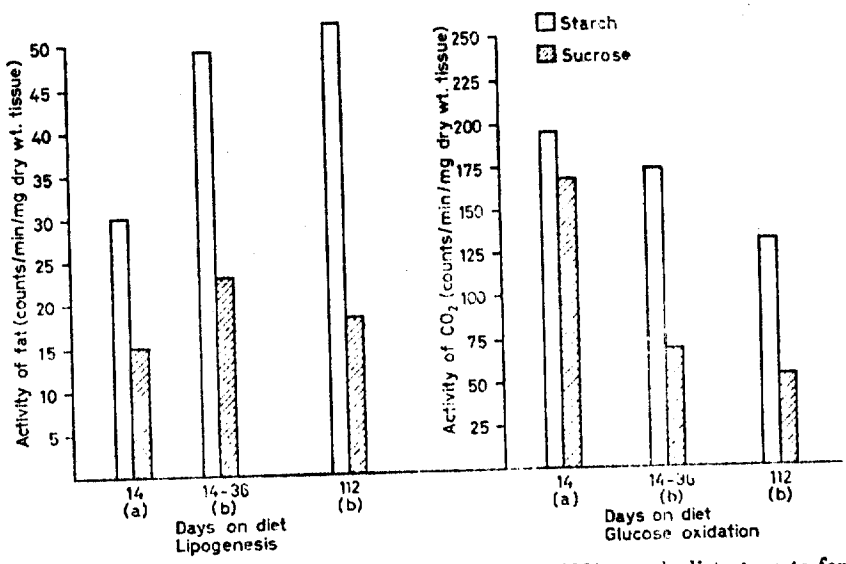

Fig. 1. Liver response after feeding 60% sucrose or 60% starch diets to rats for varying periods, (a) incubated with (1-^{14}C) glucose, (b) incubated with (U-^{14}C) glucose.

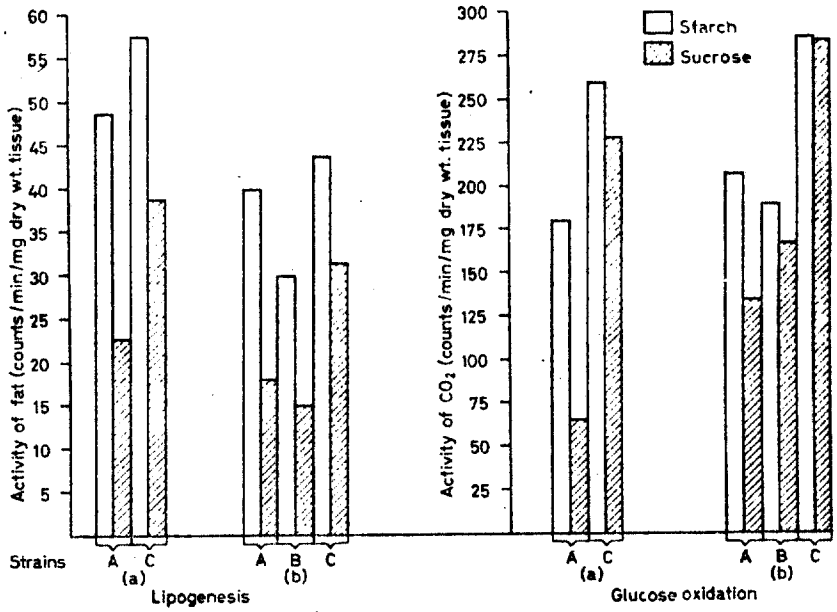

Fig. 2. Liver response in three strains of rats after feeding 60% sucrose or 60% starch diets. Strain A: Medical Research Council colony; strain B: Queen Elizabeth College Lister colony; strain C: Roebuck Farms Ltd. (a) Incubated with (U-^{14}C) glucose, (b) incubated with (1-C^{14}) glucose.

Experiment 2c. Strain differences: exp. 1 was repeated on rats of the Lister and Roebuck colonies and the response of these strains and that of the MRC colony from exp. 1 are shown in figure 2 (liver) and figure 3 (adipose tissue).

Liver: with regard to glucose oxidation the MRC strain was the most sensitive to sucrose, the oxidation of (1-^{14}C) glucose being reduced to 0.6, while in the Lister colony the reduction was to 0.9, and the Roebuck animals were not affected. Two of the strains were also examined with (U-^{14}C) glucose and again the MRC rats were sensitive – CO_2 count reduced to 0.4 while the Roebuck animals were not affected.

Similarly but to a less marked extent the depression of lipogenesis differed in the three strains. Lipogenesis from (1-^{14}C) glucose was most affected in the MRC and Lister strains, the fat count being reduced to 0.5, but only to 0.7 in the Roebuck animals. Lipogenesis from (U-^{14}C)

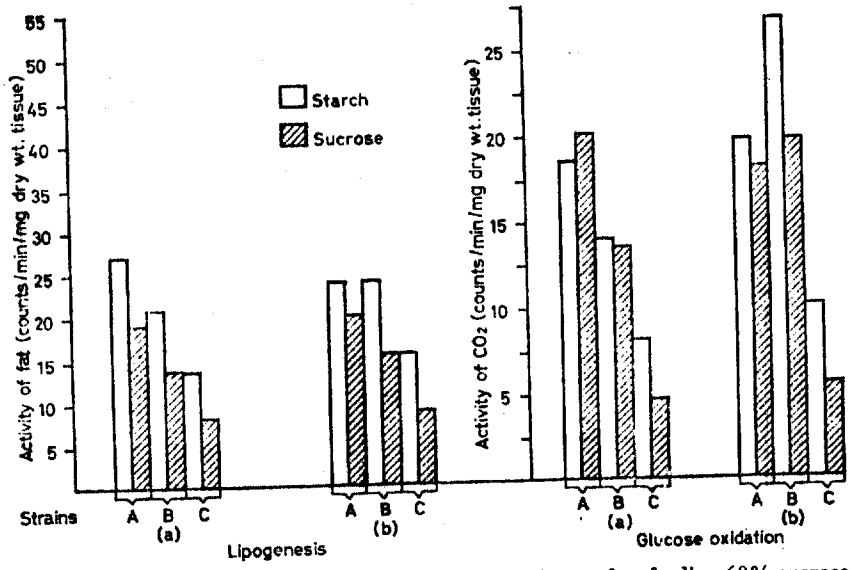

Fig. 3. Response of adipose tissue in three strains of rats after feeding 60% sucrose or 60% starch diets. Strain A: Medical Research Council black and white colony; strain B: Queen Elizabeth College Lister colony; strain C: Roebuck Farms Ltd. (a) Incubated with (U–^{14}C glucose), (b) incubated with (1–^{14}C) glucose.

glucose was reduced to 0.5 in the MRC strain and to 0.7 in the Roebuck strain.

Adipose tissue: the sensitivity of the different strains to dietary sucrose was reversed in adipose tissue. Glucose oxidation was not depressed in the MRC rats with either form of glucose; Lister rats showed a depression to 0.7 with (1–^{14}C) glucose and no effect with universally labelled glucose; the Roebuck rats were the most sensitive with a reduction to 0.6 with universally labelled glucose and 0.5 with (1–^{14}C) glucose.

The difference between the strains was not so marked with respect to lipogenesis. Both Lister and Roebuck strains shewed a significant depression to 0.6 for both forms of glucose and the MRC rats were less sensitive with a depression to 0.7 (significant) with (U–^{14}C) glucose and 0.8 (not significant) with (1–^{14}C) glucose.

Experiment 3a. Effect of fasting: in the previous experiments the animals had been allowed free access to food until the time of killing. In Exp. 3a, after feeding for 106–112 days on the respective diets,

the animals were fasted for periods of 4, 9 and 16 h before being examined. In a confirmatory experiment they were fed for 21–22 days and fasted for 24 h before being killed.

Figure 4 shows that glucose oxidation was not affected by fasting and the sucrose-depressant effect was 0.4–0.5 at each period. Liver lipogenesis of starch-fed animals fell progressively after 4, 9 and 16 h fast but as there was a proportionate fall in the metabolism of the sucrose-fed animals the sucrose-depressant effect remained at 0.35–0.5 at each period of measurement.

The results were verified in a confirmatory experiment (table II) which shows the depression after 24 h fast. Both forms of radioactive glucose were used and the ratio of CO_2 produced from the two forms (CU/C1) fell from 1.0 on the starch diet to 0.7 on sucrose-confirming the earlier finding that sucrose feeding caused a change in metabolic pathways. Lipogenesis from both forms of glucose was reduced to 0.4, $P = 0.004$ in both instances.

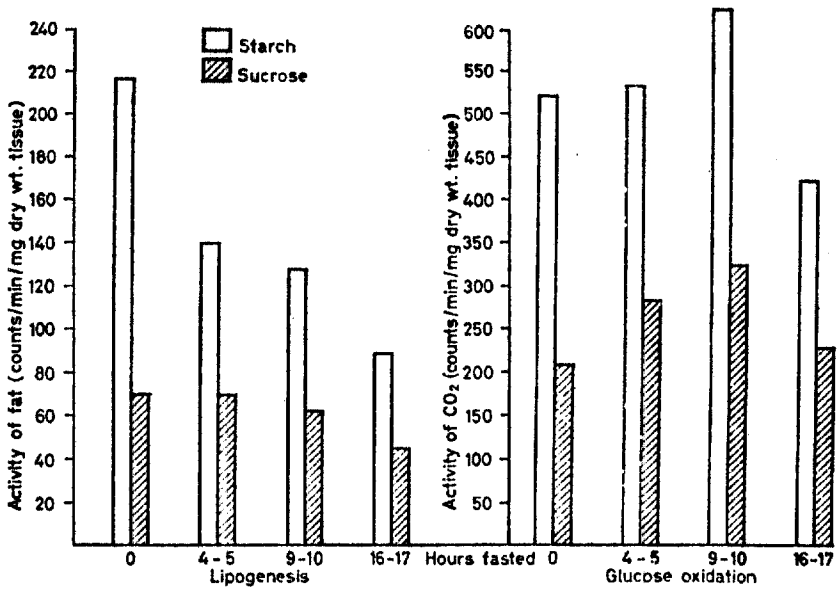

Fig. 4. Liver response after feeding 60% sucrose or 60% starch diets; effect of fasting before killing the animals and incubating the tissues. 103–112 days on diet; tissues incubated with (U-^{14}C) glucose.

Table II. Liver lipogenesis and glucose oxidation after 24 h fast following 21–22 days on diet (5 rats per group)

Diet	Lipid counts/min/mg			CO_2 counts/min/mg		
	(U-^{14}C) glucose	(1-^{14}C) glucose	Ratio CU/CI	(U-^{14}C) glucose	(1-^{14}C) glucose	Ratio CU/CI
Starch	142	89	1.6	869	897	1.0
	(110–189)	(69–133)	(1.4–2.0)	(767–1,030)	(708–1,107)	(0.7–1.2)
Sucrose	56	35	1.7	427	624	0.7
	(43–74)	(26–42)	(1.6–1.8)	(328–508)	(525–760)	(0.6–0.8)
Depression	0.4	0.4	1.0	0.5	0.7	0.7
Significance P	0.004	0.004	–	0.004	0.016	0.016

Experiment 3b. Influence of dietary fat: it was observed (unpublished) that the replacement of starch by sucrose in a fat-free diet resulted in a depressed growth rate and that this could be restored to normal by adding the small amount of corn oil, 0.56%, that is present in corn starch. In exp. 3b rats of the MRC colony were fed for 105 to 112 days on fat-free diets containing (a) starch (3 rats), (b) sucrose (4 rats), and (c) sucrose plus 0.56% corn oil (4 rats). In a confirmatory experiment an additional five rats were fed on each of the three diets for 53–64 days before being examined.

Liver: table IIIA shows that glucose oxidation and lipogenesis were depressed by sucrose both in a fat-free diet and with the addition of 0.56% corn oil, although all the differences are not significant because of the scatter of results. The confirmatory experiment verified there results and table IIIB shows that all the differences were significant.

Adipose tissue: glucose oxidation was not affected by sucrose feeding as was found previously with the same strain on a fat-containing diet (table IIIC). The addition of 0.56% corn oil gave results which were not significant. In the absence of fat sucrose had no depressant effect on lipogenesis, in contrast with the previous experiments in which 8% arachis oil had been included in the diet.

Liver and carcass composition: the livers of animals from exp. 1, 2 and 3 were analysed for fat and glycogen and the carcasses were analysed for fat and water content. Tables IV, V and VI show that the type of dietary carbohydrate did not affect these values.

Table III. Effect of feeding sucrose in a fat-free diet

Diet	No. of rats	Lipid count/min/mg			CO_2 count/min/mg		
		(U-^{14}C) glucose	(1-^{14}C) glucose	CU/CI	(U-^{14}C) glucose	(1-^{14}C) glucose	CU/CI
***A.* Liver lipogenesis and glucose oxidation: 105-112 days on diet**							
Starch	3	36 (27-45)	30 (21-35)	1.2 (1-1.4)	129 (115-144)	220 (186-264)	0.6 (0.5-0.7)
Sucrose	4	22 (18-25)	16 (12-21)	1.4 (1.2-1.5)	83 (70-96)	149 (124-197)	0.7 (0.5-0.7)
	depression	0.6	0.5		0.6	0.7	
	P	0.028	0.028	NS	0.028	NS	NS
Sucrose + 0.56% corn oil		26 (18-34)	18 (15-23)	1.5 (1.3-1.6)	87 (70-103)	183 (158-212)	0.5 (0.4-0.6)
	depression	0.7	0.6		0.7	0.8	
	P	0.028	NS	NS	0.028	NS	NS
***B.* Confirmatory experiment 53-64 days on diet**							
Starch	5	62 (43-90)	45 (26-63)	1.4 (1.3-1.6)	191 (161-211)	279 (225-324)	0.7 (0.6-0.8)
Sucrose	5	30 (22-39)	21 (11-27)	1.5 (1.3-2.0)	101 (61-155)	141 (99-176)	0.6 (0.5-0.8)
	depression	0.5	0.5		0.5	0.5	
	P	0.004	0.008	NS	0.004	0.004	NS
Sucrose + 0.56% corn oil		33 (26-41)	25 (18-37)	1.3 (1.1-1.7)	96 (79-126)	226 (139-303)	0.4 (0.3-0.6)
	depression	0.6	0.6		0.5	0.8	0.6
	P	0.004	0.016	NS	0.048	0.004	0.016
***C.* Lipogenesis and glucose oxidation in adipose tissue: 113 days on diet**							
Starch	3	15 (13-17)	15 (14-18)	1.0 (0.9-1.1)	11 (5-17)	12 (18-19)	1.2 (0.9-1.5)
Sucrose	4	16 (10-22)	17 (10-27)	1.0 (0.8-1.0)	13 (10-16)	10 (8-16)	1.1 (1.0-1.3)
Sucrose + corn oil	4	15 (11-19)	12 (7-27)	1.3 (0.7-1.6)	7 (3-10)	8 (3-15)	0.8 (0.5-1.4)
Differences		NS	NS	NS	NS	NS	NS

Table IV. Liver fat content after feeding starch or sucrose

Exp.	Animals	Percent fat in liver	
		Starch diet	Sucrose diet
1	MRC, weanling M	20.9	20.5
2 a	Roebuck, adult M	23.1	25.0
	weanling M	27.5	27.5
2 b	Lister, weanling M	20.5	18.6
2 c (part)	Lister, weanling M	22.1	21.2

No significant difference between diets

Table V. Carcass fat and water after feeding starch or sucrose (Lister colony)

Exp.	Days on diet	Diet	No. of rats	Water %	Fat %
2 c	14	Starch	3	65.6	29.5
		Sucrose	3	64.8	31.0
2 c	116–112	Starch	12	59.4	38.0
		Sucrose	12	59.5	34.0
3 a	67	Starch	9	61.2	35.5
		Sucrose	8	61.5	35.6

No significant difference between diets

Table VI. Liver glycogen content after feeding starch or sucrose (exp. 3a)

	Diet	No. of rats	Days on diet	Liver weight g	Glycogen %
Unfasted	starch	3	67	9.2 (8.0–10.4)	5.4 (4.1–6.7)
	sucrose	3	67	9.2 (8.0–10.0)	6.0 (4.7–7.4)
Fasted 24 h	starch	6	67	5.7 (5.0–6.5)	1.9 (1.4–2.5)
	sucrose	6	67	5.9 (4.0–7.4)	1.4 (1.1–1.7)
Unfasted	starch	8	22	6.1 (5.3–7.6)	5.8 (4.3–7.3)
	sucrose	8	22	6.5 (5.5–7.5)	6.0 (4.4–8.1)

No significant differences between diets

Experiment 4. Relative proportions of type of lipid synthesised: 8 rats were fed for 3 weeks on each of the two diets and samples of the epididymal fat pads were incubated with radioactive glucose. The extracted fat was separated into the main types of lipids, namely

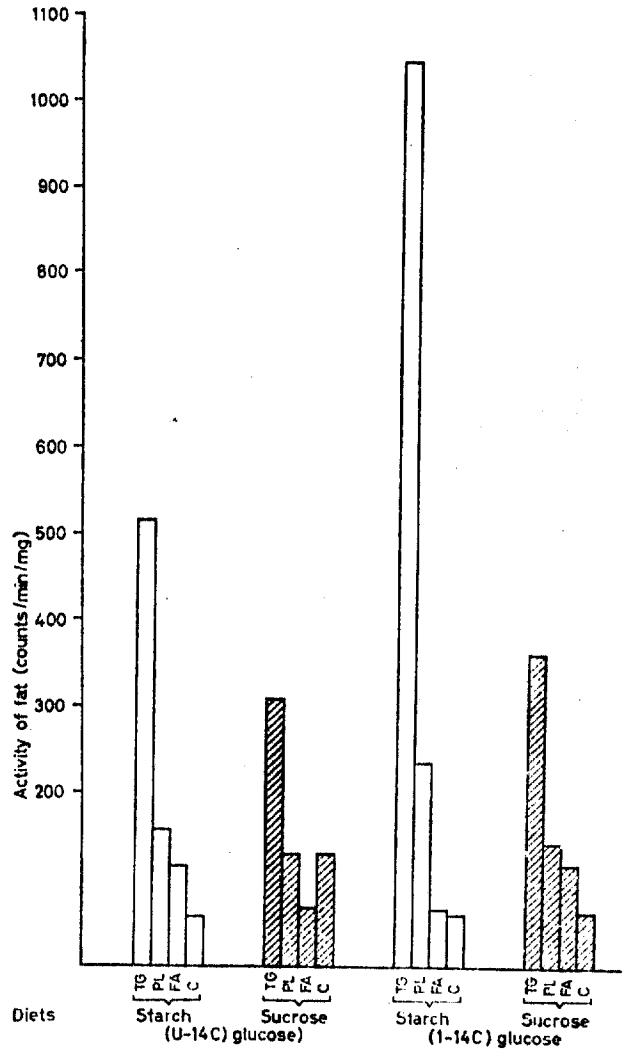

Fig. 5. Radioactivities of the different types of fat synthesised from radioglucose during the 3 hour incubation period. *TG* Triglycerides, *PL* phospholipids, *FA* free fatty acids, *C* cholesterol.

triglycerides, phospholipids, free fatty acids and cholesterol, by thin layer chromatography. The individual spots were visualised, removed from the glass plate and their radioactivity measured. Figure 5 shows the proportions of radioactivity found in each of the fractions. As, in most instances, the amount of radioactivity was very small, the counts obtained cannot be regarded as being accurate but the results indicate that the reduction in lipogenesis induced by feeding sucrose mostly, if not entirely, affects the triglyceride fraction.

Experiment 5. Lipogenesis *in vivo*: 7 rats were fed on each of the two diets for 16-31 days and then injected i.p. with radioactive glucose. They were killed 4 h later, the liver, kidneys, adipose tissue and a sample of blood were removed, the fat extracted and its radioactivity measured. Table VII shows that the count of the fat from

Table VII. Effect of dietary carbohydrate on tissue composition and *in vivo* lipogenesis (13 rats on each diet)

	Starch diet		Sucrose diet		Difference
Liver wt. (g)	6.1	(4.8–9.5)	6.9	(4.8–9.5)	NS
Percent body wt.	4.4	(3.9–4.9)	4.7	(3.9–5.6)	0.025
Fat as % dry wt.	16.4	(11.1–19.9)	16.4	(12.7–20.8)	NS
Radioactive count (/min/mg dry wt.)	35	(21–54)	33	(20–46)	NS
Kidney wt. (g)	1.18	(0.94–1.68)	1.52	(1.06–1.87)	0.025
Percent body wt.	0.84	(0.78–0.93)	0.92	(0.81–1.01)	0.001–0.01
Fat as % dry wt.	22.7	(20.6–25.1)	22.0	(19.4–26.1)	NS
Radioactive count (/min/mg dry wt.)	36	(21–48)	25	(16–43)	0.01–0.001
Carcass water (%)	69.3	(65.3–71.5)	69.6	(67.7–71.4)	NS
fat (%)	24.5	(16.2–35.2)	21.5	(13.5–26.1)	NS
Adipose tissue					
Radioactive count (/min/mg dry wt.)	220	(116–525)	102	(29–326)	0.01–0.001
Blood lipids					
Radioactive count (/min/ml)	318	(204–374)	366	(123–798)	NS

the liver and blood was not affected by sucrose feeding but that of the kidney and adipose tissue were significantly less, being 70% and 46% respectively, lower than on the starch diet.

Table VII also shows that sucrose feeding significantly increased liver weight and the weight of the kidneys (when expressed as a percentage of body weight). The fat content of these organs and of the carcass was not affected by sucrose feeding, in agreement with the results of the earlier experiments shown in tables IV, V and VI.

Discussion

The relative importance of the several different metabolic pathways that exist in animal tissues, such as the Embden-Meyerhof-Parnas glycolytic pathway, the glucuronic acid cycle and the direct oxidative pathway, is not known. Various dietary changes can produce considerable alterations in the levels of many of the enzymes involved in these pathways. In particular a change in the type of carbohydrate fed can do so [HILL, BAKER and CHAIKOFF, 1954; FITCH and CHAIKOFF, 1960; AITKEN *et al.*, 1967; BARTLEY *et al.* 1967]. It is possible, therefore, that the physiological effects observed after feeding sucrose might be explained by the changes in enzyme levels resulting in changes in the major routes of metabolism.

In the present series of experiments it was observed that dietary sucrose, as compared with starch, resulted in a change in the ratio of carbon dioxide produced *in vitro* from the two forms of radioactive glucose used, which indicates a change in metabolic pathways. This could be explained by the results of FITCH, HILL and CHAIKOFF [1959], namely that fructose was more effective than glucose in augmenting levels of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which might result in increased utilisation of the oxidative pathway.

That changes in the pathways might influence fat synthesis was suggested by Bloom [1955] and FELTS, DOELL and CHAIKOFF [1956] on the grounds that oxidation of glucose via the direct oxidative pathway produces reduced NADP, the form in which the coenzyme is required for fat synthesis. The present work indicates that there has been some change in pathways but there is no evidence of the direc-

tion of that change, and, at the same time, there was a depression of lipogenesis.

The observation that dietary sucrose depressed the rate of oxidation of glucose might be explained by a decrease in the pool of glucose-6-phosphate as several workers have shown that fructose, but not glucose, increased the levels of glucose-6-phosphatase [FREEDLAND and HARPER, 1957, 1958; FITCH *et al.*, 1959; LANDAU, HASTINGS and ZOTTU, 1958]. FREEDLAND and HARPER [1957] also showed that fasting increased the level of glucose-6-phosphatase in the liver and in the present experiments lipogenesis fell on fasting.

In vivo observations. The depression of liver metabolism induced by dietary sucrose that was observed *in vitro* was not apparent *in vivo*. This may have been due to the different techniques. *In vitro* the tissue is supplied with a surplus of nutrients and its maximum ability to function is being measured. If the tissue is not functioning at maximum capacity *in vivo* then any reduction below its maximum would not become evident until it fell low enough to limit the rate of reaction. On the other hand the depression of function of the adipose tissue was observed both *in vivo* and *in vitro*, and that of kidney, which was not examined *in vitro*, was depressed *in vivo*.

Effect of dietary fat. Liver metabolism, as measured both by *in vitro* lipogenesis and glucose oxidation, was depressed by feeding sucrose in diets with and without fat. In adipose tissue, however, lipogenesis was not depressed by a dietary sucrose on the fat-free diet in contrast with diet containing 8% arachis oil. (The addition of 0.56% corn oil did not cause any significant change.)

These results in adipose tissue are in general agreement with those of GIORGIO, BONANNO and HEGSTED [1962] who showed that small amounts of fat in the diet, 5%, depressed fat synthesis in adipose tissue *in vitro*. These authors showed that fat synthesis proceeded at its maximum rate when the diet contained 0.8% of fat but lower levels were not examined.

Other workers have shown that dietary fat affects lipogenesis in the liver. For example JANSEN, ZANETTI and HUTCHINSON [1966] showed that dietary fat depressed lipogenesis and CARROLL [1963] reported that while dietary sucrose increased the levels of certain hepatic enzymes the extent of the change was influenced by the type of fat in the diet. Sucrose plus corn oil increased the level of glucose-6-phosphatase by 61%, but with hydrogenated coconut oil the increase

was only 43%. Fructose-1-6-diphosphatase was increased by 35% by sucrose plus corn oil but was not affected by sucrose when the dietary fat was hydrogenated coconut oil.

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Evidence for a Monoclonal Origin of Human Atherosclerotic Plaques

(glucose-6-phosphate dehydrogenase/heterozygotic females/aorta)

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ABSTRACT **The main cellular elements of atherosclerotic plaques are smooth muscle cells. Because these plaques differ from their precursors in the underlying artery wall in several ways, we have asked the question: Are human atherosclerotic plaques polyclonal or monoclonal in their origin? The X-linked glucose-6-phosphate dehydrogenase (EC 1.1.1.49) in heterozygotic females has been used to obtain an answer. 30 Plaques of different degrees of complexity and 59 samples of normal aorta and iliac artery walls from four females, 25–79 years old, were investigated. The data show that fibrous caps even of relatively large atheromatous plaques, 0.5 cm or greater in diameter, are composed of cells that produce solely or predominantly one enzyme type, whereas samples of artery wall media and intima as small as 0.1 mm³ are regularly composed of a mixture of cell types. If plaques were a response to injury akin to a healing wound, a reaction to a growth stimulant, or formed due to an organization of a mural thrombus, they would be expected to be polyclonal. Hence, the results imply that atherosclerotic plaques in human beings arise by another mechanism. The mechanism compatible with the monoclonal nature of atherosclerotic plaques is mutation, and the likely causes are chemical mutagens or viruses.**

Atherosclerosis is a progressive disease of major arteries in human beings. The characteristic lesion of atherosclerosis is the fibrous plaque composed mainly of smooth muscle cells surrounded by a dense collagenous stroma. In advanced lesions it has an underlying layer of lipid debris resembling in its composition the blood lipids. Virchow ascribed the origin of this formation to irritation and subsequent inflammation of the lining of the vessel wall. Rokitansky believed that organization by connective tissues of a mural thrombus, built originally of fibrin, leads to plaque formation. The evidence that cholesterol and/or other blood lipids are altered in some persons with atherosclerosis and coronary artery disease has led to the notion that lipid penetration into the artery wall is the source of the irritant. Chemical, mechanical, and nutritional manipulations have been used in animals in an effort to reproduce lesions like those of atherosclerosis in man; none of these experimental lesions yields wholly satisfactory copies of lesions of the human disease.

Spontaneous atherosclerosis occurs in chickens and, as we have found, produces lesions that strikingly resemble those of man (1, 2). Comparison of the naturally occurring atherosclerotic plaques of human beings and birds with the response of the mammalian aorta to irritation by a suture (3) or in more recent experiments by mechanical injury with a balloon catheter, led us to consider an alternative to the injury–repair hypothesis based on the following considerations: cells of spontaneous lesions differ from cells of normal artery wall and cells populating a repair site. They differ in size, in composition of associated extracellular material, e.g., a preponderance in fibrous plaques of collagen as opposed to elastin, and in the absence of intercellular junctions. These features suggest two possibilities: either the cells of atherosclerotic plaques derive from a population of cells different from those of the normal arterial media or they are transformed cells. If the latter is so, cells of atherosclerotic plaques, like those of the benign smooth muscle tumors of the uterus, could be expected to be monoclonal.

Linder and Gartler examined the nature of the cell populations in benign uterine smooth muscle tumors by investigating the pair of X-linked glucose-6-phosphate dehydrogenase (EC 1.1.1.49) isoenzymes (4). Use of the isoenzymes in the analysis depends upon the fact that there is random inactivation of one or the other of the two X chromosomes in female cells early in embryonic development. Thus, the mammalian female is a mosaic of two cell types, each type having one or the other of the pair of X chromosomes active. About one-third of black females in the population are heterozygous for glucose-6-phosphate dehydrogenase and exhibit mixtures of the A and B enzymes in tissue extracts as the expression of this cellular mosaicism. Because the two cell populations reproduce true to type throughout somatic growth, it is possible to use them as tracers in various studies of development in embryonic systems and of origins of pathologic new formations (5). We report here that individual atherosclerotic plaques from various regions of the human aorta and common iliac arteries exhibit enzyme patterns consistent with their origin as monoclonal growths.

PATIENTS AND LESIONS

Patient 1 was a 31-year-old woman who died of the renal complications of disseminated lupus erythematosus. The lower abdominal portion of the aorta and the common iliac arteries showed early, extensive, typical atherosclerotic intimal plaques having lucent, pearly-grey fibrous character with obvious patches of yellow lipid. Microscopically the lesions were composed of masses of smooth muscle lying entirely on the intimal side of the internal elastic membrane. Some of the smooth muscle cells and a few cells resembling macrophages were shown to contain lipid by fat stains; focal areas of calcification were present in many of the plaques. Cholesterol crystals were present in some cells, and small amounts were detected in some extracellular locations. *Patient 2* was a 79-year-old woman, dead of an endometrial carcinoma. A segment of upper abdominal aorta, received frozen, had raised atheromatous plaques with thick fibrous caps and underlying

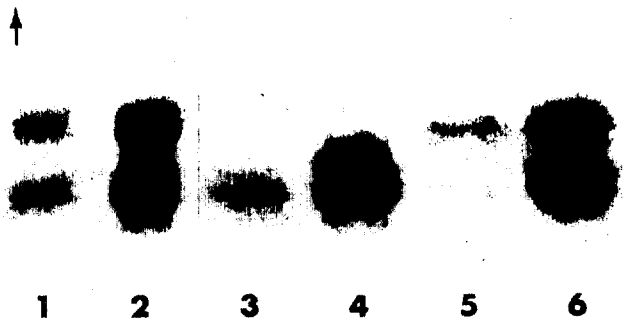

FIG. 1. Zymograms on cellulose acetate of samples from (1) blood; (2) normal tissue; (3–6) atherosclerotic plaques of *Patient 1*.

lipid debris containing cholesterol crystals. Flatter yellowish plaques were present, and more than half of the surface had discrete, grossly visible lesions. *Patient 3* was a 25-year-old woman who died with extensive sarcoidosis. The upper aortic abdominal segment had no grossly discernible atherosclerotic lesions. *Patient 4* was a 62-year-old woman who died of hypertensive cardiovascular disease with left ventricular hypertrophy and had half the surface of her upper abdominal aortic intimal surface studded with typical atheromatous plaques.

METHODS

Aorta and common iliac arteries removed at autopsy 20 hr after death from *Patient 1* were freed of most of the adventitial fat and opened, and gross clots were removed from the endothelial surface. The surface was gently wiped free of any residual blood clots with surgical gauze sponges. Upper and lower segments of the aorta, separated at the level of the diaphragm, were each placed, adventitial side down, on a piece of cardboard. Exposed intimal surfaces were covered with Parafilm to protect them from drying, and the tissues were kept in a covered plastic container placed on ice or in the refrigerator between samplings. Small pieces of blotter, sparingly moistened with water, were placed in the ends of the chamber to prevent gross desiccation of the tissue. Water condensation on the Parafilm covering the vessel surface was removed periodically to prevent its dripping onto the surface. Kept in this fashion, the material yielded useful results for 6 days after death and satisfactory histology after fixation.

Under 3-times magnification with a fine-pointed forceps and iridectomy scissors, samples 1–3 mm^3 in size were removed from various portions of plaque, from grossly plaque-free intima, and from media of the vessels. To the samples in small, round plastic culture dishes, 2–5 μl of membrane buffer (pH 9.1)* was added. Membrane and chamber buffers were those described by Sparkes *et al.* (6), diluted to 10/11 of the described concentration to increase resolution of enzyme bands. Samples were then frozen in the covered dish and kept on dry ice. Enzyme in samples so prepared was stable for more than 6 hr; it was extracted by thawing and refreezing the tissue three times. Once extracted, the enzyme was less stable. Electrophoresis of enzyme on cellulose acetate was performed in a Beckman Microzone apparatus at 400 V for 50 min. Applied sample size ranged from 0.25 to 1.25 μl. Enzyme was developed with the substrate mixture applied to filter paper (6).

Segments of the aorta from *Patients 2, 3,* and *4* were placed on cardboard and frozen immediately at autopsy, packed in dry ice for shipment, and maintained in the frozen state with dry ice during sampling at −15°. Pieces of tissue (0.1–3.0 mm^3) were cut out of the surface with a pointed scalpel blade. In several instances, much larger samples (10–30 mm^3) were removed from the fibrous cap of plaques. Samples were treated otherwise in the same manner as the fresh tissue from *Patient 1*. For quantitative measurements the patterns were scanned in a Beckman Analytrol, modified to take the small cellulose acetate strips.

OBSERVATIONS

Examples of the enzyme patterns from blood, normal tissue, and four individual plaques from *Patient 1* are shown in Fig. 1. These patterns reveal the following: The patient's blood and a sample of media, shown in panels **1** and **2**, respectively, contained equal proportions of A- and B-type enzymes, indicating equal numbers of A- and B-type cells. The plaques sampled for patterns **3** and **4** clearly present enzyme largely of the B type, whereas the tissue enzyme shown in panel **5** is clearly derived mainly from cells having the A-type enzyme. The sample shown in panel **6**, derived from a fourth plaque, is an example of a mixture of nearly equal proportions of enzymes.

81 Samples from 30 plaques and 59 samples from uninvolved intima and media have been assayed. The results are given in Table 1. As a means for comparison of variation in the composition of the aortic wall and for measurement, in some plaque samples, of the relative proportions of enzymes, 59 normal wall samples and 44 plaque samples were scanned photometrically, and the relative areas of the two peaks were determined by planimetry. 19 Normal samples from *Patient 1* yielded a mean value of $A = 0.52 \pm 0.066$ (SD), 7 samples from *Patient 2* had a mean of $A = 0.25 \pm 0.050$, 13 samples from *Patient 3* yielded a mean of $A = 0.36 \pm 0.065$, and 12 samples from *Patient 4* gave a value of $A = 0.42 \pm 0.075$. Enzyme patterns showing alterations equal to or exceeding three-times the standard deviation on either side of the mean were considered different from normal and substantially of

TABLE 1. *Phenotypes of normal vessel wall and fibrous caps of atherosclerotic plaques*

Patient number	Number of sites showing enzymic phenotype							
	Normal				Plaques			
	AB	A	B	Total	AB	A*	B*	Total
1	25	0	2	27	3	4	8	15
2	7	0	0	7	2	1	6	9
3	13	0	0	13	—	—	—	—
4	12	0	0	12	1	3	2	6
Total	57	0	2	59	6	8	16	30

* A plaque was judged to be solely or predominantly of one type, *A* or *B*, when one or more samples yielded an *A* enzyme fraction ≥3 standard deviations from the mean value for the case. —, no plaques present.

* Buffer contains 124 mM Tris–3.6 mM EDTA (disodium salt)–14 mM boric acid–65 μM NADP.

one population. Four plaques from *Patient 1*, three from *Patient 2*, and four from *Patient 4* were considered solely of one population type (>95% of one enzyme type).

In order to be certain that the apparent diminution or absence of A enzyme in several of the plaques from *Patient 2* was not merely because of its relatively low concentration, samples from three large plaques of 20–30 mm^3 were removed, minced, and treated with $^1/_2$ their volume of membrane buffer, and several applications were made to the cellulose acetate strip. The B-enzyme band was strongly stained, but insignificant amounts of type-A enzyme appeared in two of the samples and none in the third.

A direct comparison was made of tissue enzymes from fibrous caps and media underlying the yellow atheromatous material of two plaques from *Patient 4*. In both instances, the media yielded both A and B enzymes, whereas the plaques clearly contained one enzyme type. Additionally, in these assays tissue sample size was reduced to 0.1–0.2 mm^3 to try further to refine the patch size.

DISCUSSION

The experimental question posed at the outset was: "Do atherosclerotic plaques exhibit the features of an homogeneous, single-cell-derived population, or do they represent a mixed population similar to that of the person's cell mixture?" The answer depends in part upon the size of mosaic patches in the individual and her specialized tissues. Sizes of mosaic patches have been shown to vary among different tissues (7): the epidermis has a higher variability than other tissues; blood and connective tissues appear least variable. Mosaic patterns in vessel walls have not previously been examined. Our data from all four patients presented above indicate that the mosaic patch size in the aorta is small, <0.1 mm^3, and at least as small as that of the myometrium (4). Against this background, the appearance in atherosclerotic plaques of a marked preponderance or sole presence of one or the other cell type appears significant. Statistical evaluation of the data from *Patients 1*, *2*, and *4* was made by the Chi-square test of independence (8); P values were obtained for *Patient 1* of <0.0001, for *Patient 2* of <0.001, and for *Patient 4* of <0.005. The tests were performed using Yates' correction, and hence are conservative.

The data seem clear, although it was originally anticipated that blood contamination and leukocyte or macrophage infiltration might frustrate the investigation. To some extent, this background noise is seen in the zymograms of plaque material as trace contamination with the second enzyme. It may have also, in some instances, led to assignment of a plaque to the "mixed" category.

Appearance of a monoclonal enzyme pattern in 2 of the 27 samples taken from presumptively normal artery wall of *Patient 1* is somewhat deviant from the remaining control data. Histologic examination of the sites in both instances revealed slight intimal thickening, but not a definitive plaque. The other data from nonatherosclerotic arterial wall argue strongly against these being mosaic patches. The possibility exists that we were looking at two very small initial foci of plaque formation, a supposition consistent with the widespread early disease in this patient.

One may raise the question whether or not wound healing is initiated from proliferation of a single cell. Several pieces of evidence argue that it is not: it is the general observation that wounds heal by simultaneous proliferation from all margins. Furthermore, in the case of a very small wound in the mammalian aorta we have observed simultaneous mitoses in many cells isolated from each other by the elastic laminae (3). Finally, it has been found that a hypertrophic scar of the skin is polyclonal (Linder, personal communication).

It can be argued that the reason for the single-cell type in the plaque is not its monoclonal origin, but is due to some process selecting from one or the other of the two cell types. Although this is possible, the fact that some plaques are composed of only A cells and others of only B cells argues against the activity of a process of selection involving cells with these enzyme types.

Another concept is reasonable to consider, namely, that during proliferation of cells in response to injury, a single cell develops a selectional advantage, and its progeny thereafter dominate the process. This type of process seems quite compatible with the finding of a monoclonal character of the atherosclerotic plaques. The commonly accepted reason for development of such a selectional advantage is an alteration in a cell genome, i.e., a mutation. The observation that cells of atherosclerotic plaques differ, as pointed out above, from cells of the normal arterial wall in regard to size, to the proteins that they synthesize and deposit locally (i.e., collagen rather than elastin), and the apparent deficiency of cell to cell contacts coupled with their monoclonal character seems to fit with the mutation concept.

Along with the appearance of a monoclonal character of a given plaque, several features consistent with known aspects of atherosclerosis became evident during the study. For example, in *Patient 1* attention was directed to small plaques by appearance in samples of single enzyme types; their presence was verified by histological examination. Apparent confluence of plaques is one of their well-known pathological features. One site originally appeared to be a single large plaque; it yielded two distinct monoclonal patterns, A and B. On closer examination of the plaque, two interdigitated plaques became evident. This also offers an explanation for the plaques of a fully mixed pattern (e.g., sample **6**, Fig. 1): Plaques frequently exhibit layering. This has been ascribed to organization of mural thrombi. Our interpretation of these is that the layers occur in three dimensions, just as we found with interdigitations in two dimensions on the surface.

Certain sites of predilection for plaque formation in atherosclerosis exist. These are near branch orifices and on certain aspects of particular arteries. At least some of these regions now appear to be sites of high turnover of endothelial cells (9), and there is evidence for assuming higher turnover of muscle cells in the underlying wall. While this higher rate of mitotic activity may not by itself be the cause of true plaque formation, anything that increases the rate of proliferation may increase the risk of induction of cell transformation by another agent, such as virus or chemical mutagen. Acceleration of atherosclerosis in the presence of elevated concentrations of blood lipids can be viewed in this way, since it has now been found that elevation of blood cholesterol in young swine by feeding is followed in the arterial wall by increased proliferation of endothelium and smooth muscle cells (10), and we now believe that the mechanical stress of hypertension may do likewise.

In addition to the morphological and functional alterations of cells, the progressive enlargement of atherosclerotic plaques

and their tendency to degenerate and to ulcerate are all compatible with the presence of a transformed cell population.

Although more extensive data are needed, our observations make it reasonable to reconsider the theories of etiology and of pathogenesis of progressive atherosclerosis. The monoclonal character of atherosclerotic plaques indicates that consideration should be given to factors that transform cells, such as chemical mutagens or viruses, or both.

We thank Dr. S. M. Gartler for his helpful and critical comments during the work and Dr. H. Roman for reading and commenting on the manuscript. Dr. Ruth Wong of the Illinois School of Medicine and Dr. Paul Szantos of Cook County Hospital generously provided us with some of the tissues for the investigation. Supported in part by USPHS Grants HE-03174, GM-13543, and the Greenwald Fund.

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Preliminary Communications

Sugar Consumption in Acne Vulgaris and Seborrhoeic Dermatitis

Brit. med. J., 1967, 3, 153–155

There is little concrete evidence that diet plays a part in the aetiology of disorders of the skin, except in nutritional deficiency diseases such as pellagra and kwashiorkor. Nevertheless, many authorities implicate diet—dietary excess rather than dietary deficiency—as an aetiological agent, notably in acne vulgaris and, to a less extent, in seborrhoeic dermatitis. Consequently, changes in diet are frequently recommended as part of the treatment of these diseases. Of 10 recent textbooks, diet is mentioned in 9 in connexion with the cause or treatment of acne, and in 6 in connexion with seborrhoeic dermatitis. In neither disease are the authors unanimous; most frequently mentioned, however, is an excessive intake of fat or of carbohydrate, or of both. In addition chocolate is several times specifically mentioned in connexion with acne.

There are three criteria by which one tests the hypothesis that a dietary component is implicated in the causation of a disease (Yudkin, 1953). Firstly, there should be evidence that the diet of persons with the disease differs significantly from that of persons without the disease. Secondly, the symptoms and signs should be those that are known to be, or at least are plausibly suspected of being, caused by dietary imbalance. Thirdly, correction of the dietary imbalance should result in correction of the signs and symptoms.

In this paper we bring evidence of the first sort: that patients with seborrhoeic dermatitis consume significantly more sugar (sucrose) than persons without the disease. We believe that this is the first demonstration of a dietary difference in such patients. We have also shown that there is no such difference in patients with acne.

If the excessive amounts of a dietary constituent are thought to be a possible cause of disease, there are several reasons—evolutionary, historical, and metabolic—why sugar should be regarded as a likely candidate (Yudkin, 1963). It happens, too, than in practice it is more feasible to assess sugar intake than the intake of any other dietary constituent (Yudkin and Roddy, 1966). For these reasons, we measured the sugar intake of patients and control subjects with a short questionary that could be completed in 10–15 minutes by each of the subjects (see Appendix by J. Yudkin below).

The patients were referred to one of us at the skin clinics held at two hospitals. Seborrhoeic dermatitis was diagnosed when the patient presented with severe scaling of the scalp, together either with itchy scaly patches of the sternal or interscapular region or about the eyelids or nasolabial folds, or with intertrigo of the axillae, groins, or retroauricular areas, or with both types of lesions. All the patients had had previous

attacks, and the current attack had lasted at least one month. All except one of the 16 patients were males; the ages ranged from 14 to 68.

Acne was diagnosed on the basis of comedones on the face, back, or chest, together with papules, pustules, or cystic lesions. There were nine male and seven female patients, aged between 15 and 27.

The patients with acne and dermatitis were all those who were seen during the period of study, except that, as usual, we did not include in our final assessment those whose sugar consumption, by admission or by implication, had changed.

As control subjects we used two different groups of people. The first were patients suffering from warts and attending the same skin clinics. Again, they were selected only on the basis of their diagnosis and on the apparent constancy of their sugar consumption. They were listed in the order in which we saw them, and from the list we then selected those who matched, as well as possible, the age and sex of the patients with acne or the patients with dermatitis. This was important, because we have found a difference in sugar intake between men and women and at different ages (Salter and Yudkin, to be published). It was, however, possible to find only 13 control patients of appropriate age for the 16 patients with seborrhoeic dermatitis. Moreover, it was not possible to match the distribution of male and female control patients with the distribution of the nine male and seven female patients with acne. For these reasons we used a second group of controls for each disease. These were apparently healthy men and women, either from a London factory or from a large London office. Their sugar intake had been measured during a large-scale survey that was being carried out for other purposes. This second group of controls was also selected from the list of subjects, made in the order in which they were seen, by choosing the first subject in the list that matched in age and sex the first patient with acne or dermatitis, then the first subject that matched the second patient, and continuing until all the patients were matched with apparently healthy control subjects.

Results

The intake of sugar by the patients with acne was not significantly different from that of either group of control patients (Table I). On the other hand, the intake of sugar by the patients with seborrhoeic dermatitis was significantly higher than that of either group of control patients (Table II). This was true both for total sugar and for the sugar taken in tea and coffee ("beverage sugar"). Statistical analysis was carried out with the Mann–Whitney test for Control group 1, and with the Wilcoxon Matched-Pairs Signed-Ranks Test for Control group 2 (Siegel, 1956).

TABLE I.—*Sugar Intake of Subjects With Acne and of Control Subjects (Control 1: Patients With Warts; Control 2: Healthy Factory and Office Workers)*

Group	No.	Age (Mean, Years)	Sugar Intake (g./day)			
			Total		Beverage	
			Mean	Median	Mean	Median
Acne ..	16	19·5	121	103	65	70
Control 1	16	20·0	111	97	47	48
,, 2	16	19·7	120	118	63	55

No significant differences in sugar intake between groups ($P > 0·05$).

TABLE II.—*Sugar Intake of Subjects With Seborrhoeic Dermatitis and of Control Subjects (Control 1: Patients With Warts; Control 2: Healthy Factory and Office Workers)*

Group	No.	Age (Mean, Years)	Sugar Intake (g./day)			
			Total		Beverage	
			Mean	Median	Mean	Median
Dermatitis	16	38·2	170	185	104	109
Control 1	13	36·5	108	109	65	56
,, 2	16	38·1	110	110	61	57

Significant differences.—Total sugar intake: dermatitis and control 1—$P < 0·01$. Dermatitis and control 2—$P < 0·02$. Beverage sugar.—Dermatitis and control 1—$P < 0·02$. Dermatitis and control 2—$P < 0·05$.

Discussion

The numbers of patients with either skin disease was small, chiefly because we made every effort to choose those in whom the diagnosis was not in doubt. Apart from this, there was no selection either of the patients with skin disease or of those acting as controls. The selection of the second group of control subjects from the factory and the office was only on the basis of age and sex. The lists of the patients and of the healthy control subjects had been drawn up in the order in which they were seen. This latter list was scanned from the beginning until a subject was found that matched in age and sex the first patient. This procedure was repeated with the next patient, and continued until all the patients had been matched.

In spite of the small number of subjects, we found a significantly higher intake of sugar in patients with seborrhoeic dermatitis than in control subjects. We believe, therefore, that this represents a real difference. We cannot of course say whether or not this relation between disease and sugar intake is a causal one. The findings, however, do suggest that it would be worth while to determine in a controlled experiment the effect of a reduction of dietary sugar on the course of the disease.

Our findings indicate that patients with acne have an intake of sugar no different from that of control subjects. This does not of course mean that diet is not involved in the causation of the disease or that changes in diet would not affect its progress. But further and more elaborate investigation would be required to test this possibility.

Summary

The consumption of sugar (sucrose) by patients with seborrhoeic dermatitis was found to be significantly higher than that of two groups of control subjects. On the other hand, the consumption of sugar by patients with acne vulgaris was found to be no different from that of control subjects.

Mrs. Jill Morland was in receipt of a personal grant from the Medical Research Council while this work was being carried out.

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Appendix: Measurement of Sugar Consumption by Questionary

We have reported several studies in which we have measured the consumption of sugar (sucrose) by a method based on a simple questionary. The original method involved the completion of the form by an interviewer (Yudkin and Roddy, 1964). More recently, the questionary has been simplified further so that it can be completed by the subject himself (Yudkin and Morland, 1967). We have given reasons for our belief that the method is reliable (Yudkin, 1964) and we have more recently tested its reliability against the method that

...lees the subject recording in a diary all the food and drink ...med over a period of seven days (Yudkin and Roddy, ...).

The simplified questionary is set out below. In all of our ...es we have attempted to consider only those persons who, so ... as we can ascertain, have had a constant sugar consumption ... many years. For this reason we include questions relating ... "special" diets, and we eliminate from our final assessments ... subjects who we have reason to believe have changed their ...ar consumption.

The calculation of the amount of sugar in prepared foods ... drinks is made from analyses published in food tables or ... figures supplied by manufacturers. As for sugar itself, ... take a heaped teaspoon as containing 6 g., a level teaspoon ..., a heaped dessertspoon 15 g., and a level dessertspoon 10 g.

I am grateful to Janet Roddy and Jill Morland, who helped to ...vise and test this questionary, and to the Medical Research ...uncil, who supported the work.

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DIETARY QUESTIONARY

NAME ..... AGE .....

N.B.—IF THERE IS AN ALTERNATIVE MARKED* PLEASE CROSS OUT THE ONE WHICH DOES NOT APPLY

If your weight has increased in the last few years, are you making a serious effort to check or decrease it? Yes/No*

If "Yes," are you restricting sweet or sugary foods, or sugar? .....
.....
.....
.....

If you are on a special diet *now* why are you on this diet? .....
.....

How long have you been on this diet? .....

Have you *ever* been on any other special diet? Yes/No*

If "Yes"
Why were you on this diet? .....
Approximately when did you start this diet? .....
How long did it last? .....

Did your eating habits have permanently changed as a result of being on the diet, in what way? .....
.....

Go through an average day in your mind, and write down how many cups of tea and coffee you consume:

	Tea	Coffee	Other hot beverages (Cocoa, chocolate, etc.)
Before breakfast	..... cups	..... cups	..... cups
At breakfast	..... cups	..... cups	..... cups
Mid-morning break	..... cups	..... cups	..... cups
Midday meal	..... cups	..... cups	..... cups
Teatime	..... cups	..... cups	..... cups
Evening meal	..... cups	..... cups	..... cups
Bedtime	..... cups	..... cups	..... cups
Other	..... cups	..... cups	..... cups

How much sugar do you take in tea? .....teaspoons
Are the spoons level or heaped? .....
How much sugar do you take in coffee? .....teaspoons
Are the spoons level or heaped? .....
Have you always taken the same amount of sugar in these beverages? Yes/No*
If "No," how much did you take before? .....
When did you change? .....
Do you *regularly* use artificial sweeteners, e.g. saccharine, saxine, etc. Yes/No*
How long have you used them? .....
How much of the following do you eat or drink *per week*?
Sweets, toffees, and fancy chocolates .....lbs. .....oz.
No. of 2-oz. chocolate bars .....
Fizzy drinks, non-alcoholic .....glasses
(inc. tonic water, ginger-beer, etc.) .....small bottles
Fruit squash .....glasses
Fruit juice (tinned or bottled) .....glasses
How many a *week* do you have of the following:
Porridge .....portions
Plain breakfast cereals .....portions
Sugar-coated breakfast cereals .....portions
Jam or marmalade .....teaspoons
Sweet biscuits .....number
Pudding or sweet at midday (including tinned fruit) .....portions
Pudding or sweet for tea or evening meal (including tinned fruit) .....portions
Individual cakes and/or slices of cake .....number
How many spoons of sugar do you take on breakfast cereals? .....
Are they teaspoons or dessertspoons? .....
Are they level or heaped? .....
How many spoons of sugar do you take on porridge? .....
Are they teaspoons or dessertspoons? .....
Are they level or heaped? .....

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Acute Oral Toxicity of Sucrose[1]

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Received June 19, 1964

Three considerations prompted us to study the acute oral toxicity of sucrose. The first was the possibility that certain toxic effects of drugs given to rats on standard vitamin-deficient diets in our laboratory were due to the high sugar content of the synthetic diets. Preliminary estimates of the LD_{50} of sucrose indicated that rats on various vitamin B-deficient diets were eating daily amounts of sucrose which approached the single oral lethal dose. Secondly, several drugs have been tested as syrups in our laboratory, and the toxic effect of large doses might have been due to the sucrose administered. In the third place, it was considered that toxicity data upon a food, sucrose, would be of value for comparison with corresponding data upon drugs.

A review of the literature revealed little information with a direct bearing upon the project. In studies of the excretion of sucrose, Kuriyama (1917) gave dosages of the order of 2–10 g/kg intravenously to dogs, with no deaths, and observed that most of the sugar was eliminated in urine. Investigating the "Grundlagen der Osmotherapie" used in Austria early in the twentieth century, Hausmann (1925) found that sucrose injected subcutaneously or intraperitoneally as a 50% solution, at dosages of the order of 30–40 g/kg, produced in mice a hypothermic "Osmonarkose" and death in about 6 hours. On the other hand, MacKay and MacKay (1924) reported that large doses of sucrose given intravenously to rabbits produced convulsions and death when the animals were subsequently permitted to drink water.

During the 1930's, some interest was generated in the comparative nutritional values of high-sugar diets for rats, and while differences between sucrose, glucose, and fructose were reported initially, Haldi *et al.* (1938) found that such differences disappeared when the animals were allowed free exercise in activity wheels. More recently, Harper and Worden (1964) fed rats a diet containing 80% sucrose for 26 weeks, and reported hypertrophy of the liver with decreased protein and increased fat deposition, hypercholesterolemia, and hypertrophy of the kidneys and heart. Shore and Shore (1960) gave rats a diet of 100% sucrose (plus vitamins) for 4 weeks and found that they lost 30% of their body weight.

Following the introduction of intravenous infusion of 50% sucrose solution for the purpose of lowering intracranial pressure and acting as a diuretic, studies in the 1930's and 1940's disclosed its nephrotoxic action (Rigdon and Cardwell, 1942; Feher *et al.*, 1942). Among these investigations is that of Helmholz and Bollman (1939), from whose data it may be calculated that death in rabbits did not appear until sucrose had been given intravenously (during 6 hours) in dosages over 50 g/kg

[1] This project was supported by a grant from the Medical Research Council of Canada.

and orally (during 1 hour) in dosages over 20 g/kg. Spector (1956) reports no data upon lethal doses of sucrose, but lists dosages of 8–20 g of glucose per kilogram as lethal by oral administration to rabbits and dogs. More recently, Janigan *et al.* (1960) located the primary renal site of sucrose uptake, swelling, and rupture as the acid phosphatase-containing lysozomes.

From the literature, therefore, it was concluded that lethal oral dosages would likely lie above 20 g/kg, and that such dosages would be likely to produce convulsions or stupor, loss of body weight, and renal tubular degeneration.

METHODS

The experiments were performed upon adult, male, albino rats, weighing 300–500 g, of a Wistar strain which has been bred in the animal quarters of this Department since 1937. They were fed a commercial diet[2] and water ad libitum. Each animal was placed in separate metabolism cage with water but no food for 16 hours before administration of sucrose. Sucrose was dissolved in distilled water and given by intragastric cannula in a volume of 60 ml/kg. Nineteen dosages of sucrose were employed, ranging from 5 to 80 g/kg. The lowest four and highest three doses were given to 4–8 animals per dose and the remainder to 16–20 animals per dose. Forty-eight controls received distilled water only.

Clinical measurements noted below were made daily, 6 days a week, for 2 weeks followed by casual observation to 1 month, by methods described by Boyd and Boyd (1962). Urinalyses were performed upon 24-hour specimens by techniques recorded by Boyd (1960). Autopsies were performed upon sucrose nonsurvivors within 30 minutes of death to avoid postmortem shifts described by Boyd and Knight (1963), and upon survivors at 2 weeks and 1 month. Gross and microscopic appearance, wet weights, and water levels were recorded upon the organs noted below. Microscopic examination was made upon tissues fixed in Lillie's buffered formalin and stained with hematoxylin-phloxine-saffron. Water content was determined upon samples of tissue dried to constant weight at 95°C in a forced-draft oven for 24 hours. Statistical methods were those of Croxton (1953).

RESULTS

There were no deaths in animals given dosages of 5–24 g/kg. The mean interval to death in animals given higher dosages varied inversely with dosage, the regression being fitted best by a quadratic equation as shown in Fig. 1. All animals receiving 60, 70, and 80 g/kg died within 5 hours. The frequency distribution of deaths vs. time in animals receiving dosages of 50 g/kg and less had a sharp peak at 4 hours, a valley at 9 hours, and a broad plateau at 12–48 hours.

There were 76 deaths at 9 hours or less, all except 1 in animals given 35 g/kg and over. The $LD_{50} \pm SE$ for this group is 42.2 ± 4.5 g/kg. There were 43 deaths beyond 9 hours, 23 of them in animals given less than 35 g/kg, and in these it is possible to calculate an $LD_{50} \pm SE$ of 29.8 ± 1.4 g/kg. Of the 43 delayed deaths, 38 occurred within 10–48 hours and 5 within 2–11 days. Two of the control rats died on the 5th day, possibly from the effects of the distilled water which, as noted below, produced other clinical signs of toxicity. The $LD_{50} \pm SE$ for all deaths from sucrose was

[2] Purina Fox Chow Checkers, Ralston Purina Company, St. Louis, Missouri 63199.

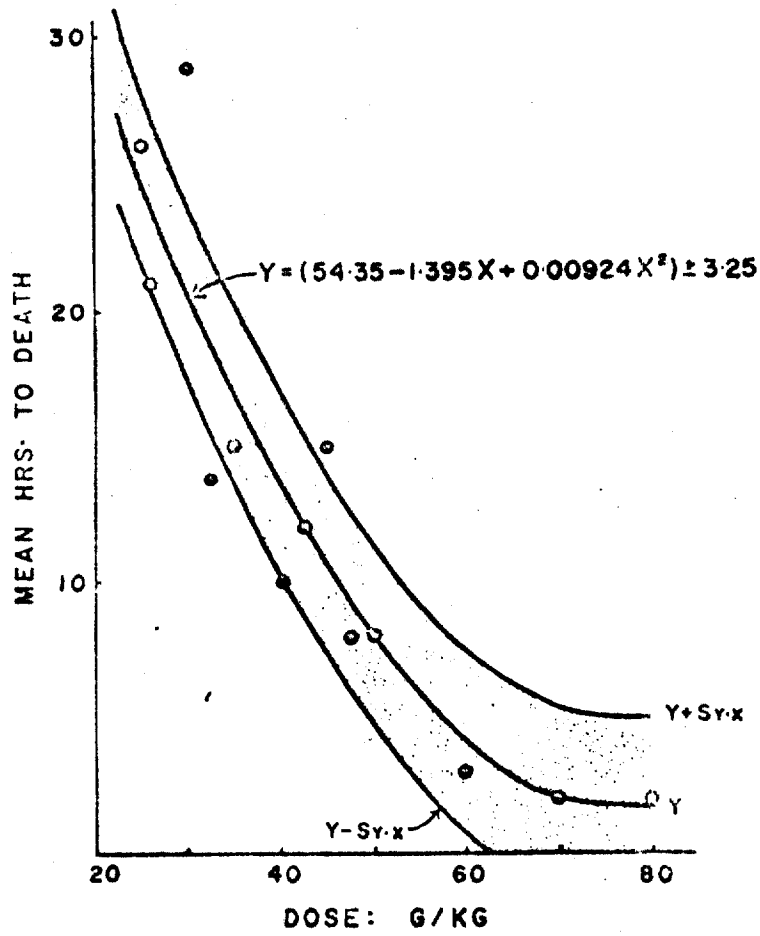

FIG. 1. Regression of mean interval to death upon dosage of sucrose administered orally to adult, male, albino rats.

35.4 ± 7.0 g/kg. The corresponding value in 30 female albino rats weighing 250–300 g was 29.7 ± 3.7 g/kg with $P = 0.02$ that the difference from males was insignificant. As in the males, the LD_{50} for early (< 10 hours) deaths was higher (35.90 ± 1.31 g/kg) than that for late (10 hours and over) deaths (27.85 ± 1.55 g/kg). The median lethal dose, therefore, was higher in animals dying in less than, than in animals dying in over, 10 hours and was higher in males than in females.

The initial clinical signs following administration of sucrose solution were hypokinesia, prostration, cyanosis, abdominal bloating, and diarrhea. All of these signs except cyanosis appeared to a lesser degree and for a lesser period of time in the controls given distilled water. They were specific for sucrose, therefore, only to the extent that they were more marked during the first 6 hours, and persisted to 24–48 hours, at which time the controls were free of these signs. The premortal signs in animals which died within 9 hours were tonic-clonic convulsions followed by stupor and respiratory failure.

During the 24 hours following administration of sucrose, food intake was depressed

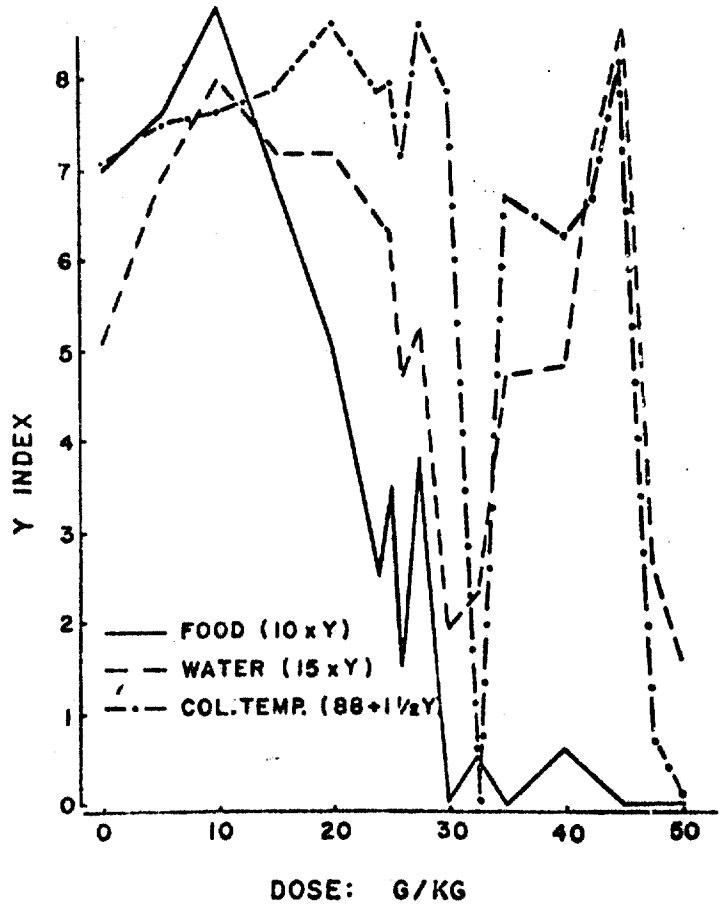

FIG. 2. Effect of increasing dosage upon mean food intake (g/kg), and mean water intake (ml/kg) over the first 24 hours after administration of sucrose to adult, male, albino rats. Colonic temperature (°F) was measured at 24 hours. The Y index is multiplied by the factors noted in parentheses to obtain the corresponding statistics.

in all surviving rats given dosages of 20 g/kg and over (Fig. 2). Water intake rose in rats given dosages up to 20 g/kg and thereafter varied erratically, as shown in Fig. 2. Body weight was significantly ($P < 0.001$) lower at 24 hours in animals given over 20 g/kg of sucrose than in controls given distilled water. Loss of body weight at this time was due to anorexia and diarrhea. Colonic temperature rose at 24 hours in rats given dosages up to 30 g/kg (Fig. 2), and thereafter was erratically lower, with the mean for all dosages above 30 g/kg significantly ($P = 0.005$) lower.

Measurements upon urine in survivors at 24 hours are indicated by results shown in Fig. 3. Doses up to about the LD_{50} produced a dose-dependent increase in the volume of urine and the daily output of reducing substances calculated as glucose, and a decrease in urinary pH. Thereafter the responses were not dose dependent. There was no significant change in the daily urinary output of protein.

At autopsy upon 16 sucrose nonsurvivors there was found a loss of wet weight in carcass and in certain organs, particularly the liver, relative to weight in 12 controls

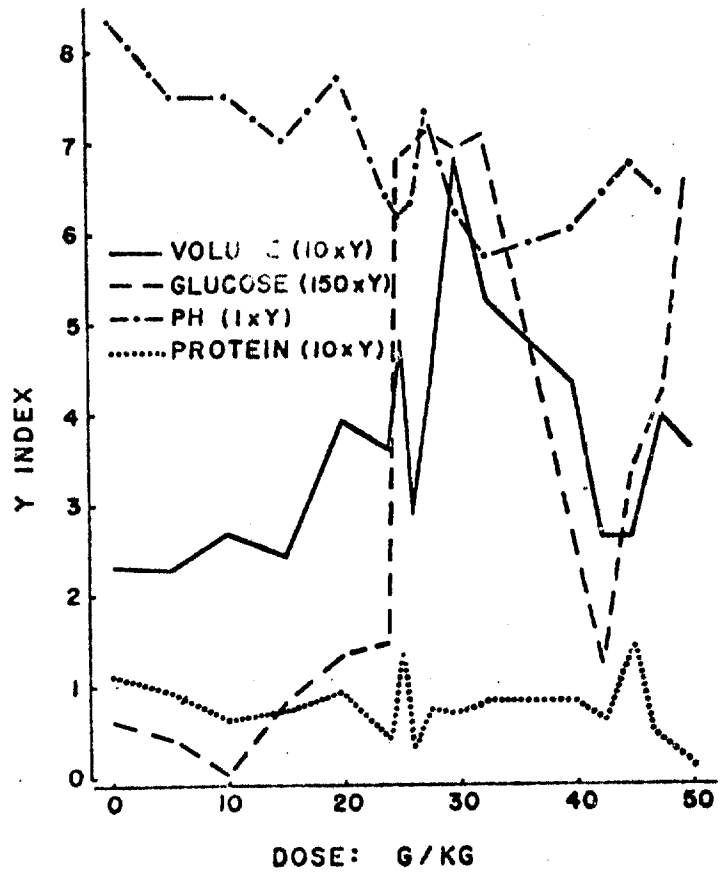

FIG. 3. Effect of increasing dosage upon mean urine volume (ml/kg), mean excretion of urinary reducing substances calculated as glucose (mg/kg), and mean excretion of urine protein (mg/kg) over the first 24 hours after administration of sucrose to adult, male, albino rats. (The kilograms in the preceding measurements refer to body weight.) The pH is that of the total 24-hour volume. The Y index is multiplied by the factors noted in parentheses to obtain the corresponding statistics.

(Table 1). The weight of the adrenal glands and parts of the alimentary canal was significantly increased. Water levels were decreased in carcass and in many organs (Table 2). Accompanying the more or less generalized dehydration and loss of weight, there was a severe but temporary gastroenteritis, a temporary mild hepatitis, a marked nephritis, a marked encephalitis, focal necrosis of the heart, moderate stressor reactions in the adrenal and thymus glands, inhibition of spermatogenesis, and an arteriolitis. The parenchyma of the lungs, pancreas, salivary glands, spleen, and skin was normal.

The gastroenteritis appeared at 1–3 hours, and disappeared at 1–4 days; it was confined mostly to the stomach and small bowel. Cardiac stomach, which has been found fairly resistant to lethal oral doses of most drugs in this laboratory, was markedly involved, with capillary, arteriolar, and venous congestion of the lamina propria and submucosa, and areas of lysis of the surface layers of stratified squamous

TABLE 1

SHIFTS IN THE WET WEIGHT OF ORGANS AFTER ORAL ADMINISTRATION OF SUCROSE[a]

Organ	Nonsurvivors	Survivors at 2 weeks	Survivors at 1 month
Adrenal glands	+ 30.9 (0.05)	+ 35.5 (0.005)	— 1.8 (0.9)
Brain	+ 4.3 (0.3)	— 3.2 (0.2)	— 5.8 (0.2)
Gastrointestinal tract			
Cardiac stomach	+ 18.4 (0.05)	+ 29.4 (0.001)	— 0.4 (1.0)
Pyloric stomach	+ 0.1 (0.9)	+ 25.9 (0.005)	— 1.5 (0.8)
Small bowel	+ 16.4 (< 0.001)	+ 52.7 (< 0.001)	— 2.5 (1.0)
Cecum	+ 16.0 (0.1)	+ 66.3 (< 0.001)	— 5.4 (0.6)
Colon	— 7.5 (0.2)	— 3.2 (0.7)	0.0 (1.0)
Heart	— 11.2 (0.05)	— 14.6 (0.01)	0.0 (1.0)
Kidneys	— 5.4 (0.3)	— 12.8 (0.01)	— 1.9 (0.7)
Liver	— 42.6 (< 0.001)	— 14.4 (0.02)	— 26.1 (< 0.001)
Lungs	+ 5.9 (0.5)	+ 1.7 (0.9)	— 9.1 (0.3)
Muscle[b]	+ 1.7 (0.9)	+ 27.8 (< 0.001)	— 3.1 (0.7)
Skin	— 8.3 (0.5)	— 3.6 (0.7)	+ 3.0 (0.4)
Spleen	— 5.0 (0.7)	— 20.5 (0.02)	— 7.8 (0.5)
Testes	— 7.2 (0.02)	— 0.3 (0.8)	— 3.3 (0.5)
Thymus gland	+ 7.5 (0.5)	+ 18.2 (0.3)	— 15.3 (0.3)
Residual carcass	— 13.9 (< 0.001)	— 3.2 (0.7)	+ 1.7 (0.8)

[a] The results are expressed as $[(\bar{X}_s - \bar{X}_c)/\bar{X}_c] \times 100$ (P that $\bar{X}_s$ equals $\bar{X}_c$) where $\bar{X}_s$ is the mean weight in grams in animals given sucrose and $\bar{X}_c$ in control animals given distilled water only.

[b] Abdominal wall.

TABLE 2

SHIFTS IN THE WATER LEVELS OF ORGANS AFTER ORAL ADMINISTRATION OF SUCROSE[a]

Organ	Nonsurvivors	Survivors at 2 weeks	Survivors at 1 month
Adrenal glands	+ 8.1 (0.4)	— 12.7 (0.1)	+ 7.8 (0.3)
Brain	— 5.3 (0.1)	— 3.7 (0.005)	+ 1.8 (0.5)
Gastrointestinal tract			
Cardiac stomach	+ 17.6 (0.1)	— 3.8 (0.2)	— 0.3 (0.9)
Pyloric stomach	— 39.6 (< 0.001)	— 7.2 (0.05)	— 0.5 (0.8)
Small bowel	— 25.7 (< 0.001)	— 4.5 (0.3)	— 4.3 (0.3)
Cecum	— 43.5 (< 0.001)	+ 1.3 (0.9)	0.0 (1.0)
Colon	— 27.2 (< 0.001)	+ 0.5 (0.9)	— 1.7 (0.7)
Heart	— 2.2 (0.8)	— 4.8 (0.5)	— 2.8 (0.4)
Kidneys	— 4.3 (0.1)	— 3.2 (0.3)	0.0 (1.0)
Liver	— 6.3 (0.3)	— 5.4 (0.2)	— 0.9 (0.9)
Lungs	— 22.5 (< 0.001)	— 0.6 (1.0)	— 5.1 (0.2)
Muscle[b]	— 19.6 (< 0.001)	+ 3.7 (0.4)	0.0 (1.0)
Skin	— 24.2 (< 0.001)	— 2.1 (0.8)	— 7.8 (0.4)
Spleen	— 1.5 (0.8)	— 10.8 (0.05)	— 3.5 (0.05)
Testes	— 15.8 (< 0.001)	— 4.6 (0.3)	+ 4.1 (0.5)
Thymus gland	+ 57.1 (0.1)	0.0 (1.0)	— 28.0 (0.01)
Residual carcass	— 28.9 (< 0.001)	— 10.0 (0.1)	— 4.1 (0.4)

[a] Water levels are calculated as grams water per 100 g dry weight of organ. The results are expressed as $[(\bar{X}_s - \bar{X}_c)/\bar{X}_c] \times 100$ (P that $\bar{X}_s$ equals $\bar{X}_c$) where $\bar{X}_s$ is the mean in animals given sucrose and $\bar{X}_c$ in control animals given distilled water only.

[b] Abdominal wall.

epithelium. Along with a similar vascular reaction in the pyloric stomach, there was a marked lysis of surface columnar epithelium and mucous neck cells, somewhat less lysis of the parietal cells, and a shrinkage of the zymogens with swelling of the lamina propria at the base of the gastric glands. The villi of the small bowel were congested, the lamina propria edematous, and the columnar epithelium completely denuded in many areas with, however, very little hemorrhage into the lumen. The inflammatory reaction in the cecum was confined to capillary congestion and edema of the lamina propria, and in the colon to a mild capillary congestion. The latter two structures were normal in animals which died at 24 hours, while the upper part of the alimentary canal was largely normal, except for occasional mild congestion in the submucosa and some edema of the arteriolar walls, in animals which died at 4 days.

The marked loss of weight of the liver was found to be due to a shrinkage of the hepatic cells. The sinusoids were distended and filled with red blood cells. The Kupffer cells were shrunken and often lysed away from the lining of the sinusoids. These changes were most marked at 3 hours. By 24 hours the liver appeared microscopically normal.

The kidneys showed abnormalities as early as 1 hour after a lethal dose of sucrose. At this time the glomeruli were shrunken, the subcapsular space increased, the proximal tubular cells shrunken and distorted, and granules were prominent at the base of the brush border. At 3 hours the proximal convoluted tubular cells had swollen and obliterated the lumen, the loop cells were edematous, and the distal tubular cells were shrunken with casts in the lumen. Vacuolar degeneration of the proximal tubular cells appeared at 24 hours, and bits of tubular debris could be seen refluxed into the glomerular space beneath Bowman's capsule. At this time, the squamous epithelium lining the descending limb of the loop of Henle was swollen, and there was some necrosis of the cuboidal epithelium lining the ascending limb. The inflammatory reaction was less marked in the distal convoluted and collecting tubular cells were shrunken with casts in the lumen. Vacuolar degeneration of the died at 4 days.

The coronary arteries were dilated and the arterial wall was swollen and pale-stained at 3 hours. At the same time there was marked capillary vasodilatation of the myocardium with minute areas of capillary hemorrhage. At 1–4 days, minute areas of focal necrosis with leucocyte and fibroblastic infiltration were found scattered throughout the heart muscle.

The adrenal glands exhibited either congestion or collapse of the cortical sinusoids at 1–3 hours. Subsequently, they were normal in appearance but somewhat enlarged. The microscopic appearance of the thymus gland varied; mild capillary and venous congestion and some loss of thymocytes were the more common findings.

At 1 hour, the seminiferous tubules of the testes were shrunken. When death was delayed to 4 days, the spermatogenic cells were found separated from the tubular wall, the sperm were shriveled and wrinkled, and there was a leucocytic infiltration of the interstitial tissue.

Toxic changes were seen in arterioles throughout the organs in both early and delayed death. In the lung there was a periarteriolar edema as early as 1 hour after a lethal dose. At 3 hours, the intima and media of the splenic arterioles were

edematous and pale-stained. In the heart and mesentery, the edema extended to the tunica externa. There were large numbers of leucocytes in the blood stream.

Minor changes were encountered in other organs. Phagocytosis was active in macrophages of the thymus gland and spleen. The M disks of striated muscle were prominent in animals dying at 1 hour; later the muscle fibers were swollen in some animals and shrunken in others. The subcutaneous tissues were shrunken. The pancreas, salivary glands, and lung parenchyma were normal.

Insofar as measurements indicated, recovery was complete in animals which survived. By the third day, hypokinesia, prostration, cyanosis, abdominal bloating, diarrhea, anorexia, glycosuria, and weight loss had disappeared. At this time there were present a polydipsia and polyuria, a slightly but significantly elevated body temperature and some alkalinuria. The latter signs apparently represented a reaction to the initial toxic effects of the large doses of sucrose; they had disappeared by the 6th day.

Fourteen survivors and 12 controls were autopsied on the 14th day. The organs appeared grossly normal. The adrenal glands, stomach, small bowel, cecum, and striated muscle weighed more in survivors than in controls, while the heart, kidneys, liver, and spleen weighed less (Table 1). Water levels were significantly low in brain, pyloric stomach, and spleen (Table 2). No microscopic examination was made upon the 2-week survivors, but examination of nonsurvivors at 4 days indicated a reactive hypertrophy of the gastrointestinal mucosa which apparently accounted for the increase in weight of the alimentary organs at 14 days.

At 1 month, 13 survivors and 11 controls were similarly autopsied. By this time the liver was still small in survivors (Table 1), and the thymus gland had lost water (Table 2). Otherwise all measurements were within the range of the controls.

DISCUSSION

The median lethal dose of sucrose given orally to albino rats was found to be in the range of 30–35 g/kg, which are dosages of the same order as those found to produce death in rabbits by Helmholz and Bollman (1939). While this LD_{50} is higher than that of most drugs, it is actually not beyond the range of possible human intake of sugar. For example, it is the dosage a child weighing 25 pounds would receive by eating—and retaining—a pound of candy.

The administration of doses higher than the LD_{50} tends to produce a sudden death in less than 10 hours, apparently as a result primarily of marked capillary congestion of the brain and meninges. The fulminating gastroenteritis may be a contributing cause to the early occurrence of death. Results suggesting that the intravenous lethal dose of sucrose (Helmholz and Bollman, 1939) and glucose (Spector, 1956) may be higher than the oral lethal dose, also indicate that gastrointestinal inflammation may have been an important factor in early deaths from oral administration.

The administration of doses lower than the LD_{50} produces a delayed death, mostly in the interval between 10 and 48 hours. At this time, the renal lesion is marked, and may have been an important cause of death. The swelling and vacuolar degeneration of the convoluted tubules is similar to that reported some twenty years ago from intravenous injection of 50% sucrose solutions (Feher *et al.*, 1942).

In view of reports on the effects of high sucrose diets on the chemical composition

of the liver (Haldi *et al.*, 1938; Harper and Worden, 1964), it is of interest that this organ was found to recover rapidly from the effects of toxic oral doses of sucrose and to be normal in histologic appearance at 24 hours. At 1 month, however, its weight was still significantly depressed (Table 1).

The focal cardiac necrosis noted in some of these rats was similar in microscopic appearance to Selye's electrolyte-steroid cardiopathy with necrosis. Sucrose is not listed as an aggravating agent of this lesion in Prioreschi's (1963) recent review.

Calculated in terms of millimoles per kilogram of body weight, the $LD_{50} \pm SE$ of sucrose is 103 ± 20 which is significantly ($P < 0.001$) higher than the figures of 64.1 ± 7.4 for sodium chloride and 40.5 ± 1.9 for potassium chloride (Boyd and Shanas, 1963). All three substances produce in common an acute gastroenteritis, dehydration, varying degrees of vascular congestion, and many deaths within 10 hours.

The administration of large doses of sucrose in solution required the administration of large volumes of water. Control rats given the equivalent volume of distilled water, 60 ml/kg, were hypokinetic, stuporous and had mild diarrhea and a bloated abdomen for several hours. This dose of water is one-fifth to one-tenth that found by Rowntree (1923) to produce death from water intoxication in dogs, cats, rabbits, and guinea pigs.

SUMMARY

The $LD_{50} \pm SE$ of sucrose given orally was found to be 35.4 ± 7.0 g/kg in male, and 29.7 ± 3.7 g/kg in female, albino rats. The initial clinical signs of toxicity were hypokinesia, prostration, cyanosis, abdominal bloating, and diarrhea. Death from doses larger than the LD_{50} tended to occur within 10 hours, following tonic-clonic convulsions, stupor, and respiratory failure, and were accompanied by a marked gastroenteritis, arteriolitis, mild hepatitis, early nephritis, myocarditis, and capillary and venous congestion of the brain and meninges. Death from doses smaller than the LD_{50} occurred mostly within 10-48 hours following anorexia, severe diarrhea, loss of weight, hypothermia, diuresis, glycosuria, aciduria and were accompanied by some residual gastroenteritis and encephalitis, marked tubular nephritis, arteriolitis, focal necrosis of the myocardium, some adrenal hypertrophy and inhibition of spermatogenesis. Sublethal doses produced anorexia, polydipsia, hyperthermia, diarrhea, and loss of weight for 1-3 days. Survivors of lethal doses developed polydipsia, polyuria, alkalinuria, and some hyperthermia between 3 and 6 days. At 2 weeks the dehydration of organs seen in nonsurvivors had largely disappeared, and at 1 month the weight and appearance of most organs were normal.

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100-Day LD_{50} Index of Chronic Toxicity*

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INTRODUCTION

The principals involved in uniposal predictive toxicometrics or the measurement of acute single-dose toxicity are reasonably well defined. If the discipline of factorial toxicometrics, or factors which affect toxic reactions, is thoroughly understood, estimates of acute toxicity can be duplicated with reasonable accuracy. The study of multiposal predictive toxicometrics, or methods of measuring the toxic effects of repeated daily dosing, has advanced at a slower pace, possibly because considerably more work is involved in multiposal than in uniposal toxicometrics.

The basic question to be answered in multiposal predictive toxicometrics is how much of an agent can be given daily for how long with or without producing what toxic effects. Because these studies are long and costly, selection of daily doses is extremely important. Three methods have been used to select the daily dose: (a) multiples of the estimated daily therapeutic dose, (b) increasing fractions of the acute LD_{50}, and (c) decreasing fractions of the acute LD_{50}. It has been our experience that method (c) yields the most information in the least amount of time.

The next question to consider is the route and method of administration.

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The discussion is confined to the two routes we have studied in multiposal tests, namely the parenteral and the oral. The repeated daily subcutaneous (e.g., Ref. [5]) or intramuscular (e.g., Ref. [1]) injection of large doses of drugs has, in our hands, invariably produced inflammatory reactions at the site of injection. The effect of these site sores complicates interpretation of the effects of the drug under study. We have, therefore, used the oral route of administration in most of our studies on multiposal toxicity. The gastrointestinal mucosa is capable of adapting to the presence, day after day, of large amounts of drugs and usually appears normal or super-normal on microscopic examination.

It has been our practice to administer the drug under study by intragastric cannula rather than by adding it to the animal's diet. If large amounts of a drug make the diet unpalatable, dietary administration may be more toxic than intragastric and if not it is usually less toxic. Phenacetin, for example, is more toxic given by daily intragastric dosing than by addition to the diet as shown by Boyd, Ciampi, and Krijnen [7].

The next question is how long to give the drug under study. Like most toxicologists, we have used periods of varying duration. In 11 studies we used an interval of 100 days. Most of these studies were performed upon albino rats and a period of 100 days was arbitrarily selected as being approximately one-tenth of the expected lifespan of the animal. It is the purpose of this communication to review these studies and to determine the validity of the 100-day interval.

THE 100-DAY LD_{50} INDEX

Our first step in a 100-day toxicity study is to determine the acute oral $LD_{50\ (1\ dose)}$ and associated clinicopathological syndrome of toxicity. Secondly, we give a high fraction of the $LD_{50\ (1\ dose)}$, usually 90%, daily by intragastric cannula until death occurs. Results with this fraction suggest what next lower fractions should be used and each of these is given daily for 100 days or until death occurs. At least two and usually three or more doses are given for 100 days. The third step consists of calculating the daily dose that kills 50% of the animals after administration for each successive weekly interval. Fourthly, these weekly LD_{50} values are plotted against duration in days and the $LD_{50\ (100\ days)} \pm$ S.E. calculated. The fifth and final step is to express the $LD_{50\ (100\ days)}$ as a percentage of the acute oral $LD_{50\ (1\ dose)}$ to obtain a quotient which we term the 100-Day LD_{50} Index.

Table 1
Values for the 100-Day LD_{50} Index

Agent	100-Day LD_{50} Index ± S.E.	Reference
Phenacetin[a]	8.6 ± 3.1	[6]
Atropine[b]	13.2 ± 0.8	[1]
Aspirin[c]	16.4 ± 0.5	[2]
Pilocarpine[c]	17.1 ± 2.2	[11]
Dicophane (DDT)[c]	17.6 ± 0.4	[12]
Paracetamol[c]	20.6 ± 0.5	[9]
Phenacetin[c]	27.1 ± 0.5	[10]
Benzylpenicillin[c]	61.8 ± 3.0	[13]
Sodium chloride[c]	71.7 ± 3.2	[4]
Caffeine[c]	78.2 ± 1.6	[8]
Sucrose[c]	80.6 ± 3.7	[14]

[a] Drug given orally to guinea pigs.
[b] Drug given intramuscularly to rabbits.
[c] Drug given orally to albino rats.

The $LD_{0\ (100\ days)}$ and $LD_{100\ (100\ days)}$ may be calculated in the same manner. It is our usual practice to report these and the clinicopathologic syndrome of toxicity at $LD_{0\ (100\ days)}$, $LD_{50\ (100\ days)}$, and $LD_{100\ (100\ days)}$.

Available values for the 100-Day LD_{50} Index are assembled in Table 1. It may be noted that there is considerably less variation from agent to agent in the 100-Day LD_{50} Index than in the $LD_{50\ (100\ days)}$. The $LD_{50\ (100\ days)}$ varied from as low as 0.065 gm/kg/day for dicophane to as high as 28.5 gm/kg/day for sucrose, a variation of over 400-fold. The maximal variation in the 100-Day LD_{50} Index was ninefold. These results indicate that the chronic lethal dose is a direct function of the acute lethal dose under the conditions of this test. The 100-Day LD_{50} Index of substances that are part of the daily diet, such as sodium chloride, caffeine, and sucrose was higher than that of drugs such as atropine, pilocarpine, and dicophane, as might be anticipated. The relatively low indices for aspirin, paracetamol, and phenacetin explains why toxicity in man is found

Table 2

The $LD_{0\ (100\ days)}$ and $LD_{100\ (100\ days)}$ as percentages of the $LD_{50\ (100\ days)}$[a]

Agent	$\frac{LD_{0\ (100\ days)}}{LD_{50\ (100\ days)}} \times 100$	$\frac{LD_{100\ (100\ days)}}{LD_{50\ (100\ days)}} \times 100$
Pilocarpine	0.8	163
Phenacetin-rat	33	119
Aspirin	40	168
Phenacetin-guinea pig	53	149
Paracetamol	54	138
Benzylpenicillin	58	144
Atropine	62	150
Dicophane	66	150
Sucrose	69	125
Caffeine	73	128
Sodium chloride	77	120

[a]Calculated from data in the references cited in Table 1.

in persons who chronically abuse these freely available analgesics. The main advantage of the 100-Day LD_{50} Index is that it presents as estimate of chronic toxicity in one figure.

As shown by data assembled in Table 2, the $LD_{0\ (100\ days)}$ of most agents studied has varied from between 33 and 77% of the $LD_{50\ (100\ days)}$. The agents commonly present in human diets, such as sucrose, caffeine, and sodium chloride, produced no deaths in daily doses about 25% less than the $LD_{50\ (100\ days)}$. The corresponding figures for the freely available analgesics such as aspirin, paracetamol, and phenacetin was 1/3 to 1/2 the $LD_{50\ (100\ days)}$, which again is indicative of the danger of abuse of these analgesics. The lowest $LD_{0\ (100\ days)}$ was that for pilocarpine which was less than 1% of the $LD_{50\ (100\ days)}$.

The $LD_{100\ (100\ days)}$ varied from 19 to 68% higher than the $LD_{50\ (100\ days)}$, as indicated in Table 2. It is of interest that the narrowest range of lethal doses was for sucrose, caffeine, and sodium chloride--25%

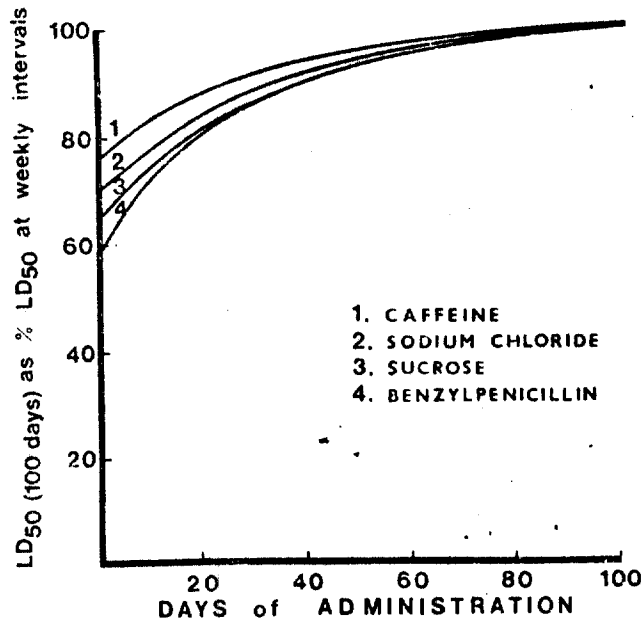

Fig. 1. The regression, on days of administration, of values for the $LD_{50\ (100\ days)}$ of caffeine, sodium chloride, sucrose, and benzylpenicillin expressed as a percentage of the LD_{50} calculated at weekly intervals.

less than the $LD_{50\ (100\ days)}$ killed no animals and 25% more than the $LD_{50\ (100\ days)}$ killed all animals. While these dietary items are relatively safe for chronic administration, there is a narrow range of daily doses over which they rapidly become 100% lethal.

THE INTERVAL OF ADMINISTRATION

It will be recalled that 100 days of drug administration was an arbitrary interval selected when these studies were first undertaken. We have therefore examined our data to determine if the same information could be obtained from a shorter period of administration or if, indeed, the period should be lengthened beyond 100 days. To do this, the $LD_{50\ (100\ days)}$ was expressed as a percentage of the LD_{50} calculated at weekly intervals, and the results plotted against days of administration. In this way, it was possible to find at what interval the $LD_{50\ (100\ days)}$ was approximated.

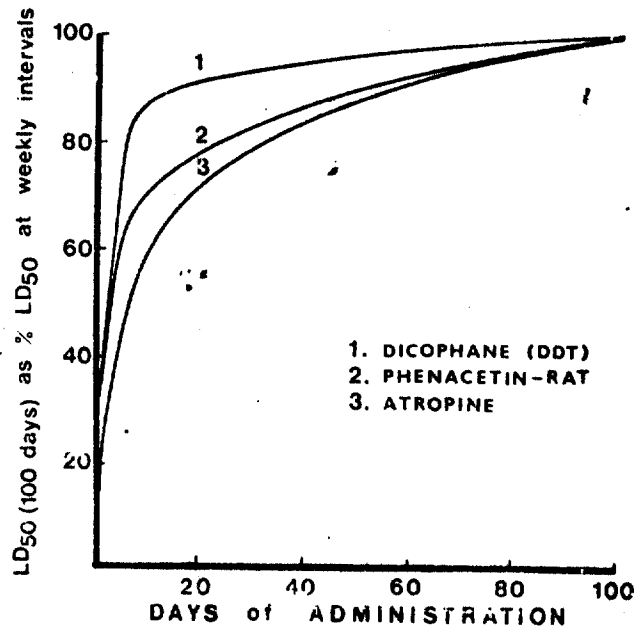

Fig. 2. The regression, on days of administration, of the $LD_{50\ (100\ days)}$ of dicophane, phenacetin (in albino rats), and atropine expressed as a percentage of the LD_{50} calculated at weekly intervals.

Three types of regression were obtained. As shown in Fig. 1, the regressions for caffeine, sodium chloride, sucrose, and benzylpenicillin were almost horizontal. This was anticipated from the high value of the 100-Day LD_{50} Index of these four agents. It is apparent from Fig. 1 that little new information about lethal effects of these agents was obtained after about six weeks of administration. It would also appear that extension of the interval of administration beyond 100 days would contribute little new information.

Regressions for the remaining seven agents, which had relatively low 100-Day LD_{50} indices, fell into two divisions. The regression for dicophane, phenacetin (in the rat), and atropine are shown in Fig. 2. The curves rose steeply during the first three or four weeks and then flattened. Most deaths from these three agents occurred during the first three or four weeks suggesting that survivors had developed a tolerance to the lethal effect of the drugs after this period. Boyd, Ciampi, and Krijnen [7] tested this hypothesis and found that albino rats do, in fact, become resistant to the toxic effects of phenacetin after an interval of daily administration of

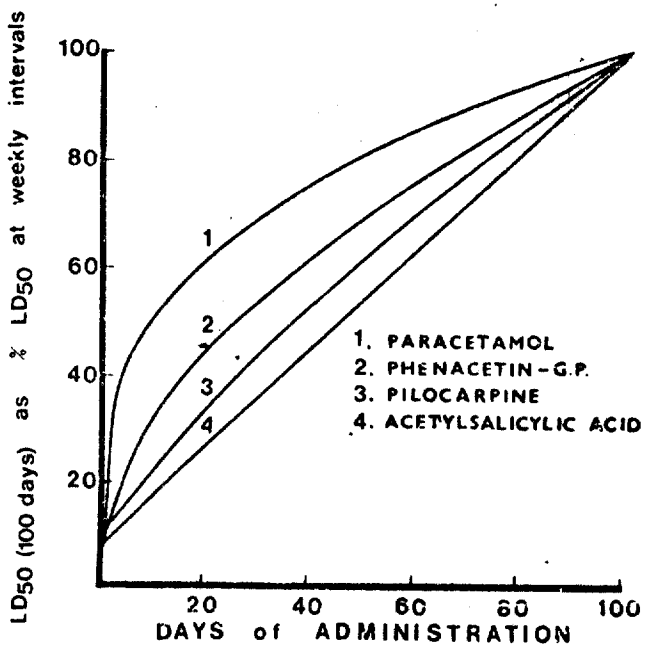

Fig. 3. The regression, on days of administration, of the $LD_{50\ (100\ days)}$ of paracetamol, phenacetin (in guinea pigs), pilocarpine, and acetylsalicylic acid expressed as a percentage of the LD_{50} calculated at weekly intervals.

sublethal doses. The regressions shown in Fig. 2 suggest that few if any further deaths would occur beyond 100 days of daily dosing. This hypothesis has also been tested recently by Boyd [3] who found that an oral $LD_{0\ (100\ days)}$ of phenacetin produced no deaths in albino rats when given for a period of 220 days.

The regressions for paracetamol, phenacetin (in the guinea pig), pilocarpine, and acetylsalicylic acid are shown in Fig. 3. These regressions were less asymptotic [16] and approached linearity, especially in the case of pilocarpine and acetylsalicylic acid. The results suggest that little or no tolerance was formed to the agents shown in Fig. 3 and that continued administration would have produced deaths after 100 days. In the case of pilocarpine, but not of the other three drugs shown in Fig. 3, this might have been predicted from the low value of the $LD_{0\ (100\ days)}$ indicated in Table 2.

These results differ from those recently described by Weil et al. [17] who found a fairly constant relationship between 7-day, 90-day, and 2-year

minimal effect levels of some 20 agents added to the diet of rats. The criterion of minimal effect was mainly inhibition of growth or loss of body weight. The difference appears to be related to our giving the agents by intragastric cannula, a method that rarely affects food intake in doses up to the $LD_{50\ (100\ days)}$. On the other hand, our results are somewhat similar to those of Hayes [15] who added drugs to the diet of rats and divided the $LD_{50\ (1\ dose)}$ by the dose which killed 50% of animals at 90 days to obtain a "chronicity factor," which is basically a reciprocal of the 100-day LD_{50} index.

CONCLUSIONS

It was hoped that this analysis of data on 11 drugs would have revealed that daily oral administration of decreasing fractions of the acute oral $LD_{50\ (1\ dose)}$ could be stopped at some interval less than 100 days and yet disclose an equivalent amount of information about chronic toxicity. Insofar as death rates are concerned, administration of the agents shown in Figs. 1 and 2 could have been stopped at perhaps six weeks. To obtain an estimate of the chronic nonlethal dose of the agents shown in Fig. 3, administration should have been continued beyond 100 days. The required duration of daily administration, therefore, varies from drug to drug. The results suggest that daily dosing should be continued for six weeks with weekly calculations of the LD_{50}. If the LD_{50} (or LD_0) is appreciably decreasing during the third to sixth weeks, administration should be continued until it ceases to fall. At the point where the weekly LD_{50} remains stationary for some three weeks, administration may be stopped.

In this review, clinicopathologic signs of toxicity other than death are not discussed. Some of these nonlethal signs do not appear until after a certain period of daily administration. An $LD_{0\ (100\ days)}$ of phenacetin, for example, produces sterility in male rats but not significantly so until administered daily for over 100 days. Signs of this nature would be missed if daily administration were stopped before they could appear.

The only general conclusion, therefore, is the time-honored one that the more thorough the study the less likely are signs of chronic toxicity to be missed. If routine tests are limited to six weeks, then conclusions must apply only to this interval and not be extrapolated to longer periods.

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DEVELOPMENT OF SUCROSE VACUOLES FROM LIVER-CELL LYSOSOMES

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PLATES XCIX–CII

ALTHOUGH the changes produced in the renal tubular cells by the injection of sucrose are well known, little attention has been paid to the vacuolation developing in the liver cell in similar circumstances. In this paper we describe how such vacuoles develop. There has been a tendency to assume that vacuoles appearing in the liver cell after a wide variety of experimental procedures such as anoxia, intravenous injection of hypertonic fluids, and partial hepatectomy, develop in the same way (Aterman, 1958). We have evidence that this is not so, but in this paper we confine our attention to sucrose vacuolation.

MATERIALS AND METHODS

Albino rats (250–300 g.) were given an intraperitoneal injection of 6–8 ml. of 50 per cent. sucrose after water had been withheld for 24 hr. They were killed at intervals of 6–24 hr by opening the thorax under ether anaesthesia, and incising the heart to avoid anoxia and congestion. Many experiments were performed: three rats, two injected and one control, were used at each time-interval. Specimens obtained only 6 hr after injection were the most useful for determining the early stages of the development of the vacuoles. Tissue was fixed in formalin for the preparation of paraffin sections for light microscopy, in buffered formalin for the demonstration of acid phosphatase by Holt's (1959) modification of Gomori's method, and in buffered osmium tetroxide and buffered potassium permanganate for electron microscopy. Osmic-fixed sections were stained with lead by Karnovsky's method (1961). The method of Holt and Hicks (1961) was used to demonstrate acid phosphatase for electron microscopy.

RESULTS

In animals killed 6 hr after the intraperitoneal injection of sucrose the liver appears pale on naked-eye examination. Light microscopy shows widespread vacuolation of liver cells (fig. 1) evenly distributed throughout the lobule. Within the cell the vacuoles show no obvious tendency to be concentrated around either the sinusoidal border or the bile canaliculi. The vacuoles appear empty; they do not stain for fat and contain no periodic acid-Schiff-positive material.

In the normal liver cell stained for acid phosphatase by Holt's method fine particles of fairly even size are concentrated about the bile canaliculi (fig. 2). After the injection of sucrose these particles increase considerably in size and some have developed into vacuoles (fig. 3).

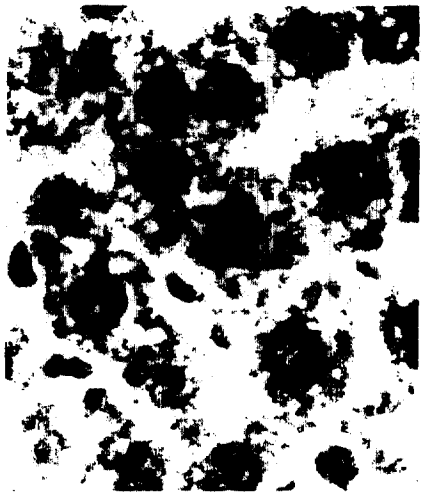

FIG. 1.—Rat liver 6 hr after sucrose injection: widespread vacuolation of liver cells. Van Gieson. ×900.

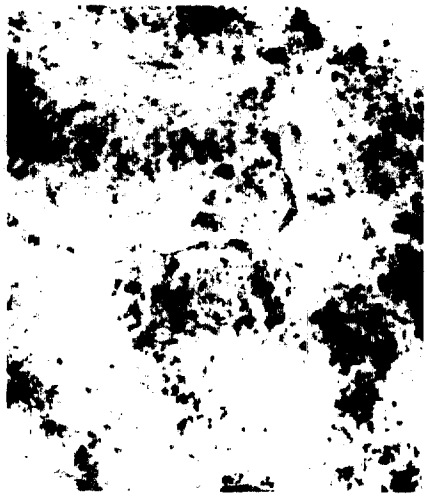

FIG. 2.—Control: small particles containing acid phosphatase ("lysosomes") are present in the liver cells. Holt's method. ×900.

FIG. 3.—The particles are much larger and some have developed into vacuoles. Holt's method. ×900.

FIG. 4.—Electron micrograph (EM). The liver cells include large vacuoles which contain a fine granular structureless material. Osmium tetroxide. ×7500.

Rat liver 6 hr after sucrose injection.

Electron microscopy confirms the presence of cytoplasmic vacuoles (fig. 4) and shows that they are not empty: they contain finely granular structureless material and often also round or oval bodies containing or surrounded by membrane (fig. 5). The lining membrane of the vacuole is clearly seen after permanganate fixation, and may have little indentations or projections into the vacuole (fig. 5). After lead-staining of material fixed in osmium tetroxide, the structure inside the vacuole appears dark with multiple clear areas within it (fig. 6).

The mitochondria, endoplasmic reticulum, nucleus, microbodies, bile canaliculi, and sinusoidal cell border all appear normal (fig. 7). The material in the vacuoles that appears dark after staining with lead in some instances closely resembles that in lysosomes. Lysosomes are rather variable in structure: they are dark oval bodies that commonly have within them smaller darker bodies (figs. 8 and 9), and occasionally there are also sharply defined clear areas. After sucrose injection these clear areas increase in number and size (fig. 10) producing an appearance suggestive of intermediate stages in the development of vacuoles. An origin of the vacuoles from the lysosomes would also give rise to the appearances seen with the light microscope, in preparations demonstrating acid phosphatase.

Holt and Hicks (1961), adapting the acid phosphatase method for electron microscopy, demonstrated the localisation of acid phosphatase activity in the lysosomes. Using their method on araldite-embedded material we have confirmed that acid phosphatase activity is sharply localised to the lysosomes (fig. 11). All other cytoplasmic bodies, in particular the microbodies, show no activity. In the sucrose-injected animals the acid phosphatase activity is localised to the rather variable structures within the vacuoles (figs. 12 and 13) confirming that this material is derived from lysosomes altered in the process of vacuole formation.

Discussion

Our results show that the vacuoles that develop after intraperitoneal injection of sucrose have morphological and cytochemical features in common with lysosomes—from which, therefore, it seems to us most likely that they develop. de Duve and his colleagues (1955) first discovered these cytoplasmic particles by examining the enzyme content of various cell fractions isolated by differential centrifugation. They found that one fraction contained high concentrations of acid phosphatase, cathepsin, acid ribonuclease, acid deoxyribonuclease, and β-glucuronidase. They called them lysosomes because of their high content of hydrolytic enzymes. Later de Duve *et al.* (1960) described another biochemically distinct type of cytoplasmic particle distinguished by a high content of uricase, catalase and D-amino acid oxidase. The identification of these particles with the electron microscope has led to some confusion, but it would now appear that there are two

distinct cytoplasmic particles corresponding to the two biochemically identified entities.

The lysosome or peribiliary body is about 0·4 μ in diameter with a simple limiting membrane; the contents appear dark, and often contain vacuoles that vary much in size and shape. The microbody is of similar size and more constant in appearance: it is bounded by a simple membrane and contains a single central darker mass about half the diameter of the whole microbody. This dark central mass has an ill-defined border. The two bodies may be further readily distinguished electron-microscopically by the fact that lysosomes contain acid phosphatase.

After the injection of sucrose the microbodies remain normal, further support for the view that they are distinct entities.

The function of the lysosome, or for that matter of the microbody, remains obscure. Bennett (1956) suggested that lysosomes might represent segregated phagocytosed material, and their increase in number after the injection of bilirubin (Essner and Novikoff, 1961) provides some support for this. de Man *et al.* (1960) also found that the vacuoles that formed in the liver cell after the injection of dextran sometimes had lysosomes in their wall; more recently Daems (1962) has shown that these structures contain acid phosphatase. The changes produced by de Man *et al.* resemble somewhat the changes we have produced, but in their experiments the lysosomal material is pushed to one side of the body, which appears to contain dextran. In our vacuoles the remnants of the lysosome remain within the vacuole. It may be that sucrose is stored within the lysosome, ultimately reaching so great a concentration that water is drawn into it causing it to swell and so form a vacuole.

However the vacuole is formed, the change seems to result simply from the lysosome swelling, and nothing appears to be added to it. This is an important distinction from the changes described by Ashford and Porter (1962) in the liver of rats after in-vitro perfusion with glucagon, and by Novikoff and Essner (1962) after intravenous injection of Triton WR-1339; both papers report bodies like lysosomes containing mitochondria.

Oudea (1963) has very recently examined anoxic vacuoles with the electron microscope, and suggests that they are a consequence of infolding of the membrane of the sinusoidal border of the cell. We have independently made some preliminary observations of this type of vacuolation, which to some extent support his view. These studies seem to indicate that vacuoles induced by anoxia develop by a different mechanism from that involved in sucrose vacuolation.

Summary

Electron-microscope studies of the vacuoles induced in the liver cells of the rat by intraperitoneal injection of sucrose show that they have morphological and cytochemical features in common with

lysosomes and, in particular, both structures are rich in acid phosphatase. On these grounds it seems probable that sucrose vacuoles develop from lysosomes.

Acknowledgements are due to the Wellcome Foundation who provided the electron microscope.

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FIG. 5.—EM. Some vacuoles contain oval bodies surrounded by membrane. Permanganate. × 20,000.

FIG. 6.—EM. The structures inside the vacuole appear dark with multiple clear areas. Osmium tetroxide. Lead stain. × 30,000.

FIG. 7.—EM. Sucrose vacuoles and normal appearance of organelles other than lysosomes. Permanganate. × 15,000.

Rat liver 6 hr after sucrose injection.

SUCROSE VACUOLES AND LYSOSOMES IN LIVER

FIG. 8.—EM. Control: normal lysosomes showing dark inclusions and lining membrane. Osmium tetroxide. Lead stain. × 30,000.

FIG. 9.—EM. Control: normal lysosomes containing small dark bodies with clear areas in them. Osmium tetroxide. Lead stain. × 30,000.

FIG. 10.—EM. 6 hr after injection. Clear areas within lysosomes have increased in size and number. Osmium tetroxide. Lead stain. × 30,000.

FIG. 11.—EM. Control: lysosomes containing acid phosphatase. Holt and Hicks' method. × 30,000.

SUCROSE VACUOLES AND LYSOSOMES IN LIVER

FIG. 12.—Acid phosphatase localised to material within a vacuole. ×30,000.

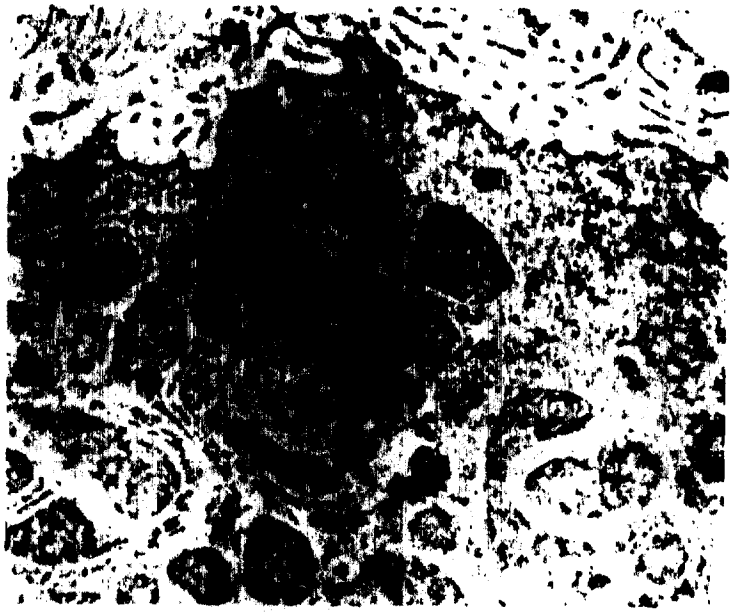

FIG. 13.—Acid phosphatase within vacuoles. Sinusoidal border of liver cell above. ×20,000.

EM. 6 hr after injection. Holt and Hicks' method.

Arch. Dis. Childh., 1964, 39, 431.

HEREDITARY SUCROSE INTOLERANCE: LEVELS OF SUCRASE ACTIVITY IN JEJUNAL MUCOSA

BY

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From The Queen Elizabeth Hospital for Children, London

(RECEIVED FOR PUBLICATION APRIL 7, 1964)

Though the amylase of duodenal juice hydrolyses the polysaccharides of the diet to oligosaccharides and disaccharides, the final cleavage of the latter to the monosaccharides, which is essential for absorption, is almost entirely effected by specific enzymes located in the intestinal mucosa. An inherited deficiency of intestinal lactase was first suggested as a possible cause of chronic diarrhoea and failure to thrive in a 13-month-old infant, by Durand (1958) and later by Holzel, Schwarz, and Sutcliffe (1959) for a similar condition in two sibs. Since then a number of cases, not only of lactase deficiency but also of sucrase deficiency, have been reported. The diagnosis has almost always rested upon severe diarrhoea following ingestion of the carbohydrate, an inability to metabolize the sugar as shown by the presence of the carbohydrate in the urine and faeces and the failure of the blood glucose to rise after oral administration of the disaccharide.

It has long been known that extracts of the intestinal mucosa of mammals have invertase activity. Following his characterization of several specific disaccharidases in hog intestinal mucosa, Dahlqvist (1961) has demonstrated the specific characteristics of the disaccharidases in human intestinal mucosa as well as the overlapping specificities of some of them. He has shown in particular that human intestinal sucrase (invertase), like the corresponding enzyme in the pig, has also some maltase activity.

Anderson, Messer, Townley, Freeman, and Robinson (1962) and Anderson, Messer, Townley, and Freeman (1963) have demonstrated the absence of sucrase in biopsy specimens of duodenal mucosa in two sibs with sucrose intolerance. Mucosal homogenates were incubated with sucrose and the incubation mixture was subsequently examined by paper chromatography. Only a visual estimate of the enzyme activity as judged by the amount of monosaccharide produced by hydrolysis was possible by this method.

In the present article, we present 7 further cases of an inherited absence of intestinal sucrase, including 2 in one family. The initial diagnosis was made on clinical grounds, and confirmed by the usual laboratory findings. In each case, a biopsy specimen of the jejunal mucosa has been obtained and the enzyme activities to a number of carbohydrate substrates, including sucrose, determined quantitatively. Only on this basis is it possible to diagnose with certainty the specific sucrase defect, and to differentiate it from the acquired carbohydrate intolerance that may occur in disease involving the small intestine. It is likely that cases of hereditary sucrose intolerance are not uncommon, as those now described were detected within a relatively short period.

Though the ultimate prognosis may be good even in untreated cases, it is nevertheless desirable to eliminate as soon as possible the responsible carbohydrate from the diet, otherwise weight gain and progress in the affected infant will be unsatisfactory.

Case Reports

Case 1. A boy (sib of Case 2) was born at Plaistow Maternity Hospital on October 13, 1958 at term after a normal pregnancy and delivery, birth weight 5 lb. 11 oz. (2,578 g.). He was fed from the beginning on dried milk with added cane sugar. On the 9th day he developed diarrhoea with loose green stools, which became more severe, so that on the 12th day he was transferred to the Queen Mary's Hospital. On admission he appeared very dehydrated, his weight being 13 oz. (368 g.) below that at birth. He was given intravenous therapy for 24 hours and then tube fed, and made a slow recovery (Fig. 1).

When discharged at 1 month of age, weighing 6 lb. 2 oz. (2,778 g.) he was still being given dried milk feeds with added cane sugar. Six days after discharge he was admitted to the Queen Elizabeth Hospital for Children

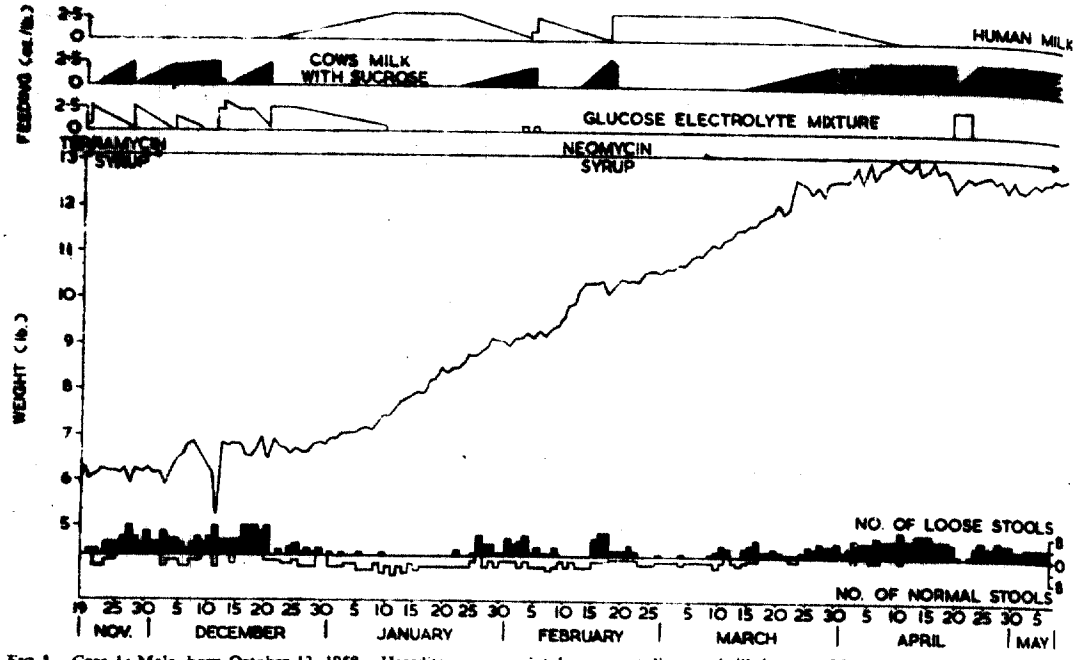


FIG. 1.—Case 1: Male, born October 13, 1958. Hereditary sucrose intolerance not diagnosed till the age of 5 years. Chart shows progress from 1 to 7 months of age.

Note (a) close correlation between intake of sucrose and the loose stools and loss of weight, (b) steady weight gain over a period of 11 weeks when sucrose was excluded except for 2 short periods when sucrose was reintroduced into the diet with resulting diarrhoea, and (c) immediate cessation of weight gain over a long period when sugar added to feeds.

with a history of cough for a day, one vomit, and refusal of feeds. On admission he appeared ill, with pallor, abdominal distension, and tachypnoea. His feeds of dried milk with sugar were continued, and on the day of admission he had a moderate diarrhoea which became more severe, with many fluid yellow stools. He became dehydrated and was given a glucose electrolyte mixture by mouth. No cause for the diarrhoea was revealed by laboratory investigation. When his feeds were fully regraded to dried milk with added sugar, diarrhoea recurred with loss of weight. The pattern of cessation of diarrhoea on an oral glucose electrolyte mixture, followed by its recurrence on regrading to full feeds, was repeated on four successive occasions during the first month of stay. Finally he was given expressed breast milk in increasing amounts over a period of 3 weeks. He began to gain weight steadily, 1½ lb. (680 g.) in one month, and the stools improved though they still remained loose. The breast milk feeds were now gradually replaced over a period of a fortnight by an evaporated milk with added sugar. Weight gain ceased and diarrhoea again became more severe. His feeds were therefore changed to expressed breast milk, with added Horlick's milk preparation, but without added sugar. He began to gain weight and diarrhoea improved. A return to dried milk feeds with added sugar led to another relapse. Breast milk was again instituted with fruit *purées* and cereals. He gained 1 lb. 3 oz. (538 g.) in weight in the next 3 weeks, and the character of the stools became more normal. Evaporated milk feeds were now gradually introduced at the same time as his expressed breast milk feeds were decreased, until, at about 5½ months of age he was being fed wholly on evaporated milk with added sugar and with fruit *purées* and cereals. Weight gain ceased and diarrhoea returned. At 7 months of age he was discharged on a dilute evaporated milk with added sugar and mixed feeding. Although he slowly gained weight the stools were always loose. He weighed only 18 lb. (8·16 kg.) at 1 year. He was admitted at 5 years of age for carbohydrate absorption tests and jejunal biopsy. He is now, at 5½ years, a well child, but has occasional loose stools. He continues to have some sugar in his diet, though much less than the average child.

Case 2. A girl (sib of Case 1) was born on November 5, 1962 at the Mile End Hospital at term after a normal pregnancy and delivery, birth weight 5 lb. 12 oz. (2,607 g.). She was breast fed for 2 months. When she was changed to dried milk with added sugar, she refused the sweetened feeds, but readily accepted them unsweetened. Although after this, cane sugar was not added to the milk feeds, sucrose was not completely excluded from the diet when mixed feeding was later introduced. There was an unexplained short episode of diarrhoea at 11 months of age, responding satisfactorily to glucose solution by mouth. She was admitted to the Queen Elizabeth

Hospital for Children at 1 year of age for investigation as a sib of an affected child. Her milestones had been normal but her weight, 17 lb. 12 oz. (8 kg.), was below the normal for this age (Levin, Mackay, Neill, Oberholzer, and Whitehead, 1959).

Case 3. A girl was born at term on April 11, 1962 after a normal pregnancy and delivery, birth weight 7 lb. 12 oz. (3,515 g.). She was breast fed for 3 weeks and then changed to an evaporated milk feed with added cane sugar. Within a few days, she developed a severe and explosive diarrhoea, with very fluid stools. When admitted to the Queen Elizabeth Hospital for Children on the 26th day, she was a crying but somewhat lethargic baby, with severe dehydration, weighing 1 lb. (453 g.) less than her birth weight. Her temperature was 100·5 F. (38° C.), and laboratory investigations revealed a metabolic acidosis with dehydration. Stool culture was negative. She was given intravenous therapy and recovery was complicated by convulsions lasting over 6 hours. Subsequently, two attempts at regrading the feeds to a dried milk mixture with added cane sugar resulted in diarrhoea and loss of weight, necessitating further intravenous therapy. She began to gain weight only when expressed breast milk was given, though the stools were still loose. When sucrose intolerance was suspected, glucose was substituted for cane sugar in her feeds, with further improvement. Throughout this period, however, she was on antibiotic therapy, and the antibiotic was dispensed in a syrup of a high concentration of cane sugar. The complete elimination of the sugar from the medicine led to marked lessening in the diarrhoea, and a maintained or increased weight gain. Several attempts since then to introduce sugar in the form of sweets etc. have led to relapse. At 14 months of age, she was readmitted for carbohydrate absorption tests and jejunal biopsy. She was then a well child, with normal milestones, weight 19 lb. 10 oz. (9 kg.), i.e. still below the normal weight for her age.

Case 4. A boy was born at the German Hospital on February 25, 1958 at full term after a normal pregnancy, birth weight 7 lb. 12 oz. (3,515 g.). He was breast fed for 2 weeks when he was gradually changed to dried milk with added cane sugar. When on full artificial feeds he developed diarrhoea, became irritable with crying, and refused his feeds. Believing that cane sugar was the cause of the diarrhoea, the mother largely replaced it by glucose, with cessation of diarrhoea. Cereal was introduced at 6 weeks and vegetable *purées* at 9 weeks. At 2 months of age cane sugar was reintroduced but led to refusal of milk feeds (Fig. 2).

At 9 weeks of age he again developed diarrhoea and was seen in the casualty department at the Queen Elizabeth Hospital. He was given an electrolyte mixture with cane sugar. Next day he had to be admitted because of continual screaming, refusal of feeds, vomiting, and diarrhoea. He was pale, mildly dehydrated, with no abdominal distension. He was treated with a glucose electrolyte mixture and an antibiotic. Faeces cultures were negative. When he was back on full feeds of dried milk with added cane sugar, diarrhoea recurred and he lost weight. This sequence of improvement in diarrhoea on an oral glucose electrolyte mixture followed by deteriora-

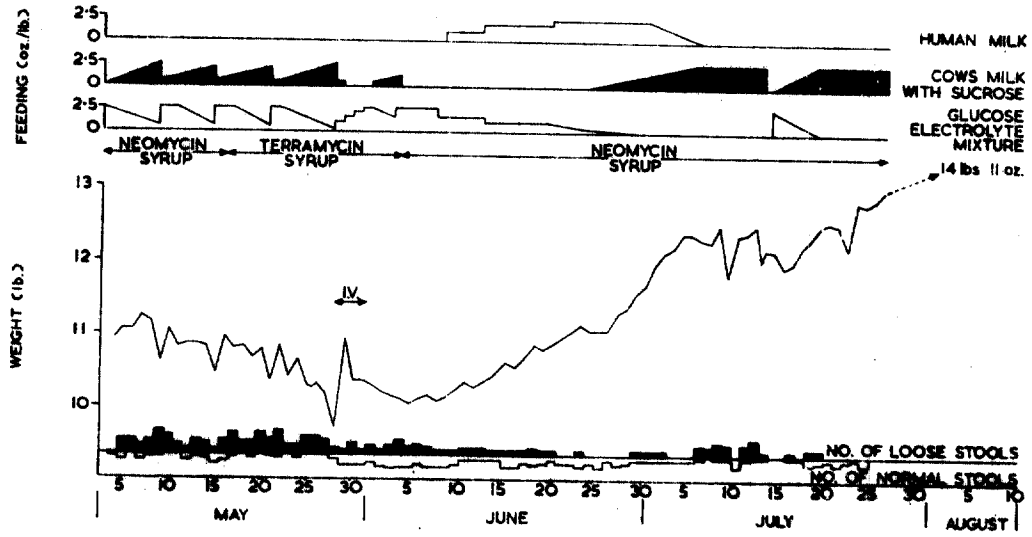


FIG. 2.—Case 4: Male, born February 25, 1958. Hereditary sucrose intolerance not diagnosed till 5 years old. Progress from 2 to 5 months of age.

Note (a) the sequence, in 4 consecutive periods, of improvement of diarrhoea and gain in weight on treatment with glucose electrolyte mixture; loose stools and loss of weight when full feeds of cows' milk with added sucrose are attained, (b) moderate weight gain over period of one month when sugar excluded from feeds; but loose stools persist because of the sucrose in the neomycin syrup.

[illegible] on [illegible] foods was repeated on four successive occasions in the first month of his stay in hospital. On the last occasion diarrhoea and dehydration were severe, necessitating intravenous therapy, after which he was given a glucose electrolyte mixture by mouth with added apple powder and casein hydrolysate for 11 days. Expressed breast milk was now introduced with gradual increase of feeds over 10 days. He began to gain weight steadily, and his stools improved though they still remained loose. Gradual replacement of breast milk by dried milk with added cane sugar resulted in failure to gain weight and increased diarrhoea.

He was discharged from hospital at 5 months of age on mixed feeding, but when home his mother again substituted glucose for cane sugar; diarrhoea ceased and an excellent weight gain followed (1 lb. 10 oz. (746 g.) in 17 days). Since then he has been a well child except for occasional loose stools. He was readmitted at the age of 5 years for the present investigation. His milestones were normal. At that time, he was still being given glucose for sweetening purposes. Sucrose in the form of sweets, chocolates, and ice-cream still caused loose stools, with some abdominal pain, though tolerance to small amounts of cane sugar had improved.

Case 5. A boy was born at home on February 5, 1960 after a normal pregnancy and delivery, birth weight 8 lb. 4 oz. (3,741 g.). He was breast fed for 9 weeks, after which he was weaned on to dried milk with added cane sugar, and cereals were introduced. Within two days of this diet the stools became loose and frequent, and this persisted in spite of a change to a half cream dried milk, though weight gain was satisfactory. His abdomen was noticed to be distended at the age of 7 months. Though there was some improvement in his diarrhoea as he grew older, it was made worse when sweet foods were eaten, and since abdominal distension persisted he was referred at 2 years of age to the Queen Elizabeth Hospital for Children. He appeared then to be a rather hypotonic child with a moderately distended abdomen, but both by weight and height he was only on the 25th percentile for his age. His appetite was very good, even excessive. He was passing 4-6 loose stools per day. These showed moderate numbers of fat globules on microscopic examination and a 3-day stool collection showed a raised fat excretion, 4·7 g. per day, the stools being fluid and bulky. A xylose absorption test proved normal. A radiograph of the abdomen was reported, 'This looks like a combination of Chilaiditi's disease and coeliac disease,' (Dr. C. J. Hodson). A barium meal (non-flocculating medium) revealed, 'clumping of medium in the small bowel,' (Dr. C. J. Hodson) and a radiograph of the wrist showed a bone age corresponding to his chronological age. A diagnosis of Chilaiditi's syndrome was made, though steatorrhoea is not normally considered part of this disorder. Because of the history of the diarrhoea being made worse by sweet foods, a form of sugar intolerance was suspected and the substitution of cane sugar by glucose in his diet for 2 months at home resulted in an improvement in the diarrhoea, though the abdominal distension persisted. At 2½ years faecal fat excretion (4·1 g. per day) was still high though slightly less than before, the stools being still bulky.

On September 3, 1962 he was admitted to hospital for further investigations for coeliac disease. He was given a diet from which cane sugar was largely though not completely excluded, and episodes of diarrhoea occurred once or twice a week. Abdominal distension was unchanged. A jejunal biopsy at this time showed no histological abnormality and this was thought to exclude coeliac disease. He was discharged on a low fat, high calorie, and high protein diet, which included restriction of added cane sugar to about 15 g. per day, apart from some small amounts in the ordinary food. His clinical state remained unchanged during the next 12 months, occasional diarrhoea and abdominal distension persisting. He was readmitted on July 16, 1963 for investigation of a possible sucrose intolerance. Carbohydrate tolerance tests and a jejunal biopsy for enzyme estimation were carried out. When discharged he was given a sucrose-free diet at home. His stools are now nearly normal but abdominal distension remains. His weight at 37 lb. 8 oz. (17 kg.) is between the 50th and 75th percentile for his age, and his milestones have been normal.

Case 6. A girl was born at Hackney Hospital at term on April 16, 1962, birth weight 6 lb. 4 oz. (2,834 g.), by forceps delivery for foetal distress, after a pregnancy complicated by toxaemia. She was asphyxiated at birth, and resuscitated with difficulty. She was admitted to the Queen Elizabeth Hospital on the next day with cyanotic attacks and convulsions, from which she gradually recovered. She was given expressed breast milk at first, but after discharge at 10 days this was changed to dried milk with added sugar. Soon afterwards she developed diarrhoea and was again seen at the Queen Elizabeth Hospital for Children, and given an electrolyte mixture with added sugar and an antibiotic. Diarrhoea became more severe and she was admitted the next day when 17 days of age. She was then severely dehydrated, having lost over 1 lb. (453 g.) in the previous 7 days, and was drowsy with deep and rapid respirations. There was a metabolic acidosis with severe dehydration for which intravenous therapy was given. No cause for the diarrhoea was found. When oral feeding was resumed with dried milk and added sugar, diarrhoea recurred and she lost weight. Improvement again followed treatment with an oral glucose electrolyte mixture with subsequent relapse on dried milk with added cane sugar. Though diarrhoea persisted she did maintain a steady weight gain while in hospital. When discharged at 7 weeks of age she weighed 7 lb. 14¼ oz. (3,580 g.), but she was readmitted the next day because of severe diarrhoea. Once again improvement followed intravenous therapy with relapse when normal feeding was resumed.

Sucrose intolerance was then suspected and glucose substituted for sucrose in the feeds. In the next 2 days her diarrhoea almost ceased, and she began to gain weight. After 18 days, a trial replacement of glucose by sucrose over seven days resulted in a prompt relapse with diarrhoea and cessation of weight gain. During her whole stay in hospital, she was given prophylactic antibiotics dispensed

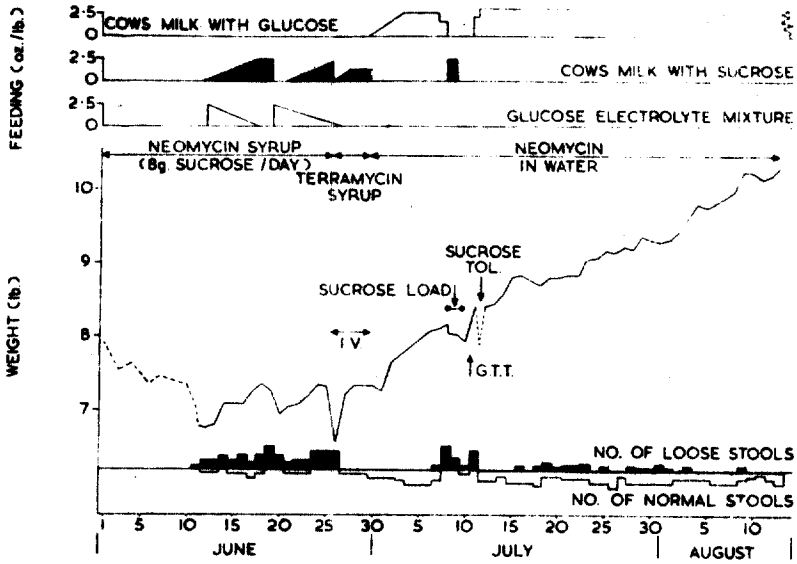


FIG. 3.—Case 7: Male, born June 1, 1963. Hereditary sucrose intolerance diagnosed and treated in early infancy. *Note* (a) improvement of diarrhoea and gain in weight on treatment with glucose electrolyte mixture; loose stools and loss of weight when full feeds of cows' milk with added cane sugar are attained, (b) severe diarrhoea on sucrose load, and (c) steady weight gain when sucrose excluded from diet.

in a syrup containing a high concentration of cane sugar. In the doses given, this constituted a considerable quantity of sugar, at least 8 g. per day.

She was discharged at the age of 3 months, weight 9 lb. 11 oz. (4,393 g.) on reconstituted dried milk with added glucose. Six weeks after discharge she had gained 2 lb. 13 oz. (1,275 g.), having been on a completely sucrose free diet. A test feed with sucrose as an out patient led to prompt relapse, which ceased when sucrose was stopped. The gradual reintroduction of sugar into her diet at 10 months of age led to intermittent but mild diarrhoea. She is still having a moderate amount of cane sugar with no more than occasional loose stools. Her milestones are normal. She was readmitted at 15 months of age for the present investigations.

Case 7. A boy was born on January 1, 1963 at the Annie McColl Maternity Hospital, after a normal pregnancy and delivery, birth weight 7 lb. 14 oz. (3,571 g.). From the beginning he was fed on dried milk with added cane sugar. On the 4th day of life he developed diarrhoea for which he was given clear fluids and neomycin orally. The diarrhoea improved but recurred on regrading the infant to milk feeds. He was admitted to the Queen Elizabeth Hospital for Children on the 10th day of life, his weight being 1 lb. 8 oz. (680 g.) below that at birth. He was lethargic and moderately dehydrated. His diarrhoea improved on a glucose electrolyte mixture with neomycin, but he relapsed as soon as he was given full feedings of dried milk with sugar added. The third sequence of improvement on a glucose electrolyte mixture therapy and relapse on full artificial feeds resulted in a diarrhoea severe enough to require intravenous therapy. Sucrose intolerance was now suspected and from the age of 1 month this sugar was excluded from his diet. His diarrhoea ceased and he began to gain weight steadily. After 8 days on a sucrose free diet, introduction of about 4 g. of cane sugar in every feed for only one day resulted in immediate diarrhoea. A tolerance test giving 11 g. sucrose resulted in the infant passing several watery, acid smelling stools four hours later. His subsequent progress on a sucrose-free diet has been good. At 9 months of age he was readmitted for jejunal biopsy and further carbohydrate tolerance tests. He is now a well child, weighing 19 lb. 6 oz. (8·78 kg.) and his milestones are normal (Fig. 3).

Laboratory Investigations

Methods. Blood glucose was determined, after precipitating the protein by a neutral reagent, by the glucose oxidase method, and blood sucrose by the Seliwanoff reaction as for fructose, using sucrose as standard. The latter values obtained included, of course, fructose, but this would usually be small. Urine and stool specimens were analysed for carbohydrates by paper chromatography, using butanol-acetic acid-water as the solvent system. For the tolerance tests 2 g./kg. were usually given.

Enzyme Estimations in Jejunal Mucosa. Biopsy specimens of jejunal mucosa were obtained by the use of the Crosby capsule. Fluoroscopy was used to determine the position of the capsule before it was fired. The specimens were always examined under the dissecting

microscope before enzyme determination. In every case the villi appeared normal. About 15 to 20 mg. of tissue were usually obtained on each occasion, enzyme activities being determined on the fresh specimens within a short time after removal. For the estimations the biopsy was well washed in ice-cold saline, blotted dry, and weighed. It was then homogenized in ice-cold distilled water in a Ten-Broeck grinder, using 15 strokes of the plunger. For sucrase, two concentrations of homogenate were taken, 1 in 40 and 1 in 100. For cellobiase and lactase a concentration of 1 in 40 was used, for palatinase, 1 in 100, and for maltase, 1 in 400.

Principle. The glucose liberated by hydrolysis of the disaccharide by the enzyme in the homogenate after incubation for 20 minutes was determined by the glucose oxidase method.

Procedure. 25 μl. of 0·2 M. potassium phosphate (KH_2PO_4) buffer adjusted to *p*H 6·0, 50 μl. of 0·1 M. of the appropriate disaccharide, and 50 μl. homogenate were incubated at 37° C. for exactly 20 minutes in a 2 in. × ⅜ in. glass test-tube. A control tube containing only buffer and homogenate was included for each estimation. Following incubation, 50 μl. of a 0·1 M. solution of the appropriate disaccharide were added to each control tube, and then from both test and control 50 μl. of the mixture were taken and incubated for a further 20 minutes with 1 ml. glucose oxidase reagent prepared by dissolving glucose oxidase (Boehringer) in 0·05 M. tris buffer, *p*H 7·0. The use of tris buffer rather than water is necessary to inhibit disaccharidase activity in the glucose oxidase reagent (Sols and de la Fuente, 1961). After centrifuging, the optical density was read at 436 mμ. The enzyme activity was calculated as μmoles disaccharide hydrolysed per min. per g. wet weight of tissue.

Carbohydrate Tolerance Tests. The blood glucose response after an overnight fast to a test dose of sucrose was determined, and in most cases glucose and lactose tolerances were investigated in addition, in order to confirm the specific nature of the failure to hydrolyse sucrose. In view of the ability of sucrase to act upon maltose, though it constitutes only 25% of the total maltase activity, the effect of a loading dose of maltose and starch was also usually tested. In several cases, a palatinose absorption test was also done, in an attempt to establish whether any associated defect of isomaltase activity was present, since palatinose, like isomaltose is a 1:6 linked glucoside and is hydrolysed by isomaltase.

Results

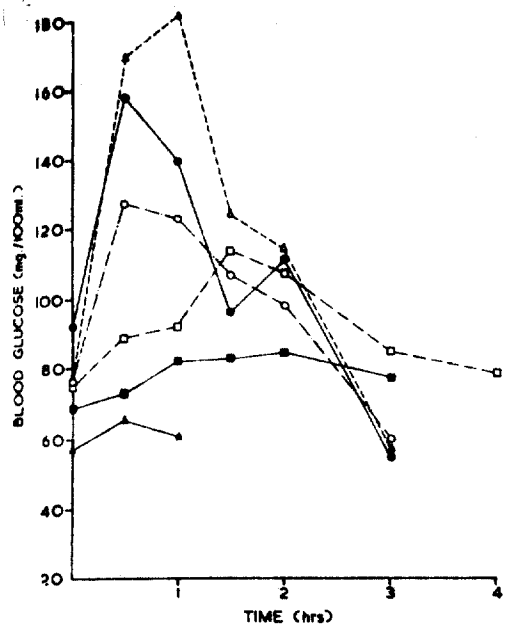
Case 1. The results of the full range of carbohydrate tolerance tests performed in Case 1 are shown in Fig. 4. There was a rise in blood glucose of between 50-100 mg./100 ml. in the first hour after the administration of glucose, lactose, or maltose. Following sucrose, the rise of glucose was only 8 mg./100 ml. Paper chromatography of urine passed 3 hours after sucrose was given revealed the presence of large amounts of sucrose. Though the blood glucose level rose 40 mg./100 ml. after oral starch administration, this did not occur until 1½ hours afterwards. Following palatinose there was a maximum rise in glucose of only 16 mg./100 ml. occurring 2½ hours after the dose. It is interesting to note that vomiting was provoked by both sucrose and palatinose test doses and a second amount had to be given. The urine obtained after administration of palatinose contained the disaccharide. Owing to an error, no stool specimen was available for chromatography. However, in contrast to the normal, the stool was a loose one.

Case 2. The results obtained in Case 2 are shown in Fig. 5 and are similar to those of Case 1. The blood glucose following lactose and maltose administration rose between 38 and 50 mg./100 ml. Whereas in Case 1, the maximum rise following a starch load was found in the second hour, that in Case 2 occurred in 30 minutes though the level achieved was about the same. As in Case 1, sucrose gave a flat blood glucose curve, the rise in the first 2 hours being only 5 mg./100 ml. The levels of blood sucrose in this test were also measured and reached a maximum of 11 mg./100 ml. 3 hours after sucrose administration. A similar result was obtained with palatinose, the rise in blood glucose over the whole period of the test amounting to no more than 6 mg./100 ml. No vomiting occurred but two diarrhoeal stools were passed during the period of the test, and these contained large amounts of the carbohydrate concerned.

Case 3. There was a rise of 60 mg./100 ml. of blood glucose following glucose administration, whereas the maximum rise was only 8 mg./100 ml. after sucrose and 14 mg./100 ml. 2½ hours after palatinose loading (Fig. 6). Both sucrose and palatinose produced loose, sour smelling, acid stools.

Case 4. The glucose absorption test showed a normal rise of 64 mg./100 ml. (Fig. 7), whereas the blood glucose rose only 4 mg./100 ml. following sucrose, which also induced loose, acid stools, containing large amounts of sucrose. A palatinose test was also attempted but vomiting ensued after about half an hour. There was, however, a significant fall in blood sugar of 5 mg./100 ml. during that time. A sib of this patient gave a normal blood glucose curve following sucrose.

Case 5. The rise in blood glucose following glucose or lactose loads was within normal limits.



IG. 4.—Case 1. Changes in levels of blood glucose after ingestion of ictose, glucose, maltose, starch, palatinose and sucrose (2 g./kg.). actose, △- - - -△; glucose, ● — ●; maltose, ○—·—·—○; arch, | — — — —|; palatinose, ■— ■; sucrose, ▲— ▲.

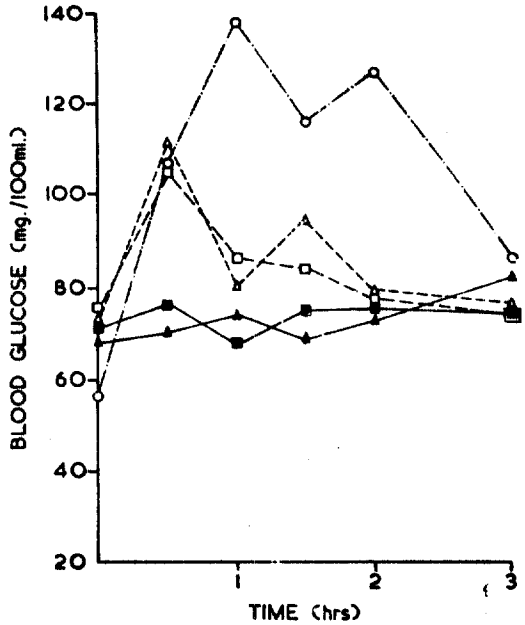
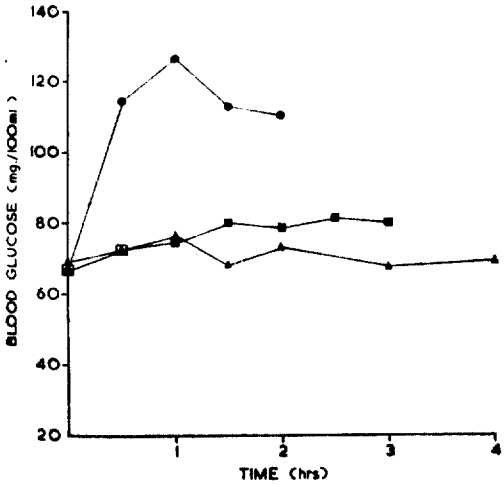


FIG. 5.—Case 2. Changes in levels of blood glucose after ingestion of maltose, lactose, starch, palatinose, and sucrose (2 g./kg.). Symbols as in Fig. 4.



IG. 6.—Case 3. Changes in levels of blood glucose after ingestion of ucose, palatinose, and sucrose (2 g./kg.). Symbols as in Fig. 4.

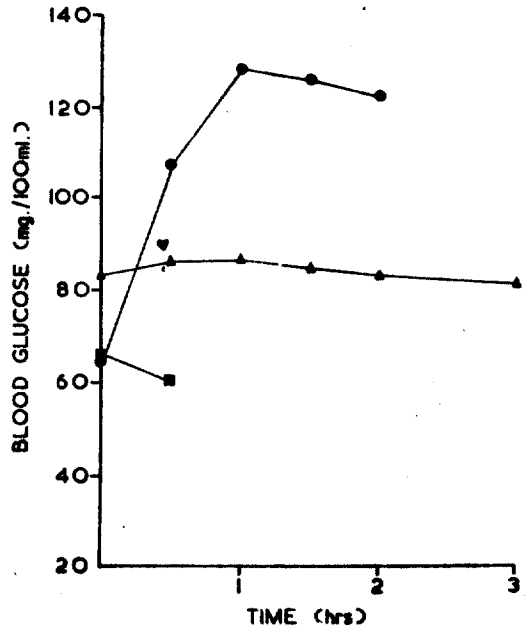


FIG. 7.—Case 4. Changes in levels of blood glucose after ingestion of glucose, sucrose, and palatinose (2 g./kg.). Symbols as in Fig. 4

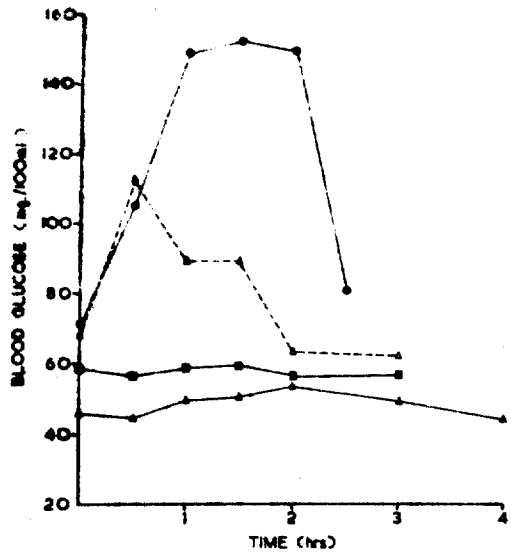


FIG. 8.—Case 5. Changes in levels of blood glucose after ingestion of glucose, lactose, palatinose, and sucrose (2 g./kg.). Symbols as in Fig. 4.

whereas there was little or no alteration in blood levels after sucrose loading (Fig. 8). In two sibs, the blood glucose rose 33 and 57 mg./100 ml. respectively half an hour after giving sucrose.

Case 6. Only the sucrose tolerance test was performed in this case. The maximum rise in blood glucose was 6 mg./100 ml. and this was only achieved at 2 hours. Examination of the urine revealed moderate amounts of sucrose. Several hours after the test, two loose stools were passed but unfortunately these were not kept for investigation (Fig. 9).

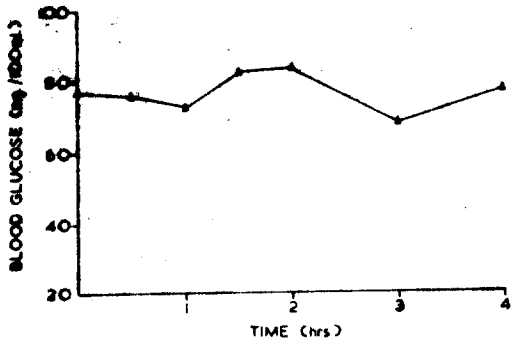


FIG. 9.—Case 6. Changes in levels of blood glucose after ingestion of sucrose (2 g./kg.).

Case 7. There was a fall in blood glucose in the first hour following sucrose administration, though there was a rise of 13 mg./100 ml. after 2 hours. Appreciable amounts of sucrose were detected in the blood after the first hour, up to 10 mg./100 ml. The urine contained sucrose and the stools passed after the test were watery and also contained sucrose. Both the maltose and lactose tolerance tests gave a normal response, though the latter did not show as great a rise of blood glucose as in other lactose tolerance tests, probably because it was performed the day following the jejunal biopsy (Fig. 10).

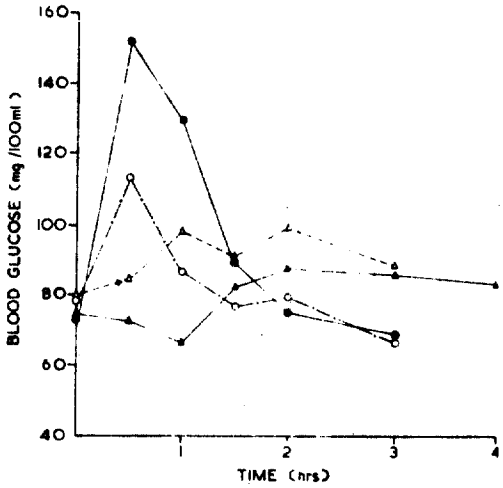


FIG. 10.—Case 7. Changes in levels of blood glucose after ingestion of glucose, maltose, lactose, and sucrose (2 g./kg.). Symbols as in Fig. 4.

Enzyme Activities. The sucrase, maltase, and lactase activities were determined in all 7 cases, the palatinase in 5, and the cellobiase was also estimated in one case. The results are given in Table 1. For comparison, the findings in 5 normal children from 1 to 6 years of age are shown in Table 2. These were mainly undertaken for diagnosis in children thought to have coeliac disease or a disaccharidase deficiency, but who from the histological examination of the biopsy as well as their subsequent progress proved not to have any condition that would seriously affect intestinal enzyme activities. All the enzymes show a wide variation in levels, except palatinase, of which, however, only 2 results were obtained.

In all the affected cases, the sucrase activities were almost absent, less than 1·5% of the mean normal value in 6 cases, and less than 3% in the remaining

TABLE 1

DISACCHARIDASE LEVELS IN JEJUNAL MUCOSA FROM PATIENTS WITH CONGENITAL SUCRASE DEFICIENCY

Case No. and Sex	Age (yr.)	Enzyme Levels (units)			
		Lactase	Maltase	Palatinase	Sucrase
1 M	5½	18·2	6·7	0·24	0·48
2 F	1¼	19·8	46·4	1·1	0·24
3 F	2	27·9	2·9	1·7	0·14
4 M	6	15·5	13·2	—	0·29
5 M	3½	18·1	7·6	1·7	0·14
6 F	2	8·8	3·4	—	0·29
7 M	1·9 mth.	20·0	27·0	2·4	0·28
Means		18·3	15·3	1·4	0·27

1 unit = 1 μmole substrate split/g. mucosa/min.

one. Where it was estimated, the palatinase activity was reduced to 15-36% of the normal, except in one instance, where it was about 3% of the normal. Maltase activity was also variable. In 3 cases, it was 10% or less of the mean normal value, in one it was approximately 20%, in one 40%, and in one it was just within the normal range. On the other hand,

TABLE 2

DISACCHARIDASE LEVELS IN JEJUNAL MUCOSA FROM NORMAL CHILDREN

Sex	Age (yr.)	Enzyme Levels (units)			
		Lactase	Maltase	Palatinase	Sucrase
F	6	8·5	63·3	—	20·2
M	3½	6·6	88·9	—	12·4
M	2	12·4	43·2	6·9	19·0
F	1	8·5	53·0	—	18·0
M	1½	7·5	64·5	6·5	17·4
Means		8·7	62·6	6·7	17·4
Ranges		6·6–12·4	43·2–88·9		12·4–20·2

1 unit = 1 μmole of substrate split/g. mucosa/min.

lactase activities were all normal and were actually considerably higher than the normal in all but one instance, suggesting possibly that our normal values are somewhat low. In the only case where it was determined, the level of cellobiase activity also was within the normal range.

Table 3 shows the enzyme activities in jejunal

TABLE 3

DISACCHARIDASE LEVELS IN JEJUNAL MUCOSA FROM PARENTS OF PATIENTS WITH CONGENITAL SUCRASE DEFICIENCY

Parent	Enzyme Levels (units)			
	Lactase	Maltase	Palatinase	Sucrase
Mother of Cases 1 and 2	16·7	44·4	5·2	9·7
Father of Case 4 ..	2·5	27·0	—	5·0
Father of Case 6 ..	10·5	50·7	5·1	16·3

1 unit = 1 μmole substrate split/g. mucosa/min.

biopsies obtained from 3 parents of our series of affected infants. In one, the father of Case 6, all the results were within our normal limits. Only in the mother of the affected sibs, Cases 1 and 2, might the sucrase be considered to be below the normal. In the third parent, the father of Case 4, the 3 enzymes determined, including lactase, all showed lower activities than normal.

Discussion

Following the reports of lactose intolerance by Durand (1958) and Holzel *et al.* (1959), a number of cases of sucrose intolerance probably due to a congenital absence of intestinal sucrase were recorded in 1961 (Weijers, van de Kamer, Dicke, and Ijsseling, 1961; Prader, Auricchio, and Mürset, 1961; Auricchio, Prader, Mürset, and Witt, 1961; Nordio, La Medica, and Vignolo, 1961; Delaitre, Fonty, Varlet, and Fourrier, 1961). Since then there have been many further reports, all but one (Anderson *et al.*, 1963) from countries in Western Europe.

Nordio and La Medica (1964), in a recent review, have summarized the clinical findings in the 26 recorded cases. A normal maltose and starch tolerance was observed in 6, though in 4 of these no palatinose or isomaltose tolerance test was done. In most of the remaining 20, intolerance to starch and maltose as well as to sucrose was also noted.

The clinical findings in our series were similar to those previously described. The presenting symptom in all was profuse watery diarrhoea, provoked as soon as sucrose was introduced into the diet. In two, this observation caused the mother to substitute glucose for sucrose in the feeds, and perhaps in this way averted the worst results. In 5 out of 7 cases, diarrhoea was severe enough to require intravenous therapy, and it is interesting to note that in these 5, cane sugar was introduced within the first 3 weeks of life, whereas those not requiring intravenous therapy were not given sugar till after 9 weeks. The early introduction of artificial feeds with cane sugar may lead to the confusion of sucrose intolerance with lactose intolerance. Diarrhoea was always sour smelling and often projectile in nature. A less frequent clinical finding was refusal of feeds, and in at least one case, this was correlated with feeds sweetened with cane sugar, though unsweetened feeds were readily accepted. In one other case, the child still dislikes sweets and sweet foods. Appetite was usually good, and in one case was even excessive. This case also showed steatorrhoea and persistent abdominal distension and has a coincidental Chilaiditi's syndrome. It is possible that this association is significant, and sucrose intolerance may be an aetiological factor in other examples of

this syndrome. Vomiting occurred in only one case and only on one occasion. In none of our cases was starch or maltose intolerance a significant feature.

Sucrose intolerance should be suspected when diarrhoea is coincident with the introduction of cane sugar into the diet and no other cause for the diarrhoea, e.g. infection, is found. It is always highly significant when, after improvement of the diarrhoea with treatment by a glucose electrolyte mixture, relapse occurs on resumption of normal feeds including cane sugar. A similar significant recovery may occur with expressed breast milk, as happened in 3 of our cases. This may lead to a suspicion of cows' milk allergy. Diarrhoea may persist even after removal of cane sugar from the feeds, and if this is so it is necessary to look for some unexpected source of this carbohydrate. In our cases, the syrup in which the antibiotics were dispensed was a concentrated solution of sucrose.

The clinical diagnosis must be confirmed by carbohydrate tolerance tests, including examination of urine and stools obtained during and immediately afterwards. In infants, the stool passed after a test dose of sucrose or palatinose was always fluid, and usually very acid when tested with *p*H papers. However, this was not invariably so, possibly because the time interval was too short for large amounts of acid to be formed or because the routine antibiotic therapy given reduced the bacterial content in the bowel. In the case of the older children, when loose but not fluid stools were passed, *p*H papers were unsatisfactory for direct testing for acidity, and no quantitative determinations of lactic acid content were done. These tests should be carried out after sucrose has been excluded from the diet, and there is no longer diarrhoea. It may be necessary for enzyme levels in the jejunal mucosa to be determined.

The progress of the untreated or incompletely treated child may be judged from 3 of our cases who were not definitely diagnosed until the age of 4 or 5 years. Tolerance to cane sugar improved with age, stools became more formed, and less frequent. Weight gain, which was usually unsatisfactory in the first year, also improved later. The condition may become less noticeable to the parents, who may accept it as normal. There appears to be no simple explanation for the improvement. The increased tolerance to sucrose with age may be more apparent than real. Infants on artificial feeding often have as much as 7 or 8 g. sucrose per kg. body weight per day, whereas the older child is unlikely to be given a similar proportional amount.

Carbohydrate Tolerance. In our series of cases, the maximum rise in blood glucose following administration of sucrose was no more than 8 mg./100 ml. except in two instances where it was 14 mg./100 ml., a level attained, however, only at the end of the second or third hour and this may have been due to some hydrolysis of sucrose by bacteria. In normal children, the maximum rise was usually between 30 mg. and 60 mg./100 ml. and this was always in the first 30 minutes. Though a normal rise in blood glucose following sucrose loading will exclude hereditary sucrose intolerance, the failure to rise does not necessarily confirm the diagnosis, since the cause may be malabsorption of varying aetiology, including a temporary or acquired depression of intestinal enzyme activity (Sunshine and Kretchmer, 1963).

Similar results were obtained when palatinose was given, with the maximum glucose rise being somewhat greater, up to 16 mg./100 ml., and always in the second hour. This slightly greater rise was probably due to the fact that the level of palatinase activity in most of the cases was still about one-third of the normal. Indeed, it is surprising that palatinose intolerance does occur with this percentage reduction of the level of palatinase activity. This, however, may be because the normal activity of this enzyme is only one-third that of sucrase, so that even a relatively moderate reduction in its activity will have a much greater effect on palatinose metabolism than a corresponding reduction in sucrase activity will have on sucrose metabolism.

The improvement in the clinical symptoms with age is not reflected in the sucrose or palatinose tolerance tests, which gave similar results in all our patients, whose ages ranged from 1 to 5 years. The younger the child, the more severe the effect of the carbohydrate load, watery diarrhoea being always provoked in the younger infant, whereas in the older child it was much more likely to be merely a loose stool. The carbohydrate given was always detected in the liquid stool, but was not a constant finding in the more formed stool. The disaccharide was sometimes found in those specimens of urine that were passed during or after the test, but unfortunately specimens of urine were not always available for examination. Whether the urine contains the carbohydrate depends probably on the amount absorbed unhydrolysed.

By contrast, the absorption of glucose and tolerance of lactose in all those cases in which these were carried out appeared normal. It is important to allow at least a week to elapse between a sucrose or palatinose test and the lactose test, otherwise there may be poor tolerance of the lactose, presumably owing to a toxic effect resulting from sucrose or palatinose loading. In 3 cases where a tolerance test

with maltose was performed, this was normal, as also were two starch loading tests. In Case 1, however, the maximum rise did not occur until the second hour, suggesting that there may have been a diminished tolerance to starch. This may possibly be correlated with the absent palatinase activity observed in this case.

Enzyme Defect. There has been much discussion as to the site of hydrolysis of the disaccharide during absorption by the intestine. Since sucrase, lactase, and maltase were found in the intestinal secretions, it was assumed that hydrolysis occurred in the lumen. On the other hand, enzyme assays on homogenates of intestinal mucosa as well as histochemical studies have demonstrated that disaccharidases are located in the intestinal mucosal cells. More recently, it has been conclusively shown that carefully collected intestinal juice contains practically no disaccharidase activity (Borgström, Dahlqvist, Lundh and Sjövall, 1957; Dahlqvist and Borgström, 1961). It seems certain, therefore, that the disaccharides are hydrolysed intracellularly and not in the lumen of the intestine.

In an investigation of the specificities of the human intestinal disaccharidases, Dahlqvist (1962, 1964) has shown by differential heat inactivation that four different maltases are present. He concluded that the whole activity of the intestinal mucosa towards both isomaltose and palatinose was present in one fraction which was termed maltase Ia (or isomaltase), and this same fraction accounted for 50% of the total intestinal enzyme activity towards maltose. The whole activity against sucrose was located in the fraction maltase Ib (or invertase), which accounted for 25% of the activity towards maltose. Maltases II and III were two separate enzyme fractions accounting for 15% and 10%, respectively, of the total activity against maltose. The whole of the activity against lactose and cellobiose was found in one enzyme, lactase, which was not associated with maltase activity. On this basis, Dahlqvist (1962) pointed out that an inherited absence of sucrase should be accompanied by a diminished activity towards maltose; as would an inherited absence of isomaltase. On the other hand, a congenital absence of either sucrase or isomaltase would not affect lactase activity.

Though the evidence in favour of an hereditary defect of sucrase in sucrose intolerance was strong, a definite proof of this required a quantitative assay of the enzyme activities in the intestinal mucosa. Anderson *et al.* (1963) showed that a homogenate of duodenal mucosa from one of their patients did not hydrolyse sucrose, and only slightly split isomaltose, whereas lactose and maltose were normally hydrolysed. Though this afforded a direct proof of the enzyme defect, the method of paper chromatographic separation of the carbohydrates obtained after incubation of the intestinal homogenate with the disaccharide, afforded only a qualitative or semi-quantitative result. Also Dahlqvist (1964) has shown that enzyme activities of duodenal mucosa may be very low in normal subjects, at least in older persons, and are much lower than in jejunal mucosa.

The enzyme activities determined in the jejunal mucosa were palatinase, lactase, and maltase, in addition to sucrase. It would have been preferable to estimate isomaltase activity but this disaccharide was not available in sufficient quantity. It was therefore decided to use palatinose as substrate since it is hydrolysed by the same enzyme as that which splits isomaltose (Dahlqvist, 1962). Lactase was selected because the proof of a specific enzyme defect should be supported not only by the observation of the very low or absent specific enzyme activity, but also on the finding that at least one enzyme activity is normal. Maltase activity was estimated because it should be depressed when sucrase is absent.

Sucrase activity in all the affected children was negligible, 2% or less of the mean normal value, and this must be accepted as indicating a complete lack of sucrase activity. Palatinase activity, which was measured in 5 affected children, was very diminished, to 15-36% of the mean normal level in 3 cases and to less than 4% in one case, the latter value being sufficiently low to be considered as absent activity. Though only two normal values for palatinase were determined in control children under 5 years of age, the results obtained in older children with treated coeliac disease, as well as in adults, suggest that these were representative values.

The results of the maltase estimations in our cases of sucrase intolerance were consistent with those predicted by Dahlqvist (1962). If the enzyme defect were confined to sucrase alone, the maltase activity should be diminished to 75% of the normal value. This was so only in Case 6. The maltase activity in Case 7 was 40% of the normal, and in 4 other cases it was as low as 11%. It is likely that the greater than expected deficiency of maltase activity can be accounted for by the lowered palatinase activity, since according to Dahlqvist (1962) this enzyme fraction also contains 50% of the maltase activity. This is supported by the fact that the decrease in palatinase activity approximately parallels the decrease in maltase activity, though the parallel is by no means exact.

Lactase, and in one case cellobiase, activity was also normal in all our affected children, indeed most

were somewhat higher. The ratio of sucrase to lactase activity was also very low in these cases, between 0·005 and 0·03, compared with a normal ratio of approximately 2.

The association of sucrose intolerance with that of isomaltose (or palatinose) has been noted by Auriocchio, Dahlqvist, Mürset, and Prader (1962, 1963) and Anderson *et al.* (1962, 1963). The presence of a diminished palatinase activity with the absent sucrase activity in our cases is difficult to explain. In the two affected sibs, one had a palatinase activity decreased to 15% of normal, whereas in the other this activity was absent (less than 4%). Neither has there been a satisfactory explanation for the simultaneous inherited absence of two enzymes, sucrase and isomaltase (palatinase), thus proved to occur in Case 1. The 'one gene—one enzyme' hypothesis (Beadle, 1945) would preclude the likelihood of this occurrence, since it would involve the simultaneous mutation of two genes, though this is not impossible. Wyngaarden (1960) has suggested that a single gene can control the synthesis of polypeptide fragments which can serve as a component of two or more enzymes, and this explanation could account for the absence of both sucrase and palatinase in our patient.

The clinical improvement with age was also not reflected in the levels of sucrase activity in the jejunal mucosa, since in the two cases of 5 years of age, no enzyme activity was detected. The absence of sucrase activity at this time supports the conclusion that the condition is an inborn error of metabolism, and not due to an immaturity of function.

Genetics. The hereditary nature of sucrose intolerance is suggested by the occurrence of affected sibs in a number of recorded cases, as well as by the fact that symptoms date from the first few days of life when sucrose had been given. Consanguinity has been noted in only one instance (Lelong and Alagille, 1963). Both males and females appear equally affected. On the basis of a history of transient diarrhoea in the father of two affected sibs and his intolerence to palatinose, Prader *et al.* (1961) suggested that the condition was inherited as an autosomal dominant. Gorouben, Bedu, Leballe, Grumbach, Yonger, Weill, and Kaplan (1963) and Rey, Frézal, Jos, Bauche, and Lamy (1963) believe that it is an autosomal recessive.

Our series of cases also shows an approximately equal sex distribution, 4 males and 3 females, and includes 2 sibs (Cases 1 and 2). The remaining sib of this family, as well as one sib of Case 4, and 3 sibs of Case 5 all showed normal sucrose tolerance. There was no history of consanguinity in any of the families. In none of our cases could a history of diarrhoea in childhood in the parents be established, though information was not complete. This may merely illustrate the difficulty of recalling in adult life a diarrhoea occurring in infancy and improving after the first year. Jejunal biopsies were performed in 3 parents of 3 unrelated families. None showed the typical enzyme pattern found in our affected cases. However, the mother of the two affected sibs showed a level of sucrase activity about half that of the mean level in normal children (Table 3), whereas the other enzyme levels were normal. One other parent, the father of Case 4, showed an even lower level of sucrase activity, but the other enzyme levels, including lactase, were equally decreased (Table 3). These are inconclusive results but they tend to support the suggestion that the condition is inherited as an autosomal recessive.

Summary

Seven cases, including two sibs, are described of hereditary sucrose intolerance, an inborn error of sucrose metabolism. They were first diagnosed at ages ranging from 1 month to 5 years. All presented with profuse watery diarrhoea as soon as cane sugar was introduced into the diet, and the earlier this occurred the more severe the diarrhoea. A less frequent finding was early refusal of feeds, especially sweetened feeds, though later the appetite was good. Vomiting and abdominal distension also occurred. Neither starch nor maltose intolerance was clinically a significant feature. Improvement of diarrhoea on breast milk or treatment with glucose electrolyte mixture, with relapse when full feeds with cane sugar are resumed, is highly significant of sucrose intolerance.

The tolerance tests showed in all cases failure of blood glucose to rise following sucrose loading and, in the 5 cases in which it was performed, following palatinose. The lactose, maltose, and glucose tolerance tests were normal. The diagnosis was confirmed by a quantitative assay of sucrase, lactase, maltase, and palatinase activities in biopsy specimens of jejunal mucosa. In all cases, there was an absence of sucrase activity, whereas lactase activity was normal. Maltase and palatinase activities were decreased and in one case, palatinase activity was absent. The significance of the enzyme pattern is discussed, in particular the association of two specific hereditary enzyme defects occurring in one patient. From a consideration of the cases diagnosed at the later ages, it appears that clinical improvement with age is accompanied neither by increased sucrose utilization nor by restoration of normal enzyme activity. The enzyme activities in the jejunal

mucosa of three of the parents were determined, the genetics of the metabolic error are discussed, and it is concluded that the condition is inherited as an autosomal recessive.

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Sugar Intake and Myocardial Infarction*

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In 1957, Yudkin showed that the mortality from arteriosclerotic heart disease in different countries was more closely related to sugar consumption than to fat consumption, and he subsequently suggested that the consumption of sugar might be a cause of the disease (1963). He tested this hypothesis by comparing the dietary histories of patients with myocardial infarction or peripheral vascular disease with those of other patients or healthy subjects interviewed at home or at work (Yudkin and Roddy, 1964; Yudkin and Morland, 1967). In two separate studies the men with myocardial infarction were found to have habitually eaten about twice as much sugar as the other men. Other investigators have failed to confirm Yudkin's findings in patients (Little *et al.*, 1965; Papp, Padilla, and Johnson, 1965); but it should be noted that they recorded the patients' dietary histories several months after the infarct had occurred, when food intake might have been modified. We have, therefore, sought to reproduce Yudkin's results by adhering as closely as possible to his method and interviewing patients while they were still in hospital.

METHOD

Men under 65 years of age were studied who were admitted to the Central Middlesex Hospital during specified periods between October 1965 and February 1968 with a diagnosis of a first myocardial infarct.

The diagnosis was accepted only if there was: (i) a history of pain at rest accompanied by Q wave changes in the electrocardiogram; or (ii) a history of at least one hour's pain at rest accompanied by S–T changes in the electrocardiogram and ancillary evidence of an infarct: that is, one or more of the following, (a) a white blood cell count of over 11,000/cu.mm.; (b) an ESR of over 20 mm./hour; (c) a temperature of 48 hours' duration, rising to at least 37·8°C. (100°F.); or (d) a serum aspartate aminotransferase (SGOT) over 40 units/100 ml. (Reitman and Frankel, 1957) or a serum hydroxybutyric dehydrogenase over 140 units/100 ml. (Rosalki, 1962).

For comparison, other men were also studied who were admitted to the general medical or surgical wards of the same hospital, who were under 65 years of age, and who provided no evidence of overt arterial disease (for example, did not have, or give a history of having had, angina pectoris, myocardial infarction, intermittent claudication, or a cerebrovascular accident). Two such men were chosen to match each infarct patient, by being in hospital at or about the same time and within 2½ years of the same age.

Bias due to national differences and the effect of disease was reduced by excluding patients from both groups if they: (i) were born outside Great Britain, or (ii) had had diabetes mellitus, a hospital diagnosis of peptic ulcer, or treatment for hypertension. Patients were not excluded if they had dieted for other purposes before admission, but the type of diet and its duration were recorded.

All patients were seen in hospital, when the medical history and diagnosis were checked, the blood pressure recorded, and the weight and height measured. Dietary histories were taken, while the patients were still in hospital, by an experienced interviewer trained as a medical social worker. A standard questionnaire was used which paid special attention to sugar consumption, but which also included questions about other relevant personal characteristics. No attempt was made to obtain a complete dietary history which would enable an estimate to be made of the calorie value of the diet or the total consumption of fat, carbohydrate, or protein. Patients were told that the object of the inquiry was to find out if there was any connexion between their diet and disease.

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TABLE I

PRINCIPAL DIAGNOSES IN CONTROL PATIENTS

Diagnosis	No. of patients
Cancer of bronchus	12
Cancer of large bowel	8
Other malignant disease	6
Varicose veins	7
Other cardiovascular disease	6
Chronic bronchitis	12
Asthma	6
Other respiratory disease	7
Hernia	19
Appendicitis	5
Gall-bladder disease	7
Other gastro-intestinal disease	9
Prolapsed disc	5
Disease of joints	11
Trauma	14
Other disease	26
All diagnoses	160

Subjects

Altogether 80 infarct and 160 control patients were studied. The principal conditions for which the control patients were admitted are shown in Table I. The comparability of the two groups of patients is shown in Tables II (age distribution), III (social class distribution), and IV (marriage status). It is evident that the groups are comparable with regard to age—as indeed was ensured by the design of the study—but that the infarct patients included a higher proportion in social classes I, II, and III, and a higher proportion who were married. This latter difference may be an artefact due to the choice of hospital patients as controls. Patients who are unmarried (whether bachelors, widowed, or separated) tend to stay in hospital longer than married patients, and are, consequently, more readily available for interview.

TABLE II

AGE DISTRIBUTION OF PATIENTS

Disease group	No. of patients aged (yr.):				Mean age (yr.)
	35 to 44	45 to 54	55 to 64	All ages	
Myocardial infarct	8	30	42	80	54·4
Other	12	59	89	160	55·1
All groups	20	89	131	240	—

$\chi^2 = 0{\cdot}501$, $n = 2$, $0{\cdot}3 < p < 0{\cdot}5$

TABLE III

SOCIAL CLASS DISTRIBUTION IN PATIENTS

Disease group	No. of patients in social class:			Total
	I and II	III	IV and V	
Myocardial infarct	18	50	12	80
Other	25	87	48	160
All groups	43	137	60	240

$\chi^2 = 6{\cdot}824$, $n = 2$, $p = 0{\cdot}03$

TABLE IV

MARITAL STATUS DISTRIBUTION OF PATIENTS

Disease group	No. of patients			Total
	Married	Widowed, separated, or divorced	Never married	
Myocardial infarct	73	4	3	80
Other	125	12	23	160
All groups	198	16	26	240

$\chi^2 = 7{\cdot}171$, $n = 2$, $p = 0{\cdot}03$

Results

Three measures have been used to assess the amount of sugar consumed: (i) the amount taken per cup of hot drink, (ii) the total amount taken in hot drinks per day, and (iii) the total amount of refined sugar consumed per day in the whole diet. To obtain these measures, items of diet were converted to grams of refined sugar by using the following conversion factors (Table V).

The distinction between heaped and level teaspoons and between large and small lumps was made in an attempt to attain greater accuracy. It is possible, however, that the attempt may have defeated itself, by increasing the amount of random variation. A simple estimate of the number of teaspoons of sugar (equating one lump of sugar with one teaspoon) may provide a better indication of the subject's taste for sugar and hence of his likely total consumption. We have, therefore, also expressed the first two measures in terms of "teaspoons" of sugar as well as in grams.

The results of the analysis show that patients admitted to hospital with a myocardial infarct took, on average, slightly more sugar in their tea and coffee than the control patients. The mean amounts were 1·40 and 1·24 teaspoons per cup, respectively ($t = 1{\cdot}60$, $n = 238$, $0{\cdot}1 < p < 0{\cdot}2$). The numbers of

TABLE V

CONVERSION FACTORS USED IN DIETARY ASSESSMENT

Item	Refined sugar (g.)
1 level teaspoon of sugar	4·0
1 heaped teaspoon of sugar	6·0
1 small lump of sugar	3·5
1 large lump of sugar	7·0
1 oz. boiled sweets, toffees or chocolates	24·0
1 oz. block chocolate	15·0
3 oz. helping of pudding	17·0
1 cake or 1 slice (1¼ oz.) of cake	12·0
1 sweet or semi-sweet biscuit	1·5
1 teaspoonful of jam or marmalade	5·0
1 teaspoonful of honey or syrup	6·0
1 glass of non-alcoholic drink, squash	10·0
1 glass of non-alcoholic drink, ribena (bottled "fizzy" drinks ignored)	17·0
1 teaspoonful of powdered Ovaltine, etc.	2·0
1 teaspoonful of sweetened condensed milk	2·0

TABLE VI

NUMBER OF PATIENTS TAKING DIFFERENT AMOUNTS OF SUGAR PER CUP OF HOT DRINK

Disease group	No. of men					
	Number of teaspoonsful per cup					All amounts
	0	< 1	1, < 2	2, < 3	3 or more	
Myocardial infarct	7	5	33	33	2	80
Other	16	14	79	49	2	160
All groups	23	19	112	82	4	240

$\chi^2 = 3{\cdot}524$, n = 4, $0{\cdot}3 < p < 0{\cdot}4$
χ^2 for trend = 1·023, n = 1, $0{\cdot}3 < p < 0{\cdot}5$

TABLE VIII

NUMBER OF PATIENTS TAKING DIFFERENT AMOUNTS OF SUGAR IN HOT DRINKS PER DAY

Disease group	No. of men					
	No. of teaspoons per day					All amounts
	< 5	5–8	9–12	13–16	17 or more	
Myocardial infarct	15	19	17	13	16	80
Other	39	43	34	34	10	160
All groups	54	62	51	47	26	240

$\chi^2 = 3{\cdot}834$, n = 4, $0{\cdot}3 < p < 0{\cdot}5$
χ^2 for trend = 3·277, n = 1, p = 0·07

patients who took different amounts are shown in Table VI. Relatively more of the infarct patients than of the control patients took two or more teaspoons per cup and relatively fewer took less than a teaspoon. A statistical test showed, however, that this trend could well be due to chance ($p > 0{\cdot}3$).

When the amounts were converted into grams, and a distinction was made between heaped and level teaspoons and large and small lumps, the difference between the groups was reduced. The means were then calculated to be 7·07 g. and 6·49 g. per cup, respectively ($t = 1{\cdot}03$, n = 238, $0{\cdot}3 < p < 0{\cdot}4$).

The effect of differences in the social class and marital status of the patients in the disease groups is shown in Table VII. Men in social classes I and II took slightly less sugar per cup than men in social classes III, IV, and V, and married men took slightly more sugar than the unmarried. Since the infarct patients included a higher proportion of married men and of men in social classes I and II, the effects of these differences will have largely cancelled themselves out. The effects were, in any case, small, and the mean amounts for each disease group, after standardizing for social class or for marital status, were almost identical with the crude figures.

TABLE VII

MEAN NUMBER OF TEASPOONSFUL OF SUGAR TAKEN PER CUP, BY SOCIAL CLASS AND BY MARITAL STATUS

Category		Mean number of teaspoons among:		
		Men with myocardial infarcts	Men with other diseases	All men
Social class	I and II	1·29	1·01	1·13
	III	1·44	1·32	1·36
	IV and V	1·42	1·23	1·27
Standardized for social class		1·41	1·24	1·30
Married		1·42	1·27	1·33
Unmarried		1·21	1·13	1·14
Standardized for marital status		1·38	1·25	1·30

Consideration of the number of cups drunk per day also made little difference to the results. On average, patients admitted with a myocardial infarct took 11·00 teaspoons of sugar in hot drinks per day compared with 9·63 teaspoons per day for the control patients ($t = 1{\cdot}18$, [illegible] $0{\cdot}2 < p < 0{\cdot}3$). The numbers of patients taking different total amounts of sugar in hot drinks are shown in Table VIII. Relatively more of the infarct patients than of the control patients took 17 or more teaspoons and relatively fewer took less than 5 teaspoons, but the trend, though somewhat [illegible] than when the number of teaspoons per cup was considered alone, was not quite statistically significant ($p = 0{\cdot}07$).

When the amounts were converted into grams and a distinction was made between heaped and level teaspoons and large and small lumps, the difference between the groups was [illegible]. The means were then calculated to be [illegible] and 51·04 g. per day, respectively ([illegible], $0{\cdot}4 < p < 0{\cdot}5$).

The estimated total amounts of refined sugar taken per day are shown in Table [illegible]. The amounts were 100·1 g. for the [illegible]

TABLE IX

NUMBER OF PATIENTS TAKING DIFFERENT TOTAL AMOUNTS OF REFINED SUGAR PER DAY

Disease group	[illegible]								All amounts
	Amount of sugar [illegible]								
	< 30	30–[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	
Myocardial infarct	4	10	2[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	80
Other	11	28	4[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	160
All groups	15	38	6[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	240

$\chi^2 = 1{\cdot}571$, n = 4, $0{\cdot}8 < p < 0{\cdot}9$
χ^2 for trend = 0·676, n = 1, $0{\cdot}3 < p < 0{\cdot}4$

TABLE X

MEAN TOTAL CONSUMPTION OF REFINED SUGAR PER DAY, BY SOCIAL CLASS

Disease group	Mean amount of sugar taken per day (g.)			Mean amount standardized for social class
	I and II	III	IV and V	
Myocardial infarct	100·7	102·2	90·8	99·1
Other	77·9	103·6	95·4	96·9
All groups	87·4	103·1	94·5	98·1

cardial infarct and 97·1 g. for the other men. The amount taken varied irregularly with social class, and standardization for social class made practically no difference to the results (Table X). Only in social classes I and II was there any appreciable difference in the total daily consumption between the affected and the control patients, but even in this subgroup the difference was not statistically significant (mean consumption, 100·7 g. and 77·9 g., $t = 1·749$, $n = 41$, $p \approx 0·1$).

Comparison of the weights of the patients is inappropriate, because some of the men in the control group were suffering from wasting disease. It may be noted, however, that about the same proportion of men in each group had attempted to control their weight by dieting (20 out of 80 infarct patients, or 25%, and 45 out of 160 controls, or 28%).

The patients' smoking habits are summarized in Table XI. Patients with lung cancer or chronic bronchitis have been omitted, because their conditions are known to be associated with smoking. The remaining results are broadly in accordance with those of many other studies (see U.S. Public Health Service, 1964, 1967) and show that a higher proportion of the infarct patients than of the control patients were current smokers before the onset of the illness that brought them into hospital.

TABLE XI

DISTRIBUTION OF SMOKING HABITS IMMEDIATELY PRECEDING THE ILLNESS THAT CAUSED ADMISSION

Disease group	No. of men					
	Non-smokers	Ex-smokers	Current smokers			All smoking categories
			Pipe or mixed	Cigarettes per day		
				1–14	15 or more	
Myocardial infarct	6	7	12	24	31	80
Other, excluding lung cancer and chronic bronchitis	16	33	13	34	40	136

Comparison between non- and ex-smokers and current smokers, $\chi^2 = 9·629$, $n = 1$, $p < 0·01$.

TABLE XII

RELATION BETWEEN SMOKING HABITS AND SUGAR CONSUMPTION: ALL PATIENTS

No. of teaspoons of sugar in hot drinks per day	No. of men					
	Non-smokers	Ex-smokers	Current smokers			All smoking categories
			Pipe or mixed	Cigarettes per day		
				1–14	15 or more	
< 5	6	15	6	8	19	54
5–8	10	20	5	15	12	62
9–12	6	7	4	17	17	51
13–16	0	5	3	13	16	39
17 or more	1	2	5	9	17	34
All amounts	23	49	25	62	81	240
Mean for all men	Daily consumption of refined sugar (g.)					
	90·6	80·8	105·4	108·2	100·7	98·1

Table XII shows that the smoking habits of the patients were, to some extent, correlated with their consumption of sugar, smokers tending to take more sugar than non-smokers or ex-smokers. For this purpose the number of teaspoons of sugar consumed in hot drinks per day was taken as the best estimate of sugar consumption, as this had provided the clearest distinction between the infarct patients and the controls. It is, therefore, important to see whether the difference in sugar consumption between the two groups could be due to an underlying difference in smoking habits, or vice versa. The results are summarized in Table XIII which shows the data separately for three categories of sugar consumption and two smoking categories. The number of infarct patients expressed as a percentage of all the patients with a given dietary and smoking history shows no trend with the amount of sugar

TABLE XIII

MYOCARDIAL INFARCT AND CONTROL PATIENTS DIVIDED BY SMOKING HABITS AND SUGAR CONSUMPTION (TEASPOONSFUL OF SUGAR PER DAY IN HOT DRINKS)

	Smoking category	No. of teaspoons of sugar		
		< 9	9–12	13 or more
No. of patients with myocardial infarct	Non-smokers or ex-smokers	9	3	1
	Current smokers	25	14	28
No. of patients with other diseases	Non-smokers or ex-smokers	42	10	7
	Current smokers	40	24	37
No. of myocardial infarct patients expressed as percent of all patients in same category	Non-smokers or ex-smokers	18%	23%	13%
	Current smokers	38%	37%	43%

TABLE XIV

DAILY CONSUMPTION OF REFINED SUGAR, BY MEN WITH AND WITHOUT MYOCARDIAL INFARCTS: COLLECTED DATA

Country	Authors	Mean daily consumption of refined sugar (g.)		Comments
		Patients with myocardial infarcts (no. in parentheses)	Other subjects (no. in parentheses)	
U.K.	Yudkin and Roddy (1964)	132 (20)	77 (25)	Controls: 13 orthopaedic patients and 12 healthy men
U.K.	Yudkin and Morland (1967)	148 (20)	90 (13)	Orthopaedic controls
			78 (20)	Healthy controls
U.K.	This paper (Burns-Cox *et al.*)	100 (80)	97 (160)	Controls: other in-patients
U.K.	Marr and Heady (1964)	—	109 (99)	Bank clerks
		—	118 (60)	Bus drivers
		—	123 (48)	Bus conductors
U.K.	Hollingsworth and Greaves (1967)	—	140 (—)	Estimated available supply per person
Canada*	Papp *et al.* (1965)	121 (20)	117 (20)	Controls: 19 in-patients and 1 healthy man
U.S.A.	Paul *et al.* (1968)	116 (66)	96 (85)	Controls: workers in same factory
Eire	Finegan *et al.* (1968)	66 (100)	69 (50)	Controls: surgical patients

* Data of Little *et al.* (1965) show lower mean values (58 coronary patients under 60 years, 48 g.; 52 healthy controls 55 g.), but they account only of sucrose used in cooking or added to food and drink.

...med within each smoking category, but it does show a substantial difference between smoking categories within each category of sugar consumption. This suggests that the slightly greater sugar intake recorded in patients with myocardial infarction may be secondary to their heavier consumption of tobacco.

Discussion

The object of our study was to see if we could reproduce Yudkin's results, by adhering as closely as possible to his method. This we have failed to do. Our data, however, do not justify a firm conclusion that sugar consumption is unrelated to the development of myocardial infarction. Retro...ve studies of hospital patients may be subject ...s both in the selection of patients and in the ...ling of the data, and it is impossible to be sure ...either our results or Yudkin's were not biased in one or other respect. In our study the infarct series consisted of groups of consecutive patients admitted to the medical wards of the hospital who survived long enough to be interviewed and who met the required criteria, with the exception only of those who were deaf or unable to speak English. The control series included similar patients with a variety of other diseases and all who were available at the time the ward was visited were interviewed. Limitation of the study to men who were born in Great Britain eliminated major national differences in dietary habits, and the exclusion of patients with a history of diabetes mellitus, peptic ulcer, treatment for hypertension, or a previous infarction eliminated the main sources of dietary differences for medical reasons. That this was effective was shown by the fact that the proportion of patients who had attempted to control their weight by dieting was similar in both groups (25% and 28%). It is conceivable that the control series included a disproportionate number of patients with conditions associated with a high consumption of sugar, but the inclusion of such a wide variety of diseases makes this unlikely.

It was not practical to conduct the interview without knowledge of the type of disease from which the patient suffered, since the infarct patients were largely concentrated in special wards. It is, however, unlikely that this influenced the recording of the data, since the interviewer had been interviewing patients for research purposes successfully, and without the apparent introduction of bias, for 20 years. It remains possible that the patient's knowledge of the condition from which he was suffering influenced the account of his dietary habits. This could most easily have affected the relatively complex history of the individual's total sugar consumption. It is less likely to have affected the simple assessment of the number of teaspoonsful of sugar taken habitually in a cup of tea.

The various results reported in earlier publications are summarized in Table XIV. All refer to middle-aged men. For comparison, an estimate has been included of the available supply of refined sugar per person in the United Kingdom in 1964. All but two show that the patients with a myocardial infarct have consumed, on average, more refined sugar than the controls with which they were compared. The extent of the difference, however, is very variable. It is possible, as Yudkin and Morland (1967) suggest, that Papp *et al.* (1965) and Little *et al.* (1965) failed to observe any appreciable excess consumption by infarct patients be-

cause the histories were not taken until several months after the infarct had occurred, when their diet (and memory of their previous diet) might have been modified. This criticism does not apply to our results nor to those reported by Paul *et al.* (1968) or Finegan *et al.* (1968). In Paul's study, moreover, the dietary histories were taken when the patients were well and before it was known which patients were going to develop an infarct. In their study the collection of the dietary histories cannot have been biased by knowledge of the nature of the patient's illness.

Smoking histories were recorded only in Paul's study and our own, and both sets of data showed a weak positive association between smoking and the consumption of sugar. In both cases the association between myocardial infarction and smoking was stronger than that between myocardial infarction and sugar consumption. In these studies much, if not all, of the excess consumption of sugar reported by patients with a myocardial infarction could have been secondary to an association between sugar consumption and smoking. The data relating sugar consumption to smoking, however, are few, and more observations are needed before the relation can be regarded as established.

We conclude only (i) that smoking habits need to be taken into account in studies of the relation between sugar consumption and disease, and (ii) that the totality of the data now available does not suggest that the consumption of refined sugar is likely to be a major and specific factor in the production of myocardial infarction.

Summary

An attempt has been made to reproduce Yudkin's finding that the dietary intake of sugar was considerably greater in patients with arterial diseases.

Comparison was made between 80 men under 65 years of age recovering from a myocardial infarction and 160 men, matched for age and country of birth, who were in hospital at about the same time with other diseases. An experienced interviewer took dietary histories from all the patients using a standard questionary.

The infarct patients were found to take slightly more sugar in their hot drinks, and slightly more refined sugar in total than the control patients. None of the observed differences, however, was statistically significant.

A greater proportion of the infarct patients than of the control patients were smokers, and smokers tended to take more sugar in their hot drinks than non-smokers or ex-smokers. The slightly increased sugar intake recorded for the infarct patients could be a reflection of their heaviest consumption of tobacco.

Miss Keena Jones undertook the dietary surveys on which this report depends, and for this we are most grateful.

We should also like to acknowledge the help of Dr. Mary Fulton and of the physicians and surgeons under whose care these patients were admitted.

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Effect of dietary sucrose and starch on oral glucose tolerance and insulin-like activity

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COHEN, A. M., AND A. TEITELBAUM. *Effect of dietary sucrose and starch on oral glucose tolerance and insulin-like activity.* Am. J. Physiol. 206(1): 105–108. 1964.—Substituting sucrose for starch, while keeping the fat, protein, salts, and vitamins of the diet constant, resulted in impaired oral glucose tolerance in rats. The time needed to develop impaired tolerance varied with the percentage of sucrose in the diet. In animals fed a diet containing 67% sucrose this took 21–40 days, whereas in rats fed diets with 40 or 33% sucrose, it took 40 and 100 days, respectively. This impairment was reversible if the animals were put back on starch diets, but only a few days were then required to redevelop the impaired tolerance when they were fed the sucrose diet. No difference was found between animals fed carbohydrates as starch and those fed the animal stock diet. Sucrose-fed animals showing an impaired tolerance had more fat in the liver and reduced insulin-like activity in the serum.

fatty liver and sucrose intake insulin reserve in rats
hyperglycemia carbohydrates and insulin reserve

DIABETES AND ATHEROSCLEROSIS are rare in recent Yemenite immigrants to Israel, while among those who have been in the country for more than 25 years the incidence is significantly higher (4, 6, 8, 13). A dietary survey of these two groups (5) showed that neither the saturated fat content of the diet nor the nature of the fat taken could explain this difference in incidence. However, most of the carbohydrates consumed in Yemen were in the form of starch, no sucrose was used, while in Israel about 40% of the carbohydrates were sucrose.

The purpose of the present investigation was to study the effect of the kind of carbohydrate consumed (sucrose or starch) on the "insulin reserve" of rats, the protein and fat intake being kept constant.

MATERIAL AND METHODS

Groups of local strain albino rats weighing 100–130 g were given either animal-house stock diet (A), or synthetic diets B, C, D, and E (composed of 67% carbohydrates, 10% butter, 18% vitamin-free casein, and 5% salts and vitamins) as specified in Table 1.

After different periods on these diets, oral glucose tolerance tests were performed: after being fasted for 16 hr the rats were given 350 mg glucose/100 mg body weight in 35% solution, by gastric tube; at 0, 60, and 90 min following the glucose load, 0.05 ml blood was taken from the tip of the tail and blood glucose was determined by the Somogyi-Nelson method (12).

The insulin-like activity (ILA) of the serum was determined on the basis of glucose uptake by the diaphragm. After being dieted for varying lengths of time, the animals were fasted for 16 hr, and an oral glucose load was given (350 mg/100 mg body weight); 60 min later the animals were anesthetized intraperitoneally by pentobarbital, opened, and bled from the aorta. The serum was separated and 1 ml used as the incubating medium. Hemidiaphragms were taken from 90 to 110-g male rats kept on a stock diet, fasted overnight, and decapitated following a blow on the head. After being weighed, one hemidiaphragm was put in the serum of an animal fed carbohydrates as sucrose, and the other in that of an animal fed carbohydrates in the form of starch. The Erlenmeyer flasks were gassed for 30 sec with a 95% O_2–5% CO_2 mixture and incubated in a Dubnov shaker at 37 C for 2 hr. The glucose uptake/100 mg of diaphragm was calculated. The water, fat (3), and nitrogen content (measured by a modification of Kjeldahl's method) of the carcasses and the total lipids of the liver (3) were also determined.

RESULTS

Glucose tolerance test. Animals fed carbohydrates in the form of sucrose (diet C) showed impaired glucose tolerance as compared with animals fed carbohydrates in the form of starch (diet B). This impairment, although suggested after 21 days (Fig. 1), became more pronounced and statistically significant after the diet had been fed for 40 days. There was no difference in glucose tolerance between the animals fed stock diet (diet A) and those fed

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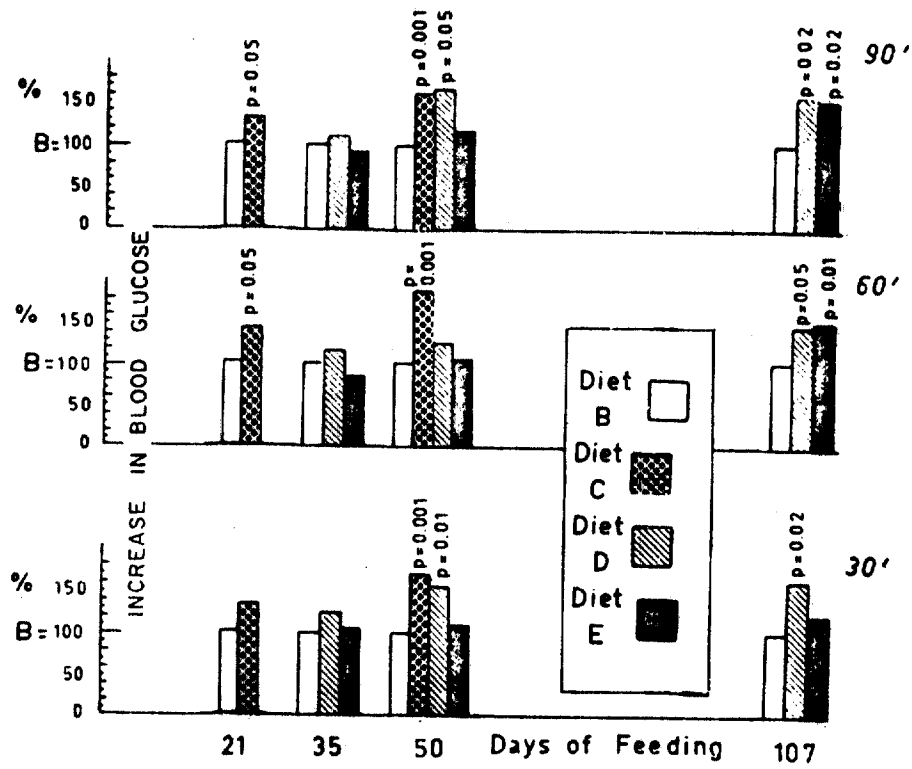

FIG. 1. Effect of change in per cent of sucrose in the diet on the oral glucose tolerance test. Contents of diet given in Table 1. Results are related to control diet B in which increase over fasting level is taken as 100%.

TABLE 1. *Contents of diets**

	Percentage by Weight			
	B starch	C sucrose	D 40% sucrose	E 33% sucrose
Vitamin-free casein	18	18	18	18
Butter	10	10	10	10
Starch	67		27	34
Sucrose		67	40	33
Salt† and vitamins‡	5	5	5	5

* Stock diet (A) was a mixture of ground whole wheat, ground alfalfa, bran, skimmed milk powder, and salts, containing 21% protein, 60% carbohydrates, 5% fat. † USP salt mixture no. II. ‡ Containing 0.2 mg thiamin, 0.3 mg riboflavin, 0.1 mg pyridoxine, 1.6 mg calcium pantothenate, 5 mg niacin, and 100 mg choline chloride per 100 g.

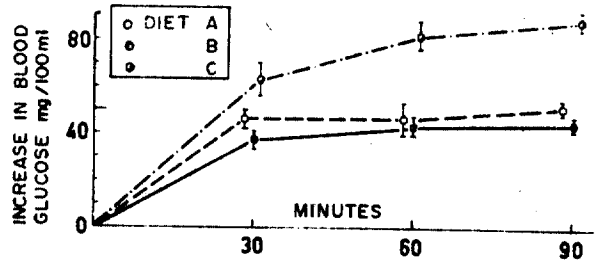

FIG. 2. Effect of kind of dietary carbohydrate on oral glucose tolerance (350 mg/100 g) after 80 days.

the starch diet (diet B) (Fig. 2). The fasting blood glucose of the three groups was not significantly different. On reducing the amount of sucrose in the diet to 40% and replacing it with a corresponding amount of starch (diet D), it took a full 40 days of feeding before glucose tolerance became impaired, and animals given a 33% sucrose diet (diet E) took between 50 and 100 days to develop impaired glucose tolerance (Fig. 1).

When animals were fed the original sucrose diet (diet C) for 78 days or more (Fig. 3) and subsequently put on the starch diet (diet B), it took 15 days to restore the glucose tolerance to the "normal" level. After 22 days or more on this starch diet, and after the glucose tolerance became repeatedly normal, these animals were put back on the sucrose diet (diet C); it now took only 6 days for fully impaired glucose tolerance to develop (Fig. 3) (as compared with a period of 21–40 days required for the "virgin animals"). These animals had thus become predisposed to the development of an impaired glucose tolerance as a result of the previous feeding on a sucrose diet.

Liver fat content. The fat content of the liver in animals fed the full sucrose diet (diet C) for 40 days was significantly higher than in animals fed the starch diet (diet B) (Table 2). This change was still found to exist after 120 days on this diet. It is to be noted that in animals fed the 40% sucrose diet (diet D) for 21 days, when the glucose tolerance was not yet impaired, the fat content of the liver was not different from that of the animals fed the starch diet (diet B).

Serum HA. HA of the sera, as measured by the diaphragm glucose uptake, was reduced in the animals fed the sucrose diet (diet C) as compared with the animals fed the starch diet (diet B) (Table 2).

There was no difference in the total-body weight gain

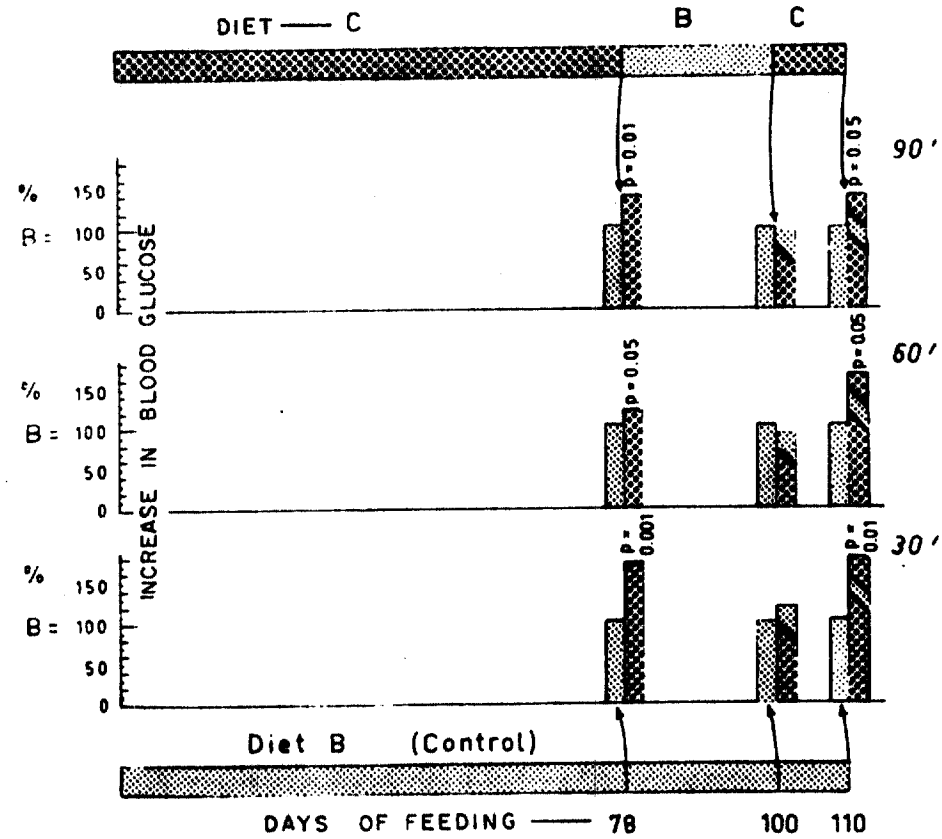

FIG. 3. Effect of the following regimen on changing oral glucose tolerance of rats: Days 1–78 on diet C, days 79–100 on diet B, and days 101–110 on diet C. Results are related to control diet B in which increase over fasting level is taken as 100%.

TABLE 2. *Effect of diet on weight gain, liver fat, and ILA of serum*

Diet	Feeding Period, days	No. of Animals	Gain in Body Wt./21 Days, g	Liver* Weight, g/100 g body wt.	Liver* Fat, %	Liver* P†	ILA* mg Glucose used/100 mg diaphragm	ILA* P†
B	21	8	78±8.9	3.2±0.34	3.7±0.31			
C		7	82±6.2	3.9±0.31	7.0±1.2	0.02		
D		8	84±5.3	3.3±0.34	3.8±0.23			
B	82	10	80±4.7	3.8±0.2	4.5±0.20		0.750±0.063	
C		9	66±5.9	4.0±0.3	6.7±0.32	0.05	0.411±0.071	0.01

Values are means ± standard error. * After being fasted 16 hr, then given glucose load. † Probability of difference between respective groups and group B.

TABLE 3. *Effect of intragastric loads of sucrose and starch, 0.5 g/100 g body wt., on the glucose blood level*

Load	Diet	No. of Animals	Fasting Blood Glucose, mg/100 ml	Excess Blood Glucose, mg/100 ml 30 min	60 min	90 min	120 min
Corn starch	A	10	67.2±2.1	28±2.5	29±2.9	24±2.9	14±2.5
Sucrose	A	9	66.1±1.2	53±1.9	76±2.0	61±1.9	62±5.1

Values are means ± standard error.

between the different groups of animals, nor in the water, fat, or protein body contents; neither was there any difference in the weight of the respective livers in terms of grams per 100 g body weight.

DISCUSSION

There is a significant impairment of glucose tolerance in the rat fed sucrose instead of starch. The time required for the sucrose-fed animals to develop this impairment was related to the amount of sucrose in the diet; even when this was reduced to 33% of the total carbohydrates (approximately the amount consumed by the average person with Western food habits (4)) impairment occurred, although only after 100 days feeding. The sera of the animals with impaired glucose tolerance showed diminished ILA, and there was a definite increase in their liver fat. These facts are compatible with a reduced insulin reserve. The facts that the impaired glucose tolerance was reversible, and that there was no change in the fasting blood glucose in the animals fed carbohydrates in the form of sucrose, indicated that there was only a relative insufficiency of the insulin reserve. When the excessive load on the insulin system caused by sucrose was relieved by feeding the animals carbohydrates in the form of starch, the glucose tolerance returned to normal. However, these animals developed impaired glucose tolerance much more rapidly when

refed the sucrose diet, suggesting that their insulin reserve was still limited and insufficient.

It was suggested by Claude Bernard in 1877 (2) that the organism's tolerance to carbohydrates is affected by previous nutritional factors, a point further elaborated by Himsworth (9). It has also been shown by several investigators (11, 14), using experimental animals under different conditions, that dietary sucrose and glucose impair the glucose tolerance, although their interpretation was different from that suggested here. Uram et al. (14) suggested a "hypoglycemic" substance in starch, an opinion that we could not substantiate since our animals fed the animal-house stock diet (diet A) had the same glucose tolerance as those fed the starch diet (diet B).

It appears that the decisive factor for the glycemic response is the rate of glucose inflow into the circulation and stimulation of the "insulin system." In carbohydrate deprivation the rate of glucose inflow is so low that the system atrophies (10) and the glucose load is met by an inadequate insulin response; hyperglycemia and glycosuria may then occur. A carbohydrate-rich diet is met by an adequate insulin response (9), but it may be assumed that repeated overstimulation may exhaust the system (7). It was shown (Table 3), in a separate experiment in normal rats fed the stock diet, that the blood glucose level was significantly higher in animals given an intragastric load of sucrose than in those loaded with an equal amount of starch. Thus, compared to the starch, the absorption of glucose from the gastrointestinal tract in the case of sucrose feeding is rapid, leading to a steep postprandial rise in blood glucose with resulting stronger stimulation of the insulin system. Such stimulation, when repeated, might possibly cause impairment of the insulin system in the sucrose-fed animals.

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Sucrose — Sucrose Toxicity

G. CONSTANTOPOULOS, E. M. BOYD[1]

Factors Affecting Sucrose Toxicity[2]

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Summary (Übersetzung siehe Karteikarte)

The following factors were found to affect the toxicity of sucrose given orally to albino rats. (1) *Food* in the stomach raised the LD_{50}; (2) *Volume of Distilled Water Vehicle:* toxicity was markedly increased when the dose of sucrose was given in a volume of 50 ml/kg body weight and over. (3) *Sex:* female animals were more susceptible than males to the toxic effects of administration of sucrose at a dose of 37.5 g/kg. (4) *Body Weight and Age:* males weighing 182 g were more susceptible than those weighing 118 g to a dose of 37.5 g/kg. (5) *Tolerance:* animals which survived daily administration for 5 weeks of 37.5 g/kg were resistant to the effect of a single lethal dose of 45 g/kg of sucrose given at intervals over a period of 31 days following abrupt cessation of daily administration of sucrose. (6) *7 Days vs 5 Days per Week:* a weekly dose divided and given each day for 5 days was more toxic than when given each day for 7 days over a period of 5 weeks. (7) *Season:* the toxic effects were similar at all seasons of the year.

Studies on the toxicity of sucrose in this laboratory were prompted by the report of BOYD, MULROONEY, PITMAN and ABLL [4] in which it was noted that benzylpenicillin toxicity was potentiated when measured in rats that had been fed a high sucrose diet. The amount of sucrose eaten per day by these rats was subsequently found to lie within the range of the acute oral LD_{50} of sucrose given as a single dose to rats with an empty stomach [3]. CONSTANTOPOULOS [7] undertook a study of the chronic toxicity of sucrose given orally to albino rats to elucidate the toxicity of high sucrose diets for animals and to provide a standard of reference for the chronic toxicity of drugs.

During the course of these studies on the chronic toxicity of sucrose it was observed that certain factors were of importance. The factors were: (1) the presence or absence of food in the rat's stomach; (2) the volume of distilled water used to dissolve the sucrose; (3) the sex of the animals; (4) the body weight and age of the animals; (5) tolerance to high doses of sucrose; (6) administration of a weekly dose of sucrose in five versus seven daily parts; and (7) the influence of season. The general literature on this subject has been recently reviewed [1].

General Methods

The studies were performed upon albino rats of a Wistar strain obtained from Canadian Breeding Laboratories of St. Constant, Quebec. They were fed Purina laboratory chow obtained from the Ralston Purina Company of Woodstock, Ontario. Their body weights, age, and sex were as noted below. The animals were brought into the laboratory from the animal quarters for clinical measurements, at which time they were placed in metabolism cages, one rat per cage. The clinical measurements made were body weight in g, food intake in g laboratory chow per kg body weight per day, water intake in ml/kg/24 hours, colonic temperature in °F, urinary volume in ml/kg/24 hours, urinary protein and glucose output in mg/kg/24 hours, urinary pH on 24 hour-samples and

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other signs as they appeared semiquantitated as 1 to 4 clinical units. The actual techniques employed have been reviewed [1].

Sucrose was administered through an intragastric cannula. It was dissolved in distilled water and given in doses and volumes noted below and expressed in terms of g/kg or ml/kg body weight. The numbers of animals are noted under description of factors studied. Statistical methods used were those of CROXTON [8] and HILL [11].

Factor Experiments

Factor 1: Food in the stomach

The acute oral LD_{50} of sucrose given as a single dose in a volume of 60 ml/kg to male rats of 300–500 g body weight and following a 16-hour fast was found to be 35.4 ± 7.0 g/kg [3]. In male rats of 180 ± 10 g (mean ± standard deviation) allowed to eat until treated, the acute oral LD_{50} determined in five groups of 20 rats per group was found to be 37.24 ± 7.3 g/kg. The LD_{50} was calculated by the linear regression method of BOYD [1]. Statistically, the median lethal dose was not significantly higher in unstarved than in starved animals. The LD_{50} is usually higher when a drug is given intragastrically to animals with a full than with an empty stomach [1].

Factor 2: Volume of Distilled Water Vehicle

Sucrose was given in a single dose of 38 g/kg to six groups of male rats of 176 ± 13 g body weight, each group consisting of 15 animals not starved to empty the stomach. Sucrose was dissolved in distilled water to final volumes of 35, 40, 45, 50, 55 and 60 ml/kg and one volume was given to one of the 6 groups. Fig. 1 shows that the mortality rates were higher in the groups that received the larger volumes. Most of the animals died between the third and seventh hour after sucrose administration irrespective of the volume of vehicle. The premortem signs were hypokinesia, listlessness, hyporeflexia, cyanosis, abdominal bloating, diarrhea, soiling, pallor, and hypothermia and their average values were more marked the greater the volume of the vehicle. Five hours after sucrose administration it was found that water intake decreased and urinary volume increased with increasing volume of the vehicle in which sucrose was dissolved. By the second and third day, survivors did not show any noticeable signs of toxicity.

Factor 3: Sex of the Animals

Sixty unstarved male rats of 181 ± 10 g body weight and 16 unstarved females of 176 ± 9 g were given 37.5 g/kg of sucrose in a volume of 50 ml/kg. Differences in mortality rates are illustrated in Fig. 2 and indicate that the death rate was higher in the female than in the male animals. Death occurred earlier in the females than in the males; 81 % of the females died within 24 hours versus 38 % of the males, with $P < 0.01$ that the difference was significant. The premortem signs were the same in both groups and were similar to those described under Factor 2 but their mean values tended to be more pronounced in the females.

Factor 4: Body Weight and Age of Animals

Eighty-two unstarved male rats of 118 ± 6 g body weight and 20 unstarved males of 182 ± 8 g were given orally 37.5 g/kg of sucrose in a volume of 50 ml/kg. The larger rats were 4 weeks older than the smaller rats. The mortality rate in the group of younger animals was 9.8 % and in the older animals 40 %, as shown in Fig. 2, with $P < 0.01$ that the difference was significant. The interval to death was more prolonged in the younger animals. The premortem signs were similar to those described for Factor 2 and in addition it was found that in survivors there was an hypothermia at 24 hours after sucrose administration.

Factor 5: Tolerance to Sucrose

Twenty unstarved rats were given sucrose at a dose of 37.5 g/kg daily, five days a week for 34 days and 10 were alive on the 35th day. On the 35th day the 10 survivors were given 45 g/kg of sucrose in a volume of 50 ml/kg, a dose which was estimated to approximate an LD_{100}. None of these rats died. The same sucrose dose of 45 g/kg given at the same time to 13 controls which had received daily water but no sucrose for 34 days produced a mortality of 85 %. The surviving controls were discarded but the 10 survivors of the sucrose-primed experimental group were again given 45 g/kg of sucrose on the 44th, 51st, 58th and 65th days or at 10, 17, 24 and 31 days after cessation of daily sucrose administration. All but 2 rats survived so

hat 8 out of 10 sucrose-primed rats survived ive administrations at approximately weekly ntervals of an LD_{85} of sucrose.

The resistance of the sucrose-primed rats could ave been due to development of tolerance to ucrose during the priming period of 34 days or t could have been due to the fact that they ere survivors. To eliminate the second possibility, the experiment was repeated in 10 rats given sucrose at a dose of 20 g/kg/day, 5 days week for 34 days and in 10 water-treated controls. The dose of 20 g/kg/day did not kill any of the rats. On the 35th day, administration of 5 g of sucrose per kg body weight killed 80 % of the controls but none of the sucrose-primed ats. The daily administration of the sublethal dose of sucrose for approximately one month had therefore produced a tolerance in rats to the lethal effect of sucrose. These results are summarized in Table 1.

Factor 6: Dosing 5 versus 7 Days per Week

Two groups, each of twenty male rats weighing 170—180 g, with eight controls for each group, were given sucrose daily without previous starvation to empty the stomach. The animals of one group were given 26.8 g/kg of sucrose orally for 7 days a week over a period of 35 days at the end of which time the mortality had reached 35 % (see Fig. 3). Animals of the other group were given 37.5 g/kg of sucrose orally for 5 days a week over the same period during which time the mortality rate reached 62.5 %. In each instance, mortality rates in the controls were subtracted from those in the sucrose-treated rats to obtain the figures quoted. The only deaths in controls occurred in the controls for the 5-day dosing group in which 2 controls died during the first week of the experiment. The two doses of sucrose were selected because they resulted in the same total amount of sucrose being given to both groups over the period of 35 days. In the 5 days/week-treatment group, most of the deaths occurred in the first week of treatment. The clinical signs observed in these latter animals were a slight increase in body weight on the two days when the animals were not treated, and a decrease in water intake, urinary volume, urinary pH and urinary

Table 1. The percent mortality to oral administration of sucrose in a dose of 45 g/kg to albino rats primed and not primed by previous administration of smaller doses of sucrose daily for 34 days.

Groups	% Mortality Induced by 45 g/kg Sucrose Day				
	35th	44th	51st	58th	65th
Control	84.6	–	–	–	–
Previously treated with sucrose for 34 days at 17.5 g/kg/day	0	0	20.0	0	0
Control	80.0	–	–	–	–
Previously treated with sucrose for 34 days at 20.0 g/kg/day	0	0	0	0	0

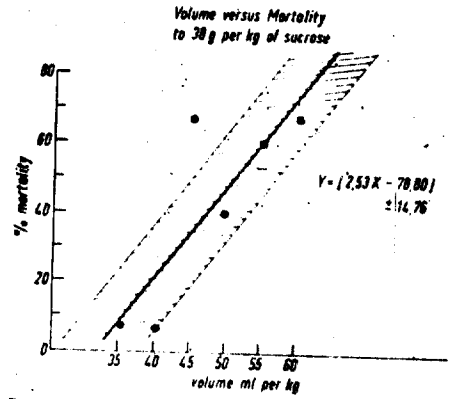

Fig. 1. The regression of percent mortality to oral administration of sucrose in a dose of 38 g/kg on volume of the distilled water vehicle in which sucrose was dissolved. The shaded area encloses the estimated value of Y plus and minus its standard error.

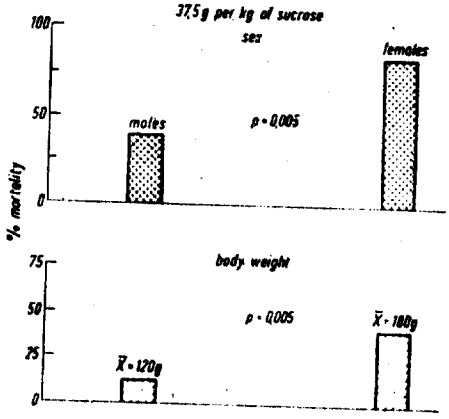

Fig. 2. The effect of sex and body weight of albino rats on the percent mortality to oral administration of sucrose in a dose of 37.5 g/kg.

Table 2. The percent mortality to oral administration of sucrose in a dose of 37.5 g/kg to albino rats at different seasons of the year

Month	Mean body weight (g)	% Mortality N = 20	Total % Mortality
June	182	40	40
January	189	40	
February	175	25	38.3
March	182	50	

protein excretion over corresponding values in animals given sucrose for 7 days a week. Other clinical signs were not significantly different between the two groups.

Factor 7: Influence of Season

Four groups of twenty rats each, with controls of 8 rats per group, having a body weight of 150–175 g, were treated with 37.5 g/kg of sucrose given orally without starvation as 50 ml/kg of a 75 % (w/v) solution in distilled water. Three of the groups were treated in the winter months of January, February, and March while the fourth group was treated in June. The mortality rates were 40 % in the summer and 38 % in the winter as shown in Table 2. There was no significant difference in clinical signs between the two seasonal groups of animals.

Discussion

In studies on acute oral toxicity, the substance is ordinarily given intragastrically to animals which have been starved to empty the stomach.

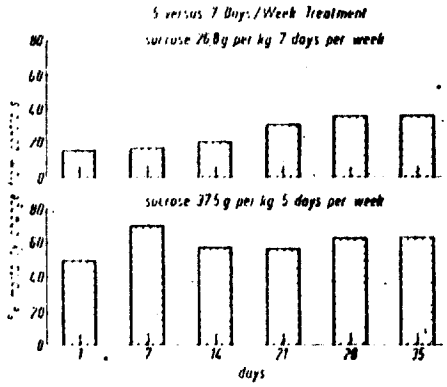

Fig. 1. A comparison of mortality rates in albino rats given the same total weekly dose of sucrose (37.5 g/kg) divided into 7 equal parts and one part given daily for 7 days and the divided into 5 equal parts and one part given daily for 5 consecutive days.

Parameters of toxicity, such as estimates of the LD_{50}, are usually more reproducible in the starved animal, presumably because the presence of varying amounts of food in the stomach can interfere in varying degrees with absorption of the agent being studied [17]. When the substance is given daily by intragastric cannula in chronic toxicity studies, the animal is ordinarily not starved before each administration. CONSTANTOPOULOS [7] found that a dose of sucrose approximately the same as that which would kill 50 % of rats when given once on an empty stomach, had to be given daily for one hundred days to kill 50 % of rats with free access to food and not starved before sucrose administration. It appeared, therefore, that the acute, single dose LD_{50} of sucrose should be higher when given intragastrically to unstarved than to starved rats. As noted above, the LD_{50} was a higher figure in unstarved rats but the difference was significant only at $P = 0.5$. Inability to demonstrate a statistically significant difference was due in part to the high standard error of estimates of the LD_{50} which, as noted by BOYD, GOBI and ABEL [4], is due to there being, in effect, two median lethal oral doses for sucrose, one for deaths within 9 hours and a second lower value for deaths after 9 hours. In view of the observation of CONSTANTOPOULOS [7] noted above, it is obvious that the LD_{50} must be higher when sucrose is given on an empty stomach. The increase is apparently so small that very large numbers of animals would be required to prove it significantly higher statistically.

It should be noted that other interpretations can be placed on the significance of a drug being more toxic when given on an empty than on a full stomach. Starvation alone produces toxic effects [18] which can augment the toxicity of, for example, caffeine [12].

The finding that oral sucrose toxicity increased with increase in the volume of distilled water used as a vehicle to dissolve it confirms similar observations on drugs reported by FERGUSON [9]. Various explanations may be proposed for increase in toxicity when a drug is given intragastrically with increasing volumes of distilled water. Distilled water itself produces measurable signs of toxicity when given intragastrically to rats in volumes of the order of 60 ml/kg body weight [3] and in larger volumes can produce death [3]. Increase in the toxicity of sucrose may have been due to a combination of the

toxicity of sucrose and that of increasing volumes of distilled water. A second hypothesis is that when sucrose is given intragastrically as a highly concentrated solution, its enzymatic hydrolysis and subsequent absorption may have been impaired, producing a lower toxicity. This was investigated in preliminary studies in which blood glucose levels were measured in 30 rats given 38 g/kg of sucrose in increasing volumes of distilled water. Increase in blood glucose was found to be higher the lower the volume of distilled water vehicle which indicated that increase in blood glucose was probably not responsible for the toxicity of sucrose in these animals.

The observation that female rats were more susceptible to sucrose toxicity than males confirms the report of Boyd et al. [3]. Most drugs are more toxic in female than in male animals [7] but there are important exceptions — for example caffeine is more toxic given orally to male than to female albino rats [16]. Zbinden [18] has noted that enzymatic detoxification of many drugs is greater in males than in females, possibly due to the production of larger amounts of anabolic steroids in the male than in the female. Differences in body reaction to a drug may explain differences due to sex of the animal — caffeine can cause death from hemorrhage due to automutilation and automutilation is more common in male than in female rats given large doses of the drug by gastric tube [16].

Older rats were found to be more susceptible to sucrose toxicity than younger animals. Similar results have been reported for other drugs such as caffeine [16]. As the animal grows older, it usually accumulates more body fat and less water per unit dry weight [14]. Since drugs are normally administered to animals in doses calculated as g/kg body weight, the older animal may be getting relatively more drug per kg of active body tissues. Drug detoxification may be less active in old than young animals [18].

The finding that rats given large doses of sucrose daily for a period of days become resistant to the lethal effects of sucrose confirms studies reported by Fiorica and Jamieson [10] who infused hypertonic sucrose solutions into rats for 5 days and found the animals then developed tolerance to the toxic effects of sucrose. Development of tolerance may explain why Constantopoulos [7] could give large oral daily doses of sucrose to rats without producing death.

Introduction of the statutory five day week in labor agreements has posed problems in chronic daily dosing of animals [11]. Those who have compared the effect of giving the total weekly dose of a drug in equal parts over 5 days with that when given over 7 days have reported some drugs more toxic and others less toxic by the latter method [11]. There appears to be no general rule. Each drug must be studied by the two methods of dosing and sucrose was found to be more toxic when the weekly dose was given over 5 rather than over 7 days.

Seasonal variation has been often found in the pharmacological [8, 9] and toxicological response of animals to drugs [1] and to diets [14]. No evidence of a seasonal variation was found in the toxic response of albino rats to oral sucrose.

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Maximal Tolerated Amounts of Sucrose Given by Daily Intragastric Administration to Albino Rats

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Abstract—Sucrose was given by intragastric cannula to young, male albino rats once daily for 100 days or until 60% of the animals had died, whichever occurred first. The $LD_{50\ (100\ days)}$ ± SE, or daily dose that killed 50% of the animals during 100 days of administration, was found to be 28·5 ± 1·3 g/kg/day. The $LD_{50\ (100\ days)}$ was 80·6 ± 3·7% of the acute oral $LD_{50\ (1\ dose)}$; this percentage is termed the 100 day LD_{50} Index. The 100 Day LD_{50} Index was higher for sucrose than for any drug studied to date. Signs of toxicity at the oral $LD_{50\ (100\ days)}$ were inhibition of growth, diuresis, polydipsia, aciduria, diarrhoea, soiling, ataxia, hyporeflexia, drowsiness, pallor, prostration, meningoencephalitis, arteriolitis, myocarditis, hepatitis, nephritis, pneumonitis, inhibition of spermatogenesis, loss of weight and degenerative changes in many body organs and hypertrophy of the gastro-intestinal tissues. These signs were less marked or absent at the maximal $LD_{0\ (100\ days)}$ which was 19·8 ± 1·9 g/kg/day. At the minimal $LD_{100\ (100\ days)}$ of 35·5 ± 0·5 g/kg/day, the signs were similar to those seen in acute (one-dose) toxicity studies.

INTRODUCTION

In a series of publications, recently summarized by Boyd (1968a), it has been proposed that the toxic effect of repeated daily administration of a drug be described as the clinico-pathological syndromes of intoxication at the maximal $LD_{0\ (100\ days)}$, the $LD_{50\ (100\ days)}$ and the minimal $LD_{100\ (100\ days)}$. The maximal $LD_{0\ (100\ days)}$ is the largest dose that can be given daily for 100 consecutive days without producing death and is practically the same as the $LD_{1\ (100\ days)}$. The $LD_{50\ (100\ days)}$ is the daily dose that kills 50% of animals during 100 consecutive days of administration and the miminal $LD_{100\ (100\ days)}$ is the smallest daily dose that kills all animals during 100 consecutive days of administration. The duration of 100 days was initially selected because it represented one tenth of the expected lifespan of the albino rats used in these studies. As noted by Boyd (1968a), most of the information obtained was reasonably obvious after 60–80 days of administration.

By expressing the $LD_{50\ (100\ days)}$ as a percentage of the acute LD_{50} or $LD_{50\ (1\ dose)}$, an index of chronic toxicity termed the 100 Day LD_{50} Index was obtained. The higher the value of this index the relatively greater is the proportion of an $LD_{50\ (1\ dose)}$ that can be given daily for 100 days without producing deaths, and therefore the greater the relative safety of a drug for long-term daily use.

As a standard of reference for studies on drugs, it was decided to determine the 100 Day LD_{50} Index for sucrose. It was anticipated that the Index for sucrose would be relatively high and this was found to be so. Sucrose was given daily in a range of amounts based upon

the $LD_{50\ (1\ dose)}$, which had been found to be 35·4 ± 7·0 g/kg for sucrose administered intragastrically to rats with an empty stomach (Boyd, Godi & Abel, 1965).

Hayes (1967) has recently proposed relating to the $LD_{50\ (1\ dose)}$ the daily amount of a drug which, when added to the diet of rats, will kill 50% of the animals in 90 days. It should be noted that a drug is usually, but not always, less toxic when added to the diet than when given by intragastric administration. Rats will show no apparent signs of toxicity when eating in their diet each day an amount of sucrose equivalent to 2–3 times the LD_{50} given intragastrically on an empty stomach (Boyd, 1968a; Richter, 1956), but they are very susceptible to the toxic effects of drugs such as benzylpenicillin and caffeine (Boyd, 1968a).

Preliminary contributions on clinical findings in chronic sucrose poisoning have been reported by Constantopoulos (1967) and on autopsy findings by Constantopoulos & Boyd (1968a). Factors that affect sucrose toxicity have been described by Constantopoulos & Boyd (1968b).

EXPERIMENTAL

Animals and diets

The work was performed upon 280 young male albino rats (initial body weight 150–175 g) obtained from the Canadian Breeding Laboratories, St. Constant, Quebec. The rats were fed Purina laboratory chow checkers (Ralston Purina Co., Woodstock, Ontario) and water *ad lib*. Since it was planned to give sucrose daily, the animals were not starved to empty the stomach prior to the first administration of sucrose.

Experimental design and conduct

Sucrose (Certified Sucrose from the Fisher Scientific Co. Ltd., Don Mills, Ontario) was given through an intragastric cannula attached to a syringe, in daily doses of 20·0, 25·0, 30·0, 32·5, 35·0, 37·5, 40·0, 42·5 or 45·0 g/kg. Each dose level was given to at least 20 animals with at least eight controls. The sucrose was freshly dissolved in distilled water and given in a volume of 50 ml/kg body weight, the controls receiving only an equivalent volume of distilled water. Sucrose was initially given each morning for 7 days/wk but this procedure was discarded in favour of daily administration for 5 days/wk when it was found that mortality rates were the same with either regimen (Constantopoulos & Boyd, 1968b). Each daily amount of sucrose was given for 100 days or until 60% of the animals had died, whichever occurred first. Calorie pair-fed controls were not introduced, since it was found that the intake (kcal/kg body weight) in the sucrose-treated rats was similar to that in the controls at the $LD_{50\ (100\ days)}$.

Clinical measurements upon each of the rats, housed singly in metabolism cages, and cage-side observations were made on the day before and at weekly (or shorter if indicated) intervals after the beginning of sucrose administration. The measurements included body weight (g), food intake (g laboratory chow/kg/24 hr), calorie intake (kcal from chow plus sucrose/kg/24 hr), water intake (ml/kg/24 hr), colonic temperature (°F), urinary volume (ml/kg/24 hr), urinary output of glucose and protein (mg/kg/24 hr), urinary pH (on 24-hr sample) and other clinical signs as they appeared, semi-quantitated in clinical units of 1+ to 4+. Colonic temperature was recorded by a Fisher Thermistemp Telethermometer with the probe inserted into the descending colon. Urine analyses were performed with Combistix manufactured by the Ames Co. of Canada Ltd., Rexdale, Ontario.

Gross and microscopic pathology were recorded on animals that died and could be autopsied within 1 hr of death to avoid post-mortem changes described by Boyd &

(1963). Sections were taken from blocks fixed in Lillie's buffered formalin and stained with haematoxylin–phloxine–saffron.

Animals that survived to 100 days or remained after 60% of other animals of the dosage group had died, whichever occurred first, were killed with chloroform and immediately autopsied. The fresh weight and water content of the heart, kidneys, liver, lungs, spleen, testes, thymus, adrenals, brain, salivary glands, sections of the gastro-intestinal tract and samples of muscles and skin were measured at autopsy and histopathological studies were made on tissues from representative animals. The contents of the lumen of the gastro-intestinal tract were removed, by a standardized process of water-washing and milking, before the tissues were weighed. The sample of skeletal muscle was the right half of the muscular layer of the ventral abdominal wall. All organs except the skin and residual carcass were weighed to 0·1 mg on a Mettler Semimicro Gramatic Balance (Fisher Scientific Co. Ltd.) while the skin and residual carcass were weighed to 0·01 g.

The water content of the organs was determined by drying aliquots to constant weight at 95°C in a Fisher Forced Draft Isotemp Oven. The sample of skin was taken from the dorso-lumbar region after the hair had been clipped. The residual carcass was cut into small pieces, homogenized in a Waring Blendor (Fisher) and a sample weighed for water analysis. Water levels were calculated as g water/100 g dry weight of tissue.

Enough of the animals given 20 or 30 g/kg/day were alive at 100 days to permit a pilot study of the effect of abrupt withdrawal of sucrose. This was done upon 13 sucrose-treated rats and 7 water-treated controls. Locomotor activity was determined in a Wahmann activity drum (Fisher) several times during the last 3 wk of sucrose administration and at intervals of 48 hr for 2 wk following abrupt withdrawal of sucrose. The clinical signs and measurements noted above were also recorded daily following this abrupt withdrawal of sucrose.

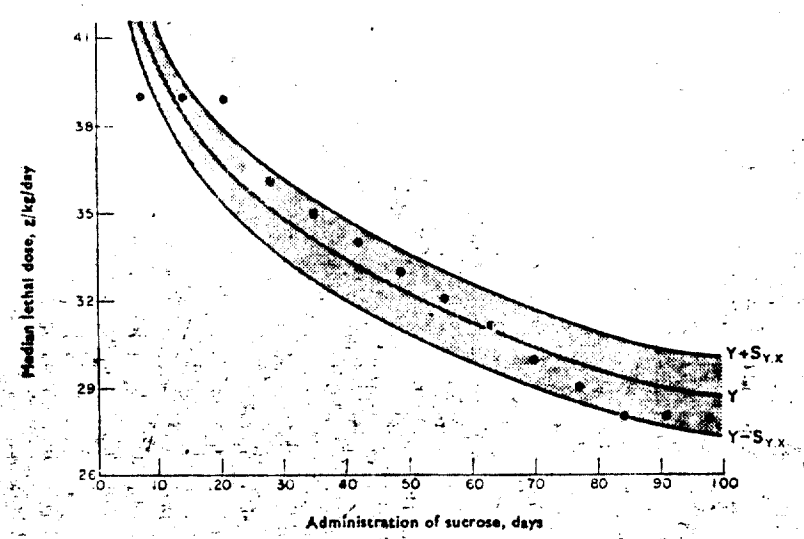

FIG. 1. The regression (Y) on days of oral administration of sucrose, of the daily dose which killed 50% of albino rats. The area enclosed by Y plus its standard error ($Y + Sy.x$) and minus its standard error ($Y - Sy.x$), calculated by the method of least squares, is shaded. $Y = (52 \cdot 1 - 11 \cdot 8 \log X) \pm 1 \cdot 32$; by substituting 2 for $\log X$, the $LD_{50\,(100\ days)}$ was calculated to be $28 \cdot 5 \pm 1 \cdot 3$ g/kg/day.

The results were analysed statistically by the application of t-tests to the significance of differences between means and by regression analysis of such differences on daily dose or duration of dosing, using statistical methods described by Croxton (1959). Values for the $LD_{50} \pm SE$ were calculated by the linear regression method of Boyd (1963).

RESULTS

The LD_{50} was calculated at weekly intervals and the results are plotted in Fig. 1 together with a regression line calculated from the equation $Y = (52 \cdot 1 - 11 \cdot 8 \log X) \pm 1 \cdot 32$ determined by the method of least squares after Croxton (1959). The $LD_{50\ (100\ days)} \pm SE$ was found to be $28 \cdot 5 \pm 1 \cdot 3$ g/kg/day. Calculated in a similar manner, the maximal $LD_{0\ (100\ days)}$ was $19 \cdot 8 \pm 1 \cdot 9$ g/kg/day and the minimal $LD_{100\ (100\ days)}$ $35 \cdot 5 \pm 0 \cdot 5$ g/kg/day.

The effect of each daily dose of sucrose on each parameter was essentially the same during each week of administration, apart from the first week when the toxicity syndrome was often marked. To compare the effects of the several doses, the mean difference from controls was calculated as $[(\bar{X}_d - \bar{X}_c)/\bar{X}_c] \times 100$, where $\bar{X}_d$ is the mean in the drug-treated (sucrose-treated) animals and $\bar{X}_c$ the mean in the controls during the entire period of administration of each daily dose.

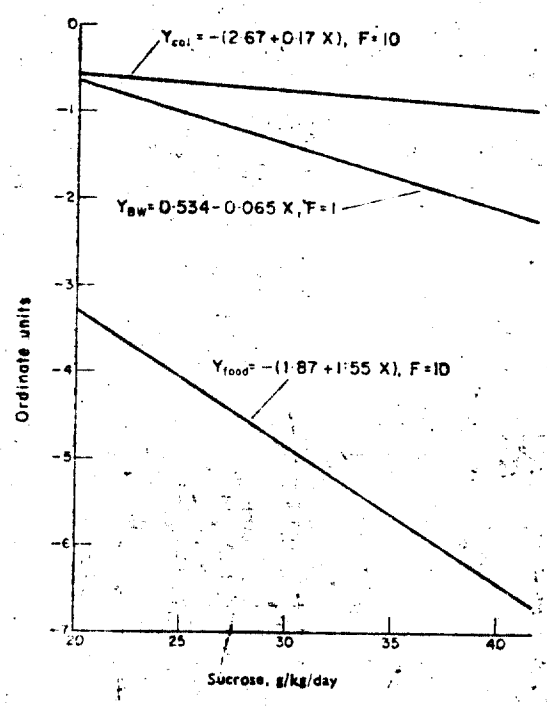

FIG. 2. The regression, on daily dose of sucrose, of the mean % change from controls in calorie intake (kcal/kg body weight/24 hr; Y_{cal}, $F = 10$), weekly % gain in body weight (Y_{bw}, $F = 1$) and food intake (g laboratory chow/kg/24 hr; Y_{food}, $F = 10$). Calorie intake was the sum of calories in laboratory chow plus calories in administered sucrose. Mean % change from controls was calculated as $[(\bar{X}_d - \bar{X}_c)/\bar{X}_c] \times 100$ where $\bar{X}_d$ is the mean in the sucrose-treated rats and $\bar{X}_c$ in their controls. The ordinate units must be multiplied by the factors (F) indicated to obtain mean % change from controls.

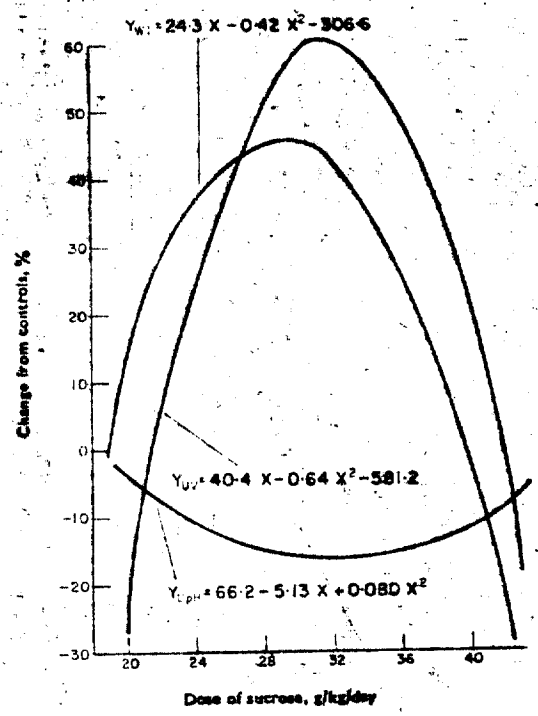

FIG. 3. The regression, on daily dose of sucrose, of the mean % changes from controls in water intake (ml/kg/day; Y_{wi}), urinary volume (ml/kg/day; Y_{uv}) and the pH of 24-hr samples of urine ($Y_{U_{pH}}$). Mean % change was calculated in the same manner as for Fig. 2.

The weekly growth rate was increasingly inhibited by increasing daily doses of sucrose (Fig. 2). Calculated as the percentage gain in body weight per wk, growth in sucrose-treated rats was of the order of 1–2%/wk less than that in the controls. At the end of 100 days, the sucrose-treated rats weighed from 9·46 to 24·7% less than the water-treated controls. The intake of laboratory chow was progressively reduced by an increase in the daily doses of sucrose (Fig. 2). When kcal/kg body weight/day taken as laboratory chow were added to kcal/kg/day administered as sucrose, the total calorie intake was not significantly decreased until the daily dose of sucrose exceeded the $LD_{50\,(100\ days)}$.

Sucrose administration caused diuresis, the daily volume of urine increasing as the daily dose was increased to approximately the minimal $LD_{100\,(100\ days)}$, after which larger daily doses produced progressively less diuresis (Fig. 3). The regression was quadratic and was best fitted by the equation $Y = 40{\cdot}4\,X - 0{\cdot}64\,X^2 - 581{\cdot}2$ as noted in Fig. 3. The diuresis was accompanied by polydipsia which reached a peak at about the $LD_{50\,(100\ days)}$ (Fig. 3). The pH of the urine decreased as the urinary volume and water intake increased. Most of the animals exhibited glycosuria which was not related to the sucrose dosage over the range of doses studied. There were no consistent or significant changes in the output of urinary protein or in body (colonic) temperature except premortally.

Clinical signs were semi-quantitated in clinical units of 1+ to 4+. The percentage of animals with clinical signs of 2+ or higher was calculated and plotted against the daily dose of sucrose and regression lines are shown in Fig. 4. All regressions were linear on employed

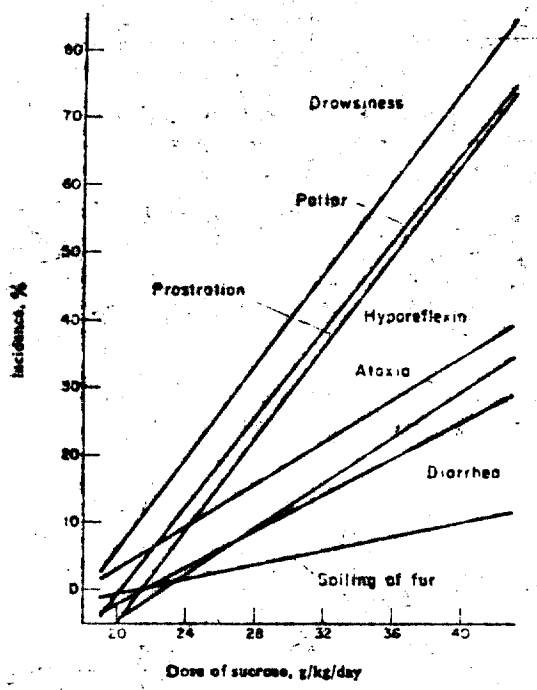

FIG. 4. The regression, on daily dose of sucrose, of percentage of animals showing clinical signs of 2+ or more clinical units. The results are shown as regression lines calculated for drowsiness from the equation $Y = 3{\cdot}45\ X - 62{\cdot}6$, for pallor from $Y = 3{\cdot}29\ X - 68{\cdot}3$, for prostration from $Y = 3{\cdot}45\ X - 73{\cdot}9$, for hyporeflexia from $Y = 1{\cdot}58\ X - 28{\cdot}5$, for ataxia from $Y = 1{\cdot}78\ X - 40{\cdot}2$, for diarrhoea from $Y = 1{\cdot}36\ X - 29{\cdot}4$ and for soiling of fur from $Y = 0{\cdot}52\ X - 11{\cdot}0$.

doses of sucrose. Signs which were statistically significant at the $LD_{50\ (100\ days)}$ and at higher daily doses included drowsiness, pallor, prostration, hyporeflexia (decreased response to stimuli), ataxia, diarrhoea and soiling of the fur. Soiling of the fur appeared to be due to the animals' having no interest in keeping their fur clean. Other signs occasionally noted, but not dose-dependent, were piloerection, irritability (especially with high doses), dyspnoea, epistaxis, cyanosis, convulsions, haemodacryorrhoea and haematuria. Death from large doses was due to respiratory failure usually following convulsions and was preceded by pallor, dyspnoea, salivation and a rise in colonic temperature. Death from smaller daily doses followed several days of hypokinetic hypothermic cachexia.

Gross pathology seen in animals which died included a congested brain, gastro-enteritis, discoloration of the kidneys and liver, pneumonitis, occasional congestion of the testes and a stress reaction in the adrenal and thymus glands and spleen.

Histopathological observations are summarized in Table 1. Deaths in the first week were associated with a marked local inflammatory reaction of the gastro-intestinal tract, extensive tissue capillary-venous congestion, hepatitis, nephritis and a stress reaction in the adrenal and thymus glands. Later deaths were associated with a meningoencephalitis, arteriolitis, myocarditis, nephritis, hepatitis, pneumonitis and degenerative changes in muscle, pancreas, salivary glands, testes and thymus gland.

Table 1. *Summary of histopathological findings at autopsy*

Organ	Deaths after treatment for	
	Less than 1 wk	9–100 days
Adrenal gland	Lipoid vacuoles prominent	Cortical hypertrophy; sinusoidal congestion and occasional haemorrhage
Aorta (thoracic)	Minor capillary-venous congestion of tunica adventitia	Occasional vacuolation of tunica media; occasional small aneurism
Arterioles (splenic)	Normal appearance	Occasional vacuolation of tunica media
Brain	Capillary-venous congestion of brain and meninges	Capillary-venous congestion and occasional haemorrhage of brain and meninges; infiltration of meninges with granulocytes; occasional brain abscess
Gastro-intestinal tract:		
Cardiac stomach	Capillary-venous congestion of lamina propria and submucosa	Hypertrophy of stratified squamous epithelium, muscularis mucosae and muscularis externa; hyperaemia of lamina propria and submucosa
Pyloric stomach	Capillary-venous congestion of lamina propria and submucosa, occasionally with haemorrhage, lysis of gastric glands and granulocyte infiltration	Mostly normal appearance; occasional capillary haemorrhage and granulocyte infiltration
Small bowel	Capillary-venous congestion of lamina propria and submucosa	Hypertrophy of villi, muscularis mucosae and muscularis externa; hyperaemia
Cecum	Capillary-venous congestion of lamina propria and submucosa	Hypertrophy of Lieberkuhn glands, muscularis mucosae and muscularis externa; hyperemia
Colon	Capillary-venous congestion of lamina propria and submucosa	Normal appearance to slight hypertrophy
Heart	Minor capillary congestion of myocardium	Occasionally capillary congestion with haemorrhage and thrombosis
Kidneys	Glomeruli shrunken; capillary-venous congestion occasionally with haemorrhage; fine vacuolation of tubules	Capillary-venous congestion, occasional haemorrhage and thrombosis; tubular cloudy swelling, vacuolation and necrosis; occasional shrinkage of glomerular tuft
Liver	Areas of granulation and vacuolation of hepatic cells	Normal appearance or sinusoidal congestion with areas of centrilobular vacuolation and necrosis
Lungs	Capillary-venous congestion and areas of venous thrombosis	Areas of capillary-venous congestion and venous thrombosis
Muscle (ventral abdominal wall)	Normal appearance	Occasional weak cross-striation
Pancreas	Acinar glands shrunken with loss of zymogenic granules and areas of necrosis	Acinar glands shrunken with loss of zymogenic granules and areas of necrosis
Salivary (submaxillary) glands	Normal appearance	Occasional atrophy of serous glands and loss of zymogenic granules
Skin	Normal appearance	Normal appearance
Spleen	Normal appearance	Contracted red pulp
Testes	Normal appearance	Varying degrees of deficiency of sperm and inhibition of spermatogenesis
Thymus gland	Minor loss of thymocytes	Varying degrees of loss of thymocytes and of capillary-venous congestion

Shifts in the fresh weight of body organs in survivors are summarized in Table 2. There was an increase in weight in most areas of the gastro-intestinal tract due in part to an increase [illegible] Table 3). Apart from a [illegible] reaction in the adrenal [illegible]

Table 2. *Fresh weight of organs from albino rats given toxic daily amounts of sucrose by intragastric cannula*†

Organ	Fresh weight (g) for doses in range of: Minimal $LD_{100\,(100\,days)}$	$LD_{50\,(100\,days)}$	Maximal $LD_{0\,(100\,days)}$
No. of controls...	28	22	5
No. of treated rats...	28	25	10
Adrenal glands	+ 4·0	+ 11·2*	+ 15·1**
Brain	− 0·9	− 3·4*	− 1·3
Gastro-intestinal tract:			
Cardiac stomach	+ 33·2**	+ 24·1**	+ 10·8*
Pyloric stomach	+ 9·7*	+ 7·2*	+ 14·2**
Small bowel	+ 5·4	+ 10·4*	+ 13·4*
Caecum	+ 5·7	+ 28·4**	+ 40·1**
Colon	− 0·1	− 0·7	+ 14·3*
Heart	− 7·3*	− 13·5**	− 9·1*
Kidneys	− 10·4**	− 16·2**	− 16·1**
Liver	− 8·2*	− 12·8**	− 9·5*
Lungs	+ 9·2	− 9·8*	− 3·5
Muscle (ventral abdominal wall)	− 9·2*	− 12·9*	− 4·8
Salivary (submaxillary) glands	+ 4·3	− 17·7**	− 1·6
Skin	− 9·7*	− 21·8**	− 14·8**
Spleen	− 20·8**	− 23·9**	− 16·5*
Testes	− 7·5*	− 4·7*	− 0·7
Thymus gland	− 22·7**	− 8·7	− 26·9**
Residual carcass	− 7·1**	− 15·6**	− 12·7**
Total body weight	− 6·6**	− 14·9**	− 10·4**

† Results are expressed as mean % change from controls, specifically as $[(\bar{X}_d - \bar{X}_c)/\bar{X}_c] \times 100$, where $\bar{X}_d$ is the mean in the sucrose-treated rats and $\bar{X}_c$ in their controls. Asterisks indicate that $\bar{X}_d - \bar{X}_c$ was significant: $*P = 0{\cdot}05$–$0{\cdot}02$; $**P = 0{\cdot}01$ or less.

organs had lost weight. Possibly because organ weights and water levels were measured on survivors, there were few or no dose-response changes (Tables 2 & 3).

Abrupt withdrawal of sucrose at 100 days was followed by no evidence of a withdrawal syndrome indicative of physical dependence upon sucrose. There was a slight increase in locomotor activity, intake of laboratory chow rose to normal and the animals gradually recovered their lost weight. The diuresis and aciduria disappeared as did the clinical signs of sucrose intoxication. Histologically, some residual inhibition of spermatogenesis was present when the testes were examined 10 days after withdrawal of sucrose. Organ weights and water contents were within the range of the controls 4 wk after cessation of sucrose administration.

DISCUSSION

The oral $LD_{50\,(100\,days)} \pm SE$ of sucrose was $28{\cdot}5 \pm 1{\cdot}3$ g/kg/day, which is $80{\cdot}6 \pm 3{\cdot}7\%$ of the median lethal dose given to overnight-starved rats by one oral administration ($LD_{50\,(1\,dose)}$) as reported by Boyd *et al.* (1965). Other values for this percentage, the 100 Day LD_{50} Index, were calculated from the literature for other drugs and the results are presented in Table 4. It will be noted that the 100 Day LD_{50} Index is high for substances such as sodium chloride, caffeine and sucrose, which are items of the normal diet of most persons. While these calculations indicate that sucrose is relatively less toxic in long-term use than many other substances, toxic syndromes can nevertheless occur from excessive use

Table 3. *Water content of organs from albino rats given toxic daily amounts of sucrose by intragastric cannula*†

Organ		Water content (g/100 g dry weight) for doses in range of Minimal $LD_{100\ (100\ days)}$	$LD_{50\ (100\ days)}$	Maximal $LD_{0\ (100\ days)}$
	No. of controls...	28	22	5
	No. of treated rats...	28	25	10
Adrenal glands		+2·6	+ 1·3	+ 4·2
Brain		−0·3	0·0	− 0·6
Gastro-intestinal tract:				
Cardiac stomach		−8·6*	+ 5·8*	+ 3·7
Pyloric stomach		+5·6*	+ 5·4*	+ 3·7
Small bowel		+3·3	+ 5·5	+17·8**
Caecum		−6·0*	+11·4*	+ 9·3*
Colon		−1·0	+ 0·1	+10·2*
Heart		+0·7	− 0·5	+ 3·3**
Kidneys		+2·8	+ 1·5	− 0·3
Liver		+5·5**	+ 2·7	+ 7·2**
Lungs		−0·6	− 0·6	− 1·8
Muscle (ventral abdominal wall)		−1·7	− 3·3	+ 2·5
Salivary (submaxillary) glands		+1·4	− 3·1	− 2·9
Skin		+6·3*	+ 3·7	− 1·7
Spleen		−0·7	− 0·7	− 0·8
Testes		+0·5	+ 0·1	− 0·7
Thymus gland		−4·1	− 0·6	+ 5·8
Residual carcass		−1·9	+ 1·5	+ 4·1

† The results are expressed as mean % change from controls, specifically as $[(\bar{X}_d - \bar{X}_c)/\bar{X}_c] \times 100$, where $\bar{X}_d$ is the mean in the sucrose-treated rats and $\bar{X}_c$ in their controls. Asterisks indicate that $\bar{X}_d - \bar{X}_c$ was significant: *P = 0·05–0·02; **P = 0·01 or less.

Table 4. $LD_{50\ (100\ days)}$ *of several drugs expressed as the 100 Day* LD_{50} *Index*

Drug	$LD_{50\ (100\ days)}$ ±SE* (g/kg)	$LD_{50\ (1\ dose)}$ ±SE* (g/kg)	100 Day LD_{50} Index
Atropine sulphate	0·078 ± 0·005 (1)	0·588 ± 0·085 (2)	13·2 ± 0·8
Acetylsalicylic acid	0·243 ± 0·066 (3)	1·48 ± 0·04 (4)	16·4 ± 0·5
Pilocarpine nitrate	0·156 ± 0·020 (5)	0·911 ± 0·111 (6)	17·1 ± 2·2
Paracetamol	0·77 ± 0·02 (7)	3·71 ± 0·83 (8)	20·6 ± 0·5
Phenacetin	1·12 ± 0·02 (9)	4·14 ± 0·71 (9)	27·1 ± 0·5
Benzylpenicillin	4·14 ± 0·20 (10)	6·7 ± 0·1 (10)	61·8 ± 3·0
Sodium chloride	2·69 ± 0·12 (11)	3·75 ± 0·43 (11)	71·7 ± 3·2
Caffeine	0·150 ± 0·003 (12)	0·192 ± 0·018 (12)	78·2 ± 1·6
Sucrose	28·5 ± 1·3	35·4 ± 7·0 (13)	80·6 ± 3·7

* All the drugs were given intragastrically to albino rats except atropine which was given intramuscularly to rabbits.

Numbers in parentheses refer to the following references: (1) Boyd & Boyd, 1962; (2) Boyd & Boyd, 1961; (3) Boyd, 1968b; (4) Coldwell & Boyd, 1966; (5) Boyd & Jarzylo, 1968; (6) Covert & Boyd, 1962; (7) Boyd & Hogan, 1968; (8) Boyd & Berezcky, 1966; (9) Boyd & Hottenroth, 1968; (10) Boyd & Selby, 1962; (11) Boyd, Abel & Knight, 1966; (12) [illegible] Knight & Sheppard, 1965; (13) Boyd *et al.* 1965.

The maximal $LD_{0\ (100\ days)}$ was found to be $19 \cdot 8 \pm 1 \cdot 9$ g/kg/day and doses in this range produced a slight inhibition of growth, some hydration of the gastro-intestinal tract, a stress reaction and loss of weight in some organs. This dose would correspond to a 10-kg child's ingestion at one time each day- and retention—of approximately 40% of a pound of candy. Laboratory chow, the normal food of albino rats in our laboratory, was eaten in smaller quantities at this daily dose of sucrose, but the decrease was equal in calories to the sucrose administered. Richter (1956) has reviewed considerable evidence of similar reductions in laboratory-chow intake by rats given alcohol, glucose, fructose, sucrose and other sugars, and again the reduction was equal to the calorie content of the substance given.

The signs of toxicity at the $LD_{50\ (100\ days)}$ included inhibition of growth, diuresis, polydipsia, aciduria, diarrhoea, soiling of the fur, ataxia, hyporeflexia, drowsiness, pallor, prostration, hydration of the gastro-intestinal tissues, a stress reaction, loss of weight in many organs, meningoencephalitis, arteriolitis, myocarditis, nephritis, hepatitis, pneumonitis, inhibition of spermatogenesis and degenerative changes in muscle, pancreas and salivary and thymus glands. The gastro-intestinal tissues tended to be hypertrophied. The $LD_{50\ (100\ days)}$ corresponds on a body-weight basis to a 10-kg child's consumption of about 60% of a pound of candy per day.

The minimal $LD_{100\ (100\ days)}$ was $35 \cdot 5 \pm 0 \cdot 5$ g/kg/day, which corresponds to the ingestion of about 75% of a pound of candy per day by a 10-kg child. At this dose there appeared convulsions which are characteristic of acute (single dose) toxicity (Boyd *et al.* 1965). It is of interest that one single administration of this dose to overnight-starved albino rats will kill 50% of the animals.

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Quantités maximales tolérées de saccharose administré quotidiennement par voie intragastrique à des rats albinos

Résumé—Du saccharose a été donné une fois par jour, à l'aide d'une canule intragastrique, à de jeunes rats mâles albinos, ceci pendant 100 jours ou bien jusqu'à ce que 60% des animaux soient morts, selon ce qui arriverait en premier lieu. On a constaté que la $DL_{50\,(100\ jours)} \pm$ S.E., ou dose journalière qui tuait 50% d'animaux pendant 100 jours d'administration, était de 28,5 ± 1,3 g/kg/jour. La $DL_{50\,(100\ jours)}$ était de 80,6 ± 3,7% de la $DL_{50\,(1\ dose)}$ aiguë orale; ce pourcentage est nommé 'indice DL_{50} à 100 jours'. L'indice DL_{50} à 100 jours du saccharose était plus élevé que celui de n'importe quel produit étudié à ce jour. A la $DL_{50\,(100\ jours)}$ orale, on a observé les symptômes d'intoxication suivants: ralentissement de la croissance, diurèse, polydipsie, acidurie, diarrhée, incontinence, ataxie, hyporéflexie, somnolence, pâleur, prostration, méningo-encéphalite, artériolite, myocardite, hépatite, néphrite, pneumonie, inhibition de la spermatogenèse, perte de poids avec altérations dégénératives de nombreux organes et hypertrophie des tissus gastro-intestinaux. Ces symptômes étaient moins prononcés ou absents pour la $DL_{0\,(100\ jours)}$ maximale, qui était de 19,8 ± 1,9 g/kg/jour. A la $DL_{100\,(100\ jours)}$ minimale de 35,5 ± 0,5 g/kg/jour, les symptômes étaient similaires à ceux observés au cours des études de la toxicité aiguë (dose unique).

Maximal tolerierte Mengen von Sucrose bei täglicher intragastrischer Verabreichung an Albinoratten

Zusammenfassung—Sucrose wurde einmal täglich an junge männliche Albinoratten mittels einer intragastrischen Kanüle entweder 100 Tage lang oder bis 60% der Tiere verendet waren verabreicht. Die $LD_{50\,(100\ Tage)} \pm$ S.E. oder tägliche Dosis, die 50% der Tiere während 100 Tagen Verabreichung tötete, betrug 28,5 ± 1,3 g/kg/Tag. Die $LD_{50\,(100\ Tage)}$ betrug 80,6 ± 3,7% der akuten oralen $LD_{50\,(1\ dosis)}$; dieser Prozentsatz wird als der 100-Tage-LD_{50}-Index bezeichnet. Der 100-Tage-LD_{50}-Index war bei Sucrose höher als bei jedem bis dahin untersuchten Medikament. Zeichen der Toxizität bei der oralen $LD_{50\,(100\ Tage)}$ waren Wachstumshemmung, Diurese, Polydipsie, Acidurie, Diarrhoe, Einschmutzen, Ataxie, Hyporeflexie, Müdigkeit, Blässe, Hinfälligkeit, Meningoencephalitis, Arteriolitis, Myocarditis, Hepatitis, Nephritis, Pneumonitis, Inhibition der Spermatogenese, Gewichtsverlust und Hypertrophie der gastrointestinalen Gewebe. Diese Erscheinungen waren bei der maximalen $LD_{0\,(100\ Tage)}$, die 19,8 ± 1,9 g/kg/Tag betrug, weniger deutlich oder fehlten. Bei der minimalen $LD_{100\,(100\ Tage)}$ von 35,5 ± 0,5 g/kg/Tag waren die Erscheinungen ähnlich denen bei akuten Dosis Toxizitätsuntersuchungen.

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Localization of the Small-Intestinal Disaccharidases[1]

ARNE DAHLQVIST, M.D.

ACCORDING TO MOST CURRENT TEXTBOOKS of physiology and physiological chemistry, the intestinal disaccharidases are secreted with the intestinal juice ("succus entericus") and exert their physiological action in the lumen of the small intestine.

This concept is, however, not true. About 10 years ago we performed a study of the intestinal digestion and absorption in the human, using the intubation technique which Blankenhorn et al. (1) had developed some time before. When we assayed the disaccharidase activity of the small-intestinal content in order to find out where the secretion of the small-intestinal juice occurred, we found that the disaccharidase activity which could be recovered from the lumen of the small intestine was so weak that it could not possibly effect the hydrolysis of the amount of disaccharide that was absorbed (2, 3).

Similarly, results obtained in animal studies, using sacs or rings of everted small intestine, have shown that the disaccharidases are not released into the incubation medium but act in the small-intestinal wall (4).

The digestion of disaccharides thus occurs intracellularly. We have tried to trace the origin of the textbook concept that the disaccharidases are secreted, but we have not been able to find it. On the contrary, we found that Cajori (5) 30 years ago beautifully demonstrated in animal experiments that the intestinal digestion of disaccharides occurs intracellularly, and as early as at the end of the last century we have found notes that the disaccharidase activity in the small intestinal mucosa is very much higher than the disaccharidase activity of the intestinal content.

The finding that the disaccharide digestion occurs in the mucosa has naturally led to curiosity about where in the mucosal cells these enzymes are located. This has been studied in several laboratories, using three principal methods: *a*) tissue fractionation methods, *b*) incubation with intestine in vitro, and *c*) histochemical staining of enzyme activity.

Tissue fractionation has been used by Miller and Crane (6, 7) who have described a method for the isolation of brush border membranes from the epithelial cells of hamster small intestine. The tissue is gently homogenized in strongly hypotonic EDTA solution and then the brush border membranes are isolated by low-speed centrifugation. The disaccharidase activities are recovered in high yield in these brush border preparations. The main objection that can be made against this method of localization of enzymes is that it is difficult to evaluate the purity of the brush border preparations. Some cytoplasmic fragments are always seen which adhere to the brush border membranes and cannot be removed by repeated washings.

Experiments based on incubation with slices of everted intestine in vitro have in most cases been performed with sucrose as the substrate. The monosaccharide compo-

[1] From Chemical Research Laboratories of the Hospital and Department of Physiological Chemistry, University of Lund, Lund, Sweden.

nents of sucrose are glucose and fructose. If a piece of surviving intestine is incubated in vitro with a mixture of these monosaccharides, glucose is rapidly absorbed and accumulated in the tissue while fructose is slowly absorbed and does not accumulate. If the intestine is incubated with sucrose, one also finds that glucose is accumulated in the tissue in large amounts while most of the fructose liberated is recovered from the incubation medium (4). If very short incubation time is used, the concentration of fructose in the tissue is somewhat higher than in the medium. Sucrose is not recovered from the tissue. These results indicate that sucrose is split rather close to the surface of the mucosal cells, since fructose diffuses back into the medium; at the same time there seems to be a certain initial resistance opposing the diffusion of fructose, which indicates that the enzyme may be separated by a membrane from the surrounding medium.

If the incubation is made in the presence of phlorhizin, the accumulation of glucose is prevented. The hydrolysis of sucrose is, however, not decreased, and both glucose and fructose are recovered from the medium when phlorhizin is present (4). These results make it probable that the hydrolysis of sucrose occurs outside the surface of the cell at which the active absorption of glucose is effected.

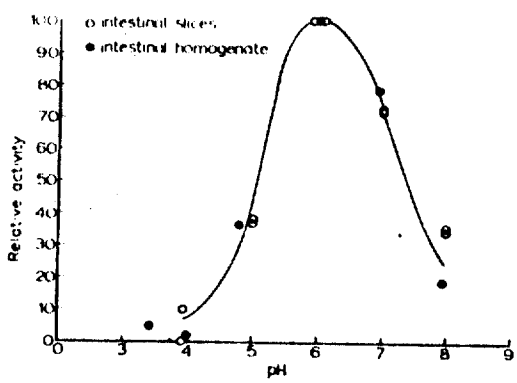


FIG. 1. The pH-activity curves for the invertase activity of intact cells of rat small intestinal mucosa (everted rings) and a mucosal homogenate (9). The two curves are identical, which points to localization of the enzyme close to the surface of the intact cell

In other experiments glucose oxidase has been included in the incubation medium (4). This enzyme does not react with sucrose but it rapidly oxidizes free glucose to gluconic acid which is not actively absorbed by the intestine. If the invertase were located on the surface of the mucosal cells in direct contact with the incubation medium the glucose liberated on hydrolysis of sucrose would have become trapped by the glucose oxidase. This did not occur: the accumulation of glucose during incubation with sucrose plus glucose oxidase was as large as on incubation with sucrose alone. This experiment too supports the concept that the intestinal invertase, although superficially located, is separated from the intestinal lumen by some kind of membrane which is impermeable to glucose oxidase.

It should be mentioned that another group has found that glucose liberated from maltose is trapped by glucose oxidase under similar conditions. This may mean that at least one of the maltose splitting enzymes is actually located on the surface of the cell (8).

The intestinal slices have also been used for enzyme kinetic experiments. Figure shows the results of an experiment in which surviving intestine has been incubated with sucrose in buffers of varying pH, and the rate of hydrolysis of sucrose has been measured (9). The figure also shows the pH activity curve for the invertase activity of mucosal homogenate. The two curves are identical. In other experiments the same activity curve has been found for solubilized and partly purified invertase. We interpret this to mean that the invertase is located so superficially in the cell that its surroundings have the pH of the incubation medium rather than the pH of the cell cytoplasm.

In other experiments the surviving intestine and the homogenate were incubated with sucrose solutions of varying concentr

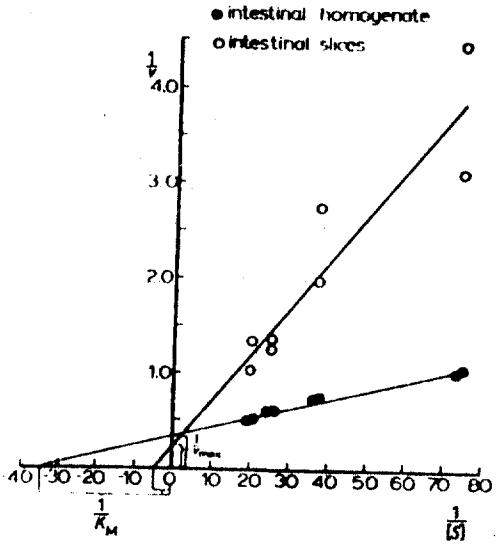


FIG. 2. Lineweaver-Burk-Dixon (10, 11) plot for the invertase activity of intact cells of rat small-intestinal mucosa (everted rings) and a mucosal homogenate (9). The apparent K_m for the enzyme in the intact cells is higher than for the enzyme in the homogenate. This is best explained by a diffusion barrier (membrane) covering the enzyme in the cell.

tion (9) (Fig. 2). A Lineweaver-Burk-Dixon (10, 11) plot showed that the K_m for the invertase activity of the intact tissue was higher than for the homogenate (the value for K_m for the homogenate was the same as for a solubilized and partially purified preparation). This result is best explained with the assumption of a diffusion barrier (membrane) which covers the enzyme in the intact cell.

The tissue incubation experiments thus indicate a superficial localization of the disaccharidases, which may very well fit with a brush border localization. They also indicate, however, that at least the invertase is not located on the surface in direct contact with the medium but rather located inside some kind of surface membrane (Fig. 3).

Histochemical staining of glycosidase activities was originally described by Seligman et al. (12) in Boston. These authors used synthetic substrates, 6-bromo 2-naphthylglycosides, and the liberated 6-bromo 2-naphthol was diazotized to give a blue-red color. There are, however, indications that such substrates may be hydrolyzed by other enzymes than the disaccharides (13). A histochemical staining method utilizing disaccharides as substrates was therefore developed in our laboratory (14). The principle of this method is seen in Fig. 1.

With this method it was possible to stain frozen-cut sections for disaccharidase activity, using disaccharides as substrates. The reaction was, however, slow; for appropriate staining it was necessary to incubate for 10–20 hr at room temperature. This caused considerable autolysis in the sections and made the localization of the stain uncertain. A granular stain was obtained throughout the cytoplasm of the epithelial cells when rat small-intestinal mucosa was stained for invertase with this method. No concentration of the stain could be seen in the brush border region, but due to autolysis this region was difficult to localize. In cooperation with Hammond and Crane (unpublished) it

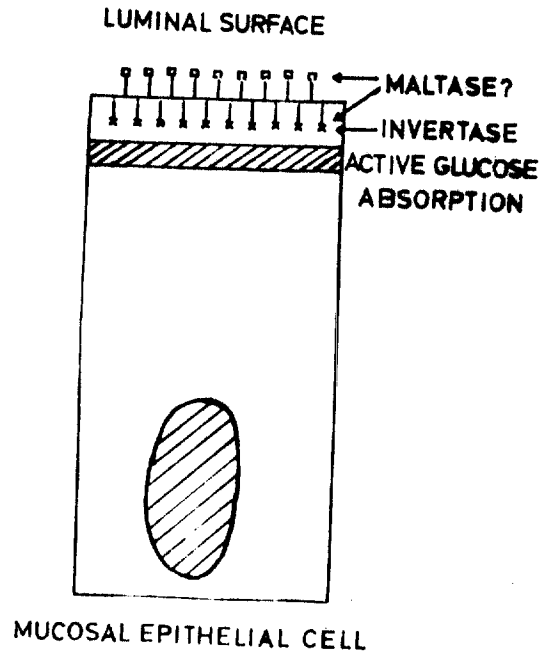


FIG. 3. Schematic drawing of the localization of the invertase and maltase activities in the epithelial cells of the small-intestinal mucosa, as indicated by in vitro incubation experiments.

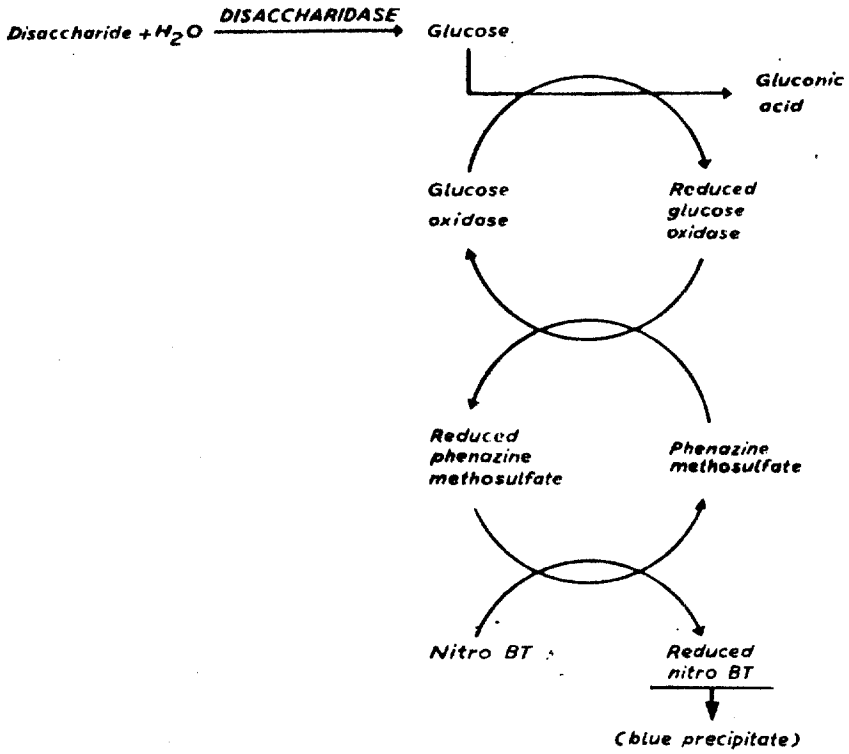


FIG. 4. Principles of the disaccharidase activity staining method of Dahlqvist and Burn (14).

was later possible to improve the method by including the staining reagents in a gelatine gel on a microscopic slide and then immerse the section attached to a cover glass into the gel. With this technique atmospheric oxygen was excluded which speeded up the reaction. Gelatine is also known to exert an accelerator effect on the reaction between PMS and tetrazolium salts. In this way staining could be performed in less than half an hour, and stain was obtained both in the brush border region and in the cytoplasmic granules with an unstained band between. However, when a glucose solution was injected into the gel immediately below the section, one could also see a diffuse but rather intense stain of the entire cytoplasm of the mucosal epithelial cells. It therefore appears that the mucosal epithelium has a great affinity for the stain, which opens the possibility of artifacts.

The modified method was never published because the investigation was no completed. Simultaneously and quite independently Jos et al. (15) in Paris have developed a much more sophisticated an superior modification of the disaccharidase activity staining method. They replaced n tro-BT with another tetrazolium salt, tetr nitro-blue tetrazolium (TNBT), which not so strongly adsorbed by the intestin mucosa. The tissue sections are placed on flat lid of a tissue culture vessel, and a sma amount of a concentrated glucose oxidase solution is placed directly on the sectio This is then covered with a piece of th filter paper, which is moistened with chr mogenic reagent (PMS, TNBT, gelatin and buffer). Then more chromogenic r agent and substrate solution are placed the vessel on the other side of the filter p per; the vessel is closed and sealed wi paraffin (Fig. 5).

With this method distinct stain is ob

tained in the brush border region, especially in the distal parts of the villi (Fig. 6-7). In this modification histochemical staining thus gives results which fit with those of the fractionation and tissue incubation studies.

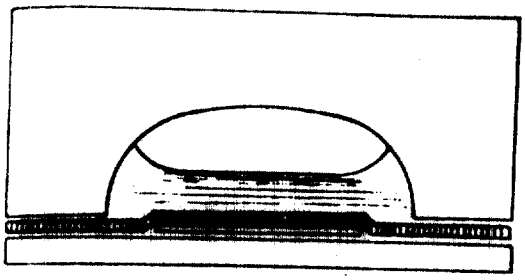
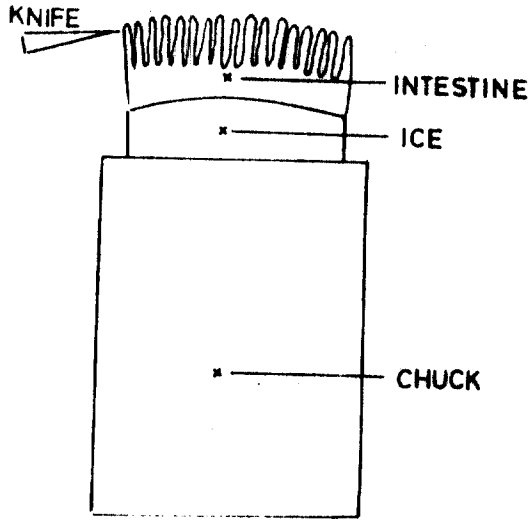


FIG. 5. Incubation vessel for disaccharidase activity staining according to Jos et al. (15).

The more intense staining obtained in the distal part of the villi may indicate that the enzyme is most active there. We have also in our staining experiments seen that the stain develops more rapidly in the distal parts of the villi. Similar observations have been made for other enzymes, such as alkaline phosphatase, nonspecific esterase, and leucine aminopeptidase (16). It has been argued, however, that this distribution of the stain for an enzyme activity need not mean that the enzyme is more active in the distal parts of the villi, but may be a reflection of membrane damages in this part of the tissue which cause an increased accessibility of the staining reagents for the enzyme stained.

FIG. 6. Staining of maltase activity in a section of human duodenum. From Jos et al. (15).

FIG. 7. Staining of trehalase activity in a section of human duodenum. From Jos et al. (15)



We have therefore developed a method fo the isolation of the different parts of th villi by cutting tissue blocks horizontally in a cryostat (17) (Fig. 8). From the tips of th villi down to the bottom of the crypt about 70 sections, each 10 μ thick, were ob tained. Every third section was used fo microscopy, the intermediate sections wer combined two and two, homogenized, an analyzed with an ultramicro method for di saccharidase activity and protein.

The distribution of the disaccharidase activity, expressed as units per gram pro

FIG. 8. Horizontal cutting of a block of smal intestinal mucosa in a cryostat according to Dah qvist and Nordström (17).

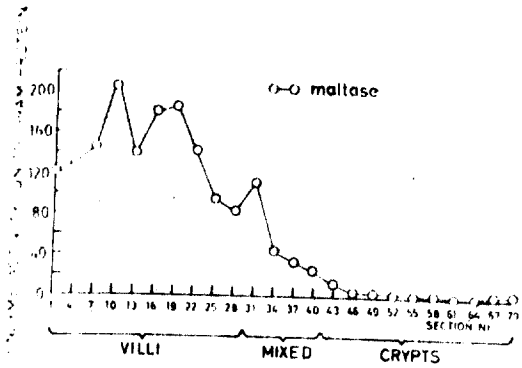


FIG. 9. Distribution of the maltase activity in the villi and crypts of rat small intestinal mucosa (17).

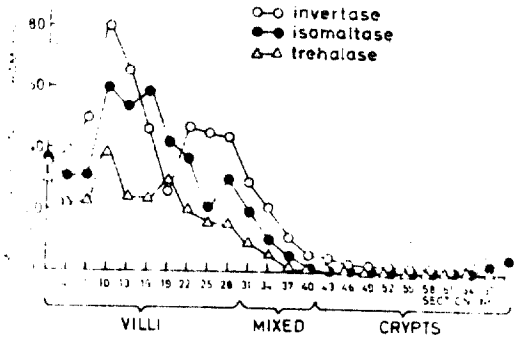


FIG. 10. Distribution of the invertase, isomaltase, and trehalase activities in the villi and crypts of the small intestinal mucosa (17).

cell, are seen in Figs. 9–10. It appears that all the enzyme activities are highest in the distal parts of the villi. We know that the new epithelial cells are regenerated in the bottom of the crypts and migrate up the sides of the villi. The enzymes thus are developed while the cells migrate up the sides of the villi, and the highest enzyme activity is obtained in the region of the villi where we know also that the active absorption process for monosaccharides is most active. This is in good accordance with our present view of the site of the physiological action of the small intestinal disaccharidases.

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INHERITED INABILITY TO EAT SUGAR

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AMONG the varied congenital and often inherited peculiarities of taste there is one particular anomaly which consists of a *congenital incapacity to eat any kind of sweet food.* We shall further term this curious condition "aglycophagia."

The anomaly has been observed in six members of the same family, which fails to record any intermarriages. In every other respect the members of the family are perfectly sound people, only a few of them suffer from headaches and general nervousness. Individuals affected with aglycophagia have been found to occur in two series of brothers and sisters (see Figure 1.) In the first series consisting of seven children there are two cases of aglycophagia (*A* and *B*), while in the second series of five children, as many as four are affected with the anomaly (*C*, *D*, *E* and *F*).

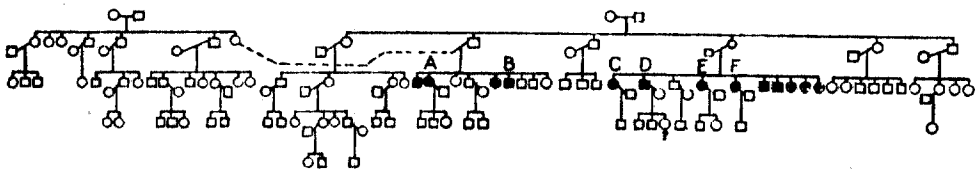
C is affected to a somewhat milder degree. The parents, as well as all of the children of those affected with aglycophagia can readily eat food of any taste. In all the cases the anomaly revealed itself in early childhood, and apparently has failed to change in the course of the individual's life.

The propositus *B*, (Figure 1), is 26 years old. He cannot remember having ever been able to eat any food that tasted sweet. His mother remembers having noticed that as a very small child he used to cry when he was made to taste a sweet bun. He cannot eat sugar, honey, jam, fancy cakes, candies, chocolate, ice-cream or anything of that kind. Nor can he eat any fruits or berries, with the exception of cranberries and unripe red bilberries. He cannot eat any kind of cake, or take any alcoholic drinks, or even mineral water. He cannot use saccharin in his food. His diet mostly consists of rye bread, unsweetened wheat bread, uncooked milk and other dairy products. He can eat potatoes, unless they have been frozen, which gives them a slightly sweet taste, so that he can no longer eat them. His "ideal of tasty food" is: tea with milk and no sugar and a slice of rye bread with a cucumber pickled in brine. At the same time he definitely states that he does not object to the taste of anything sweet. On the contrary, he sometimes even takes a candy or a lump of sugar into his mouth and sucks it for a moment. He invariably spits it out, however, and then rinses his mouth. He can never make himself swallow anything sweet as "it is as bad as taking the most repulsive kind of medicine." Several times, when there was nothing else to be had, he had forced himself to eat sweet food, which invariably resulted in nausea and a most unpleasant physical weakness; he also had a feeling of there being something "abnormal in the whole of his body," felt "apathetic," and "got tired easily." He states that he was not able to run quickly or even clench his fists with his usual strength. He had a feeling "as though he wanted to wriggle and writhe."

In every other respect he is a perfectly healthy man, very capable and active, physically strong, but slightly nervous. From time to time he suffers from eczema which affects his arms and legs, but this is no way connected with his having eaten anything sweet.

He consented to enter the clinic for a short time to undergo a more thorough examination, which failed to reveal any deviations from the normal standards either of the nervous system or the viscera. His blood contained 36.9 mg. p.c.K, and 10.0 mg. p.c. Ca. The amount of diastase contained in blood and the saliva was normal, but that contained in the urine was reduced one half.

The subject under observation was



FAMILIAL INHERITANCE OF AN INABILITY TO EAT SUGAR

Figure 1

Pedigree showing the two groups of cousins who were unable to eat sugar in any form. The one individual (*B*) who underwent extensive clinical tests became flushed, feverish, and complained of pain in his stomach when given sugar at the clinic. His conception of the perfect meal was tea without sugar, rye bread and brine pickles. The affected individuals lived mostly on rye bread and dairy products. The crossed symbols indicate death in infancy.

given 50.0 c.c. of cane sugar and this brought on a very severe condition: his face became flushed; he felt feverish, and suffered from suffocation and complained of pressure in the region of the pit of the stomach. His pulse became arythmic, the chronaxia of the flexors and of the extensors showed a manifest tendency towards levelling (0.12 σ and 0.16 σ instead of his usual 0.12 σ and 0.2 σ; the sugar-curve of the blood became atypical and gave a tardy rise. Before the intake of sugar the sugar content amounted to 94 mg. p.c.; in half an hour it was 102; in an hour 98; in an hour and a half 94; and in two hours it rose to 176 mg. p.c.). Four hours after the test the patient's usual condition was restored, chronaxia gradually became normal (0.09 σ and 0.2 σ), but for a long time after that he could not think of sugar without a feeling of repulsion, so that he even threw away the lumps of sugar which remained on his night table and refused point blank to undergo any further experiments of this kind.

According to the patient's statements, his eldest sister, *A*, was characterized by the same anomaly as himself.

His cousin, *F*, whom we had occasion to observe, had exactly the same diet as our patient to the extent that she was likewise unable to eat potatoes that had been frozen because of their being slightly sweet. If she ate anything sweet, for instance, a tiny piece of chocolate, she "felt unwell," had nausea and to avoid this unpleasant feeling had to eat a piece of rye bread with salt and drink some water. She could not say what might happen to her if she ate a larger quantity of anything sweet, for she had never been able to make herself do so: "better starve than eat a sweet bun" she said. Contrary to her cousin, the propositus, she dislikes the taste of anything sweet, and though she thinks the smell of honey pleasant, she says she "could never think of eating any." Her teeth, as well as those of her cousin, are marvelously sound and intact.

Her elder brother *D* has the same peculiarities, though he can drink beer and lemon-vodka; he tried to eat saccharin but could not swallow it. Her sister *E*, who suffers from eczema, has exactly the same peculiarities as regards anything sweet as *B* and *F*. Her eldest sister *C* has the anomaly in a somewhat milder form; she is able to take a small quantity of tea with a little sugar, as well as to eat a slightly sweetened bun and take alcoholic drinks. But she cannot eat honey, fruit, jam or sweet dishes any more than the others.

The inner mechanism of this peculiar anomaly does not seem to be perfectly clear. The fact that those affected with it are unable to take saccharin seems to indicate that it is not so much the incapacity for eating substances of a definite chemical structure as the impossibility of eating anything which tastes sweet that we have to deal with in these cases, unless we assume that the incapacity of these individuals to eat mono- and disaccharids which is due to the chemical properties of the latter may have spread to any substance that tastes sweet on the principle of the conditioned reflex. Certain bio-chemical anomalies,

such as a reduced quantity of diastase in the urine or an atypical sugar-curve, might, apparently, be regarded as having resulted from the peculiarities of a continued absence of sugar from the diet. The capacity for eating polysaccharids which do not taste sweet and which are then converted into glucose, likewise seems to indicate that the anomaly is not due to disturbed carbohydrate exchange. Thus, we are led to believe that it is the taste of anything sweet which is the incitant of the pathologic condition. The symptoms of the latter hardly give sufficient grounds to regard the entire syndrome as a kind of allergy. The syndrome is highly peculiar and seems to rank closest to family myoplegia, the atypical rudimentary forms of which are well known to us, as well as its connection with this or the other form of diet. In this respect it is of interest to note the statement made by the subject of our observations as regards his incapacity for running fast or clenching his fists with strength after he has eaten something sweet, as well as the objective changes observed in his motor chronaxia following the intake of sugar.

The above described syndrome does not wholly resemble any of the clinical affections so far known to us and, therefore, we have thought it fit to publish the results of our observations.

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GRANULATED SUGAR AS TREATMENT FOR HICCUPS IN CONSCIOUS PATIENTS

To the Editor: Although seldom more than an annoyance, hiccups can, if it persists, cause great distress. We have observed an exceedingly simple, benign and successful remedy. One teaspoonful of ordinary white granulated sugar, swallowed "dry," resulted in immediate cessation of hiccups in 19 of 20 patients. Twelve of these patients were otherwise in good health, had suffered from their hiccups for less than six hours and came either to the emergency room or to one of us directly with their complaint.

The remaining eight patients had suffered from persistent hiccups for 24 hours to six weeks; five of them had received other forms of therapy (breathing into a paper bag, phenothiazines) without success. One patient was otherwise in good health. The remainder had conditions known occasionally to stimulate hiccups: two were recuperating from strokes, two from intra-abdominal surgery, and one from open-heart surgery, and two had symptomatic hiatus hernias. One patient (with hiatus hernia) failed to respond to sugar, but hiccups ceased spontaneously within eight hours. Three suffered recurrence of their hiccups 10 to 24 hours after an initial successful treatment; all responded to repeat treatments (one patient who did not respond did so with a third treatment 24 hours later). Such recurrences are common in the treatment of hiccups[1] and demonstrate the need to seek and eliminate the underlying cause.[2]

A review of the literature of the past 10 years reveals no mention of dry granulated sugar as treatment for hiccups, but we are told that it is known among some lay persons. The mechanism of action is not known. Local irritation of the pharynx by the granules may stimulate impulses in the vagus nerve via the pharyngeal plexus and, thus, interfere with afferent vagal impulses associated with the hiccup reflex. This is the mechanism proposed to explain the successful relief of hiccups by pharyngeal stimulation with a nasal catheter.[1] Other mechanisms, such as a metabolic or osmolar effect of glucose on the nerve endings or even an effect mediated centrally via the taste buds, cannot be excluded. However, the rapidity of action makes any but a local mechanism highly unlikely.

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Sucrose

D. W. Fewkes, K. J. Parker and A. J. Vlitos

Sucrose plays an important role in biological systems and in food technology. As a chemical raw material several hundred thousand tons are used annually. This article surveys sucrose from the standpoint of the chemist, the biologist and the nutritionist.

Introduction

Sucrose occupies a rather basic and unique position in the biosphere. Not only is it one of the major products of photosynthesis and the mobile source of energy in plants, but it is also one of the world's leading commercial commodities.

Since the compound is sweet and other of the physical properties are suited to blending with a variety of foods, sucrose continues to be an important tool in the hands of food technologists. Similarly, industrial microbiologists rely on the molecule as a source of energy in fermentation, as most microbes readily will utilize the compound.

In view of the diverse characteristics it is somewhat difficult to write on a substance which is so versatile! The simplest solution is to review each of the characteristics of the compound from the viewpoint of the chemist, the plant physiologist and the food technologist in that order.

Sucrose as a chemical

Sucrose (I) is a disaccharide comprising the two monosaccharide units α-D-glucose and β-D-fructose linked through their potentially reducing hemi-acetal hydroxyl groups. It is thus classed as a non-reducing sugar: that is, it does not reduce the ions of copper, silver or mercury in alkaline solution.

Although sucrose represents one of sixteen possible modes of combination of D-glucose and D-fructose to give a non-reducing disaccharide, it is the only one to be found naturally in living organisms.

Sucrose is strongly hydrated in aqueous solution[1] and will crystallize as the hydrate below 20°. Two crystalline hydrates have been characterized;[2] the hemipentahydrate ($C_{12}H_{22}O_{11}\cdot 2{\cdot}5\ H_2O$) and the hemiheptahydrate ($C_{12}H_{22}O_{11}\cdot 3{\cdot}5\ H_2O$). Sucrose normally crystallizes in the anhydrous form (Fig. 1). The

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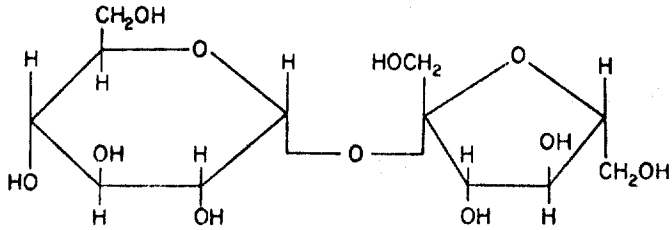


I. Sucrose, α-D-glucopyranosyl-β-D-fructofuranoside

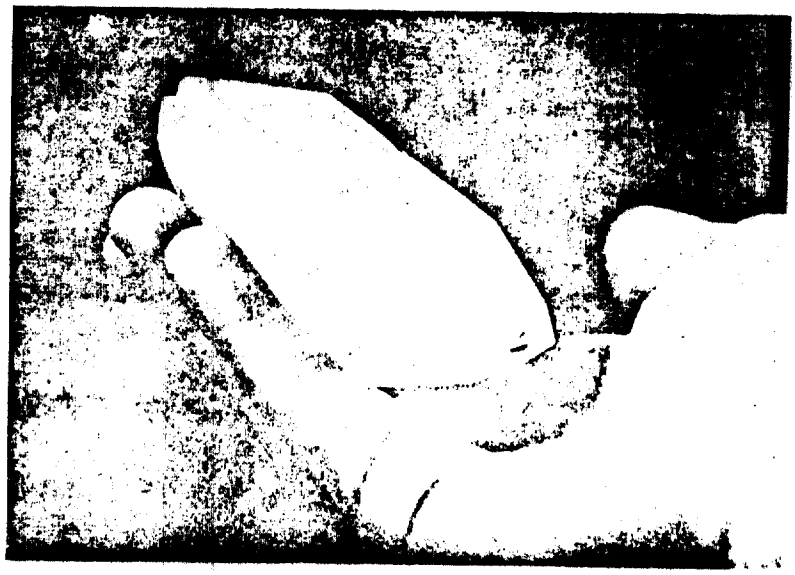
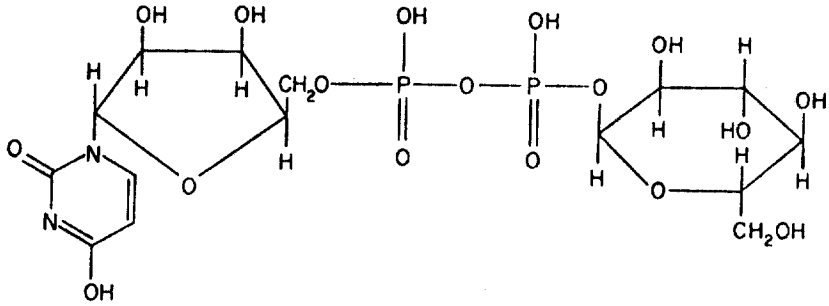


Fig. 1. A single crystal of sucrose.



II. UDPG

structure of the crystal has been precisely determined by neutron diffraction,[3] hydrogen bonds (two of which are intramolecular) utilizing every hydroxyl group except one.

Sucrose is synthesized by a number of bacterial enzymes through transglycosylation involving either free fructose and glucose-1-phosphate[4,5] or from free glucose.[6] The reaction involves the highly reactive intermediate uridine-5′-pyrophosphate glucose ester (II) with fructose in the furanose form.[7] The chemical synthesis of sucrose was first achieved in 1953 by Lemieux & Huber.[8] However, the best yield so far obtainable (6·6 per cent)[9] is unlikely to make synthetic sucrose a viable commercial proposition.[10]

The hydrolysis of sucrose to its constituent monosaccharides takes place readily in aqueous solution. The reaction is catalysed by hydrogen ions,[11] the rate being directly proportional to hydrogen ion activity. Since a molar equivalent of water is involved, the reaction is of second order.[12] The rate of hydrolysis is also a function of the temperature; the free energy of activation (26·59 kcal/mole) is independent of pH, temperature and sucrose concentration.[12] The hydrolysis rate constant (K) is given by the equation:

$$\log_{10}K = 15{\cdot}300 - \frac{5810}{T} - \mathrm{pH} + \log_{10}(C_{H_2O} - C_{SUC})$$

relating the second order rate constant to pH, absolute temperature (T) and sucrose concentration (C_{SUC}). The hydrolysis rate is sufficiently precisely measurable to allow the pH of a solution to be determined.[13] Since pH scales at different temperatures are arbitrarily defined, the rate of sucrose hydrolysis offers a practical basis for unifying the pH–temperature scale.

Sucrose is a weak polybasic acid[14,15] ($K_1 = 1{\cdot}87 \times 10^{-13}$) which will exist partly as the anion in solutions of neutral or alkaline pH. At elevated temperatures and high pH sucrose undergoes generalized decomposition with the formation of acidic products, probably as a consequence of the instability of the primary hydroxyl anion. There is no evidence for the intermediate formation of glucose or fructose, so that the reaction is not one of simple hydrolysis. Lactic acid is the predominant product of decomposition. The use of lime at high temperature results in the formation of lactic acid in high yield.[16] At moderate temperatures (65°) C_4- and C_6-unsaturated α-keto-acids have been isolated, indicating the complexity of the reactions taking place.

In anhydrous ammonia or diamino-ethane stable sodium sucrates can be obtained, though it is not possible to purify the products.[17] Complexes of sucrose, with the hydroxides of calcium, strontium or barium, of the composition $C_{12}H_{22}O_{11}{\cdot}2M(OH)_2$ are well known. These form the basis of the Steffen process for recovering sucrose from molasses.[18,19] The sucrates are not true salts but are ion-solvation complexes. Calcium chloride forms similar complex ions with sucrose, soluble in methanol.[20] Magnesium hydroxide is claimed to

form a complex only after heating to a high temperature.[21] The complex with sodium bromide ($C_{12}H_{22}O_{11}Na\ Br{\cdot}2H_2O$) is of interest in that the conformation of the furanose ring of sucrose is less distorted than in pure sucrose, and there is no intramolecular hydrogen bonding.[22]

Ferric hydroxide is extremely insoluble in water, but in the presence of sucrose it is readily soluble at alkaline pH to give a true ferrisucrate anion. The corresponding ferrosucrate is non-ionic indicating that the anion is a sucrose-solvated polyferrate. The degree of polymerization is a function of pH.[23]

A crystalline molecular complex of sucrose with the amino-acid deoxykinurenin has been isolated.[24] The complex with diazouracil, which gives a vivid bluish green coloration in alkaline solution, is diagnostic of the glycosidic linkage of sucrose.[25]

In all these interactions sucrose is acting as a polydentate donor through oxygen or by hydrogen bonding. This versatile ability to form molecular complexes with ions and polar compounds possibly reflects its unique function in biological systems, which depend on specific reversible interactions with sterically favourable receptors. This is known to be the basis of its interaction with the sweet-sensitive enzyme in the human gustatory receptors.[26,27]

Sucrose as a chemical raw material is essentially a polyhydric alcohol and will form the usual derivatives. Some of these have achieved commercial importance and form the basis of a sucro-chemical industry. Sucrose octaacetate, which is extremely bitter, has been used as a denaturant,[28] though its unpleasant handling properties are tending to diminish this use. Sucrose octabenzoate finds limited use as a plasticizer.[29] Sucrose hexaacetate di-isobutyrate finds application as a viscosity modifier in varnishes, and in inks coatings, textiles, and cosmetics.[30]

Sucrose mono-esters of long chain fatty acids, such as the stearate, are being manufactured in hundred ton quantities for use as a biodegradable surfactant though they are too costly for wide utilization.[31] The monostearate finds many applications, for instance as an emulsifier in foods, in cosmetics, and in animal feedstuffs. The distearate is an oil-soluble surfactant. The sucrose polyalkoxyether ester of tallow finds use as an inexpensive surfactant and antifoaming agent in the sugar industry.[32] Sucrose esters of linolenic acid have been proposed for use as drying oils in paint.[33]

Inorganic esters of sucrose include calcium sucrose phosphate[34] which has been proposed for use as anticariogenic agent, and the octanitrate which has limited application as an explosive.

Sucrose ethers of long chain alcohols have surface active properties. The octa-2-hydroxypropylether of sucrose is used as a cross linking agent in rigid polyurethane foams,[35] and the phosphite ester is used to introduce fire resistant properties.[36] Sucrose octacyanoethylether is used as a dielectric.[37]

The versatility of sucrose in non-food uses will be extended when derivatives of sucrose other than the simple ethers, esters and urethanes become more readily available. The replacement of hydroxyl by a halogen, thiol, azide, amino, keto or unsaturated group has already been achieved.[38] Although none of these derivatives has been shown to possess biological activity, the importance of sugars in metabolic pathways suggest that such activity might be expected.

Sucrose in biology

The prime characteristic of green plants is their ability to convert carbon dioxide and water to carbohydrate by the process of photosynthesis. This carbohydrate provides energy to drive the vital processes of the plant; energy for its own translocation within the plant, for the uptake of water and mineral salts from the soil and for the synthesis of essential metabolites such as nucleic acids, hormones and enzymes. Carbohydrates also provide the bulk of the structural material of plants, in the form of cellulose and related cell wall constituents. In addition, most plants accumulate carbohydrate reserves, often as starch, but also as inulin (e.g. *Helianthus tuberosus*, the Jerusalem artichoke)[39] and as sucrose (e.g. sugar cane, sugar beet).

Sucrose occupies a pivotal position in the carbohydrate metabolism of green plants. It is an early product of photosynthesis, the main form in which carbon is translocated in plants and an important reserve substance in some species. Sucrose also functions as a relatively stable non-reactive osmotic agent in helping to maintain the turgor of plant tissues and its presence in the cells stabilizes proteins against denaturation and protects the cells from frost damage.[40]

In animals and non-green plants sucrose is relatively less important. It is a usual although probably not an essential dietary constituent of some species, notably man and the insect pests of sugar crops, but is often replaced by other carbohydrates, particularly glucose, fructose and starch. Movement of carbohydrates in animals takes place mainly as glucose, for example, in the blood stream of man and other mammals. Sucrose is not found as a reserve substance in animals, although another non-reducing disaccharide, trehalose (α,α'-diglucoside) is stored by insects and several other invertebrates and is also found generally in fungi, in many algae and in some pteridophytes.[41] It is interesting in this respect that the alimentary enzyme which hydrolyses sucrose in animals is an α-glucosidase (or glucoinvertase) in contrast to the invertase of yeasts which is a β-fructosidase.[42]

Biosynthesis

At present sucrose cannot be synthesized artificially on a commercial scale and its production depends upon the cultivation of plants such as sugar cane

and sugar beet which accumulate sucrose as their major carbohydrate reserve. Exploitation of this characteristic by plant breeders has resulted in the selection of high yielding cultivars.[43]

Sucrose is an early product of photosynthesis and is known to be synthesized in the chloroplasts.[44] The behaviour of isolated chloroplasts is erratic in this respect, and Walker[45] suggests that sucrose synthesis may be controlled by a delicately poised mechanism involving the passage of certain metabolites into and out of the chloroplasts. In support of this he cites a similar mechanism whereby the rate of photosynthesis of isolated chloroplasts is governed by a balance between ribose-5-phosphate and orthophosphate in the medium, both of which penetrate freely into the chloroplast.

In the classical photosynthetic carbon reduction cycle, or Calvin cycle,[46] the net result of the carboxylation of three ribulose diphosphate molecules is the production of six triose phosphate molecules. Five of these are needed to continue the cycle and the sixth is available for the biosynthesis of secondary products, such as carbohydrates, fats and amino acids. The C_4-dicarboxylic acid photosynthetic pathway found in sugar cane and in other plants with dimorphic chloroplasts[47,48] differs in detail, but the end result is similar.

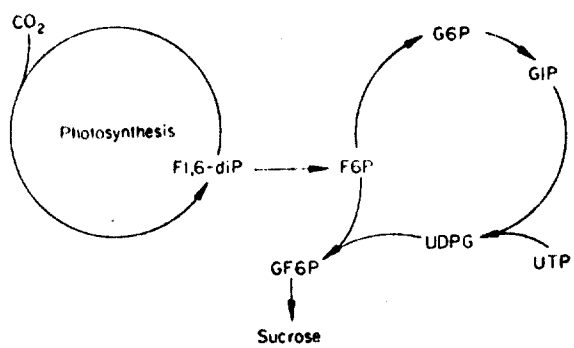


Fig. 2. The biosynthetic pathway to sucrose in the leaf.

In the biosynthesis of carbohydrates two triose phosphates condense to form fructose 1,6 diphosphate (FDP) which then loses phosphate to become fructose-6-phosphate (F6P). During the synthesis of sucrose the nucleotide sugar uridine diphosphate glucose (UDPG), itself derived from F6P and the nucleotide uridine triphosphate (UTP), acts as a glucosylating agent transferring a glucose residue to F6P under the influence of the enzyme sucrose phosphate synthetase[49] (Fig. 2). The resulting sucrose monophosphate is broken down by a sucrose phosphatase to release free sucrose and phosphate.[50,51]

Sucrose may also be synthesized in other plant tissues by the following reaction.[7,52]

$$\text{UDPG} + \text{fructose} \rightleftharpoons \text{sucrose} + \text{UDP}$$

This reaction which is catalysed by the enzyme sucrose synthetase is freely reversible and may also serve an important function in the degradation of sucrose.[53] In contrast, plant invertases act in one direction only, hydrolysing sucrose to free glucose and fructose.

Translocation

Sucrose is the main mobile energy source of higher green plants. In most species that have been investigated all carbohydrates synthesized in the leaves are transformed to sucrose before their translocation to other parts of the plant.[54] In a few cases more complex sugars (e.g. raffinose, stachyose, verbascose) are also translocated, but free hexoses do not appear to be involved.[55] As might be expected from this, the concentration of sucrose in phloem sap is generally high, ranging from 10 per cent to 25 per cent (w/v) in different species and at different times of the day.[56] Photosynthetic assimilates move from the leaves to other parts of the plant via the sieve tubes of the phloem, a series of elongated cells interconnected (mainly at their ends) by perforated areas of the cell wall, known as sieve plates. Thus the translocate must pass at high concentration through the cytoplasm of these cells where it may be subject to metabolic attack. Sucrose is much less reactive than hexoses such as glucose and fructose and is therefore more stable in this environment. Indeed, Arnold[56] has postulated that its unique role in carbohydrate translocation is as a protected derivative of glucose. In this respect it may have certain advantages over reducing sugars such as maltose and turanose which are more reactive than sucrose, and over the less easily hydrolysed non-reducing disaccharide trehalose in the sense of its subsequent utilization by the plant. Apart from these considerations, the free energy of hydrolysis of sucrose (−6·6 kcal/mol) is considerably higher than that of maltose or lactose (−3·0 kcal/mol) and stands comparison with those of high energy molecules such as UDPG (−7·6 kcal/mol) and adenosine triphosphate (−6·9 kcal/mol).[57]

An important corollary of the fact that sucrose is the major translocate of higher plants is that a few enzymes capable of splitting this sugar exercise a control over carbohydrate metabolism in the sinks to which it flows. Thus the occurrence of different groups of invertases in mature and immature sugar cane stem tissues determines whether sucrose is accumulated or utilized in further growth.[58-60] The control of invertase synthesis by hormonal activity[61,62] provides a mechanism whereby environmental factors may influence the growth of this plant through their effects upon hormone synthesis, transport and activity.[63]

Accumulation

The fate of the sugars produced during photosynthesis depends upon the sinks to which they flow. Sucrose may be translocated to the growing regions of

the plant to be used directly as the substrate for respiration and structural growth or to mature storage tissues where it is transformed to reserve polysaccharides such as starch or is stored as sucrose. Thus the organic substances of the plant are all derived directly or indirectly from sucrose.

It is evident that structural growth and storage of reserve carbohydrates are inversely related because they both draw upon the sugars produced during photosynthesis. Both are affected therefore by the interactions between genetic and environmental factors which determine the rate of photosynthesis and the rate of growth (and therefore the rate of carbohydrate accumulation) of the plant.

In the selection of high sucrose yielding cultivars of sugar cane or sugar beet the plant breeder seeks to optimise a complex, interactive series of genetic-environmental interactions. A major constraint is imposed by climatic factors which often vary widely during a year and from year to year and which are difficult or expensive to manipulate. However, it may prove possible to increase sucrose accumulation by the use of growth regulating chemicals which modify certain genetic-environmental interactions. The results of glasshouse and field tests suggest that the use of such 'chemical ripeners' may well be feasible.[63-68]

Sucrose as a food

As a foodstuff, sucrose is a source of energy and carbon for cell tissue building. It is not an essential dietary factor, as are the vitamins, unsaturated fats, amino-acids, minerals and other essential elements such as phosphorus, sulphur and nitrogen. The normal requirement of these elements is adequately provided by the variety of foods naturally selected in the traditional diet. The balance of food intake provides a source of energy for muscular activity and heat generation. Carbohydrate is essential for the assimilation of amino-acids and protein,[69] which are otherwise deaminated in the liver to provide glucose and lipids. The average daily protein requirement of an adult is approximately 1 g/kg, and the energy expenditure between 1700 and 5000 calories according to environment, activity and basal metabolic rate. One pound of refined sucrose provides 1800 calories at 8·8d and is thus the cheapest source of assimilable energy currently available.[70]

Sucrose is more rapidly absorbed than starch, which, being insoluble, must first be digested by a two-stage enzymic hydrolysis involving ptyalin in the salivary secretion and amylase in the duodenum. Sucrose passes straight through the stomach to the small intestine where hydrolysis to glucose and fructose and assimilation occur.[71,72] Glucose is actively absorbed (i.e. it will pass into the bloodstream against a concentration gradient) at a rate approximately twice that of fructose.[73] In the absence of the hydrolytic enzymes (sucrase and iso-maltase), as in the rare congenital condition giving rise to sucrose

intolerance, sucrose is not absorbed.[74] Fructose is predominantly metabolized in the liver where it is converted to glucose, glycogen and triglycerides.

Glucose is largely metabolized in the extra-hepatic tissues, resulting in the direct deposition of fat. Consequently ingested fructose, unlike glucose, will give rise to an enhanced plasma triglyceride concentration (hyperlipidaemia).[75] Increase in serum cholesterol levels is also associated with sucrose intake.[76,77] These observations have led to the proposition that sucrose is harmful to health,[78-82] particularly in relation to the incidence of coronary disease, obesity and diabetes. However, direct evidence that sucrose, by virtue of its fructose content, is a specific contributory factor in the aetiology of these diseases has not been obtained.[83-85]

The implication of sucrose as a causative factor in dental caries, follows from the observation that the dextran component of the dental plaque is only formed by cariogenic bacteria in the presence of sucrose.[86-88] This permits localized acid fermentation and the formation of carious lesions. Nevertheless, this problem is not necessarily a consequence of sucrose intake, but rather of inadequate dental hygiene and poor dietary control.[89,90,94,95]

Fructose, and hence sucrose but not glucose, has been shown to accelerate the metabolism of ethanol.[91] This may be a consequence of the coupled oxidation of the coenzyme NADH and fructose metabolism in the liver, the site of ethanol detoxification.

Although sucrose has a lower calorie equivalent on a dryweight basis than pure starch, protein or fat, other foods contain moisture and non-digestible material. Consequently, calculated on fresh weight, sucrose is approximately equivalent to fat mutton or pork in terms of calories. It provides a concentrated, soluble and rapidly available source of muscular energy and blood sugar. Its use also avoids introducing any nutritional imbalance, such as might result from the intake of saturated animal fats of the equivalent calories level. A natural demand for sugar is indicated by the preference shown for sweet foods under conditions of physiological[92] or psychological[93] stress; for example, in athletes, cosmonauts and children. Sucrose fed to animals prior to transport and slaughter counters the deterioration in meat quality induced by stress.

Nutritionally, sucrose is a valuable, but not essential, component of the normal balanced diet, against which there is no corroborated evidence of any long-term deleterious effect on health, specifically attributable to sucrose.[94,95]

It has been produced and consumed for some 2300 years,[96] though it is only within the last century that the price has fallen sufficiently for it to be ranked as a staple food item.[97] Over the last 20 years world production and consumption of sucrose has increased steeply (Fig. 3). The proportion of beet to cane sugar has remained relatively constant at about 42 per cent of the total production.

Sucrose consumption in different countries has been suggested to be an

index of affluence.[98,99] A comparison of the annual *per capita* consumption of sucrose[70] does not support this proposition (Fig. 4). Cuba, Holland and the Republic of Ireland have the highest consumption, approximately three times that of Japan. An affluent society will tend to choose the more expensive animal derived foods such as meat, butter, cream and fish. Historical, social and commercial factors probably influence dietary traditions, while economic factors allow these preferences to be exercised. This trend is seen in the sucrose consumption of Great Britain which has been falling over the last decade, and currently stands at 112 lb/head/year (Fig. 5).

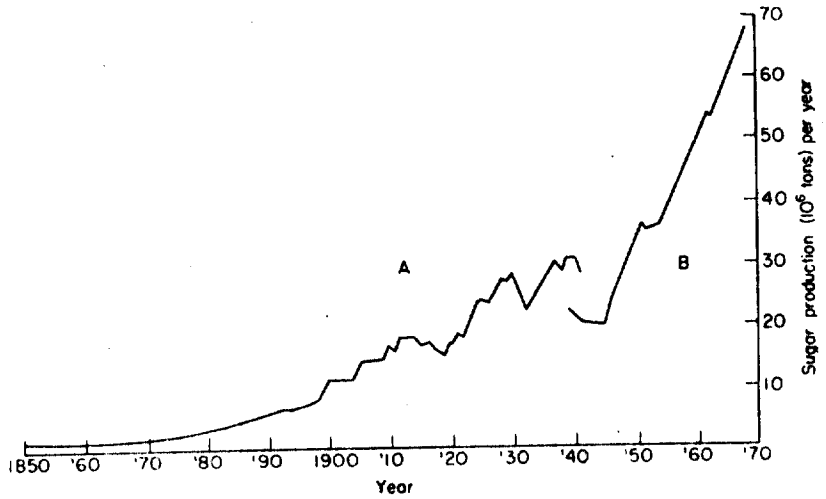


Fig. 3. Total world sugar production. A: all sugar; B: centrifugal sugar.

Apart from its nutritional value, sucrose has properties which make possible the wide variety of forms in which food is available. It is the second sweetest sugar after α-fructose,[100] and has a pure sweet taste with no secondary or after flavours.[101] This property of sweetness renders many foods palatable and attractive. Citrus fruit juices, such as lemon, orange, lime, or grapefruit, are an excellent source of vitamin C. These juices enjoy their popularity as drinks only when sweetened. Beverages benefit by being sweetened, which counters any bitter taste. Fruit, vegetables, pickles, sauces and condiments are improved by the addition of sucrose. It is a necessary ingredient in cakes, biscuits, puddings and all types of confectionery. Alcoholic beverages, such as beer, wines, liqueurs and fortified wines, are sweetened with sucrose.

Sucrose has an important preservative action and is necessary for the manufacture of fruit conserves, glazed fruits, preserved fruit and condensed milk.

The physical properties of sucrose solutions are utilized in the preparation of foods, to provide texture, body, viscosity, and moisture retention. Syneresis in

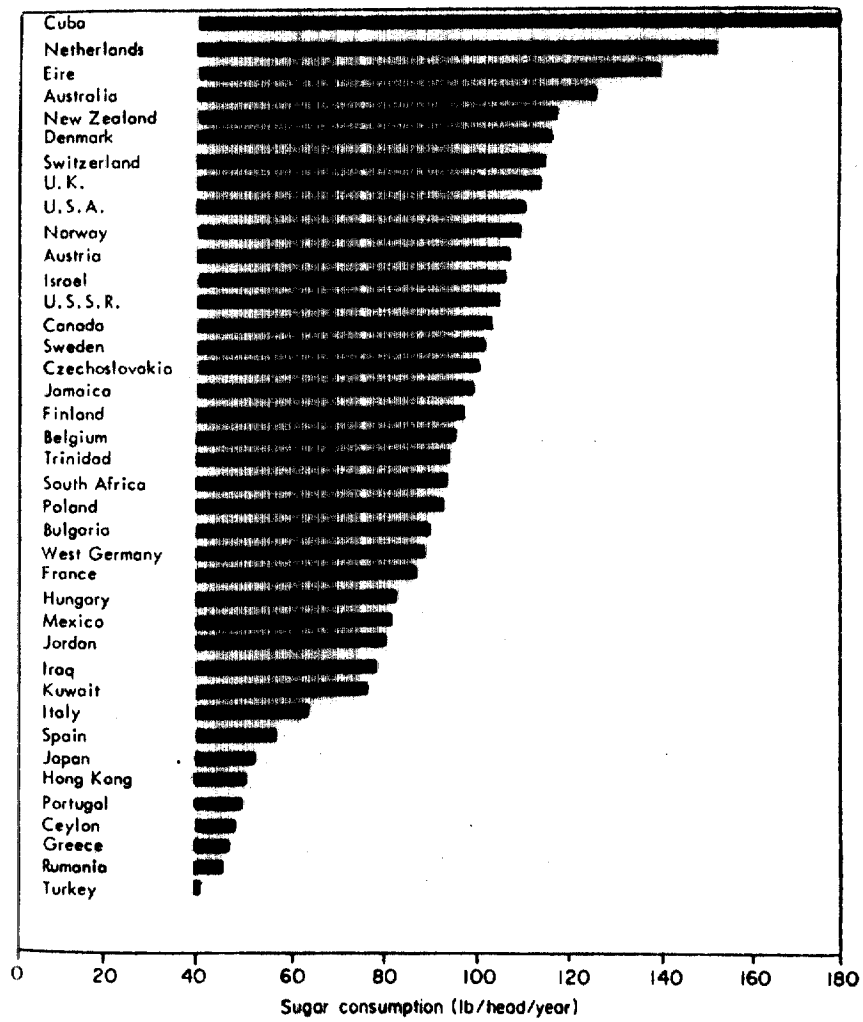


Fig. 4. Annual national consumption of sugar per head.

gels is prevented, denaturation of protein is retarded, and the emulsification of fats aided by sucrose. Flavours are developed or modified by sucrose either by autolysis or a synergistic action such as with salt[102] or citric acid.[103,104]

In Great Britain manufactured foods currently use an estimated 42 per cent of the sucrose consumed.[105] Refined sucrose provides 87·5 per cent of the total sugar,[106] 37 per cent of the total carbohydrate[107] and 17 per cent (1550 cals) of the total average calorie intake.[108]

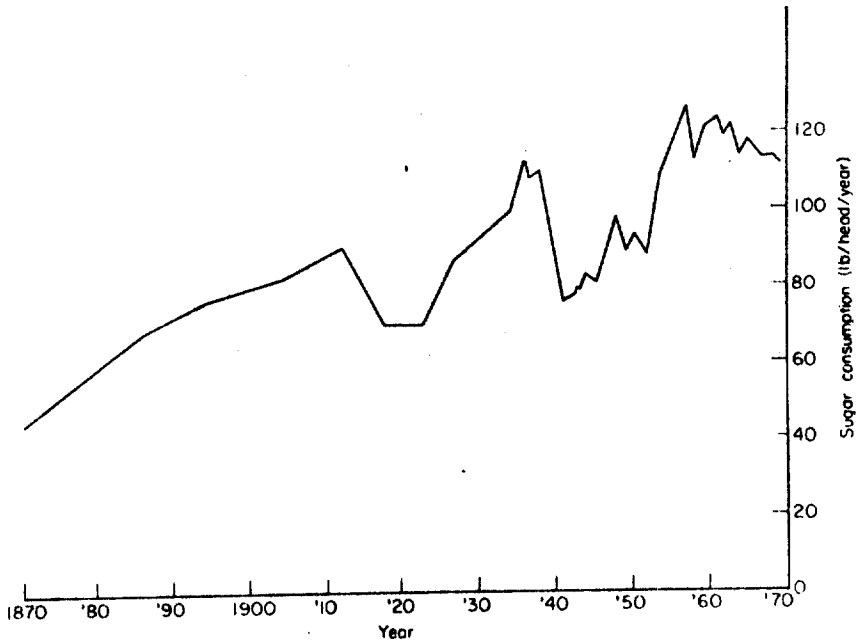


Fig 5. Annual consumption of sugar in Great Britain over the last century.

In the United States the annual *per capita* consumption of sucrose (99·7 lb) greatly exceeds the intake of sugars from all other sources (19·1 lb),[109] the ratio (89·4 per cent) being closely similar to that for Great Britain (87·5 per cent).[110] Sucrose is clearly well established as an important food ingredient which will not easily be replaced.

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THE RELATION BETWEEN SUCROSURIA, HIATUS HERNIA AND MENTAL DEFECT

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Sucrose or cane sugar is not a reducing substance and hence does not give a positive reaction with Benedict's solution. Its presence in the urine is, therefore, not detected on routine examination, and there are relatively few reports of sucrosuria in the literature. Worm-Müller (1884) reported the presence of cane sugar in the urine in normal individuals who had ingested sucrose in unusual quantity. This was confirmed by Le Goff (1911) who administered 100 g. sugar to adults, all of whom developed temporary sucrosuria. Sucrosuria has been reported in children both in health as well as in those suffering from gastro-intestinal disturbances (Lahdensuu, 1947; Reuss, 1910; Owen and Lewis, 1956; Haworth, 1960). Sucrosuria has always been assumed to be secondary to the intake of sucrose with the exception of the cases reported by Elmer, Krasowska and Ptaszek (1939) and by Hoesch (1934). In these cases cane sugar was considered to be synthesized in the body.

The methods used in detecting sucrose have varied. Both the polariscope as well as chemical methods have been employed. Latterly, urine chromatography has been the method of choice. Moreover, while some have given a measured loading dose before testing the urine, others have relied on the sugar present in the diet.

In the past few years a number of conditions have been discovered in which mental retardation is associated with errors of metabolism manifesting themselves by the presence in the urine of unusual constituents. Examination of the urine by chromatographic methods has, therefore, become an important investigation in mentally retarded children. Moncrieff and Wilkinson (1954) described a group of mentally retarded children who had sucrosuria and, in addition, hiatus hernia. In the investigation of children with hiatus hernia, who were mentally normal, they found no sucrose. They suggested, therefore, that the triad sucrosuria, hiatus hernia and mental defect constitutes a clinical syndrome. A further series of cases of the 'triad' sucrosuria, hiatus hernia and mental defect was reported from the same hospital by Woodruff (1958). Perry, Lippman, Walker and Shaw (1959) examined the urines of mentally normal and abnormal individuals, without hiatus hernia, and found that many of them passed sucrose in their urine if given a loading dose. As a result of this paper Moncrieff (1960) has altered his views. 'The biochemical aspects of sucrosuria are full of interest, and while I do not accept the view of Perry *et al.* that our cases are physiological variations, the conclusion that sucrosuria is unrelated causally to mental defect can be endorsed.' In the investigations recorded here, we came to the conclusion that sucrosuria is certainly a common finding in normal individuals. It was, however, more common in children who were suffering from hiatus hernia, but who were mentally normal. Furthermore, there is evidence to show that hiatus hernia is more common in mentally retarded children. There is, therefore, a tendency for the three conditions, hiatus hernia, mental defect and sucrosuria to occur together, although they are not causally related. It is our conclusion that the association of these three conditions, though not a syndrome, is not entirely a chance finding.

Material and Methods

The bladder was emptied and 20 g. sucrose were given with the first meal of the day (except where otherwise stated). A normal diet was then given but without the addition of sucrose. All the urine passed for six hours was collected, and whenever possible it was obtained in three separate specimens at intervals of two hours. The sugars were identified by one-dimensional paper chromatography, and their concentration was roughly estimated by comparison with known amounts of the various sugars. An effort was made to measure the total quantity whenever possible, as the concentration alone may give a misleading interpretation.

Four groups were investigated: 1. Three patients

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TABLE 1

CLINICAL DATA ON CHILDREN WITH HIATUS HERNIA

Case No.	Age	Time of Onset of Vomiting	Radiological Confirmation	Operation for Hiatus Hernia	Comment
1	11 yrs	Infancy	Yes	Yes	
2	4 mths	Shortly after birth	Yes	No	
3	5 yrs	Shortly after birth	Yes	Yes	
4	4 mths	4 weeks	Yes	No	Also had pyloric stenosis (see text)
5	1½ yrs	2 weeks	Yes	No	
6	11 yrs	Birth	Yes	Yes	
7	5 yrs	Infancy	Yes	No	
8	3 mths	Birth	Yes	No	
9	3 yrs	Birth	Yes	Yes	
10	3 yrs	Birth	Yes	Yes	
11	9 yrs	Shortly after birth	Yes	No	

showing hiatus hernia and mental defect; 2. Eleven mentally normal patients with hiatus hernia; 3. 41 relatives of these two groups; 4. 38 normal controls.

Group 1. There were three cases with hiatus hernia who were mentally defective. The case reports are given in the Appendix.

Group 2. This consisted of 11 mentally normal patients with hiatus hernia (Table 1). The diagnosis was confirmed radiologically in all and at operation in five. Cases 4 and 5 were two sisters both of whom had pyloric stenosis in infancy which was confirmed at operation, as well as hiatus hernia demonstrated radiologically later on. They have a brother, not included in this series, who suffered from the same two conditions.

Group 3. This group consisted of the relatives of patients who had hiatus hernia, both with and without mental defect. The relatives usually comprised the parents and siblings and occasionally uncles, aunts and cousins of patients. One definite case and one doubtful case of hiatus hernia were found among them. There were no relatives who were mentally defective.

Group 4. Medical students volunteered to act as controls. In addition, children in surgical and orthopaedic wards served as controls. These included children admitted for 'cold' operations but not yet operated on, and cases of Perthes' disease. No child had a general anaesthetic less than one week before the test.

TABLE 2

ANALYSIS OF RESULTS

	Total	Sucrose Present	Per Cent. With Sucrosuria
Group 1			
Patients with hiatus hernia and mental deficiency ..	3	3	100
Group 2			
Hiatus hernia, but mentally normal	11	9	82
Group 3			
Relatives of patients with hiatus hernia	41	18	44
Group 4			
Normal controls:			
Adults	20 } 38	6	} 34
Children	18 }	7	

Results

Table 2 summarizes the results in all the patients. Of the three cases who were mentally retarded and who had hiatus hernia, all showed sucrosuria at some time. Of 11 mentally normal children who were suffering from hiatus hernia (Group 2) nine showed sucrose in the urine after a loading dose. Among 41 relatives of patients with hiatus hernia (with and without mental defect) only 18 showed sucrosuria. Of the 38 controls only 13 showed sucrosuria after a loading dose. This figure is in accord with the findings of Haworth (1960). He reports that five out of 13 healthy babies and five out of 16 older children showed sucrosuria following the administration of a test dose. The incidence of sucrosuria in his series therefore is 31% as compared with 34% in ours.

The possibility that sucrose was being absorbed through the inflamed mucosa of the lower oesophagus in patients with hiatus hernia was then considered. One of the patients had a gastrostomy performed because he had a stricture of the oesophagus associated with his hiatus hernia. Sucrose, 10 g., was given through the gastrostomy tube. Over the next six hours he passed 61 mg. of sucrose. After a second test he passed 18 mg., and a third test was negative.

Two of the three cases showing the complete 'triad' had blood glucose curves investigated after a test dose of sucrose. The amount of sucrose given for the test was double the weight of glucose that would have been given for a glucose tolerance curve.

Case S.F., in Group 1, body weight 5 kg., was given 24 g. of sucrose by stomach tube at 10 a.m. The fasting level of blood glucose was 100 mg.; blood glucose at

10.30 a.m. was 145 mg.; at 11 a.m. 125 mg.; at 12 noon 105 mg.; at 1 p.m. 90 mg.; at 2 p.m. 95 mg.; and at 2.30 p.m. 90 mg.

Case S.S., also in Group 1, body weight 5 kg., was given 24 mg. of sucrose orally at 9.35 a.m. The fasting level of blood glucose was 60 mg.; blood glucose at 10.05 a.m. was 65 mg.; at 10.35 a.m. 150 mg.; at 11.05 a.m. 130 mg.; and at 11.35 a.m. 80 mg.

These results show that sucrose was rapidly absorbed.

Discussion

Our results indicate that sucrosuria is not a rare finding even among normal people who have taken no more sugar than might be added to a cup of tea. The figures do, however, indicate that sucrosuria is commoner among children having abnormalities at the lower end of the oesophagus. The incidence of sucrosuria among the latter is more than double that of normal people. Statistically the result is significant at the 0·1% level. What possible connexion there may be between hiatus hernia and sucrosuria is not known. It has been suggested that the permeability of the alimentary tract is increased at the site of the oesophagus and that this allows the passage of unsplit sugar, but this cannot always be the case as our experiment showed.

As sucrosuria is not uncommon among normal people it does not seem likely that it is causally related to mental defect. One cannot draw an analogy between sucrose and galactose, the only other of the sugars which is known to be associated with mental defect. Galactose is normally metabolized within the body. When the appropriate enzymes are absent, galactose accumulates in the body and appears in the urine. On the other hand, if sucrose has gained access to the human body without undergoing previous hydrolysis to its constituent hexoses it is entirely and rapidly excreted in the urine. There seems to be no enzyme in the body, with the exception of the digestive juice, capable of digesting this sugar (Peters and Van Slyke, 1946).

That there may be some connexion between hiatus hernia and mental defect is suggested by the report of Bonham-Carter (1956) who found six mentally defective children among 136 with hiatus hernia. Again, it does not seem that there is a specific syndrome of mental defect and hiatus hernia, but rather that mental defect from various causes predisposes to hiatus hernia.

In the literature there are conflicting reports of the occurrence of sucrosuria in various disorders. This seems to be due in part to the absence of any standard test comparable to a glucose tolerance test. The initial dose of sugar, the method of detecting it in the urine and, finally, the way of expressing the results vary from paper to paper, and it is to be hoped that some more uniform method will be adopted.

The following conclusion may be drawn. Sucrosuria is not a sufficiently specific finding for it to be regarded as an exclusive feature of any syndrome. There exists, however, an association between hiatus hernia and mental defect, and evidence is here presented that there is an increased incidence of sucrosuria in hiatus hernia. There may, therefore, be an unusually frequent association of these three features, but they do not form a specific syndrome.

Summary

One-third of normal individuals excrete sucrose in their urine after taking 20 g. of the sugar.

Of children with hiatus hernia, over three-quarters have sucrosuria in similar circumstances. There is probably an association between hiatus hernia and mental defect. The three clinical findings, sucrosuria, hiatus hernia and mental defect may therefore be frequently found together, but they do not constitute a disease entity.

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Appendix

Case histories of the three patients having hiatus hernia, sucrosuria and mental defect.

Case 1. S.F., a male, was a second child. Pregnancy and delivery were uncomplicated. Vomiting started soon after birth. At the age of 6 weeks a barium swallow showed laxity at the cardio-oesophageal opening. At the age of 1 year he was unable to sit, had poor head control and took no interest in his surroundings. He had partial optic atrophy. The urine contained no phenyl-pyruvic acid and the Wassermann reaction was negative. Sucrosuria was first noticed at the age of 11 months. It is of interest to note that a paternal aunt had suffered from cardiospasm from the age of 7 years.

Case 2. S.S., a male, was a first child. Pregnancy was normal but delivery was complicated by uterine inertia. He was delivered by the breech with forceps to the after-coming head. He had [illegible] cleft palate and was subject to cyanotic [illegible] He began to vomit a few days after birth. Vomiting persisted, and at the age of 5 months a barium swallow was performed, which showed free regurgitation at the lower end of the oesophagus with the patient in the supine position. There was no definite evidence of hiatus hernia on the films. The boy was grossly retarded mentally. The urine contained no phenyl-pyruvic acid. The toxoplasm dye and complement-fixation tests were negative, as was the Wassermann reaction. Sucrosuria was first discovered at the age of 15 months.

Case 3. D.B., a female, was a first child, and was delivered by forceps. At the age of 3 weeks she started to vomit, and this persisted up to the time she was investigated at the age of 2 years. A barium meal then showed a large sliding hiatus hernia. The child was grossly retarded mentally and had a spastic quadriplegia. The urine was negative for phenyl-pyruvic acid. Sucrosuria was noticed at the age of 2½ years.

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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 supp the existence of caries, which is related to factors other than i ietary carbohydrates.

The purpose of the ent investigation of the relationship between caries activity carbohydrate intake was therefore to find answers to the fo ing 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 below 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 sweets varied from one group to another. In other groups the subjects received a small meal of refined sugar at meals, which brought the total sugar intake 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 [illegible] the number of decayed teeth, the surfaces involved, the number [illegible] teeth extracted, not erupted or retained. Plaster models of the [illegible] 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 [illegible] caries).

3. *Pit and fissure c*[illegible] was also said to be present when the probe was "caught".

4. *Roentgen caries* [illegible] caries). Well defined decalcified areas of the proximal surfa[illegible] in the x-ray film, which could not be verified as cavities on [illegible] ection 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

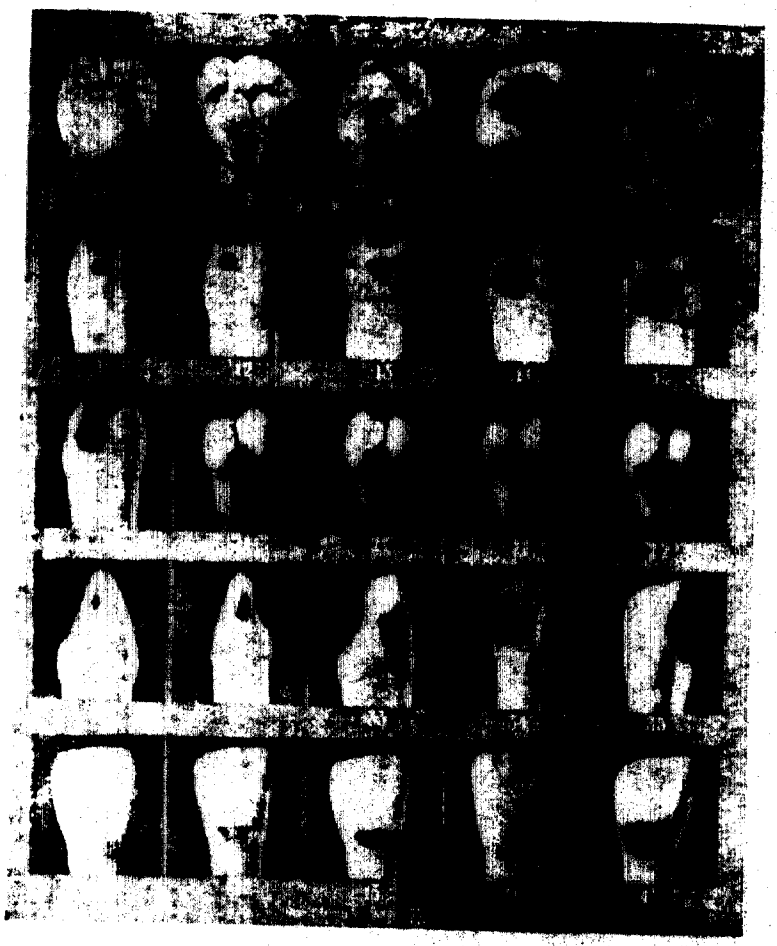


Fig. 1 a. Moulage System Series 1.

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

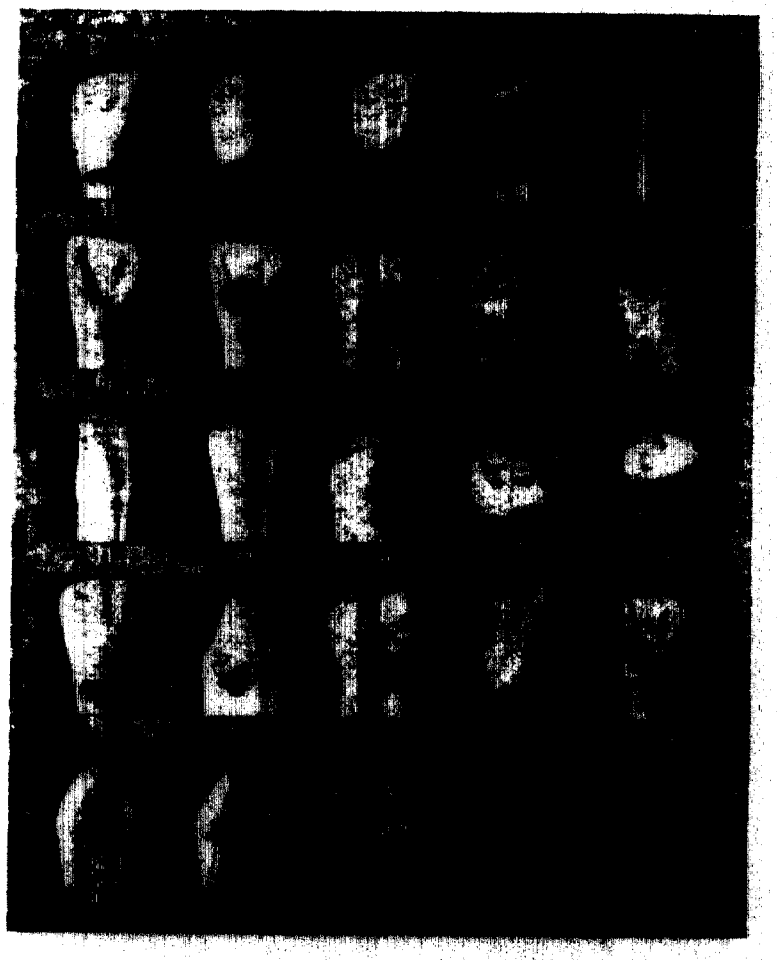


Fig. 1 c. Moulage System Series 3.

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

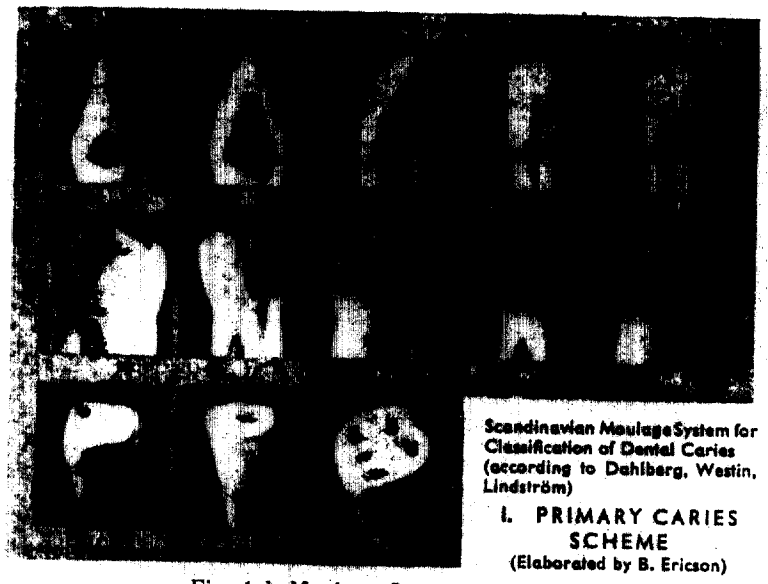


Fig. 1 d. Moulage System Series 4.

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

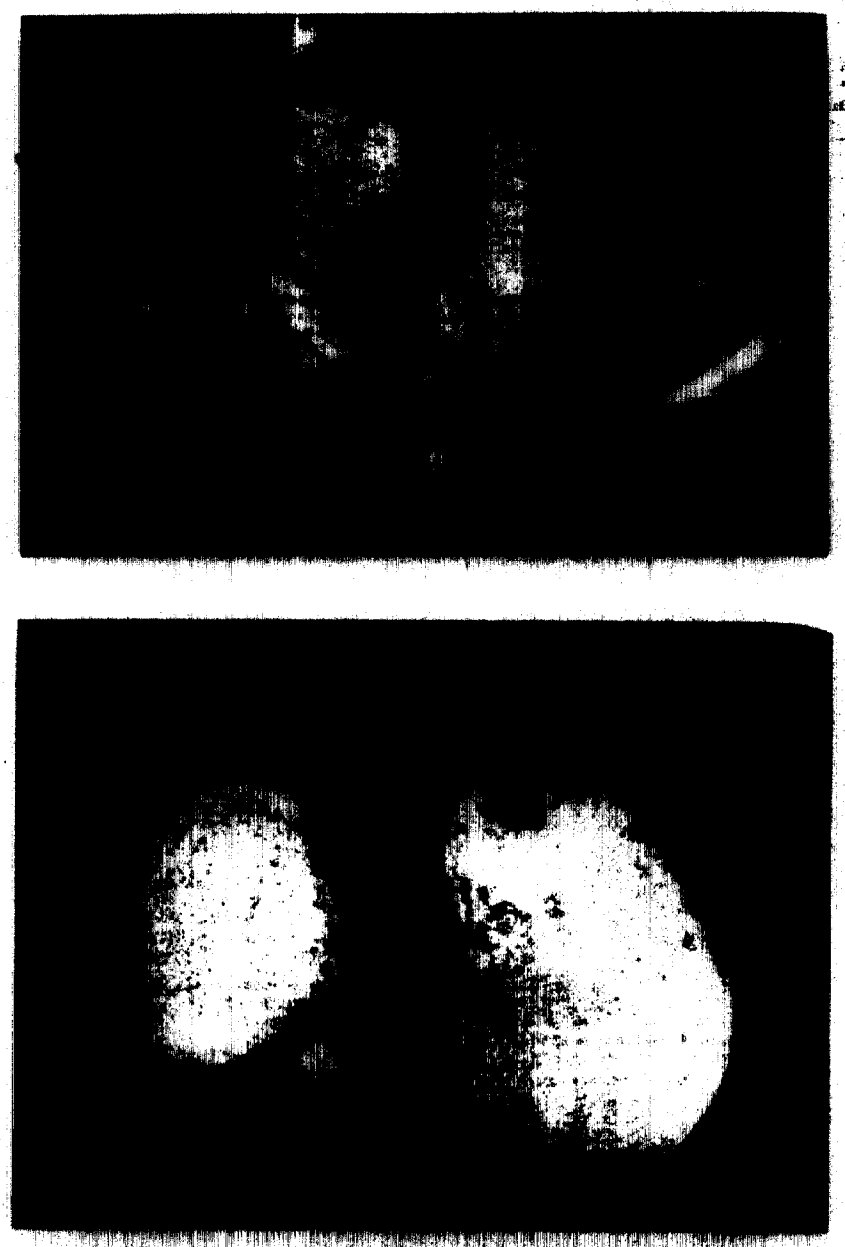


Fig. 2 a and b. Caries without defect (Cwd) intra-orally photographed. In Fig. 2 b the change has partly developed into a cavity of the type M 51.

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.

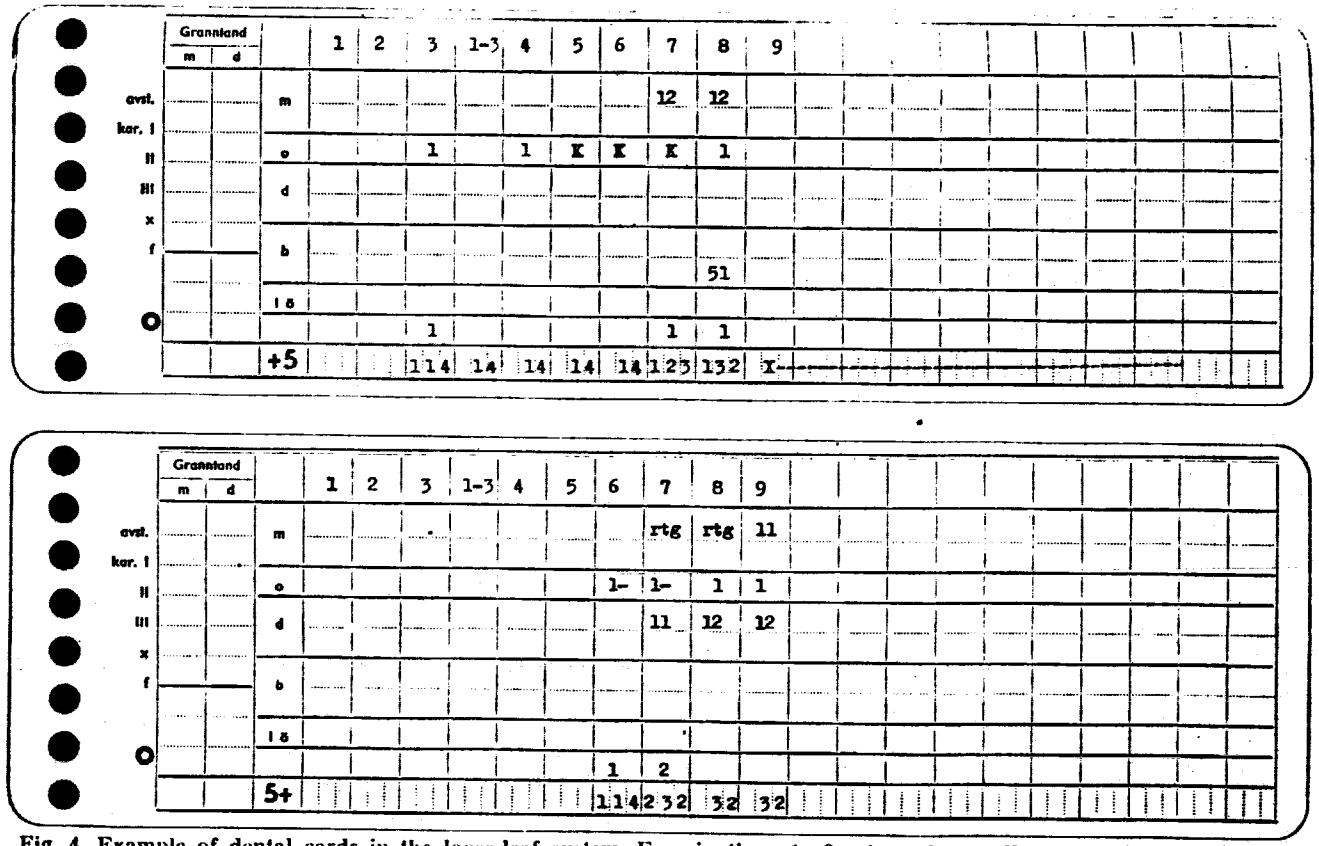


Fig. 4. Example of dental cards in the loose-leaf system. Examinations 1—9registered according to the Moulage System

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.

[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

gures 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	—	—	×	1	1	K	1	1	1	K	K	×
		d	—	—	—	—	**13**	13	16	16	×	—	—	**Rtg**	*Rtg*	13	13	16	16	×

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.

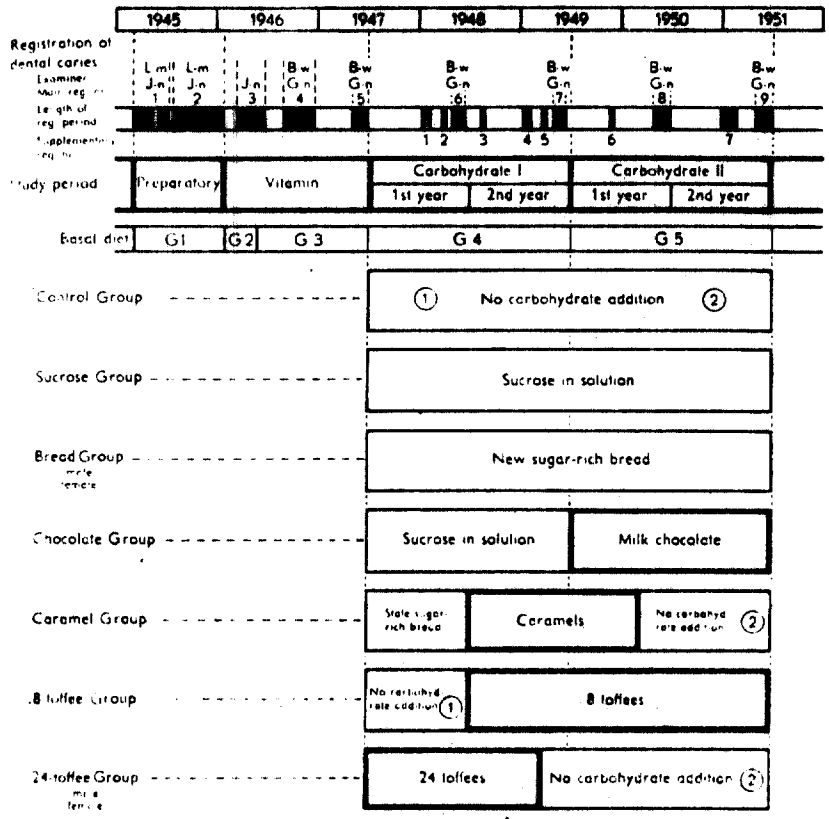
All the main dental recordings were performed by two dentists (Bonow and Grahnén) except the first three, which were carried out by two others (Lindholm and Jacobsson). In supplementary recordings three dentists participated (Bonow, Grahnén and Krasse).

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-

MAIN DIAGRAM
of the Vipeholm Dental Caries Study



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

[illegible] Preparatory Period

[illegible] final year the diet (G1) consisted of [illegible]

[illegible] Vitamin Study

[illegible] 15.1.1946 to 1.6.1946 contained 2800 [illegible] day. This diet was replaced on 1.6.[illegible] a slight modification of diet G2 [illegible] the end of the Vitamin Study Period [illegible] values of the various diets are summarised [illegible]

The [illegible] trial was to find out whether [illegible] vitamins ingested was capable of [illegible] activity. The basic diet therefore [illegible] of all nutritional constituents and the supplements [illegible] given as an extra daily dose. Thus [illegible] amount of vitamin [illegible] another of vitamin [illegible] received supplements of all the vitamins [illegible] of the amounts of vitamins. Supplementary [illegible] chloride were also studied for any [illegible] group received calcium as the lactate, one group [illegible] 1 mg. fluorine in the form of a [illegible] group received 1 mg. fluorine in the form of [illegible] spread on the bread. The control [illegible] in which the subjects received the basic diet only. The [illegible] groups and the dietary supplements are summarised [illegible]

CARBO[illegible] DURING THE VITAMIN [illegible] PERIOD

Carbohydrates [illegible] supplied mainly in the form of potatoes (500 g. per [illegible] soft bread (200 g. per day). The daily amount of [illegible] in the preparation of the basic diet was 60 g. [illegible] daily carbohydrate intake per person was [illegible] 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: 1600 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: Sugar in less sticky form	Sugar consumption during the Carbohydrate Study: Sugar in sticky form	Sugar consumption during the Carbohydrate Study: Sugar at meals	Sugar consumption during the Carbohydrate Study: Sugar also between meals
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					
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					
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					
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					
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 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 20 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 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 %	Carbo-hydrate %	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		Nr. of pers.
		Basic diet	Additions	
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 (bone 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	40 » »	
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	53	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 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	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
male	: 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
	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	—
female	: 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 sam 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-

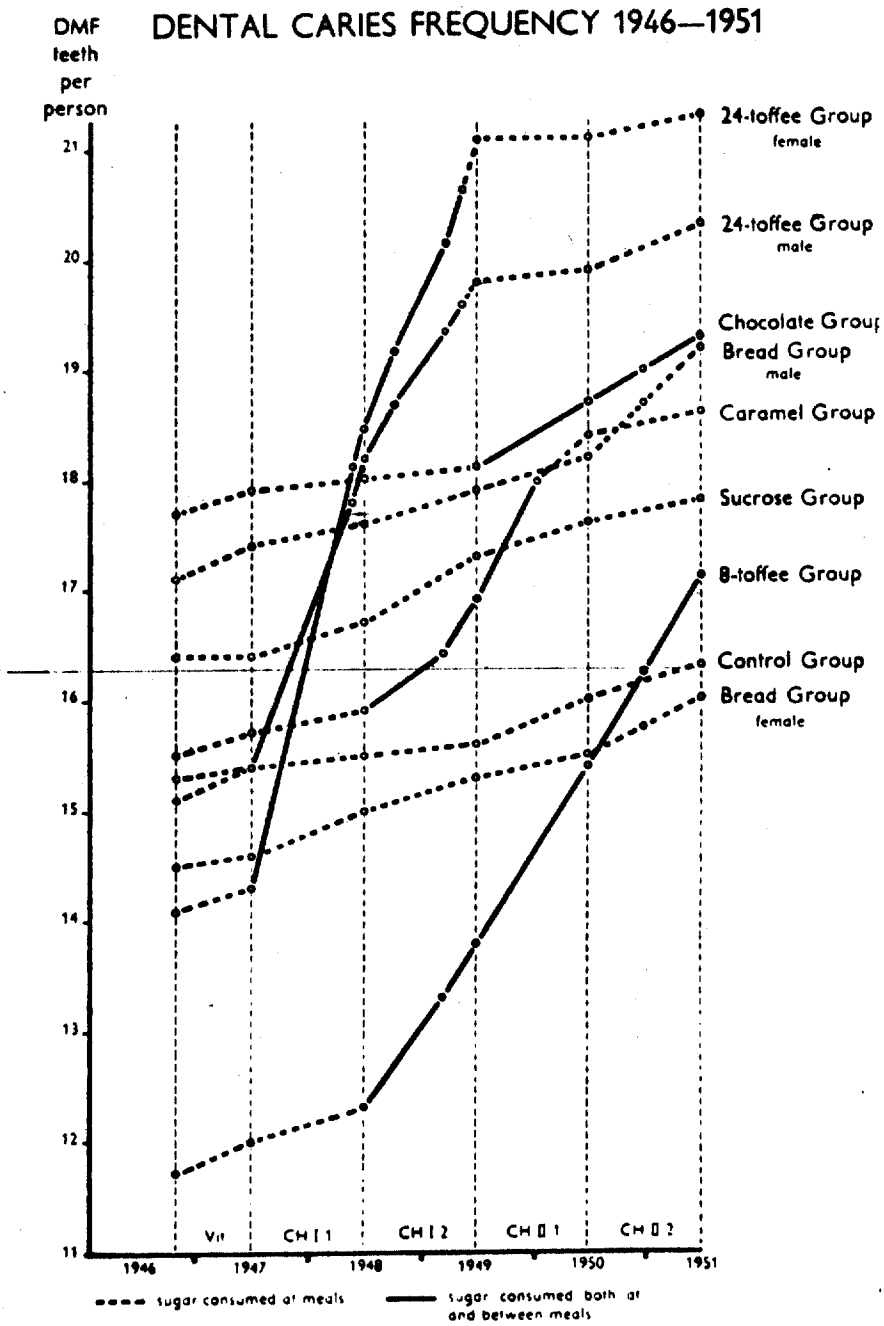

DENTAL CARIES FREQUENCY 1946—1951
DMF teeth per person
21
20
19
18
17
16
15
14
13
12
11
24-toffee Group female
24-toffee Group male
Chocolate Group
Bread Group male
Caramel Group
Sucrose Group
8-toffee Group
Control Group
Bread Group female
VII
CH I 1
CH I 2
CH II 1
CH II 2
1946
1947
1948
1949
1950
1951
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

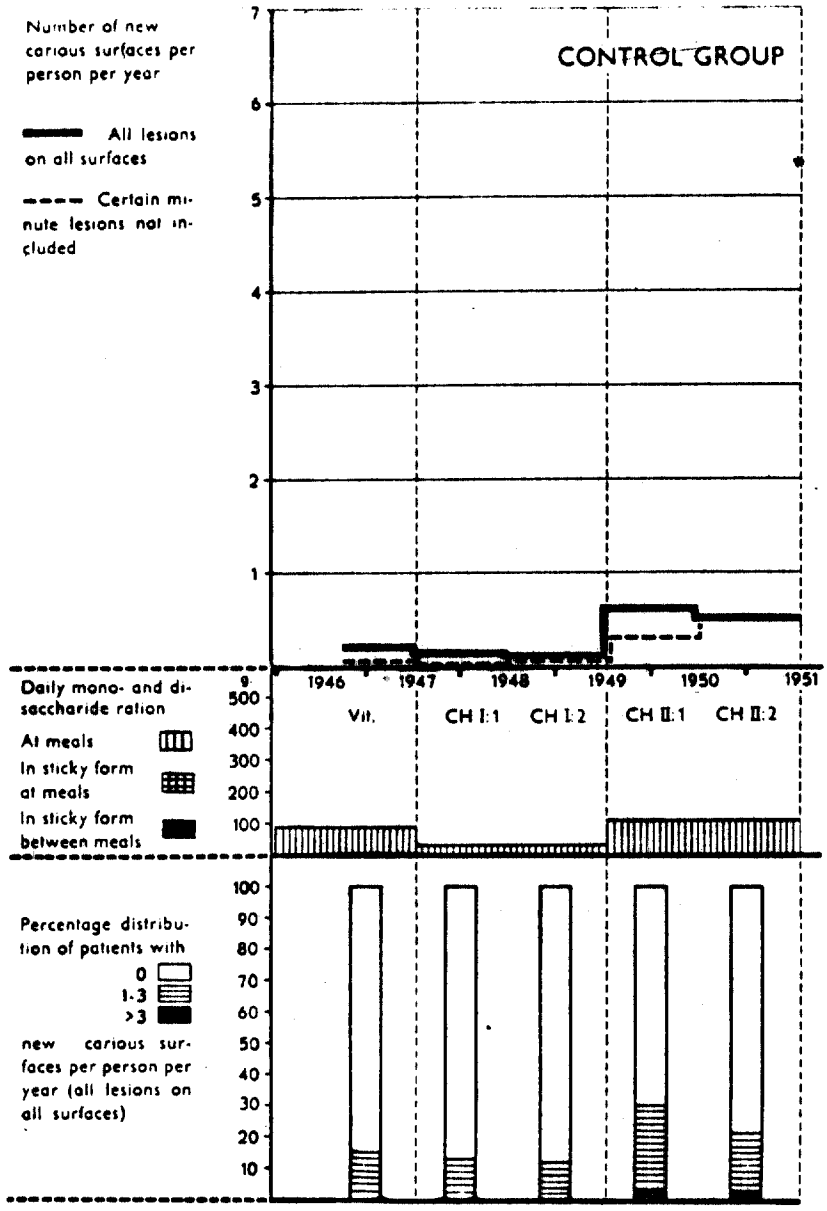
1) the margarine addition was decreased from 150 g. to 40 g.,

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



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 bern 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.

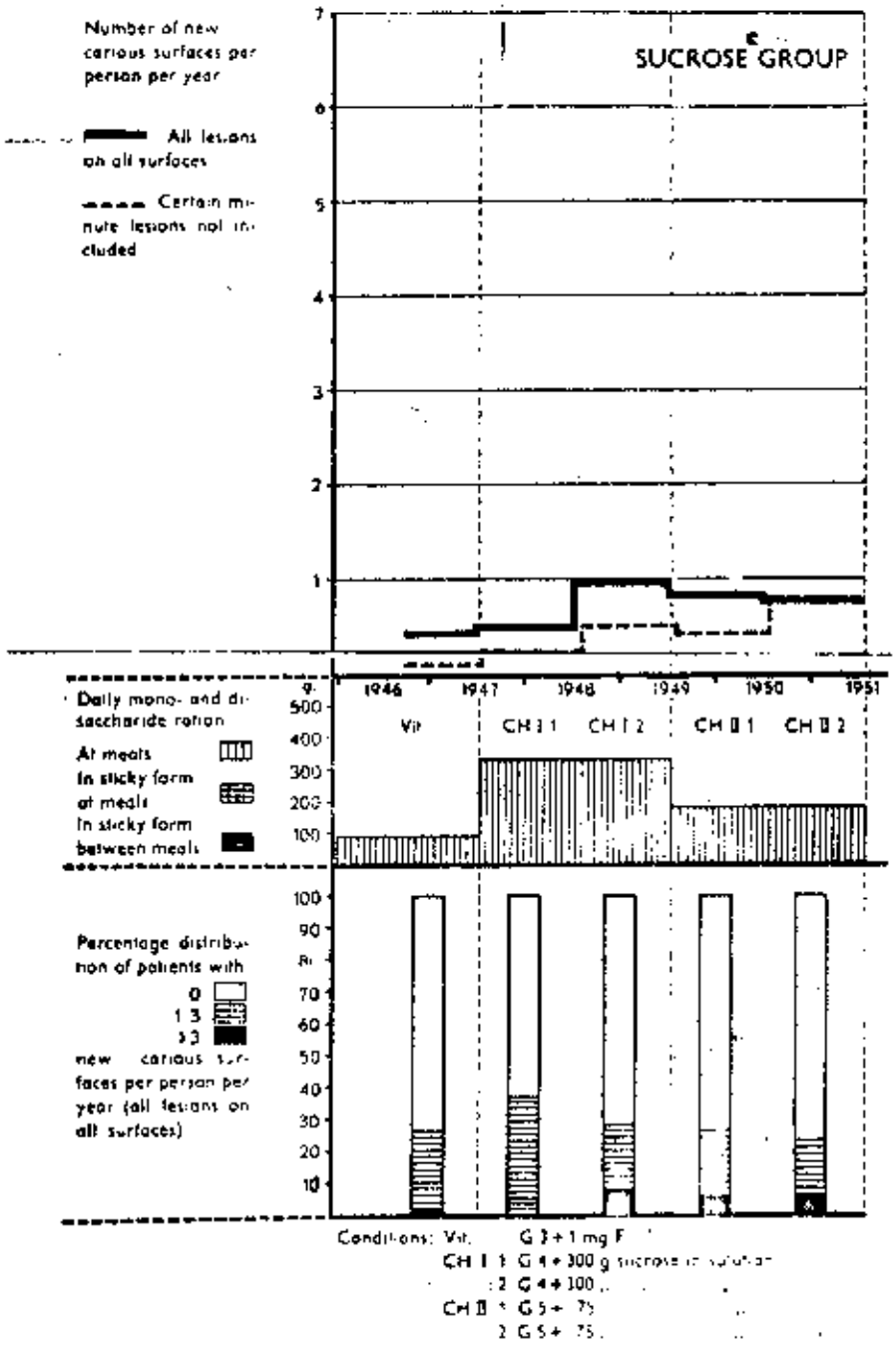

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
SUCROSE GROUP
1946
1947
1948
1949
1950
1951
Vit
CH I 1
CH I 2
CH II 1
CH II 2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1 3
> 3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions: Vit. G 3 + 1 mg F
CH I 1 G 4 + 300 g sucrose in solution
2 G 4 + 100
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., MI 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	0.15
	Mandib. inc.	0.02	0.07	0.26	0.15	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	—	0.03	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 ½ 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

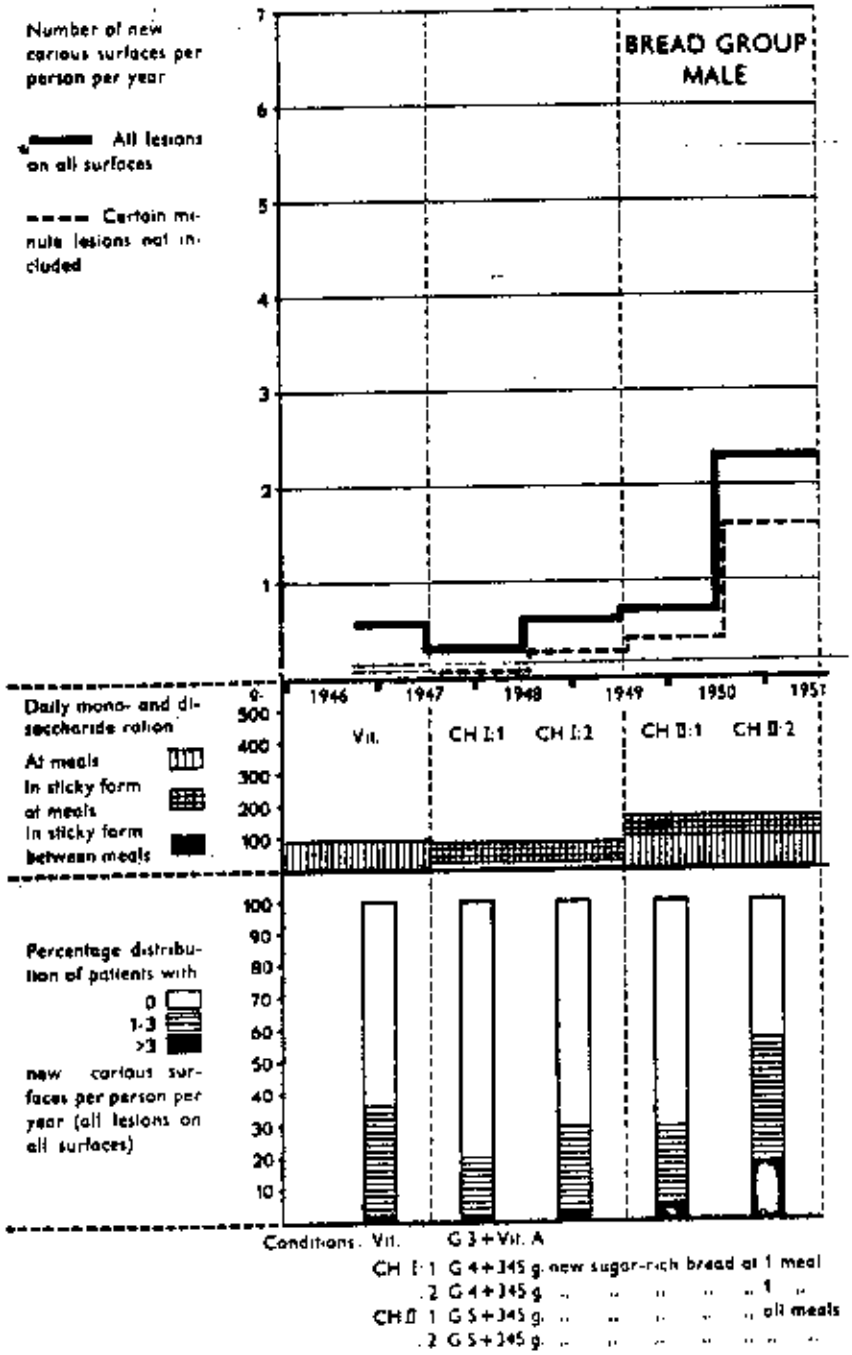

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
BREAD GROUP MALE
1946
1947
1948
1949
1950
1951
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Vit.
CH I:1
CH I:2
CH II:1
CH II:2
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
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	0.27	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., MI 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	—	—	0.07	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	0.06
	other causes	0.47	0.80	0.37	0.32	0.24
	Total:	1.32	1.71	1.34	2.17	4.51

[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	5	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

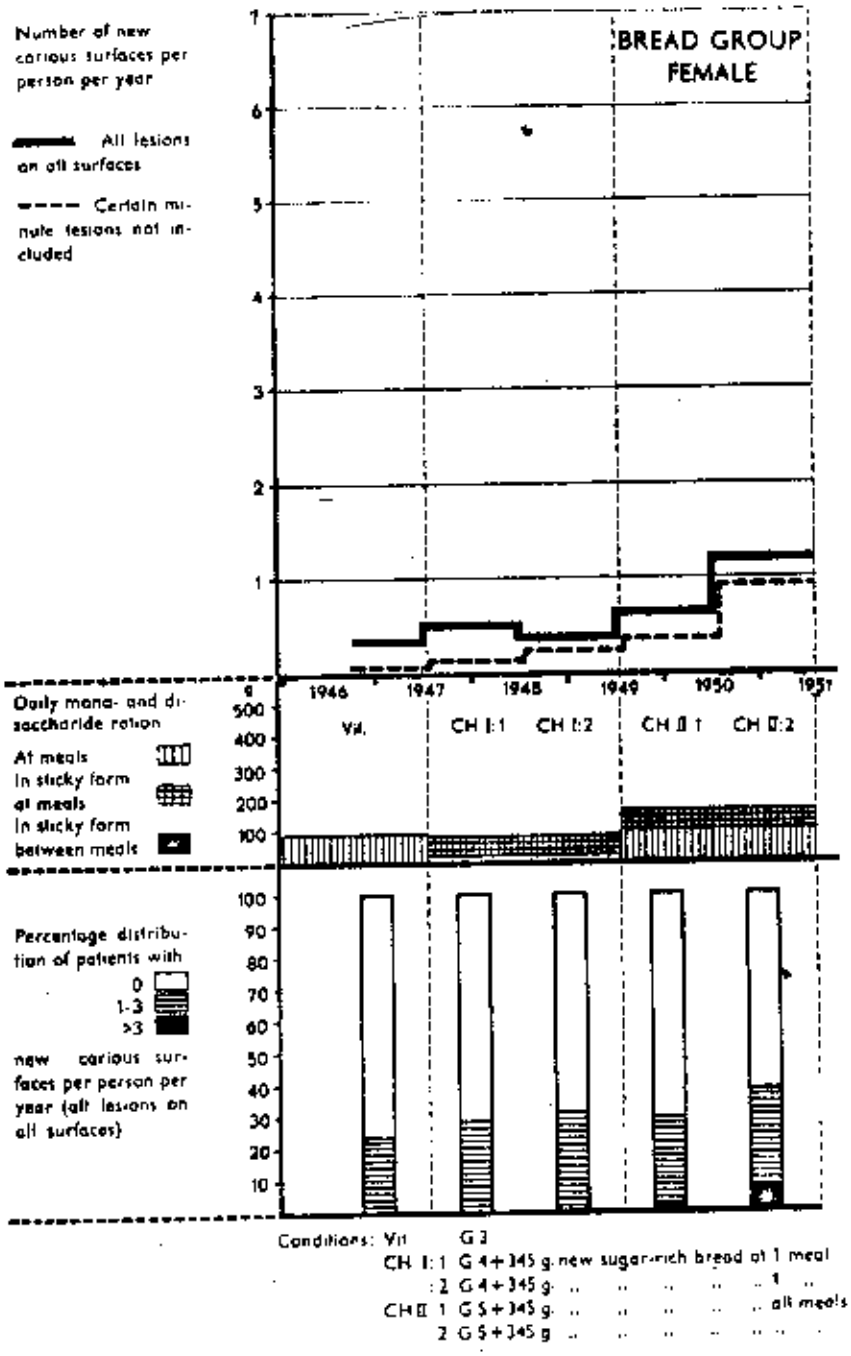

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
BREAD GROUP FEMALE
1946
1947
1948
1949
1950
1951
Vit.
CH I:1
CH I:2
CH II 1
CH II:2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions: 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., Rig., MI 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,

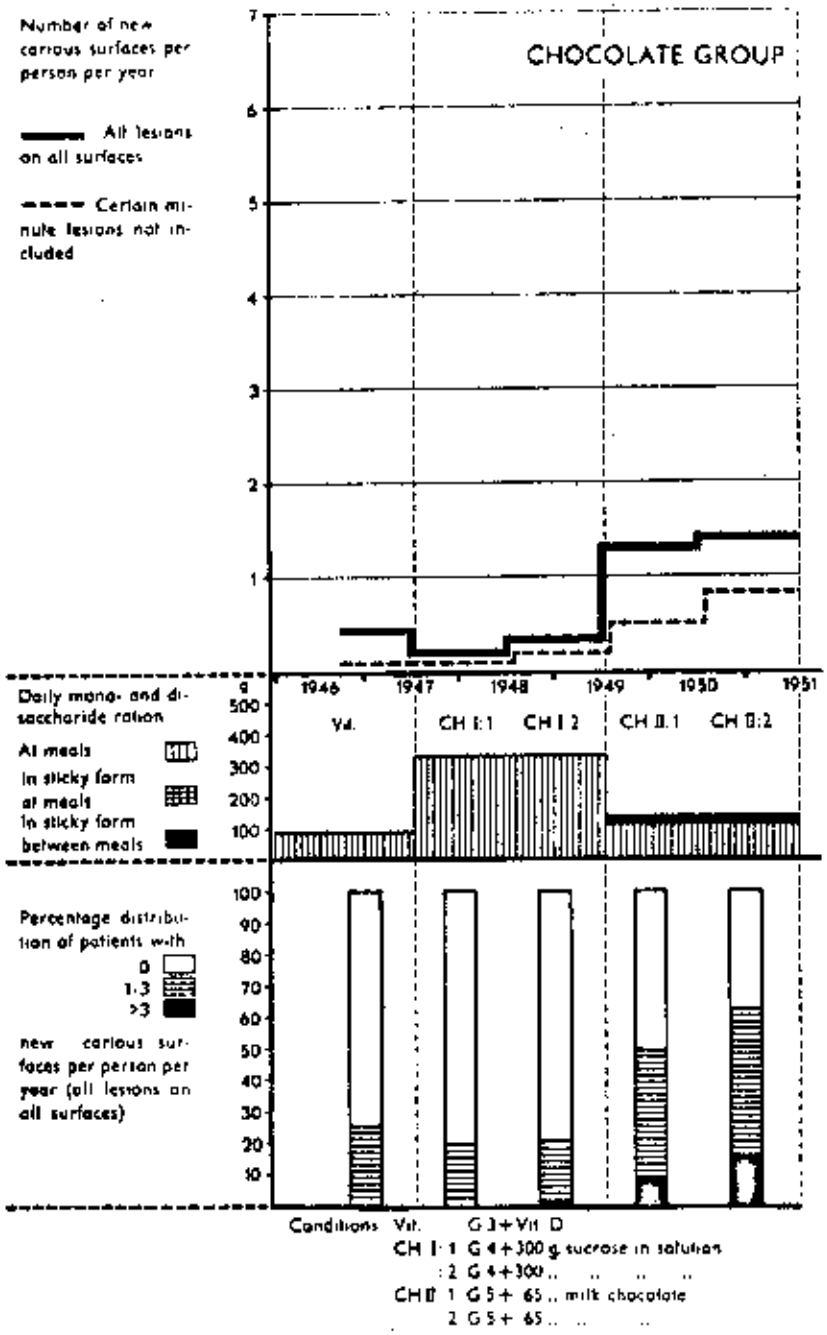

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
CHOCOLATE GROUP
1946
1947
1948
1949
1950
1951
Vit.
CH I:1
CH I:2
CH II:1
CH II:2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions 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 300 g		Milk chocolate 85 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., Rig., M1 excl.	0.13	0.13	0.19	0.51	0.82
	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	0.88	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	5	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_4 + 65 g milk chocolate	G_4 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_4 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	[illegible]	[illegible]	[illegible]	0.12	70	89
I	[illegible]	[illegible]	[illegible]	0.57	45	70
II	[illegible]	[illegible]	[illegible]	0.60	41	75
III	[illegible]	[illegible]	[illegible]	0.83	57	67
Post	[illegible]	[illegible]	[illegible]	0.64	69	70

[1] Abbreviations for periods: see Table 29.
[2] Per 1[illegible]8 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 3[illegible] gives [illegible] survey of the changes. Those persons for whom [illegible] available for two successive years were grouped according [illegible] activity in each period. Each person was assigned [illegible] 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 [illegible] chocolate period and the experimental year I in the Control [illegible] will be apparent that of 72 persons, 52 belonged [illegible] activity class during both observation periods (AA, [illegible]). Of the other twenty, 16 were graded up from lower to higher [illegible] classes (AB, AC, BC), and 4 were graded down. With [illegible] equations only, the last two figures probably counterbalance [illegible] another. The disproportion between the number graded up [illegible] number graded down denotes a significant change in [illegible] 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	3	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	32	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 —	18	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		58	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 postactive 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

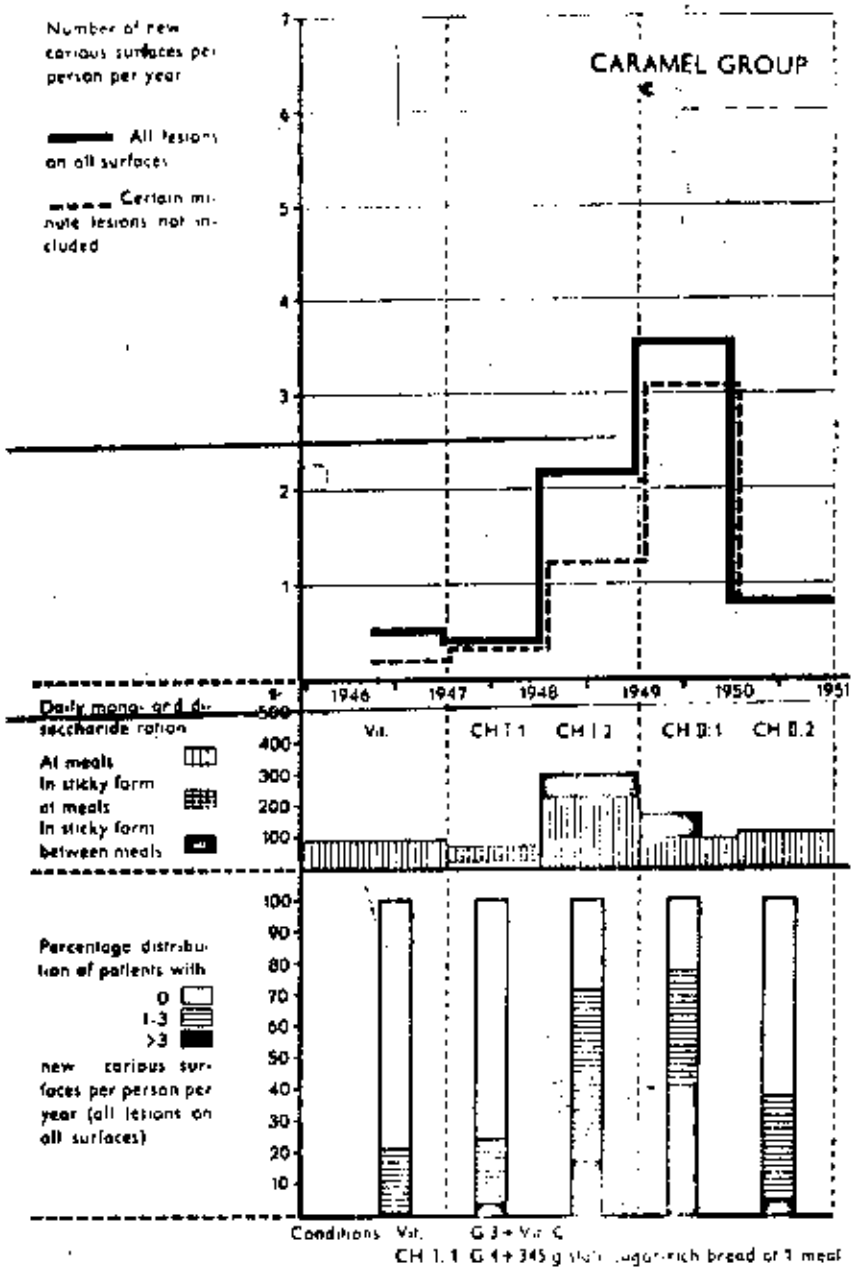

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
CARAMEL GROUP
1946
1947
1948
1949
1950
1951
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Var.
CH I 1
CH I 2
CH II 1
CH II 2
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions Var. G 3 + Var. C
CH I. 1 G 4 + 345 g
2 G 4 + 22
CH II 1 G 5 + 22
2 G 5 + 40 g

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
			22 caramels		
	Vit.C	Stale, high-sugar bread 245 g at one meal	155 g between meals + 200 g sugar in sol.	155 g in 4 portions between meals (40% red. of basic diet)	No suppl. carbohydrates Cal. compensation 40 g margarine
Number of patients who consumed					
> the supplement			—	1	
= " "			59	58	
< " "			3	3	
0 " "			—	—	
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	0.03	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

Surface losses per person per period

[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	3 % > 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-

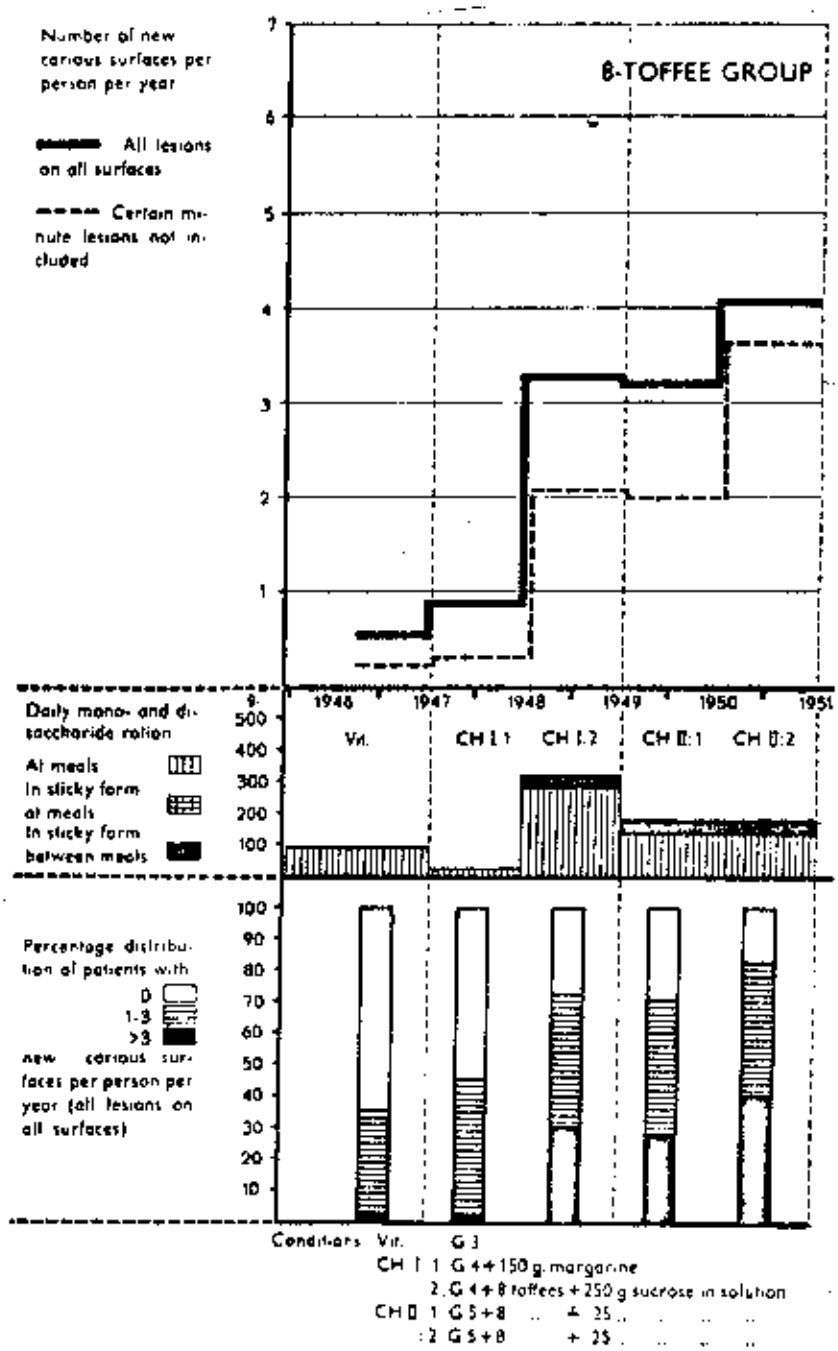

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
8-TOFFEE GROUP
1946
1947
1948
1949
1950
1951
Vit.
CH I:1
CH I:2
CH II:1
CH II:2
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions 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.	8 toffees 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., Rig., M1 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	0.50
	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 15 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

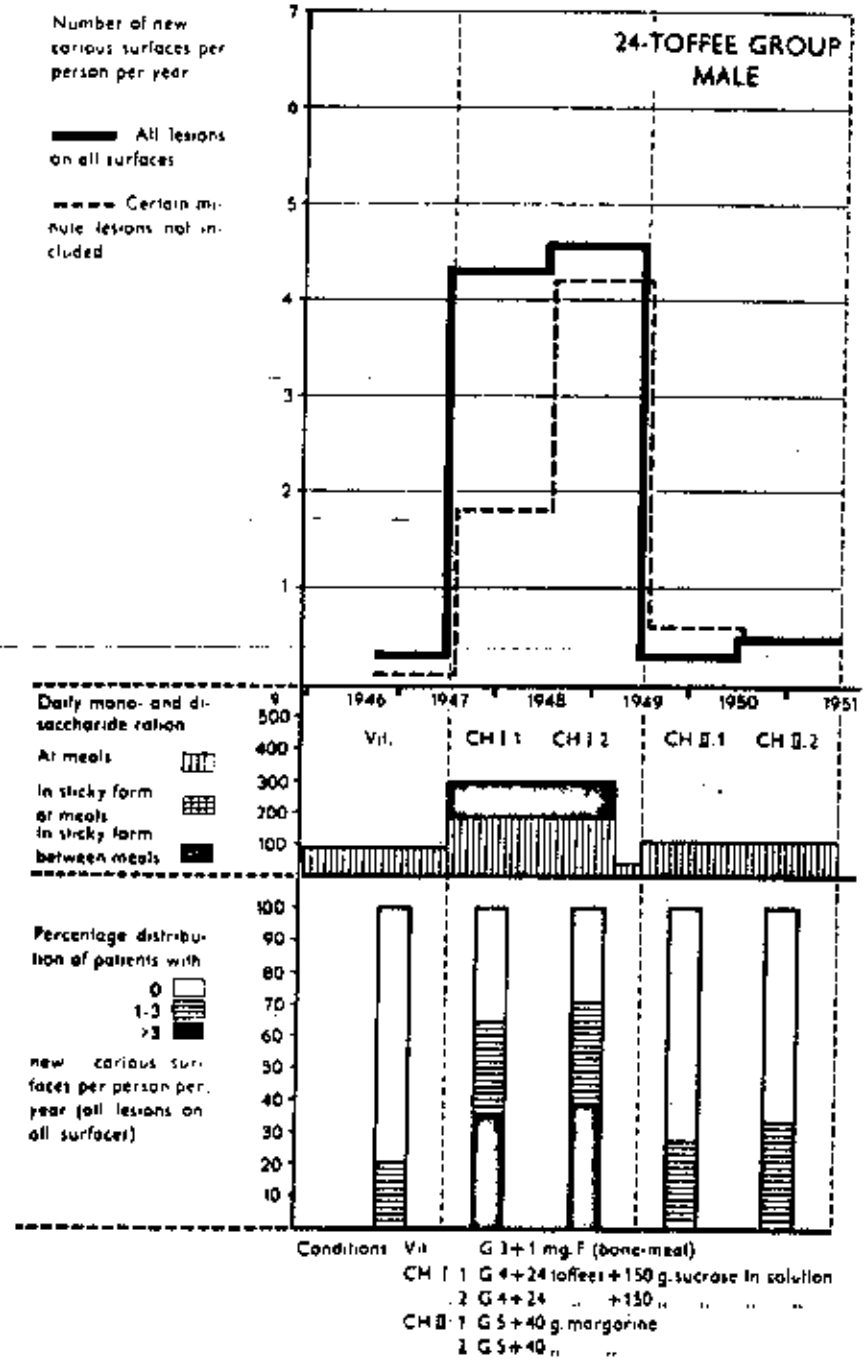

24-TOFFEE GROUP
MALE
Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
1946
1947
1948
1949
1950
1951
Vit.
CH I 1
CH I 2
CH II.1
CH II.2
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., Rig., Ml 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	2.83	1.60	0.06	0.13
	Cementum caries	—	0.60	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

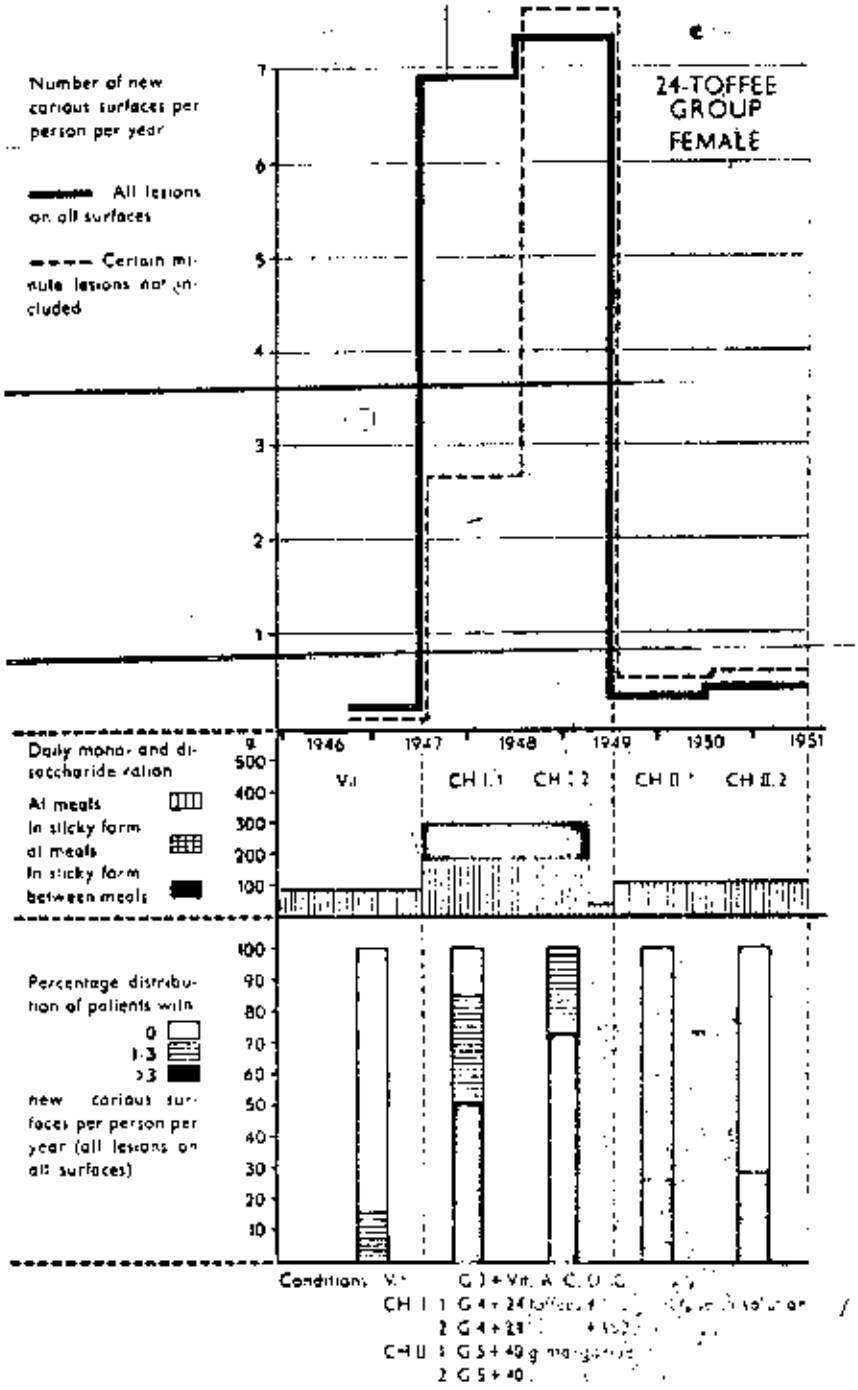

Number of new carious surfaces per person per year
All lesions on all surfaces
Certain minute lesions not included
24-TOFFEE GROUP FEMALE
1946
1947
1948
1949
1950
1951
Daily mono- and disaccharide ration
At meals
In sticky form at meals
In sticky form between meals
Percentage distribution of patients with
0
1-3
>3
new carious surfaces per person per year (all lesions on all surfaces)
Conditions

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 increase 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., Rig., Mi 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	1.40	1.31	1.15
	Loss of teeth					
	periodontitis	0.26	0.05	0.26	0.36	0.10
	other causes	0.21	0.18	0.81	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
Rig	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 Rig-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 48). 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 1940—1951. Third molars not included

Age Group	No. 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	34.8	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	5.5	326	0.79	7	2.1	0.02
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	4.01
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	85	0.85	1.00
1910—1919	77	42	93	81	0.88	0.89
1920 or later	83	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

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 ration 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	985	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 1 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	[illegible]
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	100	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	3.05
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-

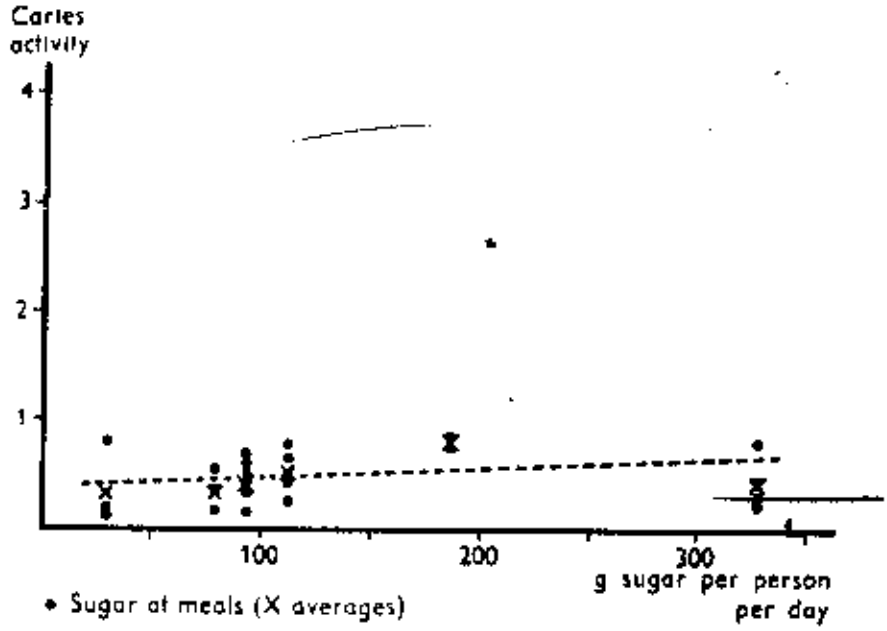


Fig. 16. The relationship between sugar consumption at meals and caries activity.

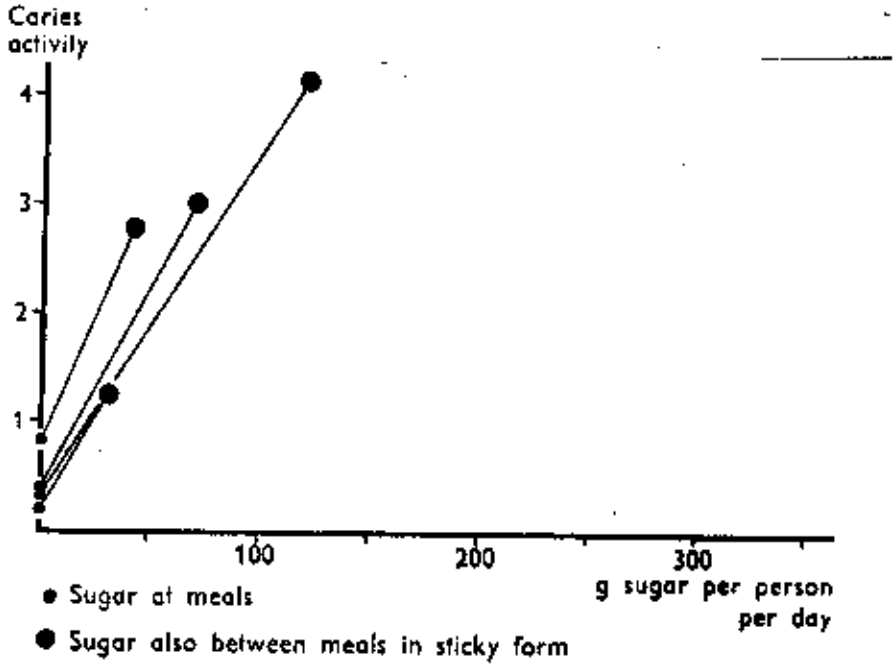


Fig. 17. The relationship between sugar consumption between meals and caries activity. Cf. p. 355.

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 21-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 1 the effect of a pronounced reduction in dietary carbohydrates on existent basic caries activity was stu-

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).

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

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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BLOOD SUGAR LEVELS AND THE BEHAVIOR PATTERN OF YOUNG HEALTHY ADULTS SEVERAL HOURS AFTER THE INGESTION OF LARGE AMOUNTS OF SUCROSE[1]

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In a previous study (1) there could be found no evidence of hypoglycemia or of the so-called hypoglycemic syndrome after a breakfast containing approximately 600 calories, 81 per cent of which was derived from carbohydrate. Similar observations were made on a group of swimmers several hours after a heavy meal in which carbohydrate provided 76 per cent of the total calories. From these studies it was concluded that the hypoglycemic syndrome which can be relieved by restriction of the carbohydrate intake is a pathological condition and not a normal physiological response to a high carbohydrate meal.

In the same volume of the Journal in which our paper appeared there was another (2) in which entirely different results from ours were reported. The ingestion of 70 to 150 grams of dextrose was found to induce the development of "hypoglycemi's" reactions in 5 out of 6 subjects approximately 50 per cent of the total number of times tested. "These hypoglycemic episodes usually began within 3 to 5 hours after ingestion of the sugar and occurred with approximately equal frequency at either ground level or altitude. The seizures were characterized by subjective sensations of nervousness, impending danger, paresthesia, weakness and hunger, and objective manifestations of pallor, sweating, tremulousness, coldness of the extremities, and a tendency in some toward a drop in oral temperature."

In view of the advisability of routine carbohydrate feeding to flight personnel suggested by the experimental evidence that a high carbohydrate intake leads to a gain in altitude tolerance (3, 4, 5), it becomes of practical importance to establish unequivocally the existence or non-existence of a relationship between a high carbohydrate intake and a subsequent hypoglycemic syndrome. It is obvious that the "occurrence of such reactions as weakness, intense hunger, and tremor, either mild or severe, may lead to disastrous results in flight and would be especially dangerous should they develop at or about the time of landing" (2).

The present study was undertaken to determine whether the hypoglycemic syndrome similar to that described by D'Angelo (2) after dextrose administration would be induced by the more common article of food, sucrose.

PROCEDURE. Forty-four medical students, three of whom were women, served as volunteer subjects. In order to avoid the possibility of suggestion playing any part in their subjective state, they were told that the purpose of

[1] The expense of this investigation was defrayed by a grant-in-aid from the Sugar Research Foundation.

the experiment was to obtain data on the sucrose tolerance curve. Care was taken to avoid any reference to the hypoglycemic syndrome.

On the day of the experiment the subjects came to the laboratory in the fasting state. Two basal blood samples were taken: arterial by finger puncture and venous by venepuncture. The subjects then drank a lemon-flavored sucrose solution containing 1.5 grams of the sugar per kilo body weight in 400 cc. water. For the remainder of the morning they continued in their usual activities reporting to the laboratory at hourly intervals for the next four hours for the drawing of blood samples. No other food was taken until the midday meal. Objective estimates were recorded by four members of the research staff on the physical and behavioral reactions of the subjects. In the course

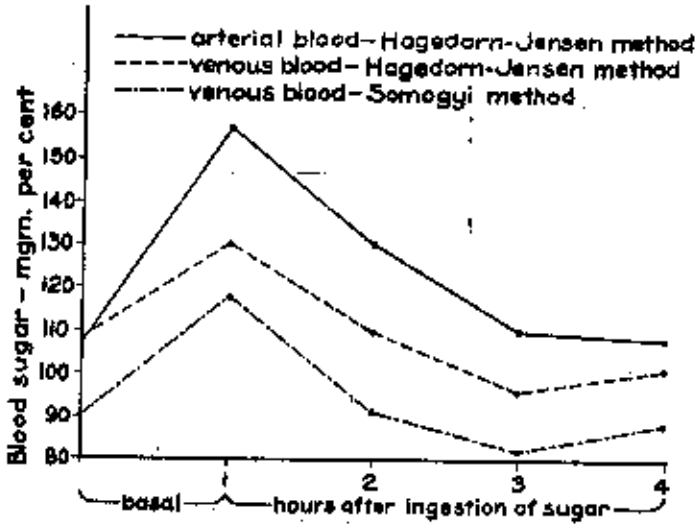

Fig. 1. Sugar concentrations in arterial and venous blood following the ingestion of 1.5 grams sucrose per kilo body weight in 400 cc. water.

of a general conversation with each subject at the conclusion of the experiment comments were elicited on how he felt. Again care was exercised to avoid suggestion.

The arterial blood was analyzed for sugar by the Hagedorn-Jensen procedure and venous blood by the Shaffer-Hartman-Somogyi method (6). In view of our previous experience that the Hagedorn-Jensen procedure in our laboratory yields values approximately 15 mgm. per cent higher than the Somogyi method it was first thought it would be satisfactory to apply a correction factor for equating the arterial and venous blood sugar concentrations. Later when the experiment was in progress it was decided to analyze the venous blood also by the Hagedorn-Jensen method in order to obtain a more accurate comparison between the arterial and venous blood sugar values. Venous samples obtained from 33 subjects were analyzed by both procedures.

Results. The average concentrations of sugar in arterial and venous blood throughout the experiment are presented in graphic form in figure 1. The

curves of venous blood sugar have the contour of those usually obtained in normal individuals on the glucose tolerance test. The concentrations which rose appreciably one hour after ingestion returned to the basal within two hours and dipped below this level the third hour. The concentration in arterial blood which was at the same level as in venous blood in the fasting state rose to an appreciably higher level in the first hour and returned more slowly to the basal than is the case with venous blood. Unlike the latter it did not fall below the basal.

Analysis of the individual data revealed that by the Somogyi method, (which gave lower values than the Hagedorn-Jensen procedure) only 9 out of 44 subjects had a blood sugar concentration below 80, 6 below 70, and 1 below 60 in the third hour after the ingestion of sugar. From these results it may be concluded that hypoglycemia, in the generally accepted sense of the term, is not a usual response to the ingestion of a large amount of sucrose.

TABLE 1

*Blood sugar concentrations after ingestion of glucose and of sucrose (1.5 grams/kilo in 400 cc. water)**

SUGAR INGESTED	BASAL	MINUTES AFTER INGESTION				
		1	5	10	15	30
	Blood sugar—mgm.%					
Glucose	95	99	108	125	134	155
Sucrose	95	100	107	122	134	148

* Each value is an average of 26 experiments.

The observations recorded by the objective observers were uniformly in agreement. In only two of the subjects were there manifest any symptoms or physical signs that bore a resemblance to the hypoglycemic syndrome. Both these subjects (one man and one woman) were somewhat nervous. Each complained of a sense of faintness. Perspiration was observed on the forehead and palms of the hands. However, it is important to note that in both these individuals these signs were present before sugar was taken. It is a reasonable assumption that fear over being stuck with a needle was responsible for the reaction. To all outward appearances the physical condition and behavioral pattern of all the other subjects were no different from those which had been observed in our previous frequent contacts with them.

Inspection of the subjective reports which were recorded after the interview showed that 31 of the 44 subjects felt "perfectly normal." Frequently the comment was volunteered by a subject that he was surprised to find he could get along just as well on the sugar as on his usual breakfast. Of the remaining 13 subjects, 8 stated that they felt hungry at 11:00 o'clock or at noon; two (as stated previously), appeared faint and nervous; one complained of headache at 12 o'clock; and two were somewhat nauseated from 10:00 o'clock on.

This discrepancy between our results with sucrose and those of D'Angelo

with dextrose suggested the possibility of a slower rate of absorption after sucrose ingestion. If such were the case a more rapid rise in the blood sugar concentration following the administration of dextrose might conceivably account for the difference in the results. This seemed improbable in view of the findings of Rabinowitch (7) that the sugar concentration of venous blood of diabetics showed an appreciable rise within 5 minutes after drinking a 5 per cent aqueous solution of sucrose containing 10 grams of sugar. However, it was considered advisable to obtain comparative determinations on the rise of arterial (finger tip) blood on normal subjects after ingestion of glucose and sucrose. One and five-tenths grams of these two sugars per kilo body weight was administered on different days to the same subjects. The sugar was dissolved in 400 cc. of water and two minutes allowed for drinking the entire amount. The averages of 26 experiments with each sugar on 9 subjects are presented in table 1.

From these observations it is apparent that the sugar concentration of the blood rises with equal rapidity after the ingestion of glucose and of sucrose. Obviously sucrose is inverted promptly in the gastro-intestinal tract.

The sugar was consumed in these experiments at 2:30 in the afternoon. No other food was eaten before the evening meal 3½ to 4½ hours later. In no instance did the subjects experience the symptoms of the hypoglycemic reaction.

CONCLUSIONS

A study of the blood sugar concentration and the behavioral response of 44 healthy young adults failed to reveal any evidence of the so-called hypoglycemic syndrome during a four hour period following the ingestion of 1.5 grams sucrose or of dextrose per kilo body weight.

The blood sugar concentration rose with equal rapidity after the ingestion of sucrose and of dextrose.

These experiments offer confirmatory evidence for the conclusion drawn from previous studies that the hypoglycemic syndrome is not the usual response of normal healthy adults to a high carbohydrate intake.

Correction in Figure one. The first point only, i. e. those on the ordinates, are the basal values. The sucrose was injected immediately after drawing the basal blood sample.

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THE RELATIVE CARIOGENICITY OF SUCROSE WHEN INGESTED IN THE SOLID FORM AND IN SOLUTION BY THE ALBINO RAT [1]

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Various studies have indicated that table sugar (sucrose) was a potent cariogenic factor in the purified diets that have been used to induce caries in the Norway rat (Sognnaes, '48; Haldi and Wynn, '52). The present study was undertaken to determine whether sucrose in the solid form is more or less cariogenic than sucrose in solution. This was possible through a slight modification of the technique employed by Kite, Shaw and Sognnaes ('50), who fed rats a complete diet by stomach tube from the time of weaning through an experimental period of 20 weeks. The present modification, where part of the diet is tube-fed and the remainder ingested orally, affords a suitable means for assessing the local etiological action of individual food items in the production of dental caries. By following that technique in this investigation, we were able to provide our experimental animals with adequate nutriment, and at the same time allow no food whatsoever to come into direct oral contact with the teeth of the rats in one group, and only sucrose in the solid form or in solution to come in contact with the teeth of the rats in other groups.

[1] The studies described in this paper have been supported to a considerable extent by grants-in-aid to the two cooperating laboratories by the Sugar Research Foundation, Inc.

EXPERIMENTAL

Twenty sets of albino rats, each set consisting of three litter mates, were selected at weaning from the colony of the Wistar strain in the Physiology Laboratories of the Emory University School of Dentistry. Weanling animals from this colony, when placed on a high sugar ration, have proved sufficiently caries-susceptible to develop some carious lesions, although their parents had been fed a stock diet. In another investigation it was observed that the purified diet used in the Harvard studies (Sognnaes, '48) was more cariogenic than the Emory diet (unpublished data). For this reason, and because of the greater ease of feeding the Harvard purified diet by stomach tube, it was chosen for these experiments. The composition of this sugar-containing diet has been described previously (Shaw, '47).

Immediately after weaning, the 20 trios of rats were distributed into three groups, with each trio having a representative in each of the three groups. For the remainder of the experiment, a period of 90 days, the control animals (group I) were fed the complete sugar-containing diet by stomach tube. All rats in groups II and III were fed by stomach tube the same purified diet except that the sucrose had been completely omitted. In addition, the rats in group II were given weighed amounts of granulated sugar in a food cup. The rats in group III received measured amounts of a 40% sugar solution in a test tube with a delivery tube attached to the side of the cage. The daily ration of sucrose, both in the granulated form and in solution, was adjusted so as to make the sugar intake of the experimental animals in groups II and III the same as that of the control animals (group I) which were fed the entire food mixture by stomach tube. Likewise the intake of the non-sugar portion of the diet was maintained at equal levels for the experimental and control rats.

The procedure described by Levin ('42) was used for the stomach tube feeding, with a few modifications. This method permits the entire operation to be carried out by one person.

After one becomes adept with the procedure, an animal can be removed from the cage, fed by stomach tube, and returned to the cage in less than a minute.

The animal is held with its back against the palm of the left hand. The thumb and index finger of the operator are clasped snugly but not tightly around the animal's shoulder. The animal's right arm is held against its body by the operator's left thumb and its left arm between the thumb and second finger. A loop at the end of a string about 20 inches long is caught over the upper incisors. The string is passed over the back of the left hand and between the 4th and 5th fingers and fastened by being wound several times around the latter. A loop at the other end of the string is caught over the lower incisors, passed down over the back of the thumb and fastened by being wound around the little finger of the left hand. The mouth can then be opened by gently pulling apart the thumb and index finger.

Of the several kinds of tubing we tried, we found most satisfactory a transparent, transflex plastic tubing, No. 16, with an inside diameter of 0.053 inch.[2] This tubing fits snugly over a No. 15 syringe needle which has been cut off to a length of about one inch.

While the tube feeding process was being conducted, the food mixture suspended in water was kept close at hand in a small beaker. It was held in suspension by constant stirring with an electric stirrer and drawn into the syringe as needed.

Water was added to the food mixtures in such amounts that 1 ml of the suspension of the sugar-containing diet provided 1 gm of the food mixture and 1 ml of the sugar-free diet suspension $\frac{1}{3}$ gm. In each milliliter of sugar-containing diet there was $\frac{2}{3}$ gm sugar. In order to equalize the food intake of the experimental and control animals, the former were given $\frac{2}{3}$ gm sugar, either in the solid or liquid form, for each milliliter of the suspended food mixture given by stomach tube. All the animals were fed by stomach tube three times

[2] Irvington Varnish and Insulation Company, Irvington, New Jersey.

a day. The first three or 4 days after weaning, they were given 1 ml of the food suspension at each tubing; then for one week 2 ml per feeding; the following week 4 ml; and from then on, 5 ml for the remainder of the experiment.

After the animals had been on the experiment for 30 days, they were desalivated in order to accelerate the carious process. Then the usual feeding procedure was continued for an additional 60 days. For the purpose of surgical desalivation, the rats were anesthetized by intraperitoneal injection of 4 mg sodium nembutal per 100 gm of body weight. An incision was made in the neck and the underlying structures exposed. The parotid duct and external jugular vein on each side were tied with a silk ligature and resected. The ducts of the submaxillary and major sublingual glands on each side and the accompanying blood vessels were also ligated and cut. The submaxillary glands, the major sublinguals and most of the parotid on each side were dissected out by teasing with a probe. A small portion of the parotid located down near the shoulder was not removed. The exorbital lachrymal gland on each side was dissected free and excised. There was very little loss of blood, and the animals appeared to suffer no ill effects from the operation.

At the end of the experiment, the animals were decapitated and the upper and lower jawbones removed. One quadrant of the upper jaw and the corresponding quadrant of the lower jaw were fixed in 4% basic lead acetate for 24 hours. They were then rinsed in distilled water and placed in 10% neutral formalin. The other two quadrants, upper and lower, were fixed immediately in 10% neutral formalin. The teeth were later examined under a dissecting microscope at a magnification of 30 diameters for the number and extent of carious lesions. Since the jaws were desired for histological studies, the caries examination was made without any grinding away of the occlusal surfaces. This has been found to be permissible in the white rat by reason of the wide and relatively shallow sulci of the rat molar as compared to those in the cotton rat. The criteria for judging and evaluating the size

of the carious lesions were those described by Shaw, Schweigert, McIntire, Elvehjem and Phillips ('44).

RESULTS

Kite, Shaw and Sognnaes ('50) have pointed out the difficulties associated with feeding young rats by stomach tube, and have described the various factors probably responsible for the fatalities which may occur. Of the 60 animals with which we started the experiment, 13 were lost within the first few days and 13 more shortly after desalivation.

The animals that survived had normal growth and manifested no evidence of malnutrition. At the conclusion of the experiment--90 days after weaning—the average weight of the three groups of females was 196, 182 and 200 gm, respectively, or an increase over their original weight of 301, 304 and 334%; the average weight of the males was 231, 232 and 252 gm or an increase of 372, 382 and 394%; respectively. These gains are of the same order of magnitude as those we have observed in other experiments in which the animals were allowed to eat the Emory high sucrose diet ad libitum. In scrutinizing the difference in average weights of the control and the two experimental groups despite the equalization of food intake, it should be borne in mind that some of the animals were lost and these weights consequently are not average weights of litter mates.

Gross dental findings

The average number of carious molars, the average number of carious lesions, and the average extent of carious lesions for the three groups in this experiment are presented in table 1. The smaller number of animals in group I at the conclusion of the experiment, as shown in the table, was due to a higher mortality in this group. This is accounted for by the greater difficulties that were encountered in feeding the entire ration by stomach tube. As we have noted in our laboratories, in keeping with the observations of others

(Sognnaes, '49), that there is no sex difference in caries susceptibility in the Norway rat, only the composite averages from the data on males and females are presented in the table.

Each of the 8 rats in group I, which received the complete diet by stomach tube, was completely free of any carious lesions which could be detected by detailed examination at a magnification of 30 diameters. Eleven of the 13 rats in group II, which received granular sucrose by mouth and the rest of the diet by stomach tube, had one or more definitely

TABLE 1

Dental caries incidence in albino rats when sucrose was fed by various methods

GROUP	METHOD OF FEEDING	NO. OF RATS	AVERAGE WEIGHT INCREASE		NO. OF CARIES-FREE RATS	AVE. NO. OF CARIOUS MOLARS	AVE. NO. OF CARIOUS LESIONS	AVE. CARIES SCORE
			Male	Female				
			gm	*gm*				
I	Entire ration by stomach tube	8	231	196	8	0	0	0
II	Granular sucrose ingested orally (remainder of ration by stomach tube)	13	232	182	2	3.5	4.9	7.5+
III	Sucrose in solution ingested orally (remainder of ration by stomach tube)	13	252	208	7	0.8	0.8	1.2+

Critical ratios[1]

GROUPS	AVE. NO. OF CARIOUS MOLARS	AVE. NO. OF CARIOUS LESIONS	AVE. CARIES SCORE
I vs. II	5.4	5.4	4.2
I vs. III	2.7	2.7	2.4
II vs. III	3.0	4.7	3.4

[1] *Critical ratio:* The critical ratio is the ratio of the difference between two means to the standard error of the difference between the means. Whenever the critical ratio is less than 2.0, the difference between the means is considered to be statistically insignificant; whenever the critical ratio is between 2.0 and 2.9, the difference is of borderline significance; when the critical ratio is 3.0 or higher, the difference is highly significant.

carious lesions, some of which were well advanced in their stage of development. Only 6 of the 13 rats in group III, which were allowed the same amount of sucrose per day in solution by mouth but the rest of the diet by stomach tube, had small and infrequent carious lesions. Grossly, the carious lesions in the molars of the rats in groups II and III were typical of those lesions previously observed in rats of the same strain which were maintained on the same diet ingested orally. The difference in size and rate of progress between the lesions of the rats in groups II and III was clearly evident in the examination of the intact teeth. The carious lesions in the rats in group II were uniformly larger, softer, lighter in color, and thereby presumably more rapidly progressing, than the lesions found in the rats of group III.

The difference in caries incidence between the animals in groups I and II was found to be highly significant by the critical ratio test whether applied to the number of carious molars, the number of carious lesions, or the caries score. The difference in caries incidence between the rats in groups II and III was also highly significant for the above three criteria. The difference in caries incidence between the rats in groups I and III was borderline in statistical significance. The latter fact, coupled with the observation that just over half of the rats in group III were entirely caries free, leads to the conclusion that in these experiments sugar in solution was much less cariogenic than dry, granular sugar.

Histological findings

Ground sections and decalcified sections of the jaws and teeth of representative animals were prepared for microscopic examination. The purpose of this examination was to determine the presence of impactions and microorganisms within the fissures of the molar teeth, the location of debris in relation to the enamel surface, the type of carious lesions, and the general appearance of the enamel, dentin, pulp and supporting structures of the teeth.

The group which received the complete diet by stomach tube, and nothing but water orally, presented histological features similar to those observed in the previous tube feeding experiment of Kite, Shaw and Sognnaes ('50). The fissures contained moderate amounts of debris, which presumably had collected there during the pre-experimental period and had been retained throughout the entire experiment. The deeper portions of the fissures of the molar teeth were relatively free from microorganisms. However, the persisting cuticular lining of the enamel which was apparent in the tube feeding experiment referred to above was not as distinct, and some microorganisms had established themselves in a few areas below impacted detritus in the sulci of the molars. No explanation for these minor differences is known, for to the best of our knowledge the procedures followed with the present group and the tube-fed group in the previous experiment were identical. Notwithstanding these observations, the enamel and dentin proved to be surprisingly intact at the microscopic level.

In animals in group II which were allowed to eat granular sugar by mouth, the ground and the decalcified sections showed definite cavities in the depth of the fissures, accompanied by surrounding pigmentation and staining of the enamel and dentin. Furthermore, the fissures were filled with microorganisms. No cuticular lining remained on the enamel surface, and invasion of the microorganisms could be observed between the enamel prisms and into the dentin to various distances. Even the interproximal surfaces of the enamel as studied in ground sections were surrounded by a multitude of microorganisms; the enamel surface adjoining these areas proved to have an increased permeability to stains, indicating some loss of the inorganic substance and resulting in an unmasking of the organic matter.

Studies of the teeth of the rats in group III, when the sucrose allowance was consumed in a 40% aqueous solution, indicated that the lesions had all the characteristics of those in the teeth of the rats in group II, with a single exception.

The degree of penetration into the tooth substance was appreciably less, which was highly suggestive of a reduced caries attack rate in this group of rats.

In all three groups of rats, the periodontal structures were judged to be normal. The pulp was also normal in every case except in those instances in group II where the carious lesions had penetrated sufficiently to produce a typical pulpal involvement.

DISCUSSION

In the experiments of Kite, Shaw and Sognnaes ('50) it was found that in the strain of Norway rats raised in the Harvard Biological Laboratories, the Harvard high sucrose cariogenic, synthetic ration had to come into direct oral contact with the teeth in order for it to produce carious lesions even in desalivated animals. These observations are confirmed by the present study on the albino rat of the Wistar strain, in which it was found that no carious lesions developed when this highly cariogenic diet was fed by stomach tube. The cariogenicity of the diet when fed after weaning must therefore be attributed to a local or environmental effect on the teeth and not to a systemic action. It should be noted, however, that when the diet is fed during the period of early tooth development, it may exert an influence on later susceptibility to caries (cf. Sognnaes, '48).

Sugar has long been suspected of playing an important role in the etiology of dental caries. Sognnaes ('48) has shown that the sugar component which constitutes 67% of the synthetic ration used in these experiments is at least an important factor contributing to its cariogenicity, for the replacement of sucrose by dextrin or whole wheat flour resulted in a much lower incidence of carious lesions. While we recognize that at the present stage of dental investigation it is not judicious to apply the results of experiments on rats' teeth directly to human teeth, nevertheless it is highly desirable to study the way in which circumstances, similar to those that obtain in human dietary practices, may modify

the cariogenic action of sugar on rats' teeth. Sugar in the human dietary is consumed both in the solid form and in solution, as in milk, coffee, tea and other beverages.

Schweigert, Shaw, Zepplin and Elvehjem ('46) and Anderson, Smith, Elvehjem and Phillips ('48) observed that fluidity of the ration was an important factor in dental decay in the cotton rat. The rats which received the solid rations in these experiments had a higher dental caries attack rate than comparable groups which received the same diets in fluid form. Our experiments, which go a step further in studying a single component of the ration with respect to its physical form, lead to similar conclusions. Anderson and her associates were seeking an explanation for the fact that liquid whole milk, which contains the sugar, lactose, did not produce carious lesions in the cotton rat. Whole milk was found to offer almost complete protection against caries even in the presence of an added 5 or 10% of fermentable sugar. The latter observations also obtain with respect to the albino rat (Shaw, '50).

Fluidity is obviously an important factor, as is evidenced by the fact that the ingestion of a dry whole milk mixture resulted in a number of carious lesions, whereas caries became almost negligible when the mixture was reconstituted with water to produce a liquid ration equivalent to whole milk. However, the authors of these studies recognized that there were other factors besides fluidity which might have contributed to their results; namely, the presence of fat in liquid milk and a possible difference in the physical state of fat in dry whole milk and in liquid milk. They call attention to the postulate of other investigators that free fat as oil in the diet may reduce dental caries by forming a mechanical coating over the tooth or food particle, thus protecting against bacterial action.

In our experiments no other food but sugar came into contact with the external surfaces of the teeth, and the only variable was the physical state of the sugar fed orally. It should be noted that conditions conducive to dental decay were most severe because the animals had been desalivated.

Yet in spite of the severity of these conditions, the number of carious lesions and the caries score, from the absolute point of view, were very low when sugar was ingested in solution. On the other hand, these same values, which are indicative of the incidence and extent of caries, were relatively high when sugar was eaten in the solid form. It therefore becomes obvious that the cariogenicity of sucrose in these experiments was greatly reduced by dissolving the sugar in water before ingestion.

SUMMARY AND CONCLUSIONS

Albino rats of the Wistar strain developed no carious lesions when they were fed entirely by stomach tube a high sucrose diet which had previously been shown to be cariogenic when ingested orally.

When all the components of this food mixture except sugar were fed by stomach tube and granulated sugar was ingested orally, the animals having been desalivated for the greater part of the experiment, there developed an appreciable number of carious areas and an appreciable caries score.

When, under the same experimental conditions, sugar was ingested in a 40% aqueous solution, the number of carious areas and the caries score were markedly less than when the same amount of sugar was eaten in the solid form. More than half of the rats were entirely caries-free.

Sugar in solution was therefore found in these experiments to be much less cariogenic than granulated sugar.

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THE FACTS ABOUT SUGAR

By T. Swann Harding

NOW that sugar bootleggers have appeared suppose we take a gander at the facts. We Americans consume about seven million tons of sugar a year. Compare that with the three million tons consumed by the much larger population of Soviet Russia. But suppose we figure on an individual basis.

In 1630 sugar was a drug, and it was so scarce that a small monthly allowance was doled out at the largest hospital in Paris by a woman who had to take oath that it left her stocks only to go into medicines compounded by physicians. In 1819 the population of Great Britain consumed only 17 pounds of sugar each per year, but a little over a hundred years later they had stepped that up to 90 pounds or so.

In such countries as Australia, New Zealand, Denmark, the United Kingdom, and the United States annual sugar consumption in recent years has run from 100 to 112 pounds a head. In short, we Americans consume about 2 pounds of sugar per week each. That covers only sugar purchased as such, and not the large quantities we also get in soft drinks, candies, cakes, preserves, ice cream, and such.

Roughly two-thirds of all the sugar is consumed in homes and in restaurants and one-third is used in processed or manufactured products. If it is assumed that an average family consists of 3.77 persons, and social scientists usually make this assumption, that means that the average American family gets away with 7.54 pounds of sugar a week. That's just too dog-gone much sugar anyway.

Take the United Kingdom for purposes of comparison. In normal times its average family of the same size consumed a little less than 5 pounds of sugar weekly as compared with our 7.54. The current sugar ration in Britain for a family of this size is 12 ounces weekly.

The current plan will ration American families at a rate of about 2 pounds of sugar weekly. Again that would be only the sugar we purchased or used as such. But it is as much sugar as a British family gets in all forms weekly, for jam, marmalade, syrup, and molasses also are rationed in Britain.

In general British sugar consumption is now about 40% of prewar levels. It is also but 70-82% of the German ration, depending upon the type of Britisher used for the comparison. Sugar is an energy food and Britishers who do heavy work get increased rations. On the other hand British medical authorities were protesting a decade ago that increased sugar consumption was leading to bad health.

Sugar is a pure chemical substance, unlike most other foods. It is highly processed and devoid of vitamins, protein, and fat. It is pure carbohydrate and tends too much to displace fat in children's diets. Our physiological tolerance for sugar is not unlimited. What is more there is good evidence that high sugar consumption leads to increased incidence of and death from catarrhal troubles, bronchitis, and pneumonia. Possibly also excess sugar adversely affects the pancreas.

Perhaps it is fortunate then that we waste a great deal of the sugar we used. A hurried survey made in New York City restaurants indicated it was a rare empty coffee or tea cup which did not still contain at least half a teaspoonful of undissolved sugar. The Secretary of Agriculture was right not long ago when he said that we were not only the most wasteful people on earth but that we had for years constantly invented new and better wastes.

We produce in the continental United States only 29% of the sugar we use. Of this 23% comes from beets and 6 from sugar cane. The continental U. S. A. sugar industry is largely an artificial one that has always required subsidy. It is uneconomical for us to produce our own sugar and any large increase in domestic continental production is unwarranted in the long run.

Under the Jones-Costigan Act of 1934, revised into the Sugar Act of 1937, the remainder of our sugar normally comes from the following sources in the percentages indicated: Hawaii 14, Puerto Rico 12, Cuba 29, the Philippines 15, the Virgin Islands 0.1, and other foreign countries less than 1%.

Early in 1942 the facts about sugar were these: If to refiners' stocks and manufacturers' reserves on hand in December, 1941, was added our anticipated 1942 continental U. S. A. sugar production we should have a base supply of 3,150,000 tons. During the past four years our average domestic production of beet sugar has been about 1,758,000 with an all-time high of 1,894,000 tons in 1940. For the

same period our average mainland production of cane sugar has been 463,000 tons a year, with an all-time high of 583,000 in 1938.

On the assumption that we would require a little over seven million tons of sugar in 1942, and that we had a base supply of 3,150,000 tons, we should have to procure nearly four million tons somewhere else. We could probably count on a million tons from Puerto Rico, four million from Cuba, and a few thousand from the Virgin Islands. That looked like over five million tons. Where then was the shortage?

In accord with original plans about a million tons of Cuban were to be used to produce industrial alcohol which we require in greatly increased amounts during war. Then the Cubans themselves consume nearly a quarter of a million tons locally, in 1941 Great Britain took over half a million tons of Cuban sugar, and Cuba sells some 75,000 tons normally to other markets.

So instead of getting over five million tons from the Caribbean it looked as if we should do well to get a little over three million. That would mean a 900,000-ton shortage on the assumption that we must consume as usual in 1942. Both Hawaii and the Philippines normally send us that much sugar but in 1942 we shall be lucky to get half the usual shipments from the former while we can count on none at all from the latter.

There are also other uncertain factors, such as the possible demands of Russia and others of the United Nations and the shipping situation. During 1941 our sugar was used so fast that more than a million tons disappeared from our Ever Normal Granary reserves. Some of this sugar was hoarded by concerns making soft drinks and other products requiring sweetening, some by panic-buying consumers.

This buying produced actual temporary shortages. No scheme the Government can set up short of strict rationing will ensure the basic needs of all because unexpected factors are always upsetting it. Even the actual rations must be changed if other urgent needs draw away the supplies.

The hope of some that sugar shortages can be avoided by using honey is false. We normally consume only about 1½ pounds of honey each per year as compared with something like 104 pounds of sugar. If the honey industry were greatly expanded it would rest on an artificial demand which would largely collapse when peace came.

The main problem here is to prevent unwise expansion and to see to it that such honey as is available goes to consumer tables, not to industry.

It is true that higher prices for sugar, increased Government payments, and removal of limitations on acreages of sugar beets and sugar cane may be expected to increase continental U. S. A. production to a considerable extent. At this writing expectations to plant sugar beets indicate a crop nearly a fourth larger than in 1942, but the labor situation is bad. In any case both beets and cane have been planted in amounts to tax our processing plants to the limit.

As fixed by the Office of Price Administration wholesale sugar prices for the refined grade are about a dollar a hundredweight higher than the average in 1938-40. The base rate of conditional payments to domestic sugar producers had been increased one-third under legislation recently approved by the President and which extended the 1937 Sugar Act for three more years.

Some time ago we purchased the entire Cuban sugar crop for the year. It was then expected to be from 4,000,000 to 4,300,000 tons. It has turned out to be nearer 4,600,000 tons. Meanwhile Mr. Batt has said that as a result of using grain for making industrial alcohol about 550,000 more tons of sugar than were anticipated will be available for other use this year. These are favorable factors.

On the other hand the United Nations will require from us at least a million tons of sugar. The chief Ethiopian in the woodpile, however, is lack of shipping. There is enough sugar, but we do not have bottoms to spare to get it to this country. Sugar is piling up right now in Puerto Rico where storage facilities are meager, though Cuba has storage space to take care of her excessive stocks.

Rationing has been invoked not because of scarcity of sugar but in part because of panic buying and largely because we lack shipping facilities to bring luxury supplies of sugar into this country. We must ration to effect equitable distribution. We shall in any case have more than enough sugar for our health's sake this year. For we normally waste and eat too much of it. We shall no doubt be far better off physically if our ration finally drops to the German or even to the British level, assuming we have an otherwise well-balanced diet sufficiently rich in energy foods.

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30. *Comparative Toxicity Studies on Glucose, Fructose, and Sucrose.* KENNETH H. HARPER and ALASTAIR N. WORDEN, Huntingdon Research Center, Huntingdon, England

There were significant differences between the effects of fructose, sucrose, and glucose when incorporated as the sole source of carbohydrate at levels of approximately 60% in the diet of rats initially 11 months of age over a 26-week period.

Food consumption was in all instances below that of a control group receiving diet 41B. Body weight gains did not parallel food consumption, but only in the case of glucose were they significantly below those of control rats. External dimensions were unaffected, but fructose markedly increased heart, liver, and kidney weight, liver fat deposition, and plasma cholesterol level and reduced total carcase water, liver water, and liver protein. Sucrose showed a similar but less marked effect, due presumably to its fructose component, but glucose had on the whole effects of a lower order. In terms of conventional chronic toxicity studies, such effects would require careful explanation.

These results are discussed in relation to the "toxic" effects of carbohydrate suggested by other workers, and to differences between the short-term effects of the three sugars.

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Are Sugars Carcinogens? An Experimental Study

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SUMMARY

Injections (s.c.) of 25% solutions of arabinose, dextrose, galactose, lactose, levulose, maltose, sorbose, and sucrose were given twice a week into the region of the nape of the neck to 480 rats and 480 mice (in groups of 60 animals) for periods up to 2 years. Only 2 rats given sorbose solutions developed sarcomas, after 21 months, at the site of injection. No tumors were found at this site in additional series of rats and mice injected similarly with water or traumatized by needle puncture.

These observations do not support the claim that hypertonic sugar solutions parenterally introduced into rats and mice exert a carcinogenic effect. It is suggested that previously reported carcinogenic effects following repeated s.c. injection of sugar solutions may be due to the presence of carcinogenic chemical impurities in the sugars used, possibly eluted from charcoal employed for the decoloration of sugar solutions.

In 1935, Nishiyama (11) first reported the carcinogenic action of glucose solutions on the subcutaneous tissue of rats. Other investigators—especially in Japan and Italy—confirmed these observations and extended them to other mono-, di-, and polysaccharides (levulose, maltose, fructose, galactose, lactose, sucrose, and glycogen (1–3, 7, 9, 11–16, 18–20, 22). While rats were used in the majority of the initial experiments, mice appeared subsequently to be equally responsive (2, 16, 18–20, 22). In fact, Nagayo (10) succeeded in transplanting a sugar sarcoma, obtained in a mouse, to rats. Only 2 investigators (8, 26) failed to corroborate the general claim that repeated s.c. injections of concentrated solutions of glucose into rats induced the formation of sarcomas at the site of their administration.

Because of the distinct scientific and practical importance of the apparent carcinogenic action of sugars and the various fundamental issues involved, it was considered necessary to test again a series of sugars under experimental conditions duplicating as closely as practicable those employed by previous investigators, while using special precautions in their administration so as to preclude the action of any carcinogenic impurities accidentally introduced into the experimental agents and procedures.

MATERIALS AND METHODS

The following sugars were tested: *l*-arabinose, anhydrous *d*-dextrose, *d*-galactose, lactose, *d*-levulose, *l*-sorbose (obtained from Mann Research Laboratories, Inc., New York), *d*-maltose, and *d*-sucrose (obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio). Only those designated as chemically or chromatographically pure were used. Different chemical structures and compositions were represented, i.e., pentoses, hexoses, aldoses, ketoses, monosaccharides, and disaccharides.

Solutions were prepared by dissolving 25 gm of a sugar in 100 ml of distilled water in a volumetric flask, and autoclaving in an Erlenmeyer flask (15 min at 15 lb of steam pressure and 250°F). The solutions of maltose assumed a slightly brownish tint upon completion of sterilization. There occurred a small increase in viscosity of most sugar solutions after autoclaving, when measured by the Hellige viscosimeter. The increases ranged from 0.1 for arabinose, sorbose, and levulose to 0.2 for dextrose, 0.4 for lactose, and 0.8 for sucrose, suggesting that some polymerization may have occurred in lactose and sucrose solutions. No viscosity change from the average value of 2.0 occurred in the solutions of galactose and maltose. New syringes were used at all times. The needles were also new and were used only once. Injections were given s.c. 3 times a week for up to 2 years.

Rats were given 2 ml of the sugar solution into the subcutaneous tissue of the nape of the neck at each injection; mice received 0.5 ml in the same area. Control groups of rats and mice were given identical amounts of distilled water. Additional control groups of rats and mice were subjected to needle trauma only, while an untreated group of mice served as strain controls. Bethesda black rats and C57BL mice were used. The rats were 3 months old and the mice were 2 months old at the start of the experiment. There were 60 rats or mice (30 male, 30 female) in each test group, including the treated control groups, while 125 mice served as strain controls.

The maximal observation period was 2 years, at the end of which all surviving animals were killed. Autopsies were performed on all animals and histologic examinations were made of those tissues which showed any abnormalities. A total of 600 rats and 720 mice was used.

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RESULTS

The rats tolerated the injections of the various sugar solutions without any untoward effects. The mice, on the other hand, not infrequently developed symptoms of acute shock, especially during the early experimental period, soon after the introduction of the sugar solutions; some died with the anatomic reactions characterizing such a response to the hypertonic agent, i.e., hemorrhagic transudates in pleural and peritoneal cavities, severe hyperemia, and hemorrhages in the lungs. In addition to these acute general reactions, mice did not usually tolerate well the repeated local deposition of hypertonic sugar solutions in the subcutaneous tissue of the nape of the neck. In most of the mice, white, cheesy, necrotic masses appeared, which ulcerated unless the injection of the sugar solutions was stopped for some time.

The principal pertinent observations made in rats and mice composing test and control series are summarized in Tables 1 and 2.

Apart from some minor brownish discolorations of the connective tissue in the nape of the neck, rats did not exhibit any abnormal local reactions.

The great majority of the benign and malignant tumors found in the test and control rats and mice were at sites remote from the nape of the neck where the sugar solutions were administered. Only 2 fibrosarcomas, found in 2 rats which received sorbose solution, were near the injection site.

TABLE 1

DEATH DISTRIBUTION AND TUMOR YIELD IN RATS RECEIVING AQUEOUS SOLUTIONS OF DIFFERENT SUGARS S.C.

There were 60 rats, 30 of each sex, in each sugar (and control) group.

Sugars and tumors	Survival time (months)							No. of tumors at:	
	0–6	7–9	10–12	13–15	16–18	19–21	22–24	Injection site	Other sites
Arabinose	1		4*	1	12	10	32		
Tumors			a, a		a, c	e, e	f, g, i, i, e		11
Dextrose	2	1	3	5	6	17	20		
Tumors			a, a, a	a	r, b	q	e, e		9
Galactose	5	3	2	5	8	9	28		
Tumors				a	i, i	d, d, s	d, h, i, e, k		11
Lactose	2	1	4	6	11	12	24		
Tumors				a	a, a, b, e, k	e, i	d, i, l, l, m		13
Levulose	8	3	2	6	13	10	18		
Tumors		a	a, a	a, d	a, c, d, k	c, d, i, k	d, e, h, i, l		18
Maltose	7	1	3	4	13	14	18		
Tumors				a	a, e, i	a, a, e, k, k, i	a, c, h, i		14
Sorbose	4	3	4	4	10	11	24		
Tumors			a	a, d	a, d	e, i, o	a, e, h, n, o, q, r	2	13
Sucrose	1	1	3	15	10	10	20		
Tumors			a, a	a, b, g		a, a, e, h	a, e, i		12
Needle-control	1	3	7	11	7	8	23		
Tumors				f, k	c		e, i		5
Water-control	1		7	3	9	7	33		
Tumors			a, a, a, f			a, b, b, q	a, e, e, e, e, h, q, t		16

* Key to tumor symbols: a, large round cell sarcoma of ileocecal lymph nodes; b, mesothelioma of the pleura; c, mesothelioma of the peritoneum; d, reticulum cell sarcoma of the liver; e, carcinoma of the uterus; f, papilloma of the urinary bladder; g, lymphangiosarcoma of the subcutis; h, cholangioma; i, adenofibroma of the breast; k, carcinoma of the maxillary sinus or gingiva; l, adenocarcinoma of the breast; m, adenocarcinoma of the pancreas; n, cholangiocarcinoma; o, fibrosarcoma of the subcutis of the back; q, leukemia; r, adenocarcinoma of the colon; s, teratoma of the testis; t, gelatinous adenocarcinoma of the stomach.

TABLE 2

DEATH DISTRIBUTION AND TUMOR YIELD IN MICE RECEIVING AQUEOUS SOLUTIONS OF DIFFERENT SUGARS S.C.

There were 60 mice, 30 of each sex, in each sugar (and control) group, except for 125 in the strain-control group.

Sugars and tumors	Survival time (months)							No. of tumors
	0-6	7-9	10-12	13-15	16-18	19-21	22-24	
Arabinose	40	10	6	4				
Dextrose	31	21	4[a]	4				
Tumor			a					1
Galactose	40	14	6					
Lactose	16	18	12	10	3	1		
Levulose	18	18	13	9	2			
Maltose	13	18	23	6				
Tumor	b							1
Sorbose	11	7	12	23	6	1		
Sucrose	26	19	5	3	5		2	
Needle-control	26	10	8	7	4		5	
Water-control	21	8	11	4	5	4	7	
Strain-control	51	22	12	16	7	7	10	
Tumor					c, d		a, b	4

[a] Key to tumor symbols: a, adenoma of lung; b, leukemia and lymphoma; c, adenoma of liver; d, spindle-cell sarcoma of groin.

Chronic hemorrhagic and purulent cystitis, associated in about one third of the cases with bladder stones, was observed in some rats and mice of both the test and control groups (18 rats, 7 mice). There was no histologic evidence of an injurious effect of the injected sugars upon any internal organ, especially the liver and kidneys, although nephrotic changes of varying degrees were noted in many animals, including controls. Rather extensive amyloidosis of the liver, spleen, and kidneys often occurred in the mice.

DISCUSSION

Almost all of the benign and malignant neoplasms seen in mice and rats of the test series occurred at sites and in numbers similar to those at which such tumors were observed in the controls, and have been seen in many previous experiments (4–6). It is, therefore, unlikely that the development of most of them was related causally to the administration of the sugar solutions. They may properly be regarded as "environmental background tumors" elicited by undetermined endogenous as well as exogenous factors. It is uncertain, however, whether this explanation safely accounts for the presence of an adenocarcinoma of the pancreas in a lactose rat, of adenocarcinomas of the colon in a dextrose and a sorbose rat, and of a cholangiocarcinoma in a sorbose rat. This uncertainty is based, in part, on the fact that such cancers have never before been seen in normal and carcinogenically tested animals of this strain of rat, of which many thousands have been employed in various types of carcinogenic experiments; in part, it is related to the occurrence of hemorrhagic and purulent cystitis in a significant number of rats and mice of both test and control series, and of papillomas of this viscus in 1 rat each of the arabinose, needle-control, and water-control series. These tumors may, therefore, be due to the recent, inadvertent introduction of an unknown carcinogenic factor into the "normal" environment of these animals. Such an agent may be contained, for instance, in the commercial feed. Since urinary calculi and cystitis were noted in test and control animals, it cannot justly be concluded that the sugar administered played an essential role in eliciting the abnormal reactions in the bladder wall. For the same reason, the administration of sugars was not responsible for the production of the bladder stones, although Vermeulen *et al.* (23) reported that rats developed urinary concretions when kept on a milk or lactose diet.

The essentially negative outcome of the present experiments on the alleged carcinogenic action of subcutaneously introduced sugar solutions in rats and mice supports the similar observations previously reported by Meier (8), who failed to induce sarcoma formation in 2 series of about 50 rats each given s.c. solutions of 50% and 5% glucose, respectively, for up to 539 days. The main local changes found by Meier (8) in the rats receiving the hypertonic glucose solutions were of a degenerative, necrotizing, and granulomatous character and thus resembled those seen in the mice similarly treated in the present experiment.

The results obtained in this investigation differ sharply from those previously reported by Japanese and Italian workers who obtained, in mice and rats receiving hypertonic sugar solutions s.c., fibrosarcomas and spindle-cell sarcomas at the site of injection in a significant percentage of animals surviving for more than 250 days: 10 rats with sarcomas among 47 survivors (14); 3 rats with sarcomas among 4 survivors (15); 4 mice with sarcomas among 8 survivors receiving galactose; 5 sarcomas among 18 survivors receiving glucose (18–20); 5 mice with sarcomas among 20 mice receiving lactose; and 4 mice with sarcomas among 20 mice receiving glucose and fructose (2).

The only point of apparent agreement between the findings of the Japanese and Italian investigators and those of the present experiment, is the occurrence of 2 sarcomas in rats at the site of sorbose injections. Because of the absence of any similar neoplastic reactions in the 7 other groups of rats and in all of the mice receiving the 8 sugars, this evidence tends to support the view that sugars are not carcinogenic. The exception to the rule seems to suggest that the carcinogenic reactions attributed to sugars by previous investigators may be due to a carcinogenic impurity present in the sugars used. Such carcinogenic impurities may be introduced into sugars, for instance, when concentrated sugar solutions are filtered for decolorizing purposes through improperly prepared charcoal, containing polycyclic hydrocarbons. Chemicals of the dibenzanthracene type are eluted from charcoal by concentrated sugar solutions, according to Druckrey (1963, personal communication); traces may be introduced in this manner and may remain in apparently chemically pure sugars.

It is unlikely that the different responses obtained by Japanese workers and by a Swiss investigator, as well as in this experiment, may be attributable to an extraordinary carcinogenic susceptibility of Japanese rats to sugar solutions introduced s.c., a property lacking in Swiss and American rats and mice (8).

Since Japanese investigators have also reported that hypertonic saline solutions given s.c. to mice and rats induce the development of sarcomas (21), whereas physiologic saline solution was said to lack such properties when similarly used (2, 14, 19), the old concept of carcinogenesis on the basis of chronic irritation and regeneration has been advanced as an explanation of sugar and salt carcinogenesis (8, 21). It is remarkable that Tokoro (19) failed, however, to induce sarcoma formation in mice receiving solutions of 5% and 25% sodium chloride, and that he was successful in this respect only when a 15% saline solution was used. Moreover, it is noteworthy that, according to several investigators, sarcomas could be produced in the subcutaneous tissue of mice by repeated s.c. injections of hot water, cold water, and water at room temperature (24, 25). Before such extraordinary claims (10, 17) can be accepted as valid, these experiments should be repeated with water and saline of unquestionable chemical purity, and should be performed under conditions which exclude secondary contamination of the injected material by any extraneous carcinogenic chemical. These precautions should be observed since the present experiments have clearly shown that distilled water given in this manner does not induce in rats and mice the development of subcutaneous sarcomas and that chronic mechanical trauma resulting from the repeated s.c. introduction of a needle was equally ineffective in this respect. The evidence reported here and elsewhere (5) militates against the acceptance of the concept that nonspecific chronic irritation represents a carcinogenic mechanism.

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PROBLEMS OF SUGAR CONSUMPTION IN A MODERN INDUSTRIAL SOCIETY, FROM A NUTRITIONAL PHYSIOLOGICAL VIEWPOINT, AS REPRESENTED BY THE SITUATION IN TWO CENTRAL EUROPEAN COUNTRIES (CZECHOSLOVAKIA AND THE GERMAN FEDERAL REPUBLIC)

by

J. Hruby and W. Wirths

With 12 tables

From the Max Planck Institute for Nutritional Physiology
(Director: Prof. Dr. B. Hess) Dortmund

(Entered on June 4, 1970)

1. Introduction

Sugar manufacture and worldwide sugar trade will be mentioned only marginally in this study. This series of problems is treated in the detailed investigations of, among others, GEERDES (11), BAXA and BRUHN (5), as well as the FAO (51).

In this investigation, the relationships between public nutrition and sugar consumption occupy the foreground. Sugar and food products rich in sugar are very attractive to the majority of consumers, because of their organoleptic and sensory properties, while, at the same time, they are associated with several disadvantages from the standpoint of health.

For some branches of the food product industry, sugar is a product with several advantages, as ALLEN and BROOK (2) showed. On the other hand, from the side of nutritional science, MASEK (23), YUDKIN (36), BALANSKY and MILLEV (4), and ZIEGLER (39) proved that for the majority of men in the modern industrial society, with its altered living conditions, sugar consumption can cause health dangers (tooth decay, adiposis, diabetes, arteriosclerosis). Concerning the connection of the last to sugar consumption, YUDKIN has described a hypothesis that has in the meantime been supported, while ZIEGLER draws our attention to its connection with acceleration.

The amount of consumption of different nationalities is very varied. Table 1 offers a survey of European countries. The survey needs a completion in relation to sugar consumption in the USSR. This could not be considered in the table, because data on animal protein consumption is not available. In

1965, sugar consumption in the USSR reached an average per capita of 34.2 kg (24). The amount of 46.5 kg per capita published in comparison by KRUSEN (22), seems to us to be too high.

In order to judge the nutritional situation, we use the supplied amount of protein of animal origin as a value standard. It is quoted many times for the evaluation of the nutritional standard of a population. Of course, sugar consumption forms only one part of the total food product consumption. The data in Table 1 show that sugar consumption increases with higher nutritional levels, and a rising chronological tendency -- in relation to 1950 -- can be seen. It is noteworthy that there is a narrow correlation between the consumption of animal protein -- a nutritive material -- and that of sugar -- a food product.

Table 1. Consumption of protein of animal origin and sugar

Country	anim. protein per capita/day 1960-62 g	sugar per capita/year 1948/9-50/1 kg	sugar per capita/year 1960/1-62/3 kg
Denmark	58.0	36.1	49.4
France	55.8	23.1	30.0
Ireland	54.9	35.3	47.7[1]
Finland	54.6	31.2	40.0
Sweden	54.3	44.4	41.3
Great Britain	53.4	38.8	49.1
Switzerland	51.3	37.6	42.8
German Federal Rep.	49.2	23.6	30.3
Norway	48.8	24.5	40.2
Austria	47.5	23.2	36.4
Belgium-Luxembourg	45.8	28.5	31.7
Netherlands	45.8	35.8	42.9
Czechoslovakia*	42.4	34.0	38.4
Poland	40.4	--	31.2[2]
German Dem. Rep.**	37.1[3]	--	30.0[3]
Hungary	37.0	--	27.6[1]
Greece	31.3	9.4	13.5[1]
Italy	29.8	11.7	22.9
Rumania	27.7	--	13.1[2]
Portugal	27.2	12.1	19.0[1]
Yugoslavia	25.5	--	16.3[1]
Spain	23.4	--	18.7

1 Period 1960-62
2 1961-63
3 1959-62

Sources: State of food and agriculture 1966 -- FAO Rome
*Statistical yearbooks of the CSSR
**J. Hruby, H.E. Schmidt: Ernahrungsforschung 11, 1966,4,645; 12, 1967,4,649.

The situation in the Federal Republic of Germany and in France cannot be brought into accord. In Sweden, a sinking tendency in sugar consumption is found during the post-war period. Czechoslovakia, on the other hand, belongs to the countries with the greatest increase in consumption.

The data used was taken from the statistics of the FAO "State of Food and Agriculture" (47), not from the "Production Yearbook" of the FAO (44), where higher values were indicated. The same is true of the statistics of the EWG (40), which were likewise not considered. The data from the FAO publication "State of Food and Agriculture" have the advantage for this study of being confirmed as accurate for the human consumption amounts by the "Food Balance Sheets" (43). Beyond that, smaller differences are caused methodically. On the one hand, there are data without syrup which, however, can be omitted for the purpose pursued in Table 1. This is also true for the data from Czechoslovakia that include syrup. The countries cited in Table 1 are ordered according to degree of intake of animal protein. The time period of 1960-61 to 1962-63 was chosen because that is the last several-year period examined.

The correlation between the conumption of animal protein and sugar consumption, with the coefficients 0.87 (for all countries) and 0.94 (without the Federal Republic of Germany and France) is highly significant statistically ($p < 0.001$). The correlation was computed for the same time period.

The increasing sugar consumption is in accord with the production and processing of sugar. Such a developmemt is optimal for this economic branch. However, what is or can be optimal for a branch of economics or for an entire national economy is not necessarily optimal for the public involved. Thus, in modern society, we have a rivalry between the economic optimum and the physiologically determined requirements of human living conditions. For this reason, there are two opposing views on this matter. The production circles would like to maintain an increase in sugar consumption, or at least maintain the present level. This can be reasonable, to a certain extent, for countries with a very low sugar consumption, as BERKOVIC (6) demonstrated for Yugoslavia.

The majority of nutrionists, on the other hand, would like to bring sugar consumption down as much as possible. It should be pointed out, in respect to this, that nutrtion-physiologically it is hardly at all necessary to use sugar. These views in relation to nutritive material intake are strengthened by earlier studies concerning optimal nutrient consumption (16) (31). In them, sugar is always revealed as an obstacle to the mathematical procedure in diets or nutrient compositions that are of full value from a nutrition physiological point of view.

We consider it indicated that the question of sugar consumption cannot be decided from these extreme positions, but must also be seen in a nutritional scientific perspective, and we have consequently striven for a symbiosis between biological-physiological and economic viewpoints. The result of these efforts are data that indicate a lowering of the present sugar consumption level. Thus, several authors recommended the reduction of sugar consumption: WIRTHS (32) for the countries of the EWG suggested about 22% reduction, WRETLIND(34) about 25% for Sweden, HRUBY (18) about 20% for Czechoslovakia.

We put aside the often used quote "high sugar consumption is an inseparable sign of the high living standard" and find the sense of this study in basing the variability of sugar consumption between individual industrial nations on the example of two countries. From the perspective of Czechoslovakia, the present high level of the average sugar consumption in the German Federal Republic is seen as surprisingly low. There are several reasons for a comparative observation of the German Federal Republic and Czechoslovakia. Besides the geographical situation, there are the similar conditions in degree of industrialization and national economy, as well as regarding population density. Furthermore, we can point out the similarity in the proportion of industrially processed food products in the average national nourishment (table 2). The present food product consumption reveals no substantial differences in the case of the most important nutritional that go beyond the borders of the EWG countries (32), but considerable ones in the sugar consumption of Czechoslovakia and West Germany. We consider this comparison purposeful, even though the living standard of the population of West Germany is higher than that of Czechoslovakia, as a Czechoslovakian study showed. (46)

As concerns the concrete results of the comparison for the two countries, along with the theoretical contribution, we see them for West Germany in the evaluation of the sugar consumption. For Czechoslovakia, the study is supposed to confirm the reality of the suggested reduction in sugar consumption.

Table 2. Individually processed food products in consumption

	Czechoslovakia	GFR
Calories	74	77
Animal protein	58	55*
Vegetable protein	78	--
Fat	53	77
Carbohydrates	78	85
Calcium	52	50
Iron	52	47
Animal vitamin A	58	37
Vegetable vitamin A	4	--
Vitamin B_1	54	39
Vitamin B_2	49	32
Vitamin C	4	6

* Total protein 64%

Sources:

WIRTHS, W. Reports on national economy 40, 1962, 4,845

SMRHA, O., Pycha, F. Possibilities of nutritive value analysis of products of the food product industry -- Research report, Prague, 1964.

2. Sugar evaluated nutrition-physiologically

In a nutritional scientific investigation, nutrition-physiologicaly conditions play a role equal to economic assumptions. The nutrition-physiological significance of sugar should be given extensive attention.

The nutritive value of sugar is the starting point. Sugar, as a pure carbohydrate -- its weight is about equal to the amount of carbohydrates -- contains in a refined state practically no other nutrients. The extremely small amounts of calcium, iron and other metabolites can be discounted. Thus, we can designate sugar as a one-sided energy carrier. Its contribution to energy is not always uniformly indicated. WIRTHS (33) ascribes 394 kcal to every 100 g, while in the Czechoslovakian tables (21), 385 kcal are ascribed to every 100 g sugar. This difference is caused by the different degree of the physiological caloric value that is considered in the tables. It does not disturb calculations and the results to be drawn from them, for the difference is onaly about 2%.

The extremely negative evaluation of sugar comes from its designation as a one-sided energy carrier. It is one of the carriers of "empty calories." Modern manners of living, especially mechanized and automatized professional labor, has led to a restriction of physical activity. For this reason, the need for energy has been reduced considerably. Under these circumstances, it is difficult to provide the full requirements of all important nutrients in a limited food intake. WRETLIND (34) concerned himself with this question in a study of Sweden. In such a situation, which is becoming more and more serious in all industrial nations, it is especially important, from the standpoint of full-value nutrition, to provide valuable food products. The significance of the carriers of "empty calories" correspondingly sinks. They are, in fact, even serious obstacles to efforts to attain a full-value diet. In addition, a higher carbohydrate consumption raises the need for thiamine.

The increased presence of sugar in food increases, moreover, the risks of damage to health. Best known and most widespread, as proved by many authors, including SOGNAES (27), NOVOTNY (25), CREMER (10), BUTTNER (7), is tooth decay.

Besides this, increased sugar consumption leads to the spread of another civilization disease, obesity, as demonstrated in Czechoslovakia by OSANCOVA (26). This knowledge follows logically from the discussion on "empty calories".

According to a hypothesis of YUDKIN (35) (36)(37), high sugar consumption is connected with arteriosclerosis and diabetes. KEYS (20) published a similar hypothesis concerning fat consumption and arteriosclerosis, which later lost much credibility. It must be admitted that further research supports the hypothesis of YUDKIN. Among this research are the investigations of COHEN (8)(9) on collectives in Yemen, and studies of HIPSLEY (12) and ANTAR (3). YUDKIN mentions: a) Sugar is not the only factor; b) mortality due to arteriosclerosis and diabetes is often incorrectly diagnosed as due to something else; c) the action of the factors of these diseases is very slow and lasts 20 to 40 years. As a means of illustration, we present in table 3 a comparison of death figures for circulatory ailments and

diseases of the coronary arteries for both of the countries. These figures would support that view (despite all reservations about the comparability of the data). The data from Czechoslovakia, the nation with the higher sugar consumption, are significantly higher. The selection of years comes from the comparability with the statistical data. Finally, we mention the opinion of ZIEGLER (39), who confirmed the relationship between acceleration and sugar consumption and determined the unfavorable effect of sugar. He considers the development important, for acceleration is becoming a serious sociological-pedagogical problem.

Table 3. Comparison of mortality due to diseases of the circulatory system[1] and the coronary arteries[2] in Czechoslovakia and the GFR (number of cases per 100,000 inhab.)

Jahre	1)		2)	
	CSSR	BRD	CSSR	BRD
1952	306,4	230,9	—	49,6
1960	306,5	283,3	220,0	101,2
1964	324,1	287,8	241,1	113,2
1965	330,4	307,0	249,4	122,5
1966	329,8	310,4	251,5	123,9

Sources: Statistical yearbooks of the German Federal Republic and Czechoslovakia.

These statements are not intended to give the impression that sugar is a harmful food. We cannot agree with the suggestion of KAPP (19), that sugar be declared a luxury food, while WANDELT (30) should not like to recognize the biological relations. The complicated biological relations cannot be simplified. We can in no way expect that an alteration in consumption of 2 kg per capita/year is coupled with a uniform change in the occurrence of tooth decay. There is no relation from which such proof can be derived, as WANDELT claims. A recommendation on the desirable level of sugar consumption made from a nutrition-physiological perspective must be indisputable.

Even though the connections between sugar consumption and damages to health are complicated and sometimes contradictory, it is irrefutable that sugar, as a carrier of empty calories, blocks the available space for food products that are of more value biologically, containing more essential nutrients. In the case of the present qualitative deficiencies in nourishment and the uniform tendencies in relation to a reduction in the energy requirement, this is in any case one of the most significant fundamental nutrition-physiological questions. For this reason alone, the nutritional scientific disciplines are obliged to devote their attention to the problems arising from sugar consumption.

3. Sugar consumption

The basis of our analysis is the consumption situation of the two countries. The consumption situation can be judged chiefly with the help of two processes: on the one hand, on the basis of various economic data, and on the other, on the basis of statements from selected groups of the population, households or persons. In the first case we deal with the amount of food products that the total population could buy over a given period of time. However, it cannot be ascertained whether the population also actually consumed these amounts. This method is widely used, even in the "food balance sheets" of the FAO (43).

In the other case, we obtain data with the help of economic calculations or other statements in a restricted circle of individuals or families. These data can be used only applying the priciple of probability, a theory of mathematical statistics. They are selected investigatons with all the possibilities of error that such a method involves.

The two methods, whose results are designated on the one hand as "indirectly determined food product consumption" and on the other as "direct or individually determined food product consumption", do yield different results, but are both absolutely necessary for consumption analyses. Both methods are applied in both the German Federal Republic and Czechoslovakia. In both countries, total food product amounts on the level of individual trade are derived according to the direct method, and as results of consumption balances.

a) Indirectly determined sugar consumption

Data for the last 50 years can be pursued with relative precision. Since 1909-13, the consumption curves reveal several irregularities. The trends can be determined mathematically. In the time between the two world wars, there is a change from a rapid increase in consumption in the first 3-4 years of the post-war period, to a stabilization in Czechoslovakia, while in the German Federal Republic there is a temporary decline and final increase . The German sugar consumption is lower, about 3 kg per capita per year on the average in this period. However, the level of sugar consumption remains at a point that is considered optimal from a nutritional standpoint.

There are data for 3 war years (1941-43) available for Czechoslovakia as well, which show a considerable rise in sugar consumption. This rise signifies about 20% of the consumption amounts of the period between the two world wars.

The development after the second world war is characterized again by a rapid short-term increase, similar in both countries. The difference in the year that the steeply rising curve is interrupted (1951) is only 1.5 kg between the two countries. After that, there was an identical two-year drop, which was especially remarkable in West Germany. The difference expanded to 5 kg. In the following 15 years, sugar consumption in both countries rose, with the difference in consumption amounts becoming ever more considerable. The peak of the curve in Czechoslovakia was reached in the years 1961-63; after that, there

was a slight reduction. The observations end with the data of 1967.

The period of 1951-54, which can be considered decisive for the present difference, shows on the one hand a reduction in sugar beet cultivation in both countries. In West Germany, there was simultaneously a restriction of imports. The drop in sugar consumption resulted in one year (1952) in a reduction in carbohydrate intake, and in the GFR, also a reduction in energy content. In Czechoslovakia, a significant change can also be observed -- the reduction in consumption of animal protein of about 5% (1953-54). However, in 1954, sugar consumption rose by about 4.5 kg per capita per year. The cause of this can be seen in currency reform and the liquidation of apportionment economics. These important measures were realized in the middle of 1953. That meant a clear price increase, especially in the case of products of animal origin, and less so in the case of vegetable products.

As concerns the nutritional situation in Czechoslovakia, it must be mentioned that the position of sugar in the development of food product consumption was very significant. The steep rise in sugar consumption was the highest in the entire food product consumption.

b) Structure of the indirectly determined sugar consumption

To evaluate sugar consumption, it is also important to determined the structural relation between household consumption and industrial consumption. These are designated respectively as "direct" and "indirect" sugar consumption. This relation is not correct. In both cases, sugar consumption takes place indirectly. Both in the household and in community feeding, sugar is used as a component of foods and beverages. Much more different is the matter of whether sugar is consumed in the form of household sugar or as a component of an industrially prepared product.

The structural composition of sugar consumption is considerably different in the two countries. In the basic data of the pre-war period, there is a difference of 7% of the total sugar consumption for processing in Germany. This difference expands a little shortly after the war, more so in the following years, and in 1965 by as much as 15.5%. In the last year of the period of the report, it was 13.5%. The large difference in sugar consumption of the two countries -- as we can confirm -- is caused by the private and institutional households, while the use in industrial processing is nearly the same. It can be supposed that more cakes and other pastries, fruit dumplings or foods made of flour and sugar are prepared in Czechoslovakia than in Germany.

In the German Federal Republic, sugar consumption in households declined, and sugar consumption in other production areas rose steeply, while in Czechoslovakia there is an increase, though different, in both cases.

As concerns the observation that community provision is included in household consumption, it should be noted that this amount, per meal portion, is not of particular significance. In Czechoslovakia, only about an average of 11 g (13) are used in noon meals at factory cafeterias, about 10 g sugar in school

cafeterias (15); in typical large cafeterias in the German Federal Republic even less is used.

In the analysis of the situation in Czechoslovakia, regional differences in sugar consumption among the 11 governmental areas can be observed (38). In comparison to the total national average, three of the districts have a higher consumption, three others a lower consumption. In the first case are included areas with a higher percentage of farming population (Southern Bohemians, Southern Moravians, Eastern Bohemians). The results of economic calculations also conform with this. Two poorer Slovakian districts, as well as the inhabitants of the capital, Prague, have a smaller consumption.

c) Directly (individually) determined sugar consumption and consumption of products rich in sugar

The evaluatable data are not entirely comparable. For the German Federal Republic we have data on households with 4 employed persons (50), and for Czechoslovakia data on laborer and white-collar worker households (48), and, where possible, results from economic calculations. Subdivision into various positions is not completely coinciding (Table 4).

A lower consumption of sugar (30%) and marmalade (60%) is shown for the households in the GFR, but not of chocolate and sweets. The GFR data are on the average 40% higher. The pastry consumption is practically the same in both countries. For the GFR it is noteworthy that in the third quarter of the year (1962 and 1963)there is a considerably higher consumption, which can be attributed to the home preparation of fruit. In Czechoslovakia, there is a difference between the sale to households and the consumption in households according to economic calculations. This difference can be explained at least partly by the data concerning a considerably higher consumption in farming households. At the same time, the consumption of other products rich in sugar is lower.

d) Consumption of artificial sweeteners

A complete consideration of the structure of sugar consumption cannot be complete without consideration of the consumption of synthetic sweeteners, or substances that are calorie-free but have sweet properties. However, this is not the place to introduce more detailed observations on this matter.

Table 4. Consumption of sugar and products rich in sugar according to the data of economic calculations (kg/capita/year)

	A. Lebensmittel / B. Jahre	1961	1962	1963	1964	1965	1966
	Zucker BRD	16,1	14,8	15,6	—	13,8	13,7
1.	Zucker CSSR a)	19,2	19,9	19,4	19,8	19,5	19,9
	Zucker CSSR b)	18,4	18,7	18,2	18,2	18,0	18,2
	Schokolade u. Süßwaren BRD a)	3,6	3,9	4,3	—	5,0	5,2
2.	Schokolade u. Süßwaren CSSR a)	1,85	2,2	2,1	2,0	2,0	2,05
	Schokolade u. Süßwaren CSSR b)	2,0	2,3	2,35	2,2	2,2	2,25
	Schokolade BRD	1,85	2,0	2,2	—	—	—
3.	Schokolade CSSR a)	0,95	1,15	1,2	1,1	1,15	1,2
	Schokolade CSSR b)	1,15	1,25	1,4	1,3	1,35	1,4
	Marmelade BRD	1,1	1,3	1,2	—	1,1	1,0
4.	Marmelade CSSR a)	2,6	3,2	2,9	3,1	3,2	2,7
	Marmelade CSSR b)	2,4	3,1	2,9	3,0	3,1	2,6
	sonst. Backwaren, Feingebäck und Dauerbackwaren BRD	5,9	6,5	6,6	—	5,9	5,8
5.	sonst. Backwaren, Feingebäck und Dauerbackwaren CSSR a)	5,6	5,8	6,4	5,7	5,6	5,6
	sonst. Backwaren, Feingebäck und Dauerbackwaren CSSR b)	6,5	6,9	7,1	6,6	6,5	6,0

Key:
BRD -- German Federal Republic -- 4-person employed households
CSSR -- Czechoslovakia -- a) laborer households
b) white-collar worker households

A. Food product
B. Years
1. Sugar
2. Chocolate and sweets
3. Chocolate
4. Marmalade
5. other baked goods, fine pastries and frozen baked goods

4. Retail trade prices of sugar and important products rich in sugar

The price of a food product is one of the deciding factors that affect the level of consumption (14). Every price comparison between the two countries is extremely problematic. This difficulty is due not only to the different price levels and price relations, but also to the generally different price formation. In Czechoslovakia, prices were heretofore determined by central organs. This fact was most clear in the case of

sugar prices. However, it should be mentioned that this practice has a long tradition. In order to make an approximate comparison, we use the figures concerning buying power, and then attempt to evaluate the price development.

Table 5. Prices of sugar and products rich in sugar (kg)

	A. Lebensmittel	B. Land	C. Einheit	1	2	3	4
a.	Zucker	BRD	DM	1,25	3,8	0,8	1,18
		CSSR	Kcs	8,0	1,05	6,35	14,0
b.	Schokolade	BRD	DM	8,30	0,56	5,0	13,0
		CSSR	Kcs	90,0	0,09	28,0	200,0
c.	Marmelade	BRD	DM	2,17	1,85	0,84	1,52
		CSSR	Kcs	6,0	1,39	6,8	8,0

Key:
1. prices of 1967
2. Amount (kg) per gross earnings of one hour's labor
3. Price of 1938
4. Price in 1950 (GFR), 1953 (Czechoslovakia)
A. Food product
B. Country
C. Monetary unit
a. sugar
b. chocolate
c. marmalade

Sources:
GFR -- Statistical yearbooks on nutrition
Czechoslovakia -- CSSR statistical yearbooks

From Table 5 it can be seen that the price of sugar, as well as the prices of chocolate and marmalade in Czechoslovakia per quantitative unit are considerably higher than in Germany. We consider this important, since we cannot ascribe a less favorable price to the lower sugar consumption of the German Federal Republic.

The food price level in Czechoslovakia can in general be considered as high. This could also be determined in a national comparison with the German Democratic Republic (17). The difference calculated in that case was 22% in the favor of the German Democratic Republic. However, this fact must be viewed in connection with other price groups in the standard of living.

The high price of sugar in Czechoslovakia can be followed historically. In the period from 1918 to 1939, sugar was one of the most important export goods. Its price on the national market always had to balance the export price. This condition still exists today. The following data concerning the relation between wholesale and retail trade prices make this clear:

A.	B.	C.	D.
Jahr	Großhandelspreis Kcs	Einzelhandelspreis Kcs	Großhandelspreis = 100
1933	0,57	6,25	1100
1953	2,0	14,0	700
1967	3,8	8,0	210

Key:
A. year B. Wholesale price C. Retail price
D. Wholesale price = 100

The present relation in Germany is 124, according to data of the statistical yearbook (49). It can be that the high sugar price has a prohibitive function. This would be desirable from the nutritional physiological viewpoint. The de facto plays no role in the determination of sugar price. On the contrary, in the comparison of the time period in Table 6 we find that the price drop and consumption increase in 1954 and 1960 coincide; from this, we can recognize a stimulating function of the price. In the period from 1953 to 1965, we can recognize a statistically very narrow correlation ($p < 0.001$). The drop in 1966, however, produced no such echo among the consumers. In West Germany, the price of sugar has a very slowly increasing influence on consumption, as Table 7 shows in comparison with other materials.

Table 6. Development of the retail sugar price in Czechoslovakia (per kg in Kcs)

1953	1954-58	1959	1960-65	1966-68
14	11	9,6	9	8

Source: CSSR Statistical yearbooks.

Table 7. Price indexes for the living standard in the German Federal Republic (1962 = 100)

		1964	1965	1966	1967
a.	Ernährung	105,8	110,6	113,3	112,7
b.	Nahrungsmittel pflanzlichen Ursprungs	100,8	106,4	108,3	107,2
c.	Nahrungsmittel tierischen Ursprungs	110,0	114,2	117,4	116,8
d.	Zucker, Süßwaren, Kakao	98,9	97,3	97,5	97,2

Key to table 7:
a. food b. food of vegetable origin c. foods of animal origin
d. sugar, sweets, cocoa

Source: Statistical yearbook on nutrition, 1968

The great difference in chocolate consumption is not due to the price of sugar. While in Germany the price of marmalade is higher than the price of sugar, the situation in Czechoslovakia is just the opposite; the price of sugar is one-third higher. The explanation of this paradox is the high tax on sugar. It does not involve sugar products. Sugar for industrial processing is bought at a lower wholesale price.

5. Consequences of sugar consumption for the nutritional situation

The general nutritional situation is influenced, under the relations described, by the sector of sugar supply. The consequences brand off into two directions; they are seen on the one hand in the nutritional value of food product consumption, including its structure, and on the other in economic features involving consumption.

a) Sugar consumption and nutritional value

The connections were already described in the description of the nutritional physiological significance of sugar. In Table 8, all the distinguishing features are summarized. Some columns show a considerable similarity. The question is raised as to what consequences the suggestions mentioned concerning the reduction of sugar consumption might have. In Table 9, we demonstrate this matter using the example of energy content. In Czechoslovakia, realization of the suggestion would mean a decline of 94 kcal/d, and a reduction of the "empty sugar calorie" portion of the total calorie amount from 12,6% to 9,6% (in the case of the same total calorie content). In this way we would obtain room for food products that are more valuable biologically. The recommended food product amounts in Czechoslovakia, in the frame of which the reduction of sugar consumption is incorporated, reckon, however, with a limiting of the total calorie amount of about 10%. The portion of "empty sugar calories" would then amount to 10.7% of the total calorie amount. We can consider the changes an improvement of the nutritional situation.

In Czechoslovakia, there is a rising tendency to use sugar in the energy intake, especially within the carbohydrate intake. The situation has not changed considerably in the last 5 years.

In Germany as well, the portion of sugar in calorie and carbohydrate amounts increase along a level that is lower absolutely than that of Czechoslovakia. The relative figures differ only a little. In the case of sugar portions in calories, they are lower in West Germany, but higher in the case of carbohydrates. This development indicates an unfavorable relation between thiamin intake and the intake of non-fat calories. The recommendation concerning this of the Committee for Nutritional

requirements of the German Society for Nutrition(41)(42): 1 μg vitamin B_1 should be contained in the food per non-fat calorie", is not realized in either country. The relation between thiamin intake and carbohydrate consumption is somewhat better in Germany, also because of the lower consumption of grain products.

Table 8. The indirectly determined sugar consumption and and the nutritional value (per capita and day)

A.

	Jahre	1936	1948	1964	1965	1966
1. Energieverbrauch*) (Kcal)	BRD	3037	2241	2943	2914	2890
	CSSR	2545	2194	3110	3060	3105
2. Zuckerverbrauch (kcal)	BRD	275	210	346	324	327
	CSSR	245	234	391	394	391
3. Anteil des Zuckerverbrauchs an der ges. Calorienmenge (v. H.)	BRD	9,1	9,4	11,2	11,1	11,3
	CSSR	9,6	10,7	12,6	12,9	12,6
4. Kohlenhydratverbrauch*) (g)	BRD	433,8	410,7	367,3	356,0	354,0
	CSSR	395,5	361,4	473,6	449,0	457,3
5. Zuckerverbrauch in Kohlenhydraten (g)	BRD	69,9	53,4	87,7	82,2	83,0
	CSSR	63,7	60,7	101,5	102,5	101,5
6. Anteil des Zuckerverbrauchs an der ges. Kohlenhydratmenge (v. H.)	BRD	16,1	13,0	25,5	22,5	23,4
	CSSR	16,1	16,8	21,4	22,8	22,2
7. Vitamin B_1-Verbrauch (mg)	BRD**)	1,487	1,538	1,402	1,451	1,386
	CSSR	1,418	1,237	1,686	1,558	1,615
8. Vitamin B_1: Nichtfettcalorien (γ Vit. B_1, je Nichtfettcalorie)	BRD	0,50	0,70	0,50	0,52	0,50
	CSSR	0,77	0,75	0,78	0,73	0,75
9. Vitamin B_1: Kohlenhydrate (γ Vit. B_1 je g Kohlenhydrat)	BRD	3,43	3,39	3,82	4,08	3,92
	CSSR	3,59	3,42	3,56	3,47	3,53

Key:
A. years
1. energy consumption
2. sugar consumption
3. portion of sugar consumption in the total calorie amount
4. carbohydrate consumption
5. sugar consumption in carbohydrates
6. portion of sugar consumption in total carbohydrate amount
7. vitamin B_1 consumption
8. Vitamin B_1: non-fat calories (γ vit. B_1 per non-fat calorie)
9. Vitamin B_1: carbohydrate (γ vit. B_1 per g carbohydrate)

Sources of table 8:
*) Statistical yearbooks on nutrition
CSSR statistical yearbooks
**) Calculations of the Max Planck Insitute for Nutritional Physiology

Table 9. Consequences of a proposed reduction of sugar consumption for the total energy amount and its composition

	CSSR	1967	A. Vorschlag
1.	Zuckerverbrauch je Kopf/Jahr/kg	37,1	28,1
2.	Calorieangehalt des Zuckerverbrauchs	391	297
3.	Unterschied		—94
4.	% Anteil der leeren Zuckercalorien an der gesamten Calorienmenge	12,6	9,6

Key:
1. sugar consumption per capita/year/kg
2. calorie content of the sugar consumption
3. difference
4. % proportion of empty sugar calories in the total calorie amount
A. proposal

b) Sugar consumption and food product consumption expenditures

Because of the difficulties mentioned in comparing the absolute monetary value data, it is more favorable to continue to work with relative data. In order to give more detailed information, we use the data of 1963. These data show no exact direction. Table 10 shows that in the course of the following years, no significant changes occurred. There are also no serious differences in the table between laborer and white-collar worker households in Czechoslovakia. For this reason, we restrict ourselves to laborer households.

Table 10 shows that the expenditures for sugar and sugar products, from which there is one very important one lacking in Czechoslovakia, namely syrup, represent one-tenth of the total expenditures for food products in the German Federal Republic, and one-eighth in Czechoslovakia. This corresponds to the same proportion, as the amount of sugar in the total calorie intake in Czechoslovakia. The difference of 1.9% between the two countries is smaller in the total sum than in the sum of sugar and sweets, where we find 2.7%. This fact is caused by the considerably higher expenditure proportion for baked goods in Germany. This position is also the only one that is higher than the corresponding one in Czechoslovakia. Especially great is the difference in the case of sugar itself. As concerns the data on the Czechoslovakian households, it should be mentioned that the data for the cooperative farmers are higher. They are about 4% more in the total food product expenditure, which corresponds to the higher proportion of retail sales. According to STILLER (29), the elasticity coefficients (average curve

elasticity coefficients) in the 3 types of Czechoslovakian households mentioned move between 0.34 and 0.45, which signifies slight dependence of sugar consumption on the income level. STAMER (28) determined the elasticity coefficients for the German Federal Republic at 0.15, or he points to WOLFRAM, who calculated 0.065-0.055. The difference between the two can be attributed to the different types of data. STAMER(28) draws our attention to the difference in the results according to various sources.

Table 10. Proportion of sugar in the food consumption expenditures (1963)

	BRD	CSSR
1. Zucker	2,5	4,9
2. Schokolade	2,4	2,5
3. andere Süßigkeiten	1,3	1,5
zusammen (1-3)	6,2	8,9
4. sonstiges Backwerk	3,8	2,5
5. Marmelade	0,4	0,9
zusammen (1-5)	10,4	12,3

Key:
1. sugar
2. chocolate
3. other sweets together (1-3)
4. other baked goods
5. Marmalades together (1-5)

Sources: BRD - 4-person employed households
CSSR - laborer households

6. Sugar production

a) Sugar beet cultivation

In both countries, the farming area has increased in comparison with 1935-38, especially in the German Federal Republic, where it is more than double the size. In 1935-38, harvests and yields in both countries were not very different. At present, however, they are considerably greater in the German Federal Republic (by nearly 50%). In Czechoslovakia, the yields remained at practically the same level as those of 30 years ago. Calculation of cultivation in relation to the population (per capita) further confirms the important role of the sugar beet in the Czechoslovakian farming economy. It should be emphasized that the farming areas in the German Federal Republic are privately owned.

In Czechoslovakia, the widespread cultivation of sugar beets per capita leads to great excesses, and therefore to cultivation for export. The situation in the world sugar market is not, however, favorable. It exercises an influence on the internal market.

b) Manufacture of sugar

In Table 11 we can observe the development since 1955. Production reveals a rising trend. In this period, sugar manufacture increased by 38% in Germany, by 45% in Czechoslovakia. Sugar manufacture in Czechoslovakia is nearly doubly so high per inhabitant than it is in the German Federal Republic. While in the latter the amount manufactured corresponds more or less to the consumption, in Czechoslovakia, an amount of sugar is manufactured, of which practically half must be exported. From time to time, the sugar supplies force the economic organs to apply pressure to the internal industry, as well as the internal market, to achieve greater sales. For this reason, the retail price fell steeply, as already mentioned. For this reason also, the wholesale price of sugar was very low up to the year 1967. It moved, since 1953, within a narrow span of 2 to 2.61 Kcs. Thus, sugar represents one of the most advantageous raw materials, price-wise, for the food product industry. However, in the German Federal Republic, sugar, also in the form of sugar beets and sugar products, is still imported. There is also a small exportation, but the balance of external trade is negative.

Table 11. Manufacture of sugar (in 1,000 t)

A				B.
Land	1955	1961/66	1961/66 1955	kg/Kopf/Jahr
BRD	1183	1636	138	28,4
CSSR	659	958	145	68,4

Key:
A. country B. kg/capita/year

Sources: Statistical yearbooks on nutrition, CSSR Statistical yearbooks

c) Manufacture of sugar products

Data on the amount of products that have a high sugar content also reveal a rising tendency (table 12). Especially high increase rates can be seen in the case of the manufacture of fruit preserves. Besides fruit preserves and marmalades, where manufacture is dependent upon harvest, the manufacture figures are growing steadily in Germany, which is not the case in Czechoslovakia. The statistics per inhabitant naturally correspond to the consumption per inhabitant.

Table 12. Manufacture of sugar products (in 1,000 t)

A.						B.
Lebensmittel		1955	1962	1961/66	1961/66 1955	1961/66 kg/Kopf/Jahr
1. Schokoladenerzeugnisse	BRD	166,1	224,3	246,6	149	4,3
	CSSR	17,5	26,3	27,7	158	2,0
2. Zuckerwaren	BRD	136,7	165,3	183,1	134	3,2
	CSSR	37,2	53,7	46,3	124,5	3,3
3. Dauerbackwaren	BRD	121	190,1	205,9	170	3,6
	CSSR	48,9	73,6	68,4	140	4,9
4. Marmeladen	BRD	82,1	93,4	93,9	114	1,6
	CSSR	22,5	29,7	26,7	119	1,9
5. Obstkonserven	BRD	45,5	75,4	92,2	203	1,6
	CSSR	19	36,3	46,0	242	3,3

Key:
A. food products B. kg/capita/year
1. chocolate products
2. sugar products
3. frozen baked goods
4. marmalades
5. fruit preserves

Source: Statistical yearbooks on nutrition, CSSR Statistical yearbooks

d) Sugar content in the products

The question of sugar content is of primary importance for a nutritional physiological evaluation. The manufacture use of sugar is about 2 kg/capita/year higher in Germany than in Czechoslovakia. According to the last year available for evaluation, it can be estimated that the sugar proportion in individual industrial branches is relatively higher in West Germany, with the exception of that used in beverages. In Czechoslovakia, in recent years, efforts were made to reduce the sugar portion in several products; however, these efforts had only slight success. In 1963 and 1965, the total production of sugar-containing products rose about 1,5% each, while at the same time, sugar consumption declined by 4%.

A comparison of the sugar portion in individual products is very problematic. Exact detailed data are available for the Czechoslovakian products. The sugar portion in the Czechoslovakian products of the group "sweets" is 60,2%, and only 55.8% in West Germany. If we apply the same ratio in Czechoslovakia as in West Germany, then we obtain a sugar proportion of 56%, thus a coinciding proportion.

In the group "marmalades and fruit preserves", there is a more significant difference. The sugar content for Czechoslovakian marmalades is 45,7%, similar to that of West Germany, including fruit preserves (44,8-46,8%). In Czechoslovakia,

the sugar content in fruit preserves is only 12.2%. Since fruit preserves represent about 65% of the weight quantity of the entire group, we get a sugar content of about 26%.

7. Further Influences

Besides production, many other factors influence sugar consumption.

Thus far, there are no detailed data concerning habits of nutrition, corresponding with sugar consumption, for Czechoslovakia. This gap can be closed by an analysis of the consumption in households. The idea that inhabitants of Czechoslovakia have a great predilection for sweet foods and sweets, which is assumed from the high consumption during holidays, especially Christmas, and other festive occasions, is completely subjective. If this were the case, several measures, as already mentioned, would contribute to it considerably. They would help in determining this custom. The rapid development of sugar consumption testifies to the fact that it is not traditionally determined custom.

Therefore, in West Germany, the consumption of household sugar is contrasted with the consumption of products containing sugar. Separate consumer groups behave completely differently, as the results of several field studies show. In households of the upper and well-to-do consumer groups, consumption of food products rich in sugar predominates, while in households of the lower and middle consumer groups, household sugar consumption is more prevalent.

In the German Federal Republic, too, a different intake in sugar can be observed in the individual Länder. In general, consumption is greater south of the Main line than it is in the north, except for some Bavarian regions, where the inhabitants manage with very small amounts.

It can be recognized from the degree of occupational difficulty of the head of a household that heavy laborers, measured for their total calorie consumption, consume relatively less sugar, and partly also sugar-rich products, than moderate and light laborers. The same can be confirmed in the case of male and female persons of all occupational difficulty groups.

As concerns the activity of social organs, we should name for Czechoslovakia on the one hand economico-political measures, and on the other enlightenment activities. Among the former, two resolutions should be mentioned, in which the high sugar consumption is designated as a deficiency in the nutritional situation. In 1958 there was decreed a Party Bureau resolution, "Proposition for Guidelines for the Development of National Nutrition", in which reduction of the high sugar consumption level was called for. Emphasized therein, not entirely correctly, was industrial consumption. In 1965 can a government resolution, No. 69 (45) "concerning the rationalization of nutrition", which placed "the reduction of the excessively high flour and sugar consumption" in first place on the list of tasks for governmental agencies up to 1970. These measures led to insiginificant improvements (the slight limitation of sugar content in products, already mentioned). The significance of both resolutions unfortunately remained largely academic.

The importance of the form of social influence achieved through enlightenment may be overvalued, even though much has been done in this direction. The principal organs of enlightenment activity in the area of nutrition in Czechoslovakia are the institutes for health education in Prague and Bratislava. These institutes have a wide field of influence. We attempted to evaluate the activity of the Prague institute from the standpoint of sugar consumption in the last 10 years. Our investigations established that no specific action directed at sugar consumption was undertaken. However, in numerous enlightenment articles there is mention of the disadvantages of sugar, especially in materials that are meant for children. These mentions were found in about 30 cases in the ten years (placards, leaflets, educational material for children, lectures).

The question is raised as to how successful these enlightenment and education activities actually were. In our analysis, we have already pointed out the lower sugar consumption in Prague, which we could possibly relate to this activity. Workers in this area proved, in their study (1), the increase in awareness of the negligible nutritional physiological value of sugar.

In West Germany, the two nutritional advisory services of the German Society for Nutrition have in recent years been able to accomplish valuable educational and advisory activity. The widespread work of the Federal Committee for economic enlightenment can also be evaluated as meaningful. At the same time, however, articles and brochures have been published in great numbers by the sugar industry and the Economic Sugar Union or its associated unions.

Summary

An analysis of sugar consumption in the German Federal Republic and Czechoslovakia shows that the difference in consumption of about 6 kg per capita and year can be attributed to the consumption in households -- sugar purchase. This is found from supply data and data of economic calculations. In Germany, a higher consumption only of chocolate and sweets, from among products rich in sugar, could be determined.

The nutrition-physiological aspect must be considered in evaluating consumption. A high sugar consumption has a negative effect on the presently desirable nutrition. It is manifested above all in the reduced consumption of food products of greater biological value that is brought about as a consequence of a higher sugar consumption.

The prices of sugar and products rich in sugar are lower in Germany and cannot indicate an inhibition of the increase in sugar consumption; the same is true of the relatively slow, in comparison with other areas of food product consumption, increase in the price of sugar. In contrast to this, the price development in Czechoslovakia can be considered one of the factors contributing to the rising sugar consumption.

The differences in consumption described cause a reverse in the content of nutritional value of food product consumption, in its composition as well as in the expenditures on food.

The production conditions in Germany reveal a sugar beet cultivation closely corresponding to consumption, while in

Czechoslovakia there is a more intensive overproduction, of which nearly half is intended for export. In both countries sugar manufacture has increased, a little less rapidly in Czechoslovakia. The same is true of manufacture of products rich in sugar. As concerns sugar content in the products, it seems to be higher in Germany, with the exception of beverages.

As concerns other factors influencing sugar consumption, few hitherto known or observed nutritional customs can be mentioned here that are affected either directly or indirectly by the national economy. In the nutrition-physiological evaluation, positive activity of societies and institutions in regard to education on this matter, economico-political measures, can be mentioned.

The situation of sugar consumption in the two countries can be characterized by the fact that the trends of consumption and production are towards increase, but that they at the same time signify a tendency toward a certain deterioration of the total nutritional situation.

Ous analysis searches for reasons for the differences in consumption levels in the two countries. In Germany, no causes besides the manner of consumption were found. In Czechoslovakia, on the other hand, we find certain moments in economic politics that formed a favorable starting point for the increase in sugar consumption.

In spite of the differences, further development is being considered in both countries; this study can provide data for such considerations. In Germany, full attention can be devoted to the industrial processing, where possibilities of restricting sugar content can be sought. In Czechoslovakia, the main point lies in private homes, thus in influencing the consumer and in the production of beverages.

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PART 2

Sugar: A Must in the Diet

People thrive on the sweet stuff that is found in carbonated beverages.

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I WOULD like to point out that the advantageous and proper use of our present national sugar supply depends on two factors (1) adequate basic nutritional education among the population to enable them to make wise dietary selections as shown above, and (2) sufficient income to purchase the ingredients of a good diet. I firmly believe that poverty and ignorance or carelessness are at the basis of nearly all malnutrition in this country.

A still further increase in the use of sugar might be possible if a part of it could be transformed efficiently into foods of other types than carbohydrates. This may not prove so impractical as it sounds. We have pointed out above that some of our excellent animal protein foods such as meat, milk and eggs are rather inefficient products insofar as land utilization is concerned. On the other hand though sugar gives an enormous return per acre, this return is nearly all in the form of carbohydrate. In recent years there has been an effort to increase the production of certain vegetable proteins which happen to be of quite high biological value. A good example is the tremendous rise in soy-bean growing. In 1914 only a thousand experimental acres were grown. By 1947, this has increased to 181,360,000 bushels. The soy-bean is prized among other things because it produces a variety of food materials in rather balanced proportions. These include a high quality protein, carbohydrate (starch), oil (fat), vitamins and minerals. Yet despite these values of the soy-bean, it does not even remotely compare with the sugar beet or cane in its total production of calories per acre.

The tops, molasses and pulp of sugar beets are often fed to stock and thus converted finally into beef, so that there is some return from this land so used, in fat, protein and vitamins in addition to the sugar. A chart (Part 1, No. II) shows the total human food return from a typical acre of such beets in comparison with the yields from soy-beans, when all possible by-products from both plants are fed to stock. Except for calories, the beet score is much lower all along the line.

There is, however, a method whereby the production of nutrients other than carbohydrate can be increased for acreages devoted to cane or beets. Molasses is an excellent substance for the growth of micro-organisms such as bacteria and yeasts. Such organisms as these are able to transform inorganic nitrogen into proteins utilizable by man. The carbon in this case is derived from the sugars in the molasses. In this way, a part of the tremendous carbohydrate yield of the sugar crops can be turned into protein with the fixation of additional nitrogen from nonagricultural sources, and in a far more efficient manner than by animals such as the steer. The microorganisms that have received the most consideration for such purposes are the yeasts, particularly *Torulopsis utilis, major*. This is not the organism or even the yeast that yields the highest known quantities of B vitamins or of protein, but it has been selected particularly because of its superior flavor. Feeding experiments have shown that the purine contents may be an obstacle to unlimited use and also that the content of sulfur-containing amino-acids is below the optimum. Altogether, it seems improbable that the present *Torulopsis* strains represent the last word in edible yeast.

Moreover, we are aware of the difficulties involved in the introduction of new foods. It is all very well to show on paper the logic or desirability of eating this or that, but to create a popular demand or even to get it eaten is quite another thing. There have been many reports about amino-acid preparations of very high biological value which even animals actually eat only with great reluctance.

On the whole, we do not expect to see a sudden popular demand for edible yeast, nor do we see the possibility of quick fortunes for the food industries. Nevertheless, the basic reasonableness of converting virtually waste carbohydrate material into protein—and vitamin-rich foods and feeds, is so great that work along these lines must be continued as a public service. As basic nutritional work proceeds and as experimental quantities of edible yeasts become increasingly available, we hope that the food processing industries will give some attention to ways and means of incorporating these products into processed foods in order to augment their protein and vitamin values. We shall be glad to serve as intermediaries in providing experimental quantities of *Torulopsis*.

Applies to Soft Drinks

The applicability of much that I have said to soft drinks should be quite obvious. Such beverages are not consumed for their nutritional value but for refreshment and pleasure. I think we have shown that there is very considerable room in our diets for the sugar that is contained by these drinks without any necessity of displacing any food that is needed primarily for its vitamin content. The positive values of soft drinks lie more in their social and psychological effects than upon a nutritional basis. They are generally used at moments of agreeable relaxation which relieve the tension and constant drive that are among the sins of our civilization. The effects of the mind upon the health of the body are gaining a new recognition, and "psychosomatic" medicine has become a prominent branch of the art. The specialists in "psychosomatic medicine" agree in their judgment that worry, haste and nervous strain play a tremendous roll in producing the prevalent conditions of hypertension, gastric ulcer, arthritis and other diseases that afflict civilized man. The moments that refresh and restore are needed by all of us. I do not think that it is necessary to seek a *nutritional* reason for the use of carbonated beverages.

Body Stores Fat

Although carbohydrate is the largest constituent of all normal diets, the body is not able to store very much of it. Something like half a pound can be stored in the muscles and a quarter of a pound in the liver. This is only enough to supply the body's requirements for about twelve hours. When these store-houses are full, any additional carbohydrate coming into the blood stream is stored as fat. Unlike carbohydrate, which is stored to such a limited extent, fat can be accumulated in almost unlimited quantities. When the muscles do work, both fat and carbohydrate are normally burned together. The fat comes out of its storehouses to the liver where it is transformed into "ketone bodies" and fed into the blood. At the same time the liver carbohydrate stores are changed into simple sugars that are fed also into the blood. The sugars and "ketone bodies" circulate together to all the working muscles where they are burned as fuel. This is the normal picture. However, we have mentioned how small a stock of carbohydrate can be stored. When this runs out, the liver can no longer supply sugar to the blood. As if to compensate for this situation, the liver produces floods of "ketone bodies" from fat and the blood becomes charged with them. This becomes the condition of "ketosis." (6) If ketosis becomes severe, the blood becomes more acid than normal, liquid and chlorides are lost rapidly from the body through the urine, general dehydration takes place and severe vomiting and violent illness may occur. Obviously the need of the body is for sugars, but if vomiting is severe there may be difficulty in keeping anything on the stomach so that the vicious circle tends to continue. Sometimes it can only be ended by injecting sugars directly into the blood. Such a series of events may occur in a person who has been dieting strenuously, particularly if he does not know how to diet wisely, and pediatricians report that it is a rather frequent experience with children.

Children have smaller livers than grownups so that they can store relatively much less carbohydrate. At the same time they have a much more rapid metabolism than adults so that they burn up fuel far more quickly. Whereas an adult may hold a stock of liver and muscle carbohydrate sufficient for twelve hours, a child often has enough reserve for only three or four hours. Hence various rather slight disturbances such as excitement or missing a meal, may precipitate an episode of ketosis. Pediatricians say that many of the vomiting-spells, upsets or so-called "bilious spells" of children are of this nature. Some children are particularly susceptible and suffer from severe periodic attacks during which they "cannot keep anything down." Such children are likely to appear pale, thin and puny. Sometimes they are given extra quantities of cod-liver oil or milk because of this appearance, but if the trouble is really due to a low liver capacity for carbohydrate, the administration of still more fat does more harm than good. Such children respond very well to between-meal feeding.

The tendency of "cyclic vomiting" is by no means rare in children and all children have such a low carbohydrate storage capacity that it is questionable whether we should expect any of them to adjust themselves to the quiet arbitrary adult concept that three meals a day constitute the only proper and respectable schedule. Carbonated beverages serve very well for the agreeable administration of between-meal carbohydrate in such cases.

Concerning Caries

I do not believe I can expect you to let me conclude until I have mentioned several specific problems that are very much in the lime-light. One of these questions is "Do carbonated beverages etch the teeth?" A good deal of publicity has been

given to studies that were made in the Naval Medical Research Institute in which rats, puppies and hamsters were allowed or compelled to drink an acidic carbonated beverage. After a period it was found that their teeth had been distinctly marked on the surfaces on account of a partial decalcification by the acid.[6] These observations are important and call for careful consideration. Nevertheless, like many animal experiments they were carried out under conditions quite different from those of normal human life, and because of these differences, one cannot hastily jump to sound conclusions as to their significance.

Fruit Juices Studied

In the first place, smaller animals such as rats ordinarily drink a great deal more liquid in proportion to their weights than do human beings. According to our conventional standards, an adult man normally drinks about six glasses of water a day or about a quart and a half. A rat weighing about 100 g. will drink from 20 to 35 cc. of liquid, that is, from 20% to 35%, of its own weight. If a man drank a comparable amount, he would consume between 15 and 26 quarts of water a day. In the experiments cited above, the rats were given nothing to drink except the acid beverages under study and ordinarily drank an amount equivalent to fifteen quarts a day for a man.

More recently it has been shown that most fruit juices such as tomato, prune, orange, pineapple, apple, grape and grapefruit produce the same kind and a similar degree of erosion as the carbonated beverage.[9] When the quantity of juice given was reduced to about a quarter of the total fluid intake, the erosive action was very considerably reduced. Even these reduced levels represented a great deal more beverage in proportion to body weight than is normally taken by humans.

It was originally stated[10] that sugar increased the erosive action of acid beverages on the teeth. This conclusion was reached because a beverage containing acid and sugar caused more severe etching than one of equal sweetness containing the same amount of acid with saccharin. It is now reported however, that the saccharin used in the experiment contained a base which neutralized a part of the acid. Hence this reported synergistic effect of sugar and acid has turned out to have no foundation in fact.

Another question that is almost certain to be asked is "Do carbonated beverages promote tooth decay?" This, of course, is only part of the larger question of the relation of sugar to tooth decay. As you are well aware, all carbohydrates are suspected of playing a role in dental caries through conversion to acids by bacterial action. Nevertheless, we know that there exists some kind of machinery that can produce resistance or immunity. Many individuals consume large amounts of sugar and other carbohydrates without suffering from appreciable decay. The factors responsible for this resistance are therefore being sought with increasing vigor. At least one has been found in fluoride, which when present at the proper concentrations in the drinking water can confer a significant degree of resistance to the teeth. There is no general agreement as to how it functions, but empirical work has established to the general satisfaction that it does function. Vitamin D is also conceded to have protective effects.

Research Progressing

Drs. Jay, Becks, Kitchin and others have reported that persons with rampant caries can be rendered relatively immune for a long time by a short period of drastic carbohydrate restriction. It was originally thought that such carbohydrate restriction merely deprived the bacteria of their material for making acid. This does not now seem to be a sufficient explanation, since after restoration of the carbohydrate material, a long period of inactivity may ensue despite the presence both of bacteria and of their fermentation material. As Dr. Jay says[11] "—patients were consuming foods that, before treatment, were associated with high oral lactobacillus counts, but after treatment the same amount of carbohydrate could be ingested without increasing the lactobacillus counts. In other words, with the same substrate, in one instance the oral flora was characteristic of high caries activity, and in the other, immunity to caries. It appears therefore, that some factor other than the substrate derived from food had influenced the growth of the bacteria.

It should be noted that when the level of carbyhydrate is reduced in a diet, something else must be increased. It is quite possible that the effects of the low-carbohydrate diets are actually due in large part to the increase of some other factor such as an amino acid which exerts a positive effect. This possibility should not be excluded since such a factor, if identified, might still be effective when superimposed upon a diet containing a normal amount of sugar and starch. A good deal more strictly controlled work is needed in which carbohydrate is not merely reduced but where complete account is taken of all dietary constituents and where the effects of variations in each one separately can be evaluated. Fortunately an increasing amount of such work is now being done.

At any rate, examples are multiplying which show that immunity is possible in the presence of ample fermentable[11] material as we have indicated in the case of the Pennsylvania children.

The considerations outlined in the initial parts of this paper indicate quite clearly, I believe, why no general mass diminution of carbohydrate intake is possible as a means of controlling tooth decay or for any other reason. We can feed the world's population only by placing heavy, and probably increasing dependence upon food

stuffs of the carbohydrate class, including sugar.

Even Dr. Robert G. Kesel of the Illinois University Dental School, who has been concentrating his attention upon the caries problem rather than upon the dilemma of global food supplies, has recognized the necessity of emphasis upon this side of the picture. As he has said recently:[9] "We must find the means of living with sugar, without the present resultant damage."

Finally I should like to offer you the solace of knowing that *fluidity* appears to be a significant factor in the caries picture.[10] There is some evidence that the same dietary combinations in liquid form are associated with less tooth decay than when taken in dry form. This may prove to be an important consideration with respect to the possible effects of carbonated beverages on the teeth.

To recapitulate, I hope that I have succeeded in sustaining the following points:

1. That sugar is a basic resource of major importance because of the very great productivity of sugar cane and sugar beets in terms of calories per acre, a productivity far surpassing that of any other plant.
2. That the world food supply is already short of the requirement for general good nutrition and that even the United States is rapidly approaching the end of an era of superabundance.
3. That there is a practical need for learning how to use as much sugar as possible in human diets in order to feed the population more adequately.
4. That the possibility of utilizing the maximum amount of sugar in balanced diets, requires the extension and intensification of nutritional research and education.
5. That the nutritional goals set up by the Food and Nutrition Board of the National Research Council furnish a valid and effective means of carrying out nutritional education.
6. That daily intake of the recommended amounts of protective foods leaves ample room for use of the present percapita sugar supply or theoretically, of considerably greater quantities.
7. That studies with both animals and human beings have demonstrated the compatibility of sugar intake even higher than present levels with well-balanced and generally excellent diets.
8. That selection of generally satisfactory diets including sugar at present percapita levels requires (1) nutritional education of the general population and (2) sufficient purchasing power.
9. That economy of land use may be achieved by conversion of sugar to food of other classes through microbiology.
10. That carbonated beverages are

valuable for social and psychological reasons rather than nutritional ones.

11. That there is physiological basis for between-meal feeding of carbohydrates to children.

12. That carbonated beverages do not etch the teeth more seriously than fruit juices, and that the full evaluation of the significance of the effect remains to be demonstrated in relation to the volume of intake.

13. That high carbohydrate or sugar intakes are not necessarily incompatible with caries immunity, through many of the significant factors have not been discovered.

14. That fluidity in foods may be a factor tending to reduce decay of the teeth.

I would like to express one additional thought that may be worthy of consideration. I hope the figures given here demonstrate conclusively that there is no competition and no need for any competition between milk and carbonated beverages. I do not hesitate to predict that if the nutritionists' dreams could be fulfilled and if every child in the United States received and drank a quart of milk every day, these same children could and gladly would still consume every bottle of carbonated beverage that they now consume. In fact, I think it very likely that their average health, vigor, appetites (and thirst) would increase to the extent that they could drink considerably more. This prediction is based upon a fair amount of personal experience with children.

I mention this because I feel sure that much opposition to carbonated beverages arises from nutrition people who fear that carbonated beverages may sometimes replace milk for children. Moreover, such nutrition workers are sensitive to their relative lack of means of reaching the population with nutritional knowledge. I believe that much of this opposition would vanish if the organized bottlers could see their way clear to participating even modestly in nutrition education through the medium of advertising or otherwise.

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Sugar: A Must in the Diet

People thrive on the sweet stuff that is found in carbonated beverages.

by DR. R. G. HOCKETT

Scientific Director
Sugar Research Foundation

I AM certain that there is not a single carbonated beverage on the market that does not contain sugar. On the other hand, soft drinks provide the outlet for something like four percent of the entire sugar distribution in this country. I should like therefore to point out a few facts about sugar that do not seem to be very well known and which have a very definite bearing upon the place occupied by soft drinks in our lives.

I do not hesitate to say categorically that sugar is one of our most important basic resources. The great question which the Sugar Research Foundation was organized to answer is this "How can we use this resource to the best possible advantage?"

In stating the basic importance of sugar as a resource I am thinking primarily of this fact—that sugar gives a far bigger yield of food *per acre of land devoted to its production* than any other product. This fact has been recognized in Europe for a long time. Such countries as Czechoslovakia, Belgium, Switzerland, Sweden and England are highly populated and do not have very ample acreages of arable land. In all these countries, the sugar beet is an important crop and its cultivation has been encouraged as a means of getting the most food possible out of the available land.

In tropical areas, our great sugar-producing plant is the cane, and as a crop the sugar cane is our most efficient converter of the sun's energy into food that can be utilized directly by human beings as a fuel for providing the essential body heat and muscular activity. The fuel value of foods, either for heat or muscular activity is measured in *calories*. A typical man weighing 154 lbs. and moderately active requires just about three thousand calories a day of food energy. In a year this amounts roughly to one million calories.

The efficiency of various crops can be stated in a striking fashion if we compare the amount of land necessary to produce in a year, the million calories required by one adult male. It is a remarkable fact that an average acre of sugar beets produces just about the same number of calories in the form of sugar as an average acre of sugar cane. There are favored spots where the production is very much greater than this average, but in making the comparisons which are to follow it seems most fair and reasonable to use a five year average for all the areas that supply the United States with sugar. We find that the average sugar beet acre supplies 8,466 pounds of refined sugar in a year while an average acre of cane supplies 4,432 pounds per twelve months. The weighted average of the two sources of sugar (one-quarter from beets and three-quarters from cane) gives us a figure of 4,230 lbs. of sugar yield annually from each acre devoted to growing sugar crops. Every pound of this sugar contains approximately 1800 calories so that the typical acre yields 7,614,000 calories per year. Since one man needs only one million calories a year, an area of 0.13 acres would

be sufficient to feed him if, of course, he could live on sugar alone.

Now, for the purpose of comparison, let us see what land areas are required for producing a million calories of other foods:

Food	Acres of Land	Ratio to Sugar Acreage
Sugar	0.12	——
Corn	0.32	2⅔ times
Sweet potatoes	0.35	3 times
Irish potatoes	0.44 (.52)	3 times
Rice	0.59	4½ times
Soy beans	0.67	5 times
Beans	0.81	7 times
Whole wheat flour	0.90	7 times
Corn meal	0.90	7 times
Refined wheat flour	1.20	8 times
Hogs (pork and lard)	2.00	13 times
Whole milk	2.8	19 times
Eggs	7.0	46 times
Chickens	9.2	62 times
Steers	17.0	113 times

These figures are dramatic without a doubt. Yet it is altogether proper that we should inquire further as to what they mean. Do we not have plenty of land in the United States so that we need not worry about such matters as the yield of calories per acre?

The answer to this question is a plain and decided "No." A number of authorities are pointing out with alarm that our population is growing so fast and so much topsoil has been lost already that we are very quickly approaching the pinch. Sir Cedric Stanton Hicks[20] has estimated that on the average we now use 3.5 acres of land per year in agriculture to feed each person in these United States. We formerly had many times that area available per inhabitant and for generations developed the habit of thinking that our resources here in the New World were inexhaustible, that hunger just could not happen here.

It is not altogether strange that we acquired such habits of thought as this. Your ancestors and mine came from countries like England, Ireland, Germany, Italy and Sweden where overcrowding had already occurred. The earliest ones arrived here to find a sparse population of Indians living very largely by hunting and the collection of wild fruits. In a typical area, Licking County, Ohio, which now feeds 70,000 souls, there were only about 176 persons being supported by that fertile soil. It is little wonder that the earlier settlers, after overcoming initial hardships, wrote to their European relatives the most glowing accounts of incredible abundance and variety of food in this hemisphere. But what is now the situation in our land of plenty?

At the present moment, we possess somewhat less than 3.8 acres apiece or 0.3 of an acre to spare over the 3.5 necessary to maintain our present living standard. When the population reaches 160,000,000 we will just break even. What will happen to us after that? Our fate will not be immediate disaster, because it is quite possible to modify our diets so that we can be well nourished on considerably less than 3.5 acres per capita. In Europe the bulk of the population must already be fed with 2.5 acres of arable land apiece.

Many past civilizations are believed to have declined because overpopulation forced the people to deficient diets that caused decrease in alertness and physical vigor so that their enemies were able to overcome them.

Taking the world as a whole, it is disturbing to note that the total cropland today only amounts to about two acres per person. From this it is evident that we Americans have far more than our pro rata share and we cannot avoid the conclusion that large sections of the world population must be receiving diets that are woefully inadequate to maintain anything like complete vigor and full mental powers.

The importance of sugar in this picture is very obvious since sugar is the great land-saver. If a man could live on sugar alone, he could be fed by 0.13 acres of land. At that rate the United States could theoretically feed a population of four billion persons! Naturally there is no such possibility in fact for we know that sugar is not a complete food and that it is not possible to grow sugar on all parts of our agricultural land. Nevertheless the principle is clear. *It is an important practical problem from the standpoint of the world population to find means of using as much sugar as possible for calories as a means of sparing land for other products such as meat and milk.* To use an extreme example, a man consuming 200 pounds of beef a year and 445 lbs. of sugar would just utilize his personal land allowance (United States rate) of 3.5 acres, 3.38 acres for the beef and 0.12 acres for the sugar! This illustrates the principle. Now

In times of mental and physical stress, people in all walks of life turn to soft drinks for a pick-up.

let us see how the problem can be attacked.

The early pioneers in nutrition study, such as Rubner, were aware that the three great components of human food are protein, fat and carbohydrate in the customary proportions of something like 11%, 30% and 59% respectively. Organic chemists put in a great deal of time in the nineteenth century learning how these three classes of compounds are put together by nature. By the last quarter of the century, they felt that they knew a great deal about these classes of substances. They had been able to separate, purify and describe quite accurately the various individual components of the fats, proteins and carbohydrates that are found in plants and animals. Inevitably efforts were made to feed animals on combinations of chemically pure fat, protein and carbohydrate respectively. Strangely enough the animals so fed could not live and thrive on these three classes of substances alone even though these materials were perfectly well digested and absorbed. Something else was still lacking.

Then began the long slow process of finding the lacking substances. This led to gradual sorting out of all the vitamins and inorganic materials that are needed by a living body. The remarkable thing about most of these substances is that such very tiny quantities are necessary and yet that they are so important to life and health. One by one the vitamins have been isolated, their molecular architecture determined and then the majority of them have been duplicated by synthetic methods. It is confidently expected that all the rest will sooner or later be obtained synthetically.

From time to time nutrition researchers try again to maintain animals on strictly purified diets consisting only of chemically pure carbohydrates, purified protein and purified fat along with a string of individual pure vitamins and mineral salts. They have never yet quite succeeded in getting satisfactory normal growth and development. It has always been necessary to add just a wee bit of liver extract or yeast or some other material which evidently contains food factors not yet separated and identified by chemists. Nevertheless, they are getting remarkably close to the goal.

Studies of this kind have given us a very great advantage over the ancients whose civilizations declined because population pressure forced them to poorer and poorer diets. Great groups of people in the Orient have been plagued with beriberi in the not very distant past. Now we can synthesize the vitamin which is the body's primary requirement for the prevention of this condition. An entire year's supply of vitamin B_1 or thiamin can now be purchased for about fifty cents! At this rate it does not seem that there is any longer any good excuse for a single person in the country ever to suffer from a deficiency of thiamin.

We have already pointed out that carbohydrate is the biggest com-

pound in all normal diets. Moreover it can replace most of the fat since only a few per cent of fat is strictly indispensable. Protein cannot be reduced below about 11% without causing trouble because protein is essential for building and rebuilding the body substance. It is quite possible however to keep animals indefinitely on diets that are 76% carbohydrate and this carbohydrate can perfectly well be sugar. Innumerable experiments of this kind have been made with mice, rats, dogs, monkeys and other animals. Such experiments are very well known to all investigators in the nutrition field and do not occasion any surprise or debate. The remaining 24% of non-carbohydrate in the diets provides plenty of room for all necessary protein, fat, vitamins and minerals.

Some Czech scientists went still further and kept rats on a 50% sugar diet for a continuous series of four generations.[6] The animals lived and developed reasonably well but so much more has been learned about nutrition in the last ten years that this experiment needs to be repeated under modern conditions. Such a study is in fact now getting under way.

It is well-known therefore that animals can live quite well on very high sugar diets and we have no reason to doubt that human beings could do likewise if they wished or if it were necessary. The point I wish to emphasize however is that *such high sugar diets can be designed successfully for animals, only because of the high degree of development that has been attained by nutritional science.* The animals can thrive on 76% sugar only because nutritinists know just what to put into the other 24% that goes with this sugar. If they new less, they could not successfully use so much sugar because they would have to include large amounts of crude and raw material by way of insurance that all the tiny traces of essential accessories would be provided.

We must conclude from this that the practical possibility of using sugar to the maximum extent in human diets depends in a very genuine way, upon the development and the dissemination of nutritional knowledge. I believe it is quite correct to argue that the producers of sugar and those who use it in their products have a bigger stake in nutrition research than almost anyone else.

It will be some time before we Americans will need to consider trying to get 76% of all our calories from sugar. We are interested in a much more immediate problem which we can frame in this way. For the last twenty-five years we have distributed in this country each year enough sugar to supply each inhabitant with approximately a hundred pounds. Some of this is used for silvering mirrors and making dynamite. A good deal more is converted into gases by the yeasts that raise our bread. However, the question is "Can we use the available sugar in such a way that in combination with other available foods, we will have a truly first-

class overall diet?" It can be answered best by considering the recommendations of the Food and Nutrition Board of the National Research Council as to what constitutes a good diet.

When the Board, by extensive study, had arrived at its recommended goals for daily intake of the various essential food materials, they faced the problem of putting their recommendations into a form that could be understood by the man in the street. You cannot reasonably expect a workman to go into the cafeteria with a book of tables in one hand and a slide rule in the other! Consequently the Board decided to group all foods into seven classes in the hope that people might learn to select something from each group each day. In their circular No. 122 on page sixteen, they set down their recommendations in the following way:

An average adult should each day consume these foods:

Milk—one pint.

Egg—one, if possible. Otherwise beans, peanuts, cheese or more milk or meat.

Meat, fish or fowl—one or more servings.

Potato—one or more.

Vegetables—two or more servings.

Fruits—two or more, one of which should be citrus fruit or tomato.

Cereals and bread—whole grain or enriched.

Other foods as needed to complete the meal.

It was the belief of the Food and Nutrition Board that if this list were selected daily, all the required vitamins, minerals and other nutrient materials would be obtained. Some might be overabundant one day and a bit low on another, but the allowances should average out properly in the course of a week or so.

It is very interesting to see how this plan actually works out. We have made out a chart to test its operation. First, the protein, fat, carbohydrate, calcium, phosphorus, iron, vitamin A, vitamin B_1, vitamin B_2, vitamin C and niacin as well as the calories in a pint of milk have been listed. These are all the materials for which a definite daily allowance was recommended by the Board. The components of an egg and a potato have been listed out similarly. To get appropriate figures for meat, we averaged the values for five different cuts of beef, four of pork and one of lamb. Similarly we derived average values for the most common fruits and vegetables. All this was done by random choice without any effort to weight the result one way or another. Cooking losses were taken into consideration. The result is very satisfactory. The totals obtained for protein, calcium, phosphorous, iron and all the vitamins except niacin are equal to or well above the recommended allowances for these nutrients. Niacin shows up as just a little low but a slightly different choice of meats or vegetables would place this vitamin also well above the recommended level. Since most of the allowances contain a pretty generous margin of safety, the deficit is not of any consequence.

Of course there are a good many vitamins and trace elements known that do not occur here upon the list of recommended allowances. What about these? As it happens these substances are all so closely associated as a rule with the other vitamins and minerals that if the ones listed on the table are generously supplied, there is a very small chance that the others can be lacking. As a form of insurance, a little liver, yeast and molasses can be added to the diet from time to time since the first two of these contain high vitamin concentrations and the last contains an accumulation of inorganic salts.

This plan for food selection thus appears to be very practical and useful for assuring an ample supply of all the protective foods. There is, however, one very great deficiency to be noted. *This list supplies only 1314 calories, or considerably less than half of the 3000 needed by a 154 lb. moderately active man.* In other words, less than half the total diet is required for supplying all the necessary protein, vitamins and inorganic salts. The additional 1686 calories must be supplied by energy producing materials—primarily fats and carbohydrates. This is where sugar plays its greatest part. Some sugar is already accounted for in the total given above, since sugar is used in baking the bread, in the cooked fruits and in the cured meats included there. It is evident that there is room for a very considerable amount of additional sugar in rounding out such a diet. If 686 calories are to be supplied by additional fat and 1000 by carbohydrate, it is evident that about 9 ounces of sugar *could* be consumed without displacement of any necessary "protective" food. This would be equivalent to over two hundred pounds a year. Of course most of us prefer to use a greater assortment and variety of foods but this method of statement will emphasize the point that 85 or 100 pounds of sugar a year *can* be accommodated into a very high-class diet. In actual practice, we may prefer to take some of the milk as ice-cream, in which case some of the sugar is combined with it. Some of the additional calories will be taken as cereal, cakes, pies, puddings and other dishes in which sugar is combined with still further quantities of some of the listed foods.

Certain extremists talk as if they thought we should eat nothing but the sort of protective foods shown in the table but this is hardly reasonable. In sheer bulk, in cost or in their requirements of agricultural land, many of these products are unsuitable for general use very far above the levels necessary for adequate supply of the vitamins and salts. It is far more reasonable to round out the caloric requirements by *proper* use of economic energy-supplying foods of which sugar is an outstanding example.

"But," some persons may be inclined to remark, "all this sounds very theoretical but I am always a little suspicious of figures. Has any such plan been tried out in actual experiments to see how it works?" The answer is that it has, both with animals and with humans.

I recall one experiment of this kind by Dr. Clive M. McCay[9] a few years ago. He placed 200 rats on a basic diet designed to supply literally all known protein, vitamin and mineral requirements. This was accomplished in less than half the amount of total food required just as in the human diet described above. Then he completed the calorie requirements of four different groups by giving them respectively:

1. Cooked corn starch.
2. Cooked corn starch 60 pts., sugar 40 pts.
3. Cooked corn starch 60 pts., dried milk 40 pts.
4. Cooked corn starch 60 pts., dried liver 40 pts.

The question he wanted to answer was, "If a person eats a truly adequate basic diet, does it matter whether he chooses the rest of his calories in the form of all starch, all carbohydrate (starch and sugar) starch and whole milk, or (4) starch and meat?" The milk and meat would, of course, supply protein and vitamins beyond the liberal quantities in the basic part of the diet.

The answer to the question *in terms of longevity* was "NO," for all groups of rats completed life spans that were not significantly different:

Dietary variable	Life span in days Males	Females	Total
1. Starch	595	705	1,300
2. Sugar	674	717	1,391
3. Milk	688	610	1,298
4. Liver	531	682	1,213

In other words, there was no benefit as far as span of life is concerned, in supplementing a truly adequate diet with even the best of natural foods such as liver and milk.

However, despite all these calculations and all these animal experiments, we thought it would be well to make specific studies along these lines and with human subjects. The work I am about to report has not yet been published and I shall therefore not mention the investigators involved.

A two-year study was made involving 234 boys in a Pennsylvania custodial institution. The prevailing diet was thoroughly analyzed at the outset and complete physical examinations were conducted. These included general medical examination, weight and skeletal measurements, X-rays analysis of bone maturity, biomicroscope examinations of eye, mouth gums and tongue, general dent[illegible] examinations, enamel solubil[illegible]ests, lactobacillus counts, X-ra[illegible] blood analysis for hemoglobin, [illegible]rocytes, hemocrit, plasma vita[illegible] A, carotene, and ascorbic acid [illegible]um protein, globulin, albumen and phosphatase, and urinary analysis for thiamine, and riboflavin.

The original diets were found to be excellent except for ascorbic acid and vitamin D. During the two-year experimental period, the ascorbic acid was increased materially but the vitamin D remained low because the school authorities would not provide codliver oil supplements. Complete examinations were made each six months.

The main objective of the work

was to see, in a practical way, whether a truly liberal diet could be maintained with respect to all requirements of essential nutrients while maintaining a sugar intake considerably above the general average for the United States population—and to assess the physical effects of such a diet.

The "free-sugar" intake for the children over 12 years of age was 125 grams a day—corresponding to an annual consumption of 100.6 lbs. This does not include the sugar in processed foods such as ham, bacon, pickles, cereal, etc. so that total sugar consumption was perceptibly greater. The total calories supplied were 142% of the Food and Nutrition Board Recommendation, protein 162%, calcium 108%, phosphorus 200%, iron 200%, vitamin A 213%, thiamine 220%, riboflavin 190%, niacin 156%, ascorbic acid 220%, vitamin D 44%.

It would be rather tiring to go through all the details of the examinations and ratings collected at the end of the experimental period. It will be sufficient to say that they were very high throughout. According to the Wetzel Grid, only 4.3% were obese, 67.1% were in the *good* class, only .9% in the *fair* class, none in *borderline* or *poor*. They progressed steadily from 67.9% in the highest class with respect to skeletal maturity at the beginning to 74.4% at the close.

The dental conditions are especially interesting. At the beginning 10.6% were in the arbitrary highest classification and 83.6% in the next two classes. At the close, 8.6% in the highest class and 82% in the next two classes. The DMF/N value increased during two years from 0.195 to 0.272, which is a *smaller* increase than for the general population. Lactobacillus counts did not show any great degree of correlation with actual caries incidence. Another school in which sugar intake was much lower and in which many other nutrigents were marginal showed a very notably poorer dental score.

The general conclusion to be drawn from this study is that an annual consumption of 100 lbs. of refined sugar is truly compatible with a liberal diet and that young human beings on such diets thrive excellently. We do not by any means claim that the sugar intake was beneficial to the teeth, but it is apparent that a relatively high sugar intake when combined with a generally superior diet is or can be compatible with having teeth far better than average.

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SUGAR:
A Must in the Diet—
(Continued next month)

International Sugar Research Foundation. 1970. p11-13.

1

Introduction

SUCROSE, commonly known as table sugar, is by far the most abundant carbohydrate found in the sap of land plants. It is one of the few organic compounds available in a state of unexcelled purity, in highly crystalline form, on a very large scale, and at low cost. It has been produced since 2000 BC from the juice of the sugar cane and since the early 1800's from the sugar beet.

The combined annual world production of sucrose from sugar cane and beet was 77 million tons in 1968. Of this total the United States (including Puerto Rico) produces six million tons and consumes ten million tons. Other major producing countries (in approximate order of size) are the USSR, Cuba, Brazil, India, France, Germany, Australia, Mexico and Taiwan.

Beets provide much of the sugar for Europe and central and western United States, and cane the rest. Other limited sources include sorghum and maple.

Sucrose is used almost exclusively by the food and other consumer goods industries for sweetening. Of the ten million tons consumed in the United States each year, 27% is sold directly to home and institutional users. The remainder goes to the baking, confectionery, ice cream, beverage, and other food industries. Pharmaceuticals, cosmetics, and other non-food users consume a hundred thousand or so tons annually.

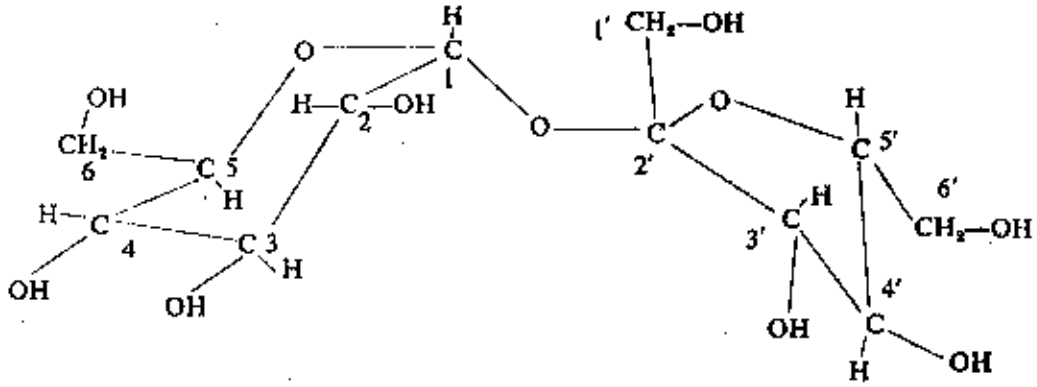
Structure and properties

Sucrose is a sweet, crystalline (monoclinic) solid which melts at 160–186°C, depending on the solvent of crystallization. It is optically active and has a specific rotation $[\alpha]_D$ of +66·53° (*c*. 26 in water).

The structure of sucrose is shown in Figure 1-1. Work on structure and configuration up to 1949 has been reviewed by Levi and Purves [*Advances in Carbohydrate Chemistry*, 4, 1 (1949)]. D-Glucose and D-fructose, the hydrolysis products, are linked through the anomeric atoms. A six-membered "pyranose" ring is present in the D-glucose portion and a five-membered "furanose" ring in the D-fructose portion. Further work with enzymes, chemical reactions, and crystallography assigned the α-configuration to the glucose moiety

and the β-configuration to the fructose portion [Koshland et al., *J. Biol. Chem.* **208**, 139 (1954); Lemieux et al., *J. Am. Chem. Soc.* **78**, 4117 (1956); ibid., **80**, 2243 (1958); *Can. J. Chem.* **31**, 949 (1953); ibid., **32**, 340 (1954)]. Part of Lemieux's work was sponsored by the Sugar Research Foundation (see References).

Fig 1-1
Structure of sucrose



α-D-Glucopyranosyl-β-D-fructofuranoside

The system of numbering the carbon atoms in the sucrose molecule was proposed by Hockett and Zief (72). The carbons in the D-glucose moiety are given simple numerals and those in the D-fructose moiety, prime numerals.

Reactivity

Sucrose has three hydroxyls in primary positions (—CH_2OH) and five in the secondary positions (—CHOH—). The reactivity of the hydroxyl groups of the carbohydrates has been reviewed by Sugihara [*Advances in Carbohydrate Chemistry*, **8**, 1 (1953)]. Little is known about the relative reactivities of the hydroxyl groups of sucrose except that the primary hydroxyls at the 6, 1′, 6′ positions show more reactivity than those at the secondary positions. As will be discussed later under sulfonic acid esters, tosylation and subsequent replacement of the tosyl groups with iodide indicate that the hydroxyls of the 6 and 6′ positions are more reactive than that at the 1′ position. Monoesterification of sucrose with fatty acids shows a similar priority of the 6 and 6′ hydroxyls, and also indicates the preference of 6′ to the 6 position by a ratio of about 2:1 (97). A brief recent study of methylation with dimethyl sulfate in 30% aqueous sodium hydroxide concludes that the fructose moiety undergoes methylation about three times more rapidly than the glucose portion (261b).

Although the three primary hydroxyls have greater reactivity in general, attempts to produce exclusively the 6, 1′, 6′ trisubstituted derivative give complex mixtures, as discussed in the chapter on sulfonic acid esters. However, selective substitution restricted to the three primary hydroxyls has recently been reported with chlorotricyclohexylsilane in excess amounts (11).

The ionization constants of sucrose and some of its derivatives and their change in alkaline medium have also been studied recently. In alkaline media sucrose and other polyhydroxy compounds undergo ionization to different extents (209, 261a).

Derivatives

The presence of five secondary and three primary hydroxyl groups in the sucrose molecule makes possible the synthesis of an enormous number of sucrose derivatives. Substitution with just one type of group could theoretically give some 255 different compounds, and with mixed groups the number becomes astronomical. For example, there are 1.6×10^7 possible octa-substituted derivatives with eight different substituents. However, the preparation of derivatives is hampered by the ease of hydrolysis (inversion) in acidic media of the glycosidic linkage to glucose and fructose. Furthermore, the steric hindrance imposed on some of the hydroxyl groups often makes complete substitution difficult.

Sucrose like other alcohols undergoes a number of reactions. Esters are the most readily prepared products. Other products include ethers, halogen derivatives, and similar compounds produced by displacement. Because of the possibility of several isomers, only the octa-derivatives and some of the partially substituted derivatives are well-defined, chemically uniform products. The number of such derivatives is probably less than 100. A significant percentage of this number have been prepared by chemists whose work was supported by the Sugar Research Foundation, Inc.

Degradation products

Sucrose is readily attacked by acids, oxidizing agents, and also by alkalies and catalytic hydrogenation. Because of the sensitivity of the molecule, sucrose is partially or wholly degraded by these reagents to compounds of lower molecular weight. The products thus formed include acids, glycols, higher polyols, and cyclic compounds. The action of a number of microorganisms—fungi, molds, yeast, and bacteria—also produces these materials. Degradation products of interest include lactic acid, levulinic acid, mannitol, piperazine, and imidazole derivatives.

Commercial significance

The commercial utilization of sucrose chemicals is increasing. Only a handful of compounds are presently used by the chemical and allied industries or other nonfood groups. Sucrose octaacetate and sucrose acetateisobutyrate are manufactured in substantial volume. Both esters are additives for specialty polymers, chiefly in adhesives. The octaacetate is a bitter chemical also used as a denaturant for alcohol. Cyanoethyl sucrose is manufactured on a limited basis for modification of specialty adhesives in the electronics industry. Mannitol is an important product in the food industry.

The Sugar Research Foundation has sponsored work on applications of sucrose chemicals. The most promising results have been obtained with sucrose ester detergents and surface coatings. Sucrose surfactants have been produced by a number of companies in the United States, Germany, Japan, and elsewhere. Although still under test, they show excellent promise as nontoxic, nonionic, highly detersive agents. Sucrose-based surface coatings also exhibit good properties, particularly those made from sucrose linseedate. Various other polymers and plastics have been prepared or modified with sucrose.

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ON THE QUESTION OF THE TERATOGENIC AND EMBRYOTOXIC EFFECTS OF CYCLAMATE, SACCHARIN AND SACCHAROSE

by

Claus Klotzsche

The use of synthetic sweeteners has increased steadily in recent years. The reason for this is not only an increase in diabetes morbidity. They are also used as calorie-free sweetening agents by people who do not wish to or are not supposed to consume many calories. This development can be observed in every country with a high living standard, and is still continuing.

It was inevitable that with the increasing significance of these sweetening agents, interest in them should also increase. This interest has been manifested in a great number of publications, both in specialized journals and in periodicals of the lay press.

Naturally, sweeteners that are added to food must, like other food additives, be subjected to a thorough examination of their innocuousness. This was already done at a much earlier time, for neither saccharin nor cyclamate are products of a recent time.

Saccharin was discovered in 1879 by Remsen and Fahlenberg (13), by accident, just as cyclamate was discovered 58 years later by Audrieth and Sveda (2). The sweetening power of these synthetic compounds is considerably greater than that of raw sugar; for instance, that of water-free refined sodium saccharin is approximately 475 times greater.

As early as 1886, extensive examinations were performed using dogs and rabbits (14), and in 1888, in France, a commission for examining the question of the harmfulness of saccharin was established (1). By now it is hardly possible to cite completely all the literature dealing with this question. A very detailed treatment, with extensive bibliographical data, can be found in Dungard (3, 4). Recent works no longer deal with saccharin, as full agreement concerning its harmlessness has been reached. Discussion has been excited concerning sodium or calcium cyclamate, which are recently risen to prominence because of its physico-chemical advantages. Its sweetening power is some 30 to 35 times greater than that of raw sugar. Cyclamate was first introduced in 1950. In 1955, the Food Protection Committee of the Food and Nutrition Board in the USA published a summarizing report (9), which concluded that the nutritively valueless sweet-

ening substances, saccharin and cyclamate, are harmless for dietetic purposes from the standpoint of health. The FDA also confirmed the harmlessness of cyclamate in several publications (18), as did the Food Addittives and Contaminants Committee in its reports of 1966 and 1967 (8). The WHO established a temporary acceptable intake of 50 mg/kg for cyclamate.(19). Recently, the FDA went beyond this, and considers 70 mg/kg as harmless (18). For adults, this corresponds to about 3.5 to 5.0 g cyclamate per day. In Europe, the average usage is very much lower than these daily amounts.

The discussion concerning cyclamate was renewed when Tanaka (16) reported about experiments in which he found an embryotoxic effect of cyclamate on mice. Interestingly enough, other Japanese animal experiments, among others those of Sumi (15), found no correspondingly extensive spreading in cases of congenital hydrocephalus in rabbits after high doses of sugar. In another publication, Tanaka attempted to set up a fetal LD_{50} (17). Lorke (6) also went into this at a later date. Then, in 1965 and 1967, Hess and Berse (10, 11) published the results of chronic feeding experiments using rats that received 5 to 10% calcium cyclamate in their food. In the generational experiments, essential differences in respect to the controls resulted both as concerned the weight of the younger animals and the number of surviving foetuses in the subsequent period, up to the twenty-first day. Palmer and Mantcher (12) could determine no significant influence of cyclamate on rabbits in doses of 650 and 1250 mg/kg daily between the seventh and eighteenth day of gestation. Absorption of the foetuses, litter size, weight at birth and deformations all agreed with the control animals.

The more recent publications concerning cyclamates and the partly contradictory results they report induced us to check the question of the teratogenic effect of the two most used synthetic sweeteners on rabbits. For purposes of comparison, saccharose was also included in the experiments.

Methodology

White New Zealand rabbits were used. The day on which sperm could be detected in the vagina was designated as 0 day. From the sixth to the eighteenth day of gestation, the animals received the substances to be tested or the solvent in the highest dose by means of a throat tube. On the twenty-ninth day, the animals were killed with pentothal and the foetuses were removed by sectional delivery. At the same time, the implantations and absorptions, as well as the Corpora lutea, were noted. The full-grown foetuses were immediately killed with ether and then exenterated, x-rayed and prepared for fixing. The skeletons were stained with alizarin red S, irradiated and poured separately into plastic resin.

Distribution of the animals was arbitrary, so that at all times, the most various doses and substances, or controls, were in the experiment. For each group, 10 animals were used.

The cyclamate amount recommended by the WHO, 50 mg/kg per day, served as a basis. To this base dose were added one twice

as large and one five times as large. Pure Na-cyclamate, which was free from by-products even in the thin layer chromatogram, was used. For saccharin and saccharose, this base dose served the purpose of establishing the doses in relation to sweetening strength. A control group received only the highest dose of the solvent alone, i.e. 20 ml water per kg, as it had to be selected for saccharose, because of the latter's solubility. In the case of cyclamate and saccharin, 5 ml water per kg were used as solvent. We added two more control groups, which remained untreated. The dosage plan can be seen from the following listing:

"Merck" saccharose, Pharmacopoe Qual.	2, 4, 10 g/kg/day
Na-cyclamate	50, 100, 250 mg/kg/day
Na-saccharin	5, 10, 25 mg/kg/day
Solvent (water)	20 ml/kg/day
Controls	untreated

Results

The results are compiled in the appended tables. In tables 1-4, besides the weight gain of the mother animals from 0 to the 29th day, there are also included in the analysis the Corpora lutea (CL), the implantations (IP), the living and dead foetuses (LF, DF), their weight and that of the placentas, as well as the number of those dead as foetuses (FD), those dead as embryos (ED) and the absorptions (AS). In tables 5 and 6, the results of the examinations of the foetuses are indicated. Under organ anomalies the slight deviations are specified, whereby the numbers under "gall bladder" indicate those animals that had no gall bladder. In the case of bone alterations, defective ossifications in the form of retardations form one group. In the case of the sternum, however, alterations determined by retardations do not occur without involving deformities. These deviations are specified separately as "anomalies". The last three columns concern the actual deformities. Clearly included in these are cleft palates and skull deformities. Under stomach lining defects, all variations from simple navel rupture to extensive hernia are included.

The mother animals revealed no peculiarities in their development. The weight development was different, but revealed no pronounced deviations from that of the controls.

The number of implantations is somewhat lower in the case of the saccharose animals with the largest dose than it is in the case of the cyclamate and saccharin animals and the controls, as is the number of living foetuses; on the other hand, the number of absorptions is greater. Statistically confirmed differences do not exist. The average weight of the foetuses is somewhat higher in the case of the saccharose animals than in the other cases, but still remains within the normal range. The same is true for the placenta weights. The number of foetal deaths is not striking; in the case of embryonic deaths, 100 mg/kg cyclamate reveals a somewhat higher rate, which is slightly above that of the controls (1.5:1.3). Aside from the fact that this

difference is not significant, there exists no dose dependency, for in the case of 250 mg/kg cyclamate, the number of embryonic deaths is on the lower limit of the normal range. The number of implantation points was much greater in the case of 100 mg/kg cyclamate than in the other cases, including the controls; the number of living foetuses, however, corresponded to that of the other groups.

From the results it can be seen that there were no essential differences between the controls and the animals treated with cyclamate, saccharin and saccharose, either as concerned the mother animals or as concerned the foetuses.

The deformities (table 6) reveal no particular distribution that would permit any conclusions concerning the effect of one of the three substances tested. One cleft palate and two skull deformities, as well as one stomach covering defect, are found only among the untreated control animals. They are distributed as follows:

No. 156, living foetus:

Microcephalus, cleft palate, defective ossification of the extremities and skull bones, stomach and large intestine not completely formed.

No. 133, dead foetus:

Missing skull, chest and stomach open, all organs exposed, front extremities have only 4 toes and are bent, very marked kyphosis/lordosis, 2 adrenals on both sides.

The third marked deformity is found in an animal that had received 5 mg/kg saccharin:

No. 03, living foetus:

Missing skull, therefore undifferentiated muscle tissue, excessive skin, defective ossification of the spinal column.

Of the stomach covering defects, only that of No. 133 is very marked, the others are slighter.

The three cleft palates (one in the case of an untreated animal and two in animals with 10,000 mg/kg saccharose) are also distributed insignificantly.

Discussion of the Results

If the results of the experiments on the rabbits are summarized, it can be determined that no one of the three substances examined demonstrates a teratogenic or embryotoxic effect under the conditions of our experiments. Neither the number of implantations nor that of the litter sizes, the weight of the foetuses and placentas, the number of foetal and embryonic deaths and that of the abortions were decreased. Deformities as a result of the treatment did not appear.

This result corresponds to the findings made by Fritz and Hess (5) on rats and Berke (7) on mice, using the same dosage. Our experiments were performed with very pure Na-cyclamate or saccharin. Thus the question is raised as to whether the findings reported by some researchers were not caused by the often extremely high doses and the impurities in the solutions. Except for England, no country has yet set up purity requirements for cyclamate. This question should in future be given more attention.

However, each individual researcher should also be sure of how pure the cyclamate he is using is, and present data concerning this fact in his publications. In this way, some negative results would certainly be explained.

Summary

Na-cyclamate, Na-saccharin and saccharose, administered orally to pregnant rabbits in increasing doses from the sixth to the eighteenth day of gestation during the period of organogenesis, lead to no teratogenic or embryotoxic effects.

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Table 1

A.	B.		C.							
					Foetus	C_1	C_2			
Präparat	Mutter-tiere Gew.-Zunahme %	CI	IP	LF ♂ ♀	TF	Gewicht g	Placenta-gewicht g	FT	ET	RS
Kontrollen I unbehandelt 1.	x̄ 12,4 s 5,60	x̄ 6,6 s 1,71	x̄ 9,9 s 2,09	56 39 x̄ 6,5 s 0,76	x̄ 6,2	x̄ 34,5 s 6,58	x̄ 6,71 s 1,17	x̄ 0,2	x̄ 0,5	x̄ 2,5
Kontrollen II unbehandelt 2.	x̄ 10,6 s 6,27	x̄ 5,7 s 2,98	x̄ 8,6 s 1,05	34 46 x̄ 8,0 s 1,76	0	x̄ 30,9 s 3,05	x̄ 5,64 s 0,805	0	x̄ 0,1	x̄ 0,5
Lösungsmittel I 20 ml/kg 3.	x̄ 9,4 s 5,56	x̄ 6,1 s 2,56	x̄ 9,6 s 1,95	56 32 x̄ 7,1 s 3,44	x̄ 0,2	x̄ 33,0 s 5,35	x̄ 6,7 s 1,27	x̄ 0,3	x̄ 0,6	x̄ 1,4
Lösungsmittel II 20 ml/kg 4.	x̄ 10,2 s 6,18	x̄ 6,7 s 3,09	x̄ 9,7 s 2,10	34 34 x̄ 6,9 s 0,97	x̄ 0,1	x̄ 32,7 s 6,93	x̄ 5,9 s 1,04	x̄ 0,1	x̄ 1,3	x̄ 1,7

Key:
A. = Preparation B. = Weight gain in % of mother animal
C. = Foetuses C_1 = weight in g C_2 = placenta weight in g
FT = FD (see text) ET = ED (see text) RS = AS (see text)
1. = Controls I, untreated 2. = Controls II, untreated
3. = Solvent I 4. = Solvent II

Table 2

A.	B.		C.							
					Foetus	C_1	C_2			
Präparat	Mutter-tiere Gew.-Zunahme %	CI	IP	LF ♂ ♀	TF	Gewicht g	Placenta-gewicht g	FT	ET	RS
Cyclamat 50 mg/kg	x̄ 13,9 s 4,55	x̄ 5,7 s 2,0	x̄ 8,9 s 2,18	51 44 x̄ 7,5 s 0,92	0	x̄ 30,1 s 9,2	x̄ 6,4 s 1,61	x̄ 0,2	x̄ 0,6	x̄ 0,4
Cyclamat 100 mg/kg	x̄ 13,9 s 5,90	x̄ 6,0 s 3,94	x̄ 10,4 s 1,77	51 44 x̄ 7,5 s 1,77	x̄ 0,2	x̄ 31,5 s 4,82	x̄ 6,8 s 1,47	x̄ 0,1	x̄ 1,5	x̄ 1,1
Cyclamat 250 mg/kg	x̄ 9,6 s 5,82	x̄ 5,5 s 2,46	x̄ 8,8 s 1,61	27 48 x̄ 7,5 s 1,26	x̄ 0,1	x̄ 32,9 s 6,58	x̄ 5,9 s 1,18	x̄ 0,2	x̄ 0,3	x̄ 0,2

For key see above.

Table 3

A. Präparat	B. Mutterliere Gew.-Zunahme %	CL	IP	C. Foeten C_1 LF ♂ ♀	TF	Gewicht g	C_2 Placentagewicht g	FT	ET	RS
Saccharose 2 000 mg/kg	x̄ 9,4 s 4,97	x̄ 7,6 s 2,17	x̄ 8,3 s 2,63	34 41 x̄ 7,5 s 3,31	0	x̄ 31,3 s 7,12	x̄ 6,9 s 1,95	x̄ 0,3	x̄ 0,6	
Saccharose 4 000 mg/kg	x̄ 8,2 s 4,99	x̄ 7,6 s 2,06	x̄ 9,9 s 1,37	35 43 x̄ 7,8 s 2,69	x̄ 0,8	x̄ 31,1 s 5,42	x̄ 6,3 s 1,29	x̄ 0,1	x̄ 0,5	x̄ 0,2
Saccharose 10 000 mg/kg	x̄ 7,4 s 7,58	x̄ 6,5 s 2,22	x̄ 7,9 s 1,97	24 31 x̄ 5,5 s 2,46	0	x̄ 30,6 s 8,54	x̄ 6,1 s 1,55	x̄ 0,3	x̄ 0,5	x̄ 1,7

For key see Table 1.

Table 4

A. Präparat	B. Mutterliere Gew.-Zunahme %	CL	IP	C. Foeten C_1 LF ♂ ♀	TF	Gewicht g	C_2 Placentagewicht g	FT	ET	RS
Saccharin 5 mg/kg	x̄ 12,3 s 8,47	x̄ 7,2 s 2,39	x̄ 8,8 s 2,74	51 47 x̄ 7,8 s 2,39	x̄ 0,1	x̄ 33,3 s 8,34	x̄ 6,7 s 2,11	x̄ 0,3	x̄ 0,2	x̄ 0,4
Saccharin 10 mg/kg	x̄ 13,6 s 9,02	x̄ 5,7 s 2,91	x̄ 8,3 s 2,91	29 43 x̄ 7,2 s 2,82	x̄ 0,1	x̄ 37,2 s 9,0	x̄ 7,4 s 1,48	x̄ 0,3	x̄ 0,1	x̄ 0,6
Saccharin 25 mg/kg	x̄ 14,1 s 8,95	x̄ 5,4 s 2,41	x̄ 9,1 s 2,6	37 37 x̄ 7,4 s 2,76	0	x̄ 33,2 s 6,13	x̄ 6,2 s 0,95	x̄ 0,3	x̄ 0,7	x̄ 0,7

For key see Table 1.

Table 5

A.		B. Dosis		C.	D. Organanomalien									E.
	Gruppe	mg/kg	ml/kg	Anzahl der Skelette	a. Auge	b. Magen	c. Dickdarm	d. Gallenblase	e. Milz	f. Niere	g. Harnblase	h. bils. 2 Nebennieren	Lidspalte offen
1.	Kontrollen I	—	—	67	—	1	1	1	—	—	—	1	—
	Kontrollen II	—	—	60	—	—	—	1	—	—	—	—	—
2.	Lösungsmittel I	—	20	73	—	—	—	1	—	—	—	—	—
	Lösungsmittel II	—	20	66	1		—	1	—	—	—	—	—
3.	Saccharose	2 000	20	75	—	—	—	—	—	—	—	—	—
	Saccharose	4 000	20	86	—	—	—	6	1	—	1	—	5
	Saccharose	10 000	20	55	—	—	—	—	—	1	—	—	4
4.	Cyclamat	50	5	75	—	—	—	—	—	—	—	—	—
	Cyclamat	100	5	77	—	—	—	1	1	—	—	—	—
	Cyclamat	250	5	76	—	—	—	—	—	—	—	—	—
5.	Saccharin	5	5	79	—	—	—	—	—	—	—	—	—
	Saccharin	10	5	73	—	—	—	1	—	—	—	—	—
	Saccharin	25	5	74	—	—	—	—	—	—	—	—	—

Key:
A.= group B.= dose C.= number of skeletons D.= organ anomalies
a.= eye b.= stomach c.= large intestine d.= gall bladder
e.= spleen f.= kidneys g.= urine bladder h.= 2 adrenals on both sides
E.=lid cleft open

1.= controls I and II 2.= Solvent I and II .
3.= saccharose 4.= cyclamate 5.= saccharin

Table 6

A.		B. Dosis		C.	D. Mangelhafte Ossifik. (Retardierung)					E. Anomalien		Mißbildungen		
	Gruppe	mg/kg	ml/kg	Anzahl der Skelette	a. Schädel	b. Wirbelsäule	c. Extremitäten	d. Sternum		E_1 Sternum	e. Gaumenspalte	f. Schädel	g. Bauchdeckendefekte
1.	Kontrollen I		—	67	2	—	1	2		—	1	2	1
	Kontrollen II		—	60	—	—	—	1		—	—	—	—
2.	Lösungsmittel I	—	20	73	—	—	—	1		—	—	—	—
	Lösungsmittel II	—	20	66	—	—	—	—		—	—	—	—
3.	Saccharose	2 000	20	75	1	—	1	—		—	—	—	—
	Saccharose	4 000	20	86	—	—	—	—		1	—	—	2
	Saccharose	10 000	20	55	—	—	—	4		1	2	—	—
4.	Cyclamat	50	5	75	—	—	2	1		1	—	—	—
	Cyclamat	100	5	77	—	—	—	3		—	—	—	—
	Cyclamat	250	5	76	—	—	—	—		—	—	—	—
5.	Saccharin	5	5	79	—	1	—	2		—	—	1	—
	Saccharin	10	5	73	—	—	—	1		—	—	—	—
	Saccharin	25	5	74	—	—	—	—		—	—	—	1

Key:
A.= group B.= Dose C.= number of skeletons D.= defective ossification (retardation) a.= skull b.= spinal column
c.= extremities d.= sternum
E.= anomalies E_1 = sternum F.= deformities e.= cleft palate
f.= skull G.= stomach covering defects

1 through 5 as above.

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Dietary carbohydrates and lipid metabolism

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The role of dietary carbohydrate in lipid metabolism has been known for some time, and this knowledge forms the basis of many agricultural practices. The conversion of carbohydrate into fat is also accepted in man and forms the mainstay of some therapeutic manoeuvres. Despite the widespread acceptance of a metabolic relationship between carbohydrate and lipid, there has been little critical analysis of this relationship. The dietary carbohydrates have been assumed to be bland constituents, all treated alike as a result of the activities of the gut and consequently, until recently, few suggestions have been made that would implicate them in disorders of lipid metabolism.

Some evidence that dietary carbohydrate may play a significant part in disease was seen in the study of kwashiorkor. The child with this malnutritional disorder not only shows the signs of protein deficiency but in addition has pathological changes which can only be due to a relative dietary excess of calories over protein, and the calories are usually supplied by carbohydrate. The large quantity of fat in the liver and the presence of reasonable quantities of depot fat mark out this malnutritional disorder from marasmus in which the child not only has a deficient protein intake but also has a similarly deficient calorie intake, the marasmic child being wasted, with no depot fat or excess liver lipid. The remarkably low serum albumin levels in kwashiorkor led to the belief that deficiency of protein was the principal dietary fault, but some experimental work in man has shown that even on a high protein intake the serum albumin is reduced by a high carbohydrate intake (Coles & Macdonald, 1964, in preparation). Thus, calories in the form of carbohydrate may have a malevolent effect in these children. It is of interest to speculate whether it is the carbohydrate or the calories that result in the changes characteristic of kwashiorkor and therefore whether calories in the form of fat would also result in the same clinical picture.

Experiments on animals

In an attempt to understand the effects of dietary carbohydrates on lipid metabolism, rabbits were placed on diets so arranged that the protein intake was kept constant but the carbohydrate (and thereby the calorie) intake varied. The results

showed that the animals with the relative excess of dietary carbohydrate lost weight more rapidly, and their livers became fatty. Also, the concentration of total serum proteins fell more rapidly in the animals eating the relative excess of dietary carbohydrate (Macdonald & Gharavi, 1960). The changes in the extent of the liver fat had been described earlier in rats (Best, Hartroft, Lucas & Ridout, 1949) and in pigs (Heard, Platt & Stewart, 1958) in which the serum protein level also fell to a greater extent with an increase in intake of carbohydrate.

Observations on children

More detailed examination of the lipids in the depot fat and livers of children with kwashiorkor revealed that the increase in lipid in the liver was mainly as glycerides and phospholipids, with no increase in sterol esters, and the fatty acid pattern changed in a similar way in both liver and depot fat (Macdonald, 1963). Examination of the serum lipids during recovery from kwashiorkor showed a marked rise in the glycerides followed by a fall to normal levels in about 3 weeks. Evidence was also obtained at the same time which made it unlikely that the accumulation of lipid in the liver was from the adipose tissue (Macdonald, Hansen & Bronte-Stewart, 1963).

Thus it seems that a relative excess of dietary carbohydrates, at least in conjunction with a low protein intake, can in children and experimental animals give rise to derangement of lipid metabolism.

Different responses to various dietary carbohydrates

It has been tacitly assumed until fairly recently that all dietary carbohydrates exert the same influence on lipid metabolism. This is now known to be incorrect, largely as a result of studies of effects of various dietary carbohydrates on serum cholesterol levels in the rat (Portman, Lawry & Bruno, 1956), rabbit (Grant & Fahrenbach, 1959), chick (Grant & Fahrenbach, 1957) and germ-free chick (Kritchevsky, Kolman, Guttmacher & Forbes, 1959). The level of serum cholesterol in the cebus monkey was not, however, influenced by the substitution of sucrose for starch in the diet (Portman, Hegsted, Stare, Bruno, Murphy & Sinisterra, 1956). The different influences of various dietary carbohydrates on growth have also been reported in the rat (Harper & Katayama, 1953) and in the chick (Monson, Dietrich & Elvehjem, 1950). In all these accounts of different effects, sucrose is the carbohydrate that seems to be associated with the highest level of serum cholesterol, and with the least growth.

To compare the effects of various carbohydrates on the amount and composition of liver and depot fat, adult rabbits were given a constant protein intake with different amounts of various carbohydrates. The results of these experiments showed that the amount of liver lipid was directly related to the mean daily carbohydrate intake. Also, weight for weight, sucrose was associated with more liver lipid than was liquid glucose BPC (a partial hydrolysate of starch), and the latter with more liver lipid than starch. Variations in the intake of sucrose and liquid glucose were associated with corresponding variations in cholesterol in the liver (Macdonald, 1962).

Experiments on man

The translation of these findings to man cannot be done with certainty and, therefore, the experimental subject must be man in order to assess the relevance of the animal work. In 1960 it was found that diets containing carbohydrates of fruits, vegetables and legumes produced lower serum cholesterol values than those containing the same number of calories supplied by sucrose and milk sugar (Keys, Anderson & Grande, 1960).

In this laboratory an experiment was undertaken on seven adult males which consisted of eating daily 500 g of one type of carbohydrate for 25 days with protein in the form of lean meat and with green vegetables and water-preserved fruit. After an interval the dietary régime was repeated with a change in the type of carbohydrate used. The two carbohydrates used were maize starch and sucrose. Weekly samples of fasting serum lipid and depot fat were analysed during the time on the diet (Macdonald & Braithwaite, 1964). The starch diet caused a fall in the level of the serum lipids whereas the sucrose diet produced a rise. The levels of sterol esters and phospholipids in the serum were reduced when the starch diet was given but were not altered by the sucrose diet. The glyceride level was not affected by the starch diet but rose on the sucrose diet (Table 1). Changes were also noticed in the fatty acid pattern of the serum and depot lipids and are summarized in Table 2. There was also some evidence in these experiments compatible with the production of liver damage while the subjects were on the starch diet.

Table 1. *Changes in the concentration of serum lipid and its fractions found in adult males on a diet low in fat and high in starch or sucrose*

	Starch diet	Sucrose diet
Total serum lipid	Decreased	Increased
Sterol esters in serum	Decreased	Unchanged
Glycerides in serum	Unchanged	Increased
Phospholipid in serum	Decreased	Unchanged
Total cholesterol in serum	Decreased	Unchanged

Table 2. *Changes in the fatty acid pattern in the serum and depot lipids in adult males on a diet low in fat and high in starch or sucrose*

	Serum lipid		Depot fat	
Fatty acid	Starch diet	Sucrose diet	Starch diet	Sucrose diet
Myristic	—	—	Unchanged	Unchanged
Palmitic	Increased	Increased	Unchanged	Unchanged
Palmitoleic	Unchanged	Unchanged	Unchanged	Decreased
Stearic	Unchanged	Unchanged	Unchanged	Unchanged
Oleic	Increased	Unchanged	Increased	Increased
Linoleic	Decreased	Decreased	Decreased	Unchanged

Acne was traditionally considered by the dermatologist to be associated with a high carbohydrate intake (Barber, 1930) and treatment was directed towards lessening the carbohydrate intake. With this empirical treatment in mind, the fatty acid composition of the sebum on the skin of the face was studied in the men while on

the diets described above. Some of the fatty acid proportions were changed by the high-carbohydrate diets and the pattern of the change was not the same for starch as it was for sucrose. There may be, therefore, some metabolic justification for the therapy of reducing the intake of carbohydrates in certain skin disorders, and the skin lesions in kwashiorkor may be, in part, the product of the carbohydrate.

One of the results of exercise is an alteration in the serum lipid level, depending on the nature and duration of the exercise (Kontinnen, 1959). Since the nature of the diet during exercise may also influence the serum lipids the opportunity was taken of studying the effect of exercise while the subjects were consuming one of two types of carbohydrate. The exercise was the 55 mile walk from London to Brighton by medical students. It was found that after walking 27 miles the students who ate glucose only had a significantly lower serum cholesterol than those eating sucrose. This difference was even more marked after 55 miles walking (Macdonald, 1964, in preparation).

Possible causes for metabolic differentiation of dietary carbohydrates

One possibility for the differences in lipid response observed may be that glucose does not follow the same metabolic pathway as fructose. Against this argument is the response by the liver to liquid glucose BPC, the amount of lipid found being intermediate between the amounts found when starch and sucrose were given (Macdonald, 1962). As liquid glucose BPC, when hydrolysed, gives glucose molecules only the response should have been similar to that with starch if the different metabolic handling of fructose was the explanation. Also rats given sucrose, glucose or fructose as the dietary carbohydrate had serum cholesterol levels that were not significantly different (Portman, Lawry & Bruno, 1956).

Can the explanation lie in the influence of these carbohydrates on intestinal microflora? In support of this hypothesis are the findings that the intestinal microflora is influenced by the type of dietary carbohydrate (Peterson, Dick & Johansson, 1953) and that the fall in serum cholesterol level in rats on a high-starch diet is eliminated when a poorly absorbed antibiotic is given (Portman, Lawry & Bruno, 1956). The hypocholesterolaemic effect of glucose in chickens is reduced by the addition of an antibiotic to the diet (Kritchevsky, Grant, Fahrenbach, Riccardi & McCandless, 1958). If this hypothesis is accepted then it is difficult to explain the fact that the reduction in serum cholesterol after about 7 h walking is greater when glucose is eaten than when sucrose is eaten. It is unlikely that any great change in gut microflora could have occurred in this relatively short time.

There is, at the moment, no adequate explanation for the different effects of dietary carbohydrates on lipid metabolism, nor any convincing leads as to the site of the metabolic differentiation. Dietary carbohydrates, it can be concluded, do exert an influence on lipid metabolism; the extent of this influence is largely unknown, as are the metabolic pathways involved. However, it is quite clear that their effect is not negligible either as a group or as individual substances. The clinical significance of these findings must await further knowledge.

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SUCROSE—WHAT ELSE BESIDES CARIES?

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SUCROSE or table sugar is a substance which has made history. It was in no small measure responsible for the slave trade, the repercussions of which seem to be magnified by time. Sucrose still is the major factor in the economy of most of the West Indies and a sudden lack of demand would lead to untold hardship (Aykroyd 1967).

In terms of disease in man the only charge that can be laid against sucrose with any expectation of a verdict of guilty is its special ability to facilitate the development of dental caries. Whether those metabolic effects of sucrose that are different from the other dietary carbohydrates are advantageous or disadvantageous to man cannot, at the present time, be answered. The various common types of carbohydrate consumed by man are set out in Table I.

TABLE I

CARBOHYDRATES COMMONLY CONSUMED AND THEIR SYNONYMS

Glucose	Dextrose, grape sugar, corn sugar
Fructose	Laevulose, fruit sugar
Maltose	Malt sugar
Lactose	Milk sugar
Sucrose	Cane sugar, beet sugar, table sugar, 'sugar'
Starch	

Before considering the special role, if any, of sucrose compared with other dietary carbohydrates, it must be borne in mind that sucrose, as a carbohydrate, is a source of calories, its importance being in the fact that it is both pleasant and cheap. As a commercial product it is remarkably pure. It has been labelled as containing 'empty calories' and this was meant in a derogatory sense, suggesting that foods should be eaten that contain not only calories but contain in addition proteins, vitamins, salts, etc., that the body can also use.

Within the past few years dietary carbohydrates have been implicated in the aetiology of atherosclerosis and thus of angina and myocardial infarction. The basis of this claim rests on the recent finding (Ahrens, Hirsch, Oette, and Farquhar 1967) that dietary carbohydrates may in some people cause a rise in

serum lipid concentrations, and high serum lipid concentrations are found more frequently in subjects with atherosclerosis. If this implication could be substantiated it would be desirable to find out which members of the community are prone to this type of carbohydrate lipid disorder and then to advise the susceptible to cut down drastically their carbohydrate intake.

Is dietary sucrose in any way metabolically special? This suggestion strikes at the roots of chemical physiology where it has long been taught that it is not possible for the body to distinguish between the types of carbohydrate eaten. It was held that all dietary carbohydrates were converted to glucose before metabolism by the tissues. It is now known that this is too rigid a concept and that quantities of fructose do get into the peripheral blood and that these quantities may have important biochemical effects.

The peasants of Strasbourg have known for years that if they force feed their geese with maize they will produce a gargantuously obese bird whose excessively fatty liver will delight an expensive palate. This must be one of the early demonstrations that dietary carbohydrate can be converted by the body to fat. The fatty liver of children in the tropics with Kwashiorkor is very likely another example of relative excess of dietary carbohydrate being converted to fat and this fat accumulating in the liver. If an excess of carbohydrate in the diet, either relative or absolute, can increase the amount of fat in the liver are all the various dietary carbohydrates similar in this ability?

Obesity is the commonest form of malnutrition seen in the U.K. today and carbohydrates have the reputation for being a major factor in the aetiology of this condition, solely due to the fact that many people find carbohydrates, and especially sucrose, pleasant to taste. However, weight for weight, carbohydrates contain less than half the calories found in fat. Theoretically, all carbohydrates make the same contribution to the calorie-balance of the body but some recent studies have suggested that this theoretical concept may not be transferred to practice. Two reports of dietary experiments in baboons have shown that sucrose or fructose seems to result in more depot fat or greater body weight than the isocaloric intake of other carbohydrates (Allen, Brook, Lister, Sim and Warwick 1986; Brook and Noel 1969). Similar results have been reported in rats (Allen and Leahy 1968). There is, at the moment, no evidence that these findings apply to man.

Sucrose has different metabolic effects as compared with the other carbohydrates. In 1968 it was found that the respiratory [illegible]

ingestion of the same amount of glucose (Cathcart and Marwitz 1927). This difference in R.Q. was consistent with the view that more sucrose than glucose had been converted to fat.

The effects of different dietary carbohydrates on the levels of the various lipids in the serum also support the hypothesis that not all dietary carbohydrates are metabolically equal. The serum lipids and their level are of especial interest because of their association with atherosclerosis and myocardial infarction. In 1961 it was found that many cases of hypertriglyceridemia were caused by the carbohydrate in the diet (Ahrens *et al.* 1961) and if the daily intake of carbohydrate was reduced to 120 g for an adult the triglyceride level in the fasting serum fell. Subsequently there has been a report that sucrose is especially potent in inducing hypertriglyceridemia (Kuo, Feng, Cohen, Fitts and Miller 1967).

Studies have been carried out, in an attempt to learn whether the type of dietary carbohydrate can influence lipid metabolism in apparently healthy persons. In one such study lasting 25 days the dietary carbohydrate consisted either of raw corn starch or of sucrose. In young men it was found that the concentration of triglyceride in the fasting serum rose when the diet contained sucrose, but did not change when the carbohydrate was starch (Macdonald and Braithwaite 1964), and comparable findings have been reported from the U.S.A. and Israel (Kuo and Bassett 1965; Kaufmann, Pozanski, Blondheim and Stein 1966.)

Sucrose may have a different effect from starch on the serum triglyceride concentration because:

i. Sucrose is absorbed more rapidly than corn starch.

ii. Sucrose contains fructose, whereas starch does not.

To test which of these factors was responsible, male volunteers were given an entirely fat-free diet for 5 days. The protein content was 50 g of calcium caseinate per day, and apart from vitamin tablets, the only other constituent was carbohydrate. The carbohydrates used were raw cornstarch, which is absorbed slowly, and liquid glucose B.P.C. (a partial hydrolysate of starch), sucrose, maltose and glucose, which are absorbed rapidly. It was not possible to use fructose as one of the test carbohydrates because at the required level of intake its relatively slow rate of absorption resulted in an osmotic diarrhoea. The results of these experiments suggested that it was the fructose content and not rate of absorption that was associated with the increase in serum glyceride concentration. To confirm that fructose was more active in glyceride metabolism than was glucose, healthy men were given [illegible] and glucose labelled with the radio-isotope ^{14}C. No ^{14}C

was incorporated in the cholesterol but following fructose ingestion, the radio-activity in the triglycerides was much greater than after glucose ingestion (Macdonald 1968).

If dietary carbohydrates, and especially fructose, do play a part in the aetiology of ischaemic heart disease, then young women should react differently to fructose compared with men and post-menopausal women should respond to sucrose similarly to men, because coronary thrombosis is rare in women before the menopause. To test this hypothesis diets similar to those given to men, were given to young women and post-menopausal women. It was found that the sucrose diet was associated with a significant reduction in fasting triglyceride levels in young women and with a significant rise in the post-menopausal women (Macdonald 1968). Thus sucrose can raise the triglyceride concentration in the fasting serum of men and post-menopausal women, but not in pre-menopausal women.

Most of the experiments in man have been carried out with a diet that contains an abnormally large amount of sucrose and it could well be that lesser amounts have no effect at all, or have an effect that is only apparent after many years. The diet we usually eat consists of fat as well as carbohydrate, in addition to proteins. Diets were therefore devised which contained sucrose with cream or with sunflower seed oil in the kind of ratio of carbohydrate : fat normally found in a Western diet. Sunflower seed oil was compared with cream because the former is a highly unsaturated fat and there is undisputed evidence that such fats will lower a raised serum cholesterol concentration. Cream on the other hand is a saturated fat and would be more likely to raise the concentration of serum cholesterol. The results of these experiments showed that a mixture of cream and sucrose caused the fasting level of serum triglyceride to rise in every subject, whereas no rise was seen after a diet of sunflower seed oil and sucrose or after glucose with cream or sunflower seed oil (Macdonald 1967).

The older textbooks of dermatology advised the patient suffering from pustular acne to reduce his carbohydrate intake and the mother of the child with infective lesions on the skin was also told to cut down starches and sugars in the child's diet (Barber 1930). This advice was presumably given on clinical observation of the success of the treatment. It has recently been found that the amount of triglyceride in the skin surface lipids is affected by the amount of carbohydrate in the diet, and further more, by the type of carbohydrate ([illegible] 1967). There is some evidence that the [illegible] have a [illegible]

SUMMARY

Dietary sucrose, a substance considered to facilitate the development of dental caries, can also give rise to metabolic responses that are unlike those in response to starch and glucose, especially in lipid metabolism. The clinical significance of these differences in metabolism cannot yet be assessed.

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SERUM-FRUCTOSE LEVELS AFTER SUCROSE OR ITS CONSTITUENT MONOSACCHARIDES

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Summary. Meals of sucrose or of a mixture of its constituent monosaccharides, glucose and fructose, were given to healthy men and women students. The serum-glucose responses to the disaccharide and the monosaccharide mixture were similar. The serum-fructose level was significantly higher after sucrose than after a mixture of its constituent monosaccharides. The fructose response was similar in the men and the women: thus the different triglyceride response to fructose found between men and young women could not be due to different rates of absorption.

Introduction

THE fasting triglyceride level of healthy men can be influenced not only by the quantity but by the nature of the dietary carbohydrate (Macdonald and Braithwaite 1964, Wilnitz et al. 1964, A[illegible] 1965, McGandy et al. [illegible] been reported in patients [illegible] and Bassett 1965, [illegible] diets containing fructose [illegible] serum-glycerides were [illegible] when glucose replaced fructose [illegible] (Macdonald 1966). Further experiments [illegible] given meals of glucose or fructose [illegible] being uniformly labelled with [illegible] greater incorporation of the isotope into the [illegible] fraction of the serum after fructose [illegible] after a glucose meal (Macdonald 1968). These results suggest that fructose may be the carbohydrate [illegible] serum-glyceride levels. [illegible] fructose can raise the fasting [illegible] but not in young women (Beveridge [illegible]).

However, before attributing the raised fasting serum-triglycerides after dietary sucrose [illegible] fructose content it is necessary to see to what [illegible] fructose rises after a load of sucrose.

Methods

The subjects were [illegible] women students. On separate occasions [illegible] first, each volunteer was given either [illegible] of sucrose, or an equivalent amount [illegible] glucose monohydrate). [illegible] first and, a week later, the [illegible] were dissolved in water, and the [illegible] per kg. body-weight, to which [illegible] increase [illegible] before, and at 15, [illegible] Serum fructose and glucose [illegible] each sample. Glucose was [illegible] glucose-oxidase method. Fructose [illegible] Roe's (1934) method, [illegible]

Results

The mean values [illegible] glucose serum-levels are shown [illegible] each volunteer the fasting value of [illegible] glucose concentration was subtracted [illegible] readings, and the results thus obtained [illegible] interval were averaged for all participants [illegible] after the disaccharide and monosaccharide [illegible] compared. The rises in blood-[illegible] monosaccharide mixture did [illegible] significantly. However, the rise in fructose [illegible] significantly higher when fructose was given as the disaccharide than when it was given in the monosaccharide [illegible] at 30, 60, 90, and 120 minutes [illegible] minutes in women (figs. [illegible] all individuals showed the [illegible] serum-fructose response.

Discussion

The results show that serum-fructose rises significantly more after a sucrose drink than [illegible] equivalent amount of a mixture of fructose and glucose [illegible].

These findings could be explained by a difference either in the rates at which the disaccharide and the monosaccharide mixtures were delivered to the duodenum or the rates at which they were absorbed. Elias et al. (1968), using as an index the volume of a test meal remaining in the stomach 20 minutes after ingestion, have shown that sucrose and an equimolar mixture of fructose and glucose are emptied from the stomach, and thus delivered to the intestine, at the same rate, though the concentration was

TABLE I—MEAN (AND STANDARD ERROR) OF SERUM-GLUCOSE LEVELS AFTER SUCROSE AND AFTER FRUCTOSE AND GLUCOSE

—	Mean (±S.E.M.) serum-glucose levels (mg. per 100 ml.) after (min.):											
	Sucrose						Fructose and glucose					
	0	15	30	60	90	120	0	15	30	60	90	120
Men ..	76 (2·9)	99 (5·0)	102 (5·3)	72 (3·9)	65 (5·0)	69 (3·9)	91 (4·5)	114 (4·6)	125 (10·5)	94 (9·1)	82 (4·0)	81 (5·3)
Women	92 (4·4)	124 (8·4)	124 (6·9)	108 (10·9)	85 (1·0)	85 (3·6)	84 (3·9)	112 (6·3)	112 (7·9)	97 (5·9)	93 (6·0)	85 (5·6)

TABLE II—MEAN (AND STANDARD ERROR) OF SERUM-FRUCTOSE LEVELS AFTER SUCROSE AND AFTER FRUCTOSE AND GLUCOSE

—	Mean (±S.E.M.) serum-fructose levels (mg. per 100 ml.) after (min.):											
	Sucrose						Fructose and glucose					
	0	15	30	60	90	120	0	15	30	60	90	120
Men ..	0·2 (0·11)	3·1 (0·69)	5·8 (0·71)	6·2 (0·84)	9·1 (1·03)	9·8 (1·25)	0·0 (0·00)	1·9 (0·45)	3·7 (0·37)	5·0 (0·42)	5·8 (0·57)	6·3 (0·5)
Women	0·5 (0·38)	2·1 (0·61)	5·6 (1·12)	7·9 (0·90)	9·3 (0·80)	8·4 (0·62)	0·5 (0·38)	2·5 (0·63)	4·1 (0·69)	5·1 (0·52)	5·4 (0·37)	4·8 (0·5)

less than that used in our experiments. Gray and Ingelfinger (1966), using a double-lumen tube to study absorption from a perfused 30 cm. segment of gut in healthy young people, found the same absorption-rates of the monosaccharides from sucrose as from an equivalent fructose-glucose mixture: the serum-monosaccharide levels were not measured. This suggests that the observed difference in the rise of serum-fructose after the disaccharide and monosaccharide meals probably cannot be accounted for by differences in the rates of absorption of the sugars from the small intestine, and must presumably be accounted for in subsequent metabolism.

The site of hydrolysis of disaccharides has been identified, by differential centrifugation of intestinal preparations, as the brush border of the mucosal cells (Miller and Crane 1961). This has been further confirmed by the use of fluorescent antibodies against sucrase and lactase (Doell et al. 1965). With this information Detheridge et al. (1966) have suggested a model of the mucosal cell in which disaccharides are hydrolysed within the outer part of brush-border membrane, and the released monosaccharides are transported across a "permeability barrier" located deeper in the inner portion of the same membrane. Although they found the same rates of absorption of fructose and glucose from sucrose as from an equivalent monosaccharide mixture, Gray and Ingelfinger (1966) demonstrated that sucrose-hydrolysis rates exceeded monosaccharide-absorption rates and that some of the glucose and fructose diffused back into the lumen of the gut (Gray and Ingelfinger 1965). Sucrose hydrolysis could therefore allow fructose to enter the mucosal cell at a greater rate than it would in the monosaccharide form. Administration of fructose as the disaccharide sucrose may facilitate its passage across the mucosal cell, and thus account for the greater rise in serum-fructose observed after a sucrose meal than after a fructose-glucose meal. Clinical evidence that this is so is seen when 100 g. fructose by mouth usually results in an osmotic diarrhœa in adults whereas 200 g. or more of sucrose rarely produces diarrhœa.

Some fructose is converted to glucose before reaching the mesenteric blood, and a variety of experiments performed on human intestinal mucosa, both in vitro and at operation, have shown widely differing rates of conversion. Ockerman and Lundborg (1965), studying human jejunum at operation, found that up to 70% of absorbed fructose was converted to glucose as measured in mesenteric venous blood, whereas White and Landau (1965) found a maximum conversion-rate of 16%, as measured by the incorporation of ^{14}C from fructose-1-[^{14}C] and fructose-6-[^{14}C] into glucose and glycogen. The differences in blood-fructose levels after meals of sucrose and the fructose-glucose mixture reported here could then be accounted for by differing amounts of conversion of the fructose present in the mucosal cell in the different forms. This seems unlikely, however, since there was no significant difference in the rise in glucose levels after the two meals.

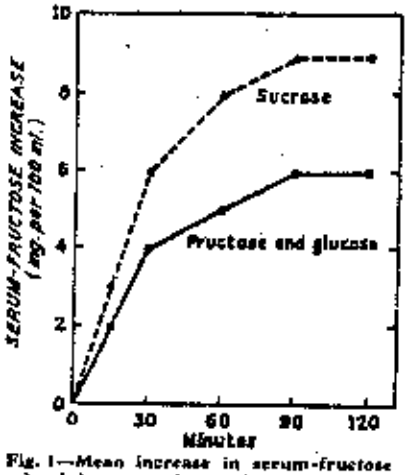

Fig. 1—Mean increase in serum-fructose level in man after sucrose and after fructose and glucose.

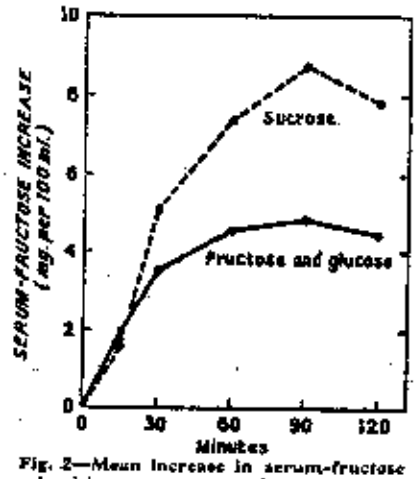

Fig. 2—Mean increase in serum-fructose level in young women after sucrose and after fructose and glucose.

Experiments similar to those described above have been carried out in rats: Dalquist and Thomson (1961) showed that rates of absorption of glucose and fructose from the intestine as measured with a double-lumen tube did not differ significantly whether the sugar was fed as the disaccharide or the monosaccharide mixture. Fructose was not converted to glucose in the rat (Kiyasu and Chaikoff 1957). However, in-vitro experiments on isolated rat small intestine by Chain et al. (1961) showed that absorption of sucrose led to a higher concentration of fructose on the serosal side than absorption of free fructose at an equimolar concentration. These results accord with our observations, and suggest that in the

as, at least, the difference in blood-fructose levels must be accounted for in the process of passage through the intestinal mucosa. We suggest, therefore, that in man the entry of fructose into the bloodstream may be facilitated by its absorption into the mucosal cells of the small intestine in the form of the disaccharide, sucrose: this suggestion would account for the greater rise in serum-fructose observed after sucrose than after an equivalent amount of the fructose-glucose mixture.

Fructose or fructose-containing compounds in the diet seem to be more glyceridogenic than many glucose or glucose-containing compounds. Though the experiments described here shed no light on the mechanism of this difference it is relevant to consider whether the extent of the serum-level of fructose is associated, in any way, with triglyceride metabolism. In fasting baboons given a single dose of ^{14}C-labelled fructose, Coltart and Crossley (1968) found that the higher the serum level of fructose attained the greater the specific activity found in the serum-triglycerides. When these animals had been on a diet containing fructose, a single dose of ^{14}C fructose now resulted in higher levels of serum-fructose and the greater the degree of rise the greater was the specific activity found in the triglycerides. Probably related to this metabolic finding is the direct association between the level of serum-fructose after a fructose-containing meal and level of fasting serum-triglyceride in men. This association is not found in premenopausal women (Crossley 1966). In rats the activity of some liver enzymes essential to lipogenesis is greater after the animals had been on a fructose diet than on a glucose diet (Bailey et al. 1968). Thus the higher level of fructose seen after ingesting sucrose may be a factor in elevation of serum-triglycerides seen in men on a high-sucrose diet. The rate of presentation might be a determining factor in inducing the increased activity of lipogenic enzymes.

The similar results in the men and young women show that the different glyceride response to dietary fructose seen in the two groups is, due not to differences in absorption into the blood, but presumably to the fact that the metabolism of fructose is influenced by sex hormones.

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THE ATHEROGENICITY OF SUCROSE

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Abstract. Feeding rats for thirty days with sucrose in feed rations with a normal calorie content evoked a disruption in cholesterol and fatty acid transport with acceleration of synthesis of the latter and some decrease in the rate of cholesterol formation in the liver and a number of other organs. An increase in amount of cholesterol was determined by its more prolonged occurrence in the blood; this increase was evidently created due to an increase in β-lipoproteid content. The accumulation of fatty acids in the blood occurs because of the increase in their complex compounds, since the amount of non-esterified fatty acids in the experimental rats does not differ from that of the control animals. The changes indicated above in lipid exchnage may cause atherogenesis.

In the last decade the question of the role of food carbohydrates in the development of atherosclerosis has been considered time and time again by a number of authors (1-3). Based on numerous statistical and epidemiological studies, it was learned that sucrose consumed along with an excess of calories, a situation which is characteristic of definite segments of the population, increases the level of blood cholesterol, which is associated with an increase in the number of cases of morbidity due to coronary atherosclerosis (4,5). In a number of clinical works as well, analogous conclusions were reached (6,7).

The question of the specific influence on atherogenesis of different food substances, among them sucrose, is debatable. At the present time there is a lot of scientific data which is evidence for essential differences in metabolism of different carbohydrates (8) and the extent of their participation in lipogenesis (9). We indicated not long ago (10) that long-term administration of sucrose in the feed ration of rats evoked some acceleration in the inclusion of 1-6 C^{14}-glucose markers in fatty acids and their increase in the liver, aorta, and blood. However the rate of marker inclusion in cholesterol in the given experiment did not change, although its level in the blood increased significantly statistically. In connection with the fact that the inclusion of radioactive carbohydrate from glucose in the molecules of fatty acids and cholesterol depends to a significant degree on the glycolysis rate, we considered it necessary to determine as well the biosynthesis of these substances from 2-C^{14}-sodium-acetate. Considering the possibility of changing the conditions of cholesterol absorption and transport in rats being kept on a diet including sucrose, we conducted a study of the distribution of tagged cholesterol in organs

and tissues and determined the activity of lipoproteid lipase and the amount of β-lipoproteid and free fatty acids in the blood.

The work was done on 150 male rats of the Vistar strain with original weights of 110-130 g and kept for thirty days on diets differing in carbohydrate quality. The animals in the control group were started on a feed ration (60 kcal per 100 g weight, necessary vitamins, and salts), in which 20% of the calorie content was provided by casein, 26% by lard, 54% by cornstarch. The experimental group of rats was fed the same ration but with sucrose instead of 73% of the cornstarch, which constituted 41% of the total calorie content.

At the end of the test feeding of the animals, they were divided into two subgroups. One was injected with 4-C^{14}-cholesterol (25 μcuries/rat) and the rats were killed after 24 and 72 hours. The other group was injected with 2-C^{14}-sodium acetate (150 μcurie/rat) and the rats were killed after 2 hours. The radioactivity of the cholesterol and fatty acids was studied in ten rat organs with a PP-8 apparatus and a BFL-T calculator. The specific radioactivity (SR) was calculated in microcuries X 10^{-4} for 1 mg of cholesterol or fatty acid. Cholesterol content was determined according the Sperry and Webb (11), fatty acid content according to Folch et al. (12). Lipoproteid lipase activity was studied as Lukasik (13) did, β-lipoproteid content according to Burshteyn and Ledvina (14), free fatty acids in the blood according to Doll (15), and blood sugar according to Hagedorn and Jenson (16).

As shown in Table 1, the cholesterol level in the blood of rats in the experimental group was somewhat increased. In the remaining studies of the organs, essential changes in its content were not found. The rate of inclusion of radioactive tags from sodium acetate in cholesterol decreased for the most part in the organs, especially in the lungs, spleen, aorta, and liver. Because of this a significant decrease occurred in tag content in blood cholesterol. An accelerated synthesis of fatty acids was found in the liver and to a lesser degree in other organs, along with an increase in their content in the blood, liver, and aorta (see Table 1).

During study of the absorption and distribution of 4-C^{14}-cholesterol during the 24 hours after enteral administration, statistically significant differences between indices in the control and experimental groups were not found (Table 2). However within 72 hours, the specific radioactivity of cholesterol in the blood and liver of rats in the experimental group was higher (21.3 + or -3, 20.3 + or - 3.7 μcurie • 10^{-4}), than that in the control group (15.6 + or - 2, 16.4 + or - 2.3 μcurie • 10^{-4}) by 35 and 26%, respectively. Somewhat of an increase in β-lipoporteid content in the blood (+47%) was found in the rats which received sucrose in the diet (control 23.4 mg %, experimenta; 34.3 mg %). The blood sugar level in experimental and control group rats was the same. The activity of lipoproteid lipase showed a noticeable increase in rats of the experimental group. The content of free fatty acids was analogous to that in the control group before stress due to heparin (Table 3).

The results presented above permit conclusions to be made concerning noticeable changes in lipid exchnage in rats which received sucrose. Above all attention must be paid to the increase in cholesterol and fatty acid content in the blood. An accelerated biosynthesis of the latter, which is found in the liver and other organs of experimental group rats, is unlikely to serve as the single explanation of the significant increase in their quantity. In the literature it is reported that the introduction of glucose in the incubation medium decrease lipolysis in fatty tissue (17). In addition the increase in triglyceride content in the blood during starch replacement by sucrose was noted in a number of works (2,7).

TABLE 1

Cholesterol and Fatty Acid Content in Organs and Tissues (in mg/g wt of dry Tissue) and Radioactivity of Cholesterol and Fatty Acids (in μcurie $\cdot 10^{-4}$), Separated from 1 g Tissue

Organs & Tissues	Cholesterol Content Control	Cholesterol Content Exper.	Radioact. of Chol. fr. Acetate-$2C^{14}$ Control	Radioact. of Chol. fr. Acetate-$2C^{14}$ Exper.	Fatty Acid Content Control	Fatty Acid Content Exper.	Fatty Acid Radioactivity fr. Acetate-$2C^{14}$ Control	Fatty Acid Radioactivity fr. Acetate-$2C^{14}$ Experimental
Blood	57 +or- 1.6[2]	70 +or- 61[1,2]	0.65 +or- 0.11	0.48 +or- 0.09	360 +or- 28[2]	730 +or- 110[1,2]	1.5 +or- 0.32	1.9 +or- 0.3
Liver	2.82 +or- 0.29	2.58 +or- 0.27	3.16 +or- 0.24	2.45 +or- 0.33[1]	27.2 +or- 1.3	32.8 +or- 2[1]	10.6 +or- 2.3	25.6 +or- 22[1]
Fat	0.49 +or- 0.06	0.61 +or- 0.06	0.21 +or- 0.11	0.07 +or- 0.01[1]	885 +or- 51	811 +or- 28	7.0 +or- 0.9	6.8 +or- 0.8
Aorta	3.4 +or- 0.74	3.7 +or- 0.43	1.0 +or- 0.13	0.20 +or- 0.04[1]	99 +or- 17	199 +or- 14[1]	1.1 +or- 0.2	0.9 +or- 0.1
Spleen	3.18 +or- 0.14	3.86 +or- 0.24	2.82 +or- 0.78	1.82 +or- 0.3	14.9 +or- 2.6	24.4 +or- 3[1]	11.0 +or- 2.3	11.0 +or- 2.7
Kidneys	2.96 +or- 0.15	3.4 +or- 0.09	3.18 +or- 0.3	2.87 +or- 0.5	24.6 +or- 1.2	33.6 +or- 3.6[1]	38.6 +or- 5.1	30.6 +or- 5.8
Heart	1.19 +or- 0.08	1.12 +or- 0.06	0.38 +or- 0.13	0.22 +or- 0.03	25.8 +or- 6	30 +or- 3.8	4.2 +or- 0.7	4.2 +or- 0.7
Brain	8.16 +or- 0.16	8.00 +or- 0.26	0.99 +or- 0.14	0.37 +or- 0.05[1]	35.2 +or- 2.2	48.0 +or- 2[1]	11.4 +or- 1.9	10.7 +or- 2.1
Adrenal Glands	22.80 +or- 2.47	13.6 +or- 4.5[1]	9.4 +or- 3.0	5.9 +or- 1.3	212 +or- 41	240 +or- 12	83.0 +or- 20	34.0 +or- 5.1
Lungs	3.95 +or- 0.23	3.85 +or- 0.14	3.45 +or- 0.24	1.37 +or- 0.21[1]	26.5 +or- 1.2	33 +or- 4.4	9.5 +or- 1.3	9.9 +or- 1.8

1. The results are statistically significant at $P > 0.5$.
2. Cholesterol and fatty acid content in mg %.

TABLE 2

Specific Radioactivity (in μcurie $\cdot 10^{-4}$/1 mg cholesterol) During the 24 hr. after Administration per os 4-C^{14}-cholesterol

Animal Group	Blood	Liver	Spleen	Lungs	Fat	Kidneys	Adrenal G.	Aorta	Brain	Heart
	Organs & Tissues									
Control	21 +or- 1.26	14.2 +or- 2.1	17.1 +or- 1.1	8.3 +or- 0.2	3 +or- 0.37	2.1 +or- 0.22	10.1 +or- 1.2	1.18 +or- 0.13	0.12 +or- 0.01	6.5 +o 1.8
Exper.	18.8 +or- 2.1	16.5 +or- 0.92	17.4 +or- 1.46	8.6 +or- 0.5	2.2 +or- 0.6	2.1 +or- 0.11	14.0 +or- 1.7	0.86 +or- 0.14	0.1 +or- 0.02	6.7 +o 0.6

Since the studies conducted do not show an increase in free fatty acids in the experimental rat group, it may be supposed that an increase in fatty acid content in the blood is caused not by an acceleration of lipolysis in organs and tissues, but by a changes in transport and metabolism of triglycerides together with an acceleration of fatty acid synthesis in the liver and other organs.

The increase in cholesterol content in the blood, as is evident from our data, is not cause by an acceleration in its biosynthesis, nor by a change in absorption and distribution of exogenic cholesterol. It becomes evident that it is evoked by changes in its transport conditions. We propose that cholesterol retention in the blood is associated with an increase in the amount of β-lipoproteid which was noted in our studies. It is possible that an increase in low-density β-lipoproteids occurs in the blood which, as is known, circulate for a longer time than high-density ones. This suggestion is corroborated by the larger specific radioactivity of blood cholesterol which is found in the 72 hours after administration of exogenic tagged cholesterol. In the literature there are data on the atherogenicity of rations without cholesterol and with the main content carbohydrates, under conditions of long-term feeding to rabbits (18). In addition an increase in content in the blood, liver, and aorta of both cholesterol and triglycerides was noted. To this it is necessary to add that, together with an increase in these components of lipid exchange, an increase in β-lipoproteid level was found in the blood.

The results obtained by us and certain data from the literature permit the consideration that changes in lipid exchange evoked by sucrose are caused by the high rate of its absorption in comparison to the same for

TABLE 3

β-Lipoproteid Level in Blood and Free Fatty Acids Before and After Stress due to Heparin

Animal Group	Before Heparin Administration		After Heparin Administration	
	β-lipoprot. in blood (in mg %)	Free fatty acids (in mg %)	β-lipoprot. in blood (in mg %)	Free fatty acids (in mg %)
Control	23.4 + or - 2.9	0.75 + or - 0.09	19.8 + or - 2.3	0.86 + or - 0.07
Exper.	34.3 + or - 4.1[1]	0.73 + or - 0.1	11.6 + or - 2.0[1]	1.05 + or - 0.10[1]

1. The results are statistically significant, P ≥ 0.05.

starch, which was used in the control diet. Besides the occurrence of the specific influence of fructose is possible, a substance which is formed during sucrose hydrolysis, on the biosynthesis of fatty acids (9.19). The long-term administration of slightly assimilated disaccharide of sucrose is capable of causing an insulin effect (20), which is characteristic of a

phenomenon in which an accelerated synthesis of fatty acids occurs and their deposition within the triglyceride content in tissues, together with somewhat of a deceleration of cholesterol synthesis (21), which was noted in the experiment we conducted. The lack of change in blood sugar content is not in contradiction to these conclusions, since these changes appear during more obvious forms of hyperinsulinism. The mechanism of the changes, which occur in β-lipoproteid content in blood in the presence of significant lipoproteid lipase activity, require further study.

CONCLUSIONS

1. Long-term feeding of sucrose to rats in a normal-calorie feed ration causes a distrubance of cholesterol and fatty acid transport in the blood during acceleration of synthesis of the latter and some decrease in formation rate of cholesterol in the liver and a number of other organs.

2. Cholesterol retention in the blood is caused by an increase in β-lipoproteid content and a more long-term entrapment of cholesterol in them.

3. The accumulation of fatty acids in the blood occurs due to an increase in their complex compounds, since the amount of non-esterified fatty acids in the rats being tested did not differ from the control.

4. The changes indicated above in lipid exchange may be considered as factors which contribute to atherogenesis.

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Dietary Factors and Coronary Heart Disease *

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Mortality data from arteriosclerotic and degenerative heart disease (AHD) and per capita consumption of total fat, saturated fat, sucrose, simple sugars, complex carbohydrates, and protein, and calorie intake for 37 countries were statistically evaluated to investigate possible relationships between dietary factors and incidence of AHD. On a geographical basis, consumption of total and saturated fats is strongly and positively correlated with the death rates, while consumption of complex carbohydrates is negatively correlated. No correlations were found with temporal changes in death rates or with differences within one country. These findings are discussed in the light of the works of many other investigators. It is concluded that the relation of diet to AHD is still controversial, and that the development and severity of the disease cannot be confidently attributed to any single dietary factor nor to blood cholesterol. The contributing effects of other factors, such as physical activity, mental stress, and affluence, are also discussed.

It is common knowledge that mortality rates from arteriosclerotic and degenerative heart disease (AHD) are much higher in the highly industrialized, technically advanced countries than in the developing countries. It is also well known that death rates from this disease have increased over the past several years, particularly in the younger age-groups: in some countries they have increased by as much as 2 or 3 times in one decade. Part of the increase is likely to be an artefact brought about by the changing fashion in certifying heart disease as a cause of death, by better diagnostic procedures, and by the longer life expectancy; nevertheless, several authors (Lew, 1957; Oliver & Stuart-Harris, 1965; Reader & Wynn, 1966) have pointed out that much of this increase cannot be accounted for simply in terms of artefacts and must be considered as real. Recent autopsy studies have confirmed that differences in aortic and coronary atherosclerosis between different countries and population groups are real and striking (Tejada et al., 1968).

In an attempt to explain these differences much epidemiological, clinical and experimental work has been carried out by numerous investigators to determine whether dietary factors play an important role in the development of AHD.

The aim of the present article is to supply up-to-date information on AHD death rates as related, on a geographical basis, to the intake of various dietary factors. Although this kind of statistical and epidemiological evidence does not permit definitive conclusions to be drawn as to the causal significance of the correlations detected, it is useful in identifying trends and in suggesting specific problems worthy of further study.

METHODS

Death rates per 100 000 population for arteriosclerotic and degenerative heart disease were taken from *World health statistics annuals* (World Health Organization, 1958, 1968). Various groups, defined by age and sex, were studied: all ages and both sexes, 45–54-year-old males, 55–64-year-old males, and 55–64-year-old females.

Dietary data were taken from the *Food balance sheets* published by the Food and Agriculture Organization (1949, 1966). When some data, such as those relating to saturated fats or to complex carbohydrates and simple sugars, were not directly available, they were calculated on the basis of the *Food composition tables* published by the Food and Agriculture Organization (1953) following the definitions of Jolliffe (1961) and of Lopez et al. (1966). Although the data in the FAO *Food balance sheets* are a crude estimate of the food consumption, they proved to be similar to other data obtained in

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accurate dietary surveys, recently carried out by a team of investigators in Finland, Greece, Italy, the Netherlands, and Yugoslavia (Den Hartog et al., 1968).

The national *per capita* income and *per capita* energy consumption data referred to in Table 1 were taken from the United Nations *Statistical yearbook* (1967) and from the United Nations *Yearbook of national account statistics* (1966).

RESULTS

Table 1 shows the death rates from AHD and the dietary consumption data for 37 countries. The statistical evaluation shows that consumption of total fat and of saturated fat (as a percentage of the total calorie intake) is strongly and positively correlated with death rates, whereas consumption of complex carbohydrates is negatively correlated. The intake of calories, sucrose, and simple sugars is correlated less strongly, and protein consumption shows a poor correlation. The values relating to the previous decade, shown in Table 2, also show similar types of correlations: for this period data were available for only 25 countries. These trends are present in all the age-groups studied; in the females 55–64 years of age, however, some of the correlations are weaker, thus suggesting the possible involvement of a sex factor. The influence of sex differences on cardiovascular mortality patterns is extensively described in the monograph by de Haas (1964).

Table 3 shows the correlation coefficients between the various dietary factors listed in Table 1. The highest correlation is between total fats and saturated fats. Contrary to Yudkin's finding (1964), the present data show only a weak correlation between consumption of fats (both total and saturated) and sugars (sucrose and simple sugars). Consumption of complex carbohydrates showed a strong negative correlation with the 4 dietary constituents mentioned above: the strong negative correlation with fat intake indicates that the negative correlation, shown in Table 1, between complex carbohydrate consumption and AHD death rates may actually be secondary to the positive correlation between fat consumption and death rates. In other words, fat may be the dietary constituent playing an important role in cardiovascular mortality while the complex carbohydrates may be just neutral bystanders.

The present results are in line with the generally accepted hypothesis that excessive intake of fats, especially of the saturated type, may have a detrimental effect on cardiovascular health and is therefore associated with higher cardiovascular mortality rates. The results agree also with those reported by Lopez et al. (1966) with the exception of those concerning simple sugars: whereas the present investigation did not reveal any strong correlation between the consumption of simple carbohydrates and cardiovascular mortality rates, Lopez et al. found a high correlation coefficient. The reason for this discrepancy may be that these authors used data relating to only 14 European countries, in which simple sugar consumption is almost the same, i.e., 13.5%–21.2% of total calorie intake, whereas in the 37 countries considered in the present investigation simple sugar consumption ranges from 5.1% to 38.5% of the total calorie intake. As there are countries, such as Colombia, Costa Rica, Jordan, Mexico and others, where death rates from AHD are low and yet the calorie contribution from simple sugars is as high as, or even higher than in the European countries, then we may assume that the relation between AHD death rates and simple sugar intake, described on a geographical basis by some authors (Lopez et al., 1966; Yudkin, 1964) is actually a secondary association for which a causal significance has not been proved yet.

Very good positive correlations are also present between AHD death rates and national *per capita* income and energy consumption, as well as between income and consumption of total fat, and saturated fat: there is also a negative correlation between income and consumption of complex carbohydrates. The implications of these findings will be discussed in more detail later.

The next step in the present study was to determine whether the increase in cardiovascular mortality rates which has occurred in practically all countries over the past several years has been accompanied by a parallel increase in dietary intake. Only for 23 countries was it possible to collect both mortality and dietary data for the past 15–20 years. Between 1955 and 1965 the death rates from AHD increased on average by 51% (all ages, both sexes); consumption of total fat, saturated fat, sucrose, simple sugar and, to a lesser extent, calorie intake increased also. Complex carbohydrate consumption decreased, and protein consumption decreased slightly (Table 4). This is a well established picture that has been observed by many investigators and it is considered to be a reflection of increased prosperity. The statistical analysis, however, reveals that the correlations between the changes observed in dietary intakes

TABLE 1

GEOGRAPHICAL CORRELATION BETWEEN DEATH RATES FROM AHD, DIETARY FACTORS, AND FACTORS ASSOCIATED WITH LEVEL OF NATIONAL DEVELOPMENT, 1986[a]

Country	AHD death rates [a]				Total calorie intake	Dietary factors (approximate percentage of total calorie intake [b])						Income [c]	Energy [d]
	Males, 55–64 years of age	Females, 55–64 years of age	Males, 45–54 years of age	Both sexes, all ages		Fat	Unsaturated fat	Sucrose	Simple sugars	Complex carbohydrates	Protein		
Jordan	49	19	18	6	2 230	18.5	3.7	11.0	18.8	67.9	11.1	192	300
El Salvador	51	28	17	8	2 030	18.5	5.5	—	13.8	65.0	11.2	236	183
Taiwan	53	63	16	10	2 350	15.3	4.6	4.2	5.1	71.8	9.9	186	706
Philippines	80	40	36	14	1 840	9.6	3.7	6.6	9.8	65.9	13.7	221	207
Guatemala	115	84	50	18	2 080	15.1	4.1	—	13.5	61.5	10.6	291	213
Mexico	122	86	48	18	2 810	24.8	7.9	—	17.8	45.7	11.0	412	997
Greece	162	81	87	38	2 940	28.5	5.4	4.9	12.7	55.1	13.0	570	831
Japan	165	92	51	58	2 280	14.5	3.4	7.3	8.6	70.8	12.4	694	1 954
Panama	170	76	80	38	2 310	22.5	6.8	8.7	19.6	52.2	10.0	425	1 143
Spain	171	71	88	68	2 800	27.9	7.5	7.0	12.7	55.4	10.9	594	1 104
France	206	89	74	82	3 050	37.0	14.5	10.4	13.8	44.6	13.0	1 445	3 019
Colombia	218	130	49	31	2 180	21.6	8.1	8.0	24.8	53.1	9.6	297	534
Costa Rica	224	180	87	44	2 430	18.5	6.4	13.1	36.8	31.8	8.9	552	312
Romania	235	152	75	141	3 140	21.3	4.4	4.3	8.0	63.7	12.3	353	2 072
Portugal	259	114	64	116	2 580	23.7	6.2	6.0	13.0	62.1	11.5	353	832
Yugoslavia	267	184	71	121	3 030	22.3	3.2	5.2	8.6	63.2	12.4	720	1 902
Poland	281	114	99	63	3 380	27.2	4.6	—	11.1	60.1	11.1	550	3 898
Chile	321	184	106	68	2 410	19.4	7.8	—	16.8	57.0	12.7	615	1 113
Mauritius	322	96	178	47	2 330	16.9	3.8	17.6	18.1	60.6	8.1	213	171
Venezuela	342	239	139	49	2 310	22.6	8.1	15.6	19.0	52.2	10.3	745	2 466
Italy	357	152	134	206	2 730	26.4	7.7	8.9	14.5	53.8	11.8	884	1 961
Switzerland	398	68	184	229	3 220	36.4	13.4	14.0	21.2	38.9	11.2	1 829	2 898
Belgium	419	124	172	149	3 040	41.1	12.9	11.0	13.5	42.6	11.2	1 428	4 636
Hungary	453	234	157	254	3 030	29.4	13.7	—	13.2	52.6	12.1	740	2 825
Austria	478	160	153	244	2 970	33.7	9.7	13.0	19.9	42.9	11.7	970	2 692
Uruguay	485	178	189	167	3 290	36.6	16.4	—	16.7	35.9	12.9	537	843
Sweden	490	152	130	313	2 890	40.6	14.5	14.6	20.1	33.2	11.2	2 000	5 080
Netherlands	503	134	190	188	2 990	39.5	11.5	15.9	20.7	38.8	10.6	1 274	3 506
Federal Republic of Germany	549	175	194	220	2 980	38.8	14.6	10.8	17.6	42.1	10.9	1 455	4 267
Norway	583	136	182	248	2 930	39.2	12.4	16.7	19.1	36.3	11.0	1 451	3 963
Denmark	588	172	178	290	3 370	41.5	16.1	18.5	18.6	35.2	11.0	1 677	4 515
Israel	628	306	201	169	2 620	28.9	6.9	12.0	20.8	43.2	11.9	1 078	2 948
United Kingdom	743	208	253	392	3 280	39.2	15.1	15.9	18.8	35.1	10.9	1 487	5 139
Canada	832	283	306	247	3 090	40.8	15.8	15.5	17.1	28.8	12.0	1 824	7 878
New Zealand	889	309	288	251	3 490	40.4	19.2	12.8	18.2	29.7	12.0	1 995	2 644
USA	933	301	357	316	3 100	41.5	14.3	14.5	20.5	27.3	11.7	2 910	9 595
Australia	942	303	327	339	3 140	38.0	16.3	14.0	21.1	30.3	11.5	1 640	4 931
Finland	1 037	252	450	272	3 110	33.0	16.2	13.6	18.8	42.7	12.1	1 393	2 836
Correlation coefficients of death rate to dietary factors, income and energy consumption													
Males, 55–64 years of age						0.80	0.74	0.80	0.86	0.31	−0.74	0.11	0.75	0.72
Females, 65–84 years of age						0.80	0.65	0.62	0.64	0.33	−0.59	0.11	—	—
Males, 45–54 years of age						0.89	0.70	0.74	0.84	0.32	−0.71	0.08	0.73	0.71
Both sexes, all ages						0.77	0.84	0.81	0.56	0.24	−0.72	0.21	0.83	0.77
Correlation coefficients of income to dietary factors and energy consumption														
						0.87	0.86	0.78	0.56	0.28	−0.78	0.18		0.89

[a] Death rates for 1986 per 100 000 population.
[b] Approximate daily *per capita* consumption, 1980–82. Figures collated from various sources.
[c] National *per capita* income in US $, 1985.
[d] National *per capita* consumption in kg of coal equivalent, 1986.

TABLE 2

GEOGRAPHICAL CORRELATIONS BETWEEN DEATH RATES FROM AHD AND DIETARY FACTORS, 1940s–1950s

Countries	AHD death rates [a]			Total calorie intake	Dietary factors (approximate percentage of total calorie intake [b])					
	Males 35–44 years of age	Males 45–54 years of age	Both sexes, all ages		Fat	Unsaturated fat	Sucrose	Simple sugars	Complex carbohydrates	Protein
Yugoslavia	92	20	33	2 144	14.3	4.3	1.8	5.2	71.1	12.1
Colombia	94	30	16	1 [illegible]	18.1	7.1	7.1	38.6	47.1	10.2
Greece	101	20	60	2 [illegible]	25.2	5.3	4.0	6.7	58.7	12.0
Poland	121	58	21	2 [illegible]	14.6	5.9	—	3.0	57.0	11.2
France	144	48	54	2 257	26.6	11.3	6.9	3.6	68.6	12.9
Japan	148	56	43	1 670	5.8	1.1	2.1	2.0	85.0	12.0
Ceylon [c]	169	68	29	1 650	15.5	3.1	8.0	6.3	66.1	11.9
Chile	282	77	45	2 352	16.2	8.6	—	13.9	62.1	12.4
Italy	317	97	157	2 249	21.3	5.7	2.1	6.0	66.1	12.6
Hungary	329	109	147	2 422	17.1	6.4	—	7.9	70.8	12.0
Uruguay	343	135	96	2 478	30.5	13.6	—	13.5	39.4	14.5
Belgium	344	132	134	2 667	23.6	12.3	10.3	12.1	58.6	11.6
Norway	355	113	153	2 [illegible]	34.1	10.6	8.3	12.1	50.9	12.5
Netherlands	367	115	168	2 693	26.6	12.2	10.2	12.6	59.6	11.5
Switzerland	377	117	236	3 106	27.6	11.3	12.2	22.1	48.7	12.0
Iceland [d]	383	148	124	3 [illegible]	37.2	18.0	18.9	12.1	40.9	14.7
Federal Republic of Germany	413	134	179	2 169	13.7	5.5	8.0	12.0	74.7	13.7
Denmark	423	129	201	3 125	36.7	14.4	11.7	14.2	48.1	13.8
Sweden	436	107	234	2 571	34.6	15.7	14.2	19.0	43.1	15.7
United Kingdom	597	191	313	2 [illegible]	31.3	12.1	13.7	16.0	48.3	12.0
New Zealand	712	224	229	3 214	38.0	18.2	14.9	16.0	32.1	12.5
Australia	788	247	244	3 292	34.3	18.4	14.3	20.3	36.1	12.9
Canada	796	277	228	3 181	38.0	17.3	13.6	21.2	35.3	12.0
Finland	847	306	261	2 617	24.8	11.3	6.1	11.0	58.7	13.9
USA	896	342	287	3 244	38.0	15.4	16.0	21.0	32.0	12.2
Correlation coefficients of death rate to dietary factors										
Males, 35–44 years of age					0.78	0.63	0.73	0.85	0.48	−0.62	0.28
Males, 45–54 years of age					0.70	0.62	0.69	0.62	0.47	−0.52	0.23
Both sexes, all ages					0.61	0.70	0.70	0.78	0.52	−0.63	0.31

[a] Death rates for 1955 per 100 000 population.
[b] Approximate daily *per capita* consumption, 1947–49. Figures collated from various sources.
[c] Not included in Table 1 due to lack of mortality data for 1955.
[d] Not included in Table 1 due to lack of dietary data for 1955.

TABLE 3

CORRELATION COEFFICIENTS BETWEEN THE CONSUMPTION OF VARIOUS DIETARY CONSTITUENTS [a]

Dietary constituents	Total calorie intake	Total fats	Saturated fats	Sucrose	Simple sugars	Complex carbo-hydrates	Proteins
Total calorie intake	1.00	0.83	0.71	0.32	0.07	−0.59	0.27
Total fats	0.83	1.00	0.90	0.60	0.30	−0.83	0.13
Saturated fats	0.71	0.90	1.00	0.59	0.34	−0.83	0.18
Sucrose	0.32	0.60	0.59	1.00	0.59	−0.79	−0.38
Simple sugars	0.07	0.30	0.34	0.59	1.00	−0.70	−0.42
Complex carbohydrates	−0.59	−0.83	−0.83	−0.79	−0.70	1.00	0.10
Proteins	0.27	0.13	0.18	−0.38	−0.42	0.10	1.00

[a] Calculated from the data given in Table 1.

and in death rates from AHD are rather poor. In other words, it seems that the death rates from AHD increased in the individual countries regardless of the changes that occurred in the calorie intake and in the consumption of the various dietary factors.

DISCUSSION

For many years investigators have sought an explanation for the differences between cardiovascular mortality in developed and developing countries, and also for the increase that took place in practically all countries over the last decades.

In is now widely held that many factors play a role in the etiology and pathogenesis of cardiovascular disease; among them, diet has attracted the greatest interest and many authors believe that dietary factors play an important role in the development of atherosclerosis and of ischaemic heart disease. However, in spite of the great amount of epidemiological, clinical and experimental work that has been carried out by numerous investigators in attempting to define the role of diet in the development of the disease, it is still unclear to what extent diet can account for the observed phenomena and which dietary characteristics are responsible for the secular changes and the regional variations. It is therefore likely that other factors besides diet, those that are associated with living patterns, might influence the temporal and geographical differences in cardiovascular mortality.

Using the findings of the present investigation as a guide-line, it will be useful to review briefly the literature and try to summarize the available knowledge on the role of diet and other environmental factors in the development of AHD. It would, however, be beyond the scope of this article to provide detailed documentation; this can be found, for example, in the book published by Bajusz (1965) on the nutritional aspects of cardiovascular disease, in the review on dietary fat and coronary heart disease that was prepared by the National Heart Foundation of Australia (1967) and in the more recent publication on the geographical distribution of atherosclerosis in relation to diets and to other factors (*Lab. Invest.*, 1968).

Total calorie intake

As shown in Table 1, calorie intake is positively associated with death rates from AHD on a geographical basis. Albrink (1965) postulated that excessive calorie intake, rather than consumption of any particular kind of food, might be responsible for the higher incidence of AHD. According to this author, from an evolutionary point of view the high fat and protein consumption of modern times is indeed a harmless reversion to the primordial carnivorous habits of man; Albrink also considered that the most important, recent change in human dietary habits is the greater calorie intake being enjoyed by certain populations.

Proteins

Most investigators consider that mortality from AHD is not related to the protein contribution to total calorie intake; however, some have demon-

TABLE 4

INCREASE IN AHD DEATH RATE AND IN CONSUMPTION OF VARIOUS DIETARY FACTORS IN RECENT YEARS

Countries	Death rates (ratio 1960: 1946)			Dietary factors (ratio 1960: 1947)						
	Males, 55–64 years of age	Males, 45–54 years of age	Both sexes, all ages	Total calorie intake	Fat	Saturated fat	Sucrose	Simple sugars	Complex carbohydrates	Protein
USA	1.04	1.04	1.09	0.88	1.09	0.99	0.97	1.00	0.84	0.98
Canada	1.05	1.10	1.08	0.95	1.06	0.94	1.12	0.81	0.86	1.00
Switzerland	1.06	1.08	0.92	1.04	1.28	1.18	1.15	0.96	0.73	0.93
Chile	1.10	1.37	1.47	1.02	1.36	1.14	—	1.31	0.98	1.02
Sweden	1.14	1.21	1.31	1.04	1.18	0.92	1.00	1.06	0.77	0.71
Japan	1.14	0.88	1.22	1.33	2.42	3.09	3.48	4.50	0.83	1.03
Australia	1.19	1.32	1.17	0.96	1.12	1.06	0.88	3.08	0.86	0.89
Finland	1.22	1.47	1.30	1.19	1.32	1.34	2.23	1.73	0.72	0.87
Italy	1.22	1.38	1.22	1.21	1.24	1.35	2.87	2.16	0.80	0.94
Belgium	1.22	1.30	0.87	1.14	1.37	1.04	1.07	1.56	0.73	0.94
United Kingdom	1.23	1.36	1.03	1.10	1.26	1.25	1.16	1.19	0.73	0.91
New Zealand	1.26	1.28	1.12	1.06	1.09	1.06	0.84	1.00	0.94	0.86
Federal Republic of Germany	1.33	1.48	1.16	1.35	2.78	2.86	1.35	1.91	0.88	0.79
Denmark	1.36	1.36	1.26	1.08	1.14	1.12	1.32	1.36	0.73	0.80
Hungary	1.38	1.44	1.31	1.24	1.70	2.14	—	1.86	0.78	1.01
Uruguay	1.41	1.17	1.68	1.30	1.23	1.19	—	1.31	0.92	0.88
Netherlands	1.41	1.58	1.15	1.09	1.34	0.94	1.56	1.61	0.68	0.92
France	1.43	1.51	1.46	1.29	1.42	1.28	1.50	1.83	0.85	1.00
Greece	1.60	2.86	1.33	1.30	1.06	1.02	1.22	1.26	0.93	1.08
Norway	1.64	1.81	1.67	1.01	1.15	1.17	1.69	1.58	0.70	0.86
Poland	2.14	1.77	3.85	1.42	1.80	0.78	—	3.86	0.86	1.00
Colombia	2.81	1.74	1.94	1.08	1.22	1.14	1.13	0.83	1.13	0.94
Yugoslavia	2.90	3.55	3.66	1.41	1.87	0.74	2.89	1.30	0.89	1.02
Mean	1.42	1.51	1.51	1.15	1.39	1.27	1.58	1.81	0.78	0.93
Correlation coefficients of death rate to dietary factors										
Males, 55–64 years of age				0.51	0.10	−0.25	0.29	0.16	0.26	0.26
Males, 45–54 years of age				0.48	−0.02	−0.31	0.24	0.07	0.20	0.30
Both sexes, all ages				0.57	0.18	−0.27	0.43	0.40	0.08	0.26

strated a positive relationship with consumption of animal protein (Yudkin, 1957; Jolliffe & Archer, 1959; Annand, 1961, 1967; Scrimshaw & Guzman, 1968). Annand (1967) considers that the consumption of heated animal proteins, particularly those found in milk, may be an important etiological factor in AHD and thrombosis. Most of the above authors, however, considered the relation between protein and AHD to be a secondary association with no etiological significance. Experimental studies on animals and in man, reported by Bajusz (1965) indicate that variations in dietary protein

intake have practically no effect on the development of atherosclerosis.

Fats

The finding by Keys (1953) that the fat content in the diet is positively correlated with the incidence of atherosclerosis and ischaemic heart disease gave rise to extensive speculation on the relationship existing between diet, the serum cholesterol level and atherosclerosis. Such a relationship does exist in experimental animals, where coronary atherosclerosis and occlusions can indeed be produced by dietary manipulations (Friedman, 1960). Whether this relationship exists in man also is, however, still controversial. Many authors believe that excessive intake of dietary fats has an influence on the incidence of AHD (Higginson & Papler, 1954; Bronte-Stewart, Keys & Brock, 1955; Keys et al., 1958; Paul et al., 1963; Epstein et al., 1965a, 1965b; Lopez et al., 1966; McGandy, Hegsted & Stare, 1967; Page & Stamler, 1968; Scrimshaw & Guzman, 1968), and strong evidence is available that the serum cholesterol level and incidence of coronary heart disease are directly correlated (Epstein, 1965; Stamler et al., 1960; Cornfield, 1962; Keys et al., 1963; Kannel et al., 1964; Morris et al., 1966; Scrimshaw & Guzman, 1968; Obeyesekere, 1968). Some investigations, however, showed no relation between the blood cholesterol level and dietary habits (Morris et al., 1963; Little et al., 1965). Several other authors, however, are critical of the role played by dietary fats in the development of the disease and they have revealed many instances in which the dietary fat intake, the blood cholesterol level, and the incidence of atherosclerosis or of coronary disease were not correlated: some examples may be found in the studies of Camara-Besa, Macapinlac & Albino (1959) on Filipino soldiers, of Karvonen et al. (1961) on Finnish lumberjacks and sedentary men, of Lapicirella et al. (1962) on certain Somali tribes, of Stuart et al. (1962) on the rural population of St. Kitts, of Shaper (1962, 1963) on the Samburu tribe of northern Kenya, of Mann et al. (1962, 1964) on Pygmies and Masai, of McDonough et al. (1965) on the high and low social classes of Evans County, Ga., USA, of Finegan et al. (1968) on cardiac patients and healthy subjects in Ireland, of Bassett & Schroffner (1968) on Chinese and Japanese men living in Hawaii, and of Brunner et al. (1969) on sedentary and non-sedentary workers in Israel.

It should be pointed out, however, that several of the population groups mentioned above are habitually engaged in strenuous physical activity, and this might favourably influence the cardiovascular function. The allegedly protective role of physical activity will be discussed later in the text.

Several authors (Sinclair, 1956; Kinsell et al., 1958; Keys et al., 1958; McGandy, Hegsted & Stare, 1967) maintain that the type, rather than the amount of fat consumed plays a role, and it has been demonstrated that saturated fats cause an increase in serum cholesterol level whereas unsaturated fats decrease it through mobilization of cholesterol (Kinsell & Michaels, 1955; Bronte-Stewart et al., 1956; Malmros & Wigand, 1957; Morris, 1960, 1961; Stamler, 1960). Christakis et al. (1966), Turpeinen et al. (1968), Dayton et al. (1968) and others have indeed demonstrated that use of a cholesterol-lowering diet, in which some of the saturated fats have been replaced by unsaturated fats, brings about a decrease in the incidence of coronary heart disease in small, well-defined population groups.

Other authors, however, do not share this view. According to the surveys carried out on Yemenites who immigrated into Israel (Cohen, Barly & Poznanski, 1961; Cohen, 1963) and on Polynesian populations (Hunter, 1962; Prior et al., 1966) neither the amount nor the type of dietary fat seems to play a major role in the etiology of ischaemic heart disease.

Antar, Ohlson & Hodges (1964) reported that the marked increase in coronary heart disease that has occurred in the USA over the past several decades was accompanied by only a slight (6%) increase in fat consumption, due mostly to an increase in unsaturated fats. The ratio of polyunsaturated to saturated fatty acids, instead, increased markedly (29%). These authors state that their data " do not fit the hypothesis that low ratios of polyunsaturated to saturated fatty acids in the diet are significantly related to high mortality rate from coronary heart disease ". Similar criticism against the hypothesis that saturated fatty acids have a primary role in the etiology of atherosclerosis had also been expressed by Annand (1961). According to Finegan et al. (1968), high calorie and saturated fat intakes may be risk factors at a population level, but they are probably risk factors of a low-grade type.

The only conclusion that can be drawn from the examination of the literature and of the data presented above is that although there is strong evidence that dietary fats, particularly the saturated ones, play an important role in the etiology of AHD, there is no proof that they are the only or the main culprit.

Carbohydrates

The relation of carbohydrate intake to atherosclerosis and ischaemic heart disease is also a source of much controversy. From an epidemiological point of view the following findings seem worthy of mention. Yudkin (1964) suggested that sugars may be the causal factor in the development of AHD and that the statistical association that several authors had demonstrated, for a number of countries, between fat intake and AHD may be only secondary to the association he found between the figures for national consumption of sugars and of fats. Osancova, Hejda & Zvolankova (1965) supported Yudkin's hypothesis by demonstrating a good relationship between cardiovascular mortality and sugar consumption among the 10 administrative regions of Czechoslovakia, whereas fat consumption was poorly correlated. These authors, however, refrained from drawing any conclusions as to the role played by sugars in the development of AHD. Cohen et al. (1961) and Cohen (1963) pointed out that the higher mortality from coronary heart disease in Yemenite settlers in Israel, as compared with their compatriots recently emigrated from the Yemen, may be related to their higher consumption of sugar. These authors postulated that sucrose may play a role in the development of ischaemic heart disease. One more piece of indirect evidence that sugars may be related to ischaemic heart disease is the finding by Aqtar, Ohlson & Hodges (1964), that the increased incidence of coronary heart disease in the USA over the past decades was accompanied by a marked increase in simple sugar consumption and by a greater decline in consumption of complex carbohydrates. These authors suggest that the changes that have occurred in the type of dietary carbohydrates may be a contributing factor to the increased incidence of coronary heart disease in the USA.

Lopez et al. (1966) studied the relationship between food consumption and mortality from atherosclerotic heart disease in Europe, and reported that " the highest correlation coefficient was found between the percentage of calories derived from simple carbohydrates and the death rate; this correlation has been positively maintained through the years covered by the study ".

More recently, higher sugar consumption in a coronary group as compared to a non-coronary group was observed also by Paul et al. (1968), although the statistical analysis showed no significant association between heavy intake of sucrose and coronary disease.

Additional, indirect, evidence also pointing to the possibility that carbohydrates may play a role in the development of atherosclerosis and myocardial infarction is the repeated finding that diabetes and decreased sugar tolerance are prominent risk factors of atherosclerotic heart disease. Clinical studies and epidemiological surveys confirmed that people affected by disorders of the carbohydrate metabolism are also more prone to myocardial infarction and to vascular lesions including atherosclerosis (Stamler et al., 1960; Waddel & Field, 1960; Pell & D'Alonzo, 1961; Sowton, 1962; Yudkin & Roddy, 1964; Epstein et al., 1965a, 1965b; Ostrander et al., 1965; De Lange, Mey & Vivier, 1965; Kingsbury, 1966; Sievers, 1967; Epstein, 1967; Bassett & Schroffner, 1968). The possibility that excessive sugar consumption may be an etiological factor in the development of diabetes and of atherosclerosis was pointed out also by Ziegler (1966, 1967a, 1967b). These views, however, are challenged by Brunner & Altmann (1968), according to whom " no obligatory relationship exists between diabetes and atherosclerosis ".

As regards the relationship of sugars to cardiovascular diseases it must be borne in mind that these nutrients have common metabolic pathways with fats. Disturbances in carbohydrate metabolism may be responsible for abnormal fat metabolism and may therefore act as a causative factor in the development of atherosclerosis and of coronary disease (De Lange, Mey & Vivier, 1965; Waddel & Field, 1960; Cohen, 1963; Falsetti et al., 1968).

Minerals

Another factor, also of nutritional importance, which it has recently been suggested may play a role in the etiology of cardiovascular diseases, is trace-element imbalance. Several authors (for references see Masironi, 1969) have repeatedly observed a statistical correlation between softness of drinking water and cardiovascular mortality rates; there is also limited evidence that various trace elements may influence cardiovascular function and are involved in the pathogenesis of various cardiovascular diseases.

Man's mineral balance is changing as a function of technological progress; industrial pollution of air and water, the use of fertilizers and of food additives, food processing and canning, all alter the mineral balance because of accumulation of some trace elements or depletion of others. Populations living in highly industrialized countries are more exposed to

artificial manipulations of the environment than are the populations of the developing countries, particularly in the past few decades in which the rate of technological progress increased markedly. It may be speculated that the differences in cardiovascular mortality rates that have been detected on an international and on an intranational basis could be due, at least partly, to technologically-induced trace element imbalance. Although still very speculative, this new field of investigation is certainly worthy of consideration.

Non-dietary factors

From the data reported above and from the examination of other authors' findings the conclusion is that coronary heart disease cannot be attributed to any one single dietary factor. Although, in some studies, heavy consumption of fats and simple sugars has been found to be associated with high prevalence of atherosclerosis and ischaemic heart disease, in several other investigations no definite relationships to dietary habits (Paul et al., 1963; Papp, Padilla & Johnson, 1965; Finegan et al., 1968; Obeyesekere, 1968) were detected.

Also in the context of a wide geographical investigation like the International Atherosclerosis Project it appears that in most population groups the sources of fats and of carbohydrates cannot be considered as the primary factors in determining severity of atheroclerotic lesions (Scrimshaw & Guzman, 1968).

Such discrepancies may be explained, at least to a certain extent, by taking into consideration other interrelated factors that are always present, in addition to the dietary ones, thus preventing a clear understanding of the relationship of diet to cardiovascular diseases. These factors are: physical activity, psychological stresses and, perhaps, some of the concomitants of affluence. It was suggested by some authors (Lowenstein, 1964; Mann et al., 1964; Sievers, 1967) that the low death rates from ischaemic heart disease detected in population groups leading a rather primitive way of life could be attributed to the fact that these peoples are regularly engaged in strenuous physical activity, possess a good degree of physical fitness, and are free from the psychological stresses of a competitive society. It is beyond the scope of this article to discuss the effects of smoking on cardiovascular health. Although none of these factors individually may explain major differences in cardiovascular mortality rates, together they might play an important protective role.

Physical activity has been reported to exert a favourable effect on the incidence of atherosclerosis and coronary heart disease (Morris et al., 1953a, 1953b; Karvonen et al., 1961; Mann et al., 1964; Brunner, 1966), possibly by lowering the blood lipid level (Lopez et al., 1966; Montoye et al., 1969).

Mental stress, instead, allegedly provokes mobilization of nutritional deposits but these are not properly used: lipid levels in the blood increase (Bogdonoff et al.;[1] Steinberg & Shafrir, 1960) and this situation, if repeated or protracted for long periods of time, might precipitate the onset of AHD in people who, because of dietary, hereditary or other factors, are already prone to the disease (Charvat, Dell & Folkow, 1964). According to Russek (1967) psychological stress may enhance the atherogenicity and lethality of a high fat diet. The work of Groen et al. (1961), on Benedictine and Trappist monks, does indeed lend support to the hypothesis that a life free from social and psychological stress has, regardless of dietary patterns and habits, a beneficial effect on cardiovascular health.

Observations on animals also showed the detrimental effects of psycho-social stresses: hypercholesterolaemia, coronary atherosclerosis, myocardial necrosis, and myocardial infarction were produced in animals by subjecting them to emotional stresses (Uhley & Friedman, 1959; Groover et al., 1963; Raab, Chaplin & Bajusz, 1964; Ratcliffe & Snyder, 1967).

It is true that the real importance of mental stress in the development of AHD has never been definitely proved, and that people in primitive societies also experience considerable psychological stress but, as Jolliffe (1959) pointed out, the mental stress in modern civilization as opposed to the " stress of the jungle " is perhaps of a new type to which man has not yet learnt to adjust fully.

Concerning prosperity, it seems to be associated with higher death rates from AHD on a geographical basis: if we take, for instance, the national *per capita* income and the national *per capita* energy consumption as indices of living standards and of technological progress, and relate them to the death rates from AHD, good correlations are found (see Table 1). Positive correlations of cardiovascular mortality to indices of national " development " such as income, number of television and radio sets, number of

[1] Bogdonoff, M. D., Estes, E. H. & Harlan, W. (1959) *Psychophysiologic studies of fat metabolism*, a paper presented at the 40th Annual Session of the American College of Physicians, Chicago, April 1959.

telephones, etc., were also found by Yudkin (1957, 1964) and by Jolliffe & Archer (1959). Even on a small population basis it has been reported that prevalence of coronary heart disease increases with economic status (Bronte-Stewart, 1958; Morris, 1956; Servotham & Berry, 1968), although this association cannot be considered as well defined.

Higher consumption of fats (particularly of the saturated type) and of refined sugars, and lower consumption of complex carbohydrates are usually associated with higher prosperity. Table 1 shows indeed that national *per capita* income correlates positively with intake of total and of saturated fats, and of sucrose, and negatively with consumption of complex carbohydrates. Therefore, it may well be that the relation between dietary factors and the incidence of AHD is secondary to other factors, which are in turn associated with living standards, and which perhaps play a more important role.

In conclusion, the relationship of diet to atherosclerosis and coronary heart disease is still a matter for further research; no single dietary factor, nor the blood cholesterol, may be held as the sole or even the main factor responsible for the development of the disease. AHD is currently regarded as a disease of multiple etiology in which dietary factors, obesity, elevated blood levels of cholesterol, triglycerides and other lipids, heredity, hypertension, diabetes mellitus, mental stress and physical inactivity are considered to be important predisposing factors; when studying the possible influence of diet on cardiovascular health and disease, we should thus consider the way of life of the people. The way of life may cause the dietary factors to be harmful for some populations while they are harmless for others. Moreover, factors which are of a high-risk type on a population basis may not be so on an individual basis, and *vice versa*.

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RÉSUMÉ

FACTEURS DIÉTÉTIQUES ET MALADIES DES CORONAIRES

On a réuni pour 37 pays les informations (1965) relatives à la mortalité par artériosclérose des coronaires et myocardite dégénérative ainsi que les renseignements disponibles (1962) concernant la consommation *per capita* des graisses totales, des acides gras saturés, du saccharose, des sucres simples et complexes, des protéines et l'apport de calories. Ces données ont ensuite fait l'objet d'une évaluation statistique afin de déceler une relation possible entre ces facteurs diététiques et l'incidence des affections des coronaires.

On relève une forte corrélation positive entre la consommation de graisses totales et d'acides gras saturés et les taux de mortalité par artériosclérose des coronaires et myocardite dégénérative. En ce qui regarde la consommation de calories, de saccharose et de sucres simples, la corrélation est moins nette et elle est quasiment nulle pour la consommation de protéines. La très nette corrélation négative existant entre la consommation d'hydrates de carbone complexes et la fréquence des maladies des coronaires représente vraisemblablement une fausse relation étant donné qu'une corrélation du même type apparaît aussi entre ce facteur et les autres facteurs diététiques, à l'exception des protéines. Des résultats similaires ont été obtenus par l'analyse de données relatives à la décennie précédente.

Les tendances exposées ci-dessus sont observées dans tous les groupes étudiés, qui présentent diverses combinaisons d'âge et de sexe. On note cependant que les corrélations sont parfois moins apparentes dans le sexe féminin. Il n'existe par ailleurs qu'une très faible corrélation entre l'évolution des différents facteurs diététiques et l'augmentation croissante des taux de mortalité par artériosclérose des coronaires et myocardite dégénérative au cours des années dans un pays donné. Les résultats de cette analyse sont examinés et discutés à la lumière des observations faites à l'occasion de nombreuses autres investigations.

Il semble que l'on puisse considérer comme établi que

la consommation exagérée de graisses, et surtout d'acides gras saturés, favorise le développement de l'artériosclérose des coronaires et de la myocardite dégénérative, mais une telle tendance ne se manifeste pas dans plusieurs groupes de population, et en particulier dans les couches sociales les plus défavorisées du point de vue économique et dans les pays en voie de développement. Il faut peut-être en chercher la cause dans le fait que les individus appartenant à ces groupes ont une activité physique quotidienne relativement importante qui assurerait une sorte d'effet protecteur sur la fonction cardio-vasculaire. Les rapports entre le régime alimentaire, d'une part, et l'artériosclérose des coronaires et la myocardite dégénérative, d'autre part, sont encore imprécis et l'apparition et le degré de gravité de ces affections ne peuvent être attribués avec certitude à l'un ou l'autre facteur diététique unique. Il importe de tenir compte d'autres éléments, comme le manque d'activité physique, les tensions psycho-sociales et le bien-être matériel, dont le rôle favorisant ne doit pas être négligé. En ce qui regarde l'influence de la prospérité et du développement technologique, on relève, sur le plan national, de fortes corrélations positives entre les taux de mortalité par artériosclérose des coronaires et myocardite dégénérative et le revenu ou la consommation d'énergie par tête d'habitant.

L'influence éventuelle sur la fonction cardio-vasculaire des oligo-éléments et le rôle du déséquilibre de l'apport en sels minéraux résultant des modifications du milieu et des manipulations subies par les denrées alimentaires sont exposés à grands traits.

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Scintillation Counting Techniques In The Isotope Dilution Analysis For Sucrose

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Introduction

The Spreckels research laboratory currently uses an isotope dilution (ID) analytical technique to determine the true sucrose content of various materials. The ID method, as originally reported (3)[2], utilized a gas flow proportional counting system to detect radiation. This system was adequate for the analysis of a small number of samples where the counting time was unimportant. However, the limited sample capacity of the proportional counter did not permit the analysis of a large number of samples.

The ID method has been modified to use a liquid scintillation counting technique. The modification has expanded the general usefulness of the method with no loss in analytical accuracy.

This paper details the scintillation counting technique used in the ID method. Included are preparation of the scintillation solvent, the activity of standards, preparation of vials for counting and counting procedures. Factors that affect the accuracy of the method are also discussed.

Method and Materials

Extraction and purification of the sucrose from samples were reported earlier (3) and are essentially unchanged i.e., barium precipitation and carbonation, purification by ion exchange treatment and crystallization from methanol.

Standards: The radioactive standard sucrose is prepared by diluting 0.5 microcuries of sucrose-^{14}C with 1000 grams of nonradioactive sucrose. The sucrose-^{14}C and sucrose-^{12}C are dissolved in the minimum amount of distilled water, concentrated on a steam bath, and recrystallized from distilled methanol. A portion of this standard is added to the extraction sample so that the resultant mixture has about a 1:1 ratio of radioactive to nonradioactive sucrose.

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[2] Numbers in parentheses refer to literature cited.

A counting standard is prepared in the same manner as the sample. In this case however, the sample is an accurately weighed quantity of pure nonradioactive sucrose. The ratio of radioactive to nonradioactive sucrose is accurately determined and held at a 1:1 ratio.

Comparison of the activities of the counting standard and the pure sucrose recovered from the sample is the basis of the isotope dilution method. The ratio of activities is a direct measure of the sucrose originally present in the material being analyzed.

Preparation of Scintillation Mixture: A sample prepared for liquid scintillation counting includes at least three components: the material being counted, a solvent and a scintillator. Often a fourth component is added to facilitate sample preparation. Usually the physical or chemical nature of the sample, rather than the isotope in question, determines the solvents and additives used in sample preparation. We have found the system(1) described below to be well suited for sucrose analysis.

Components of the scintillation mixture include: Reagent grade p-dioxane; Naphthalene-recrystallized from alcohol; 2,5 diphenyloxazole (PPO). Normal mixture composition by weight is 100 p-dioxane, 4.5 naphthalene, 1.0 PPO.

The mixture is treated with 10% by weight activated carbon(2) and filtered through a 0.45 micron Millipore filter. It is advisable to prepare a fresh scintillation solution for each new group of samples to be counted. Scintillation solutions that have yellowed or set for a prolonged period of time are not reliable.

It should be noted that the scintillation solution described does not contain a secondary scintillator e.g., wavelength shifter. We have found, that for our particular scintillation counter, inclusion of a secondary scintillator does not significantly increase our counting efficiency. This situation will not be true for all counters. Maximum counting efficiency for any counter will result when the wavelength of the light emitted by the scintillator most nearly matches the counter's photomultiplier response. Use of a secondary scintillator with our scintillation mixture will, therefore, depend on the characteristics of the counter to be used.

Preparation of Samples for Counting: The choice of the amount of sucrose that can be used in scintillation counting is restricted by the limited solubility of sucrose in the organic scintillation solvent. To increase its solubility, sucrose is dissolved in water. Water is a good solubilizer, but it is also a strong chemical quencher i.e., interferes with the transfer of energy between the site of an event and a molecule of scintillator. Inclusion of

water in the scintillation mixture will naturally lower the counting efficiency. However, if a constant amount of water is used for all samples, and the counting rate remains sufficiently high to insure good statistics, the loss in counting efficiency is of little consequence.

We have found that 0.2000 grams of sucrose dissolved in 5.0 milliliters of deionized water will form a homogeneous mixture with the scintillation solvent and will remain in solution at reduced counting temperatures e.g., 50°F.

The sucrose is accurately weighed into glass vials for counting. Five milliliters of deionized water are pipetted into each vial. The vials are capped and warmed in a water bath until the sucrose is completely dissolved. After the vials have cooled, 15 milliliters of scintillation solution are pipetted into each vial. Each vial is capped, agitated for homogeneous mixing, and wiped with lens paper. The vials are then ready to count.

Extreme care should be taken to see that insoluble material does not enter the vial with the water. To prevent this the deionized water is filtered through a 0.45 micron Millipore filter before use.

The use of a constant amount of solution in all vials requires precise delivery of both water and scintillation solvent to the vials. Nonuniform delivery will result in greater than normal variation in average count rate between consecutive vials of the same sample. Automatic pipettes with good delivery precision are essential for dispensing scintillation solvent and water.

The choice of the number of standards and samples to be counted and the counting time required for any particular accuracy of sucrose determination is based, in theory, on the activity of the radioactive component. However, certain variables can limit the precision of the test. We have found that the physical and chemical nonuniformity of all commercially available glass vials can be one such limiting error. The magnitude of this error can be reduced by using vials that have been selected for their uniformity of counting. A simple, yet novel vial selection procedure is given below. It should be remembered that vial selection is not a necessary part of the liquid scintillation technique. However, selection of vials will reduce the number of replications required and, therefore, the time necessary to achieve the desired analytical precision.

Vials can be selected for their uniformity of counting if each vial is counted with the same source of radioactivity. Variations in count rates between vials due only to the random nature of radioactive decay follow a known function and can be approximated. Therefore, it is possible to make a comparison of vials

where there is a reasonable probability that any detectable nonuniformity of counting is attributable to the physical or chemical nature of the vials.

If a weighed amount of a homogeneous radioactive scintillation solution is added to each vial, then all vials can be counted with the same source of radioactivity.

The random nature of radioactive decay is described by a Poisson distribution function and the expected variation due to this randomness is approximated by:

$$\text{Eq. [1]} \quad \sigma_p = \frac{\sqrt{\text{Total Counts}}}{\text{Total Time}}$$

The expected variation due to the random nature of radioactive decay can, therefore, be reduced by increasing the total counting time per vial.

The resultant counts of the vials will be distributed in a normal fashion. An example of such a distribution is given in Figure 1. The count interval in the distribution is twice the expected standard deviation of counts due to the randomness of radioactive decay calculated in terms of counts per minute per gram of scintillation solution. The 2σ value for the count interval allows a 95% assurance that the vials in any interval are uniform to 0.05% in counting. Vials in any one interval are considered sufficiently uniform for use.

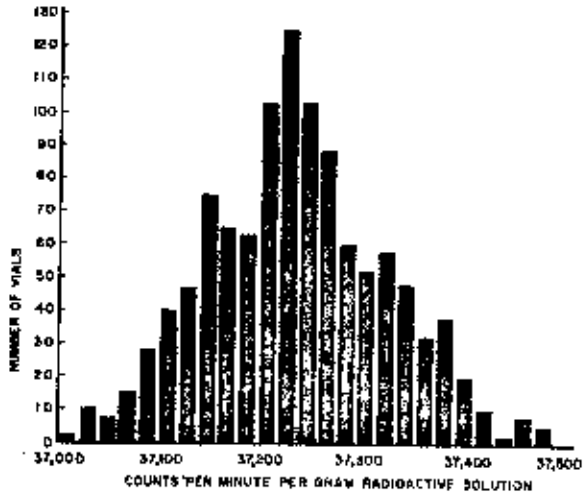

Figure 1.—Distribution of vials by count rate.

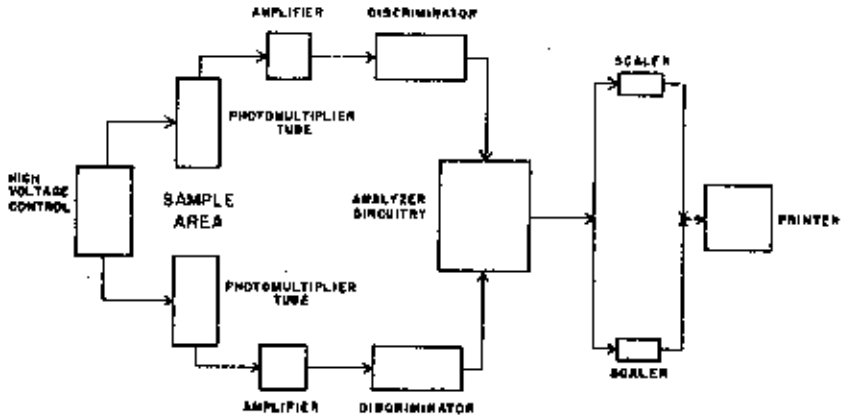

Figure 2.—A simplified schematic of a liquid scintillation counter.

Counting Procedure: Samples and standards are counted in a liquid scintillation counter. A simplified schematic of a modern scintillation counter is given in Figure 2.

It is generally accepted that a more efficient count is obtained if the sample temperature is lowered. This axiom was developed from early work in the field of liquid scintillation counting when counters were less sophisticated. Modern instruments have photomultiplier tubes that are less sensitive to thermal excitation, and ambient counting temperatures are now common. The choice of counting temperature should, therefore, be based on the particular scintillation counter.

The modern scintillation counters allow a choice of counting procedures. Two counting procedures, the channels ratio and balance point methods, are equally applicable to the ID method.

The channels ratio procedure does not give maximum counting efficiency, but it does provide a constant check on sample uniformity. This procedure is normally used for counting samples that vary in their degree of quench. If it is desired to prepare samples that are uniformly quenched, as in the ID procedure, then this counting method is a good check on sample preparation. Once sample preparation is standardized it is better to count the samples with the highest counting efficiency.

The balance point counting procedure insures maximum counting efficiency for samples of uniform composition. The energy discriminator settings used in this procedure will depend on the liquid scintillation counter to be used.

Calculation for percentage sucrose in the sample is the same as for proportional counting and has been described earlier (3). An example of typical counting data and calculations is given in Table 1.

Table 1.—Liquid scintillation counting data and percent sucrose calculation.

Sample	Molasses (beet)			Counting standard		
Vial No.	Wt sample in vial gm	Total counts 10 min	Avg CPM 0.2000 gm	Wt std in vial gm	Total counts 10 min	Avg CPM 0.2000 gm
1	0.2003	1,682,300	167,978	0.2002	1,676,510	167,484
2	0.1998	1,685,720	168,741	0.2001	1,676,870	167,554
3	0.2000	1,683,970	168,397	0.2003	1,678,090	167,558
4	0.1999	1,686,350	168,714	0.2001	1,670,350	166,952
5	0.1998	1,686,610	168,829	0.2004	1,675,020	167,168
6	0.2002	1,687,130	168,544	0.2000	1,672,060	167,206
7	0.1999	1,682,580	168,337	0.2001	1,673,370	167,253
8	0.2000	1,686,120	168,612	0.1996	1,670,230	167,558
9	0.1998	1,683,500	168,719	0.2000	1,672,630	167,266
10	0.2000	1,681,930	168,193	0.2004	1,671,990	166,865

$W^{14c} = 6.0016$ $R_1 = 167,266$ $\sigma_{R_1} = \pm 74$ $a_1 = 0.00044$

$W_s = 12.0017$ $R_2 = 168,506$ $\sigma_{R_2} = \pm 87$ $a_2 = 0.00052$

$$\% \text{ Sucrose} = \frac{100\ W^{14c}}{W_s}\left(\gamma \frac{R_1}{R_2} - 1\right) \text{ and}$$

$$\sigma_{\% \text{ Sucrose}} = \frac{100\ W^{14c}}{W_s} \cdot \gamma \frac{R_1}{R_2} \cdot \sqrt{a_1^2 + a_2^2}$$

$$\% \text{ Sucrose} = \left(\frac{6.0016}{12.0017}\right)(100)(0.98528) = 49.27$$

$$\sigma_{\% \text{ Sucrose}} = \left(\frac{6.00}{12.00}\right)(100)(1.985)(0.00063) = \pm 0.07$$

where:

W^{14c} = weight of standard sucrose added to the extraction sample
W = weight of the extraction sample
R_1 = average counting rate(CPM) of the counting standard
R_2 = average counting rate(CPM) of the sample
a_1 = relative error of the counting standard
a_2 = relative error of the sample
γ = weight ratio $W^{14c} : W^{14c} + W^{14c} = 2.0000$

A comparison of results by proportional and liquid scintillation counting techniques is given in Table 2. The data show no significant difference in percentage sucrose or analytical error between the two methods.

Table 2.—A comparison of results by proportional and liquid scintillation counting techniques in the ID determination of sucrose.

Beet sample	% Sucrose ± σ_A	
	Proportional	Liquid scintillation
1	12.44 ± 0.02	12.44 ± 0.03
2	12.52 ± 0.03	12.54 ± 0.02
3	12.75 ± 0.06	12.75 ± 0.02
4	12.66 ± 0.02	12.69 ± 0.02

Summary

The use of a liquid scintillation counting technique in the isotope dilution analysis for sucrose is described. Included are

preparation of the scintillation mixture, activity of standards, preparation of vials for counting and counting procedures. Examples of typical counting data and analytical results are also given.

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DYSGLYCIDOSES
ANOMALIES OF SUGAR METABOLISM

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Since, in 1815, Chevreul showed that the sugar extracted from the urine of diabetics is identical to grape sugar, or glucose, great progress has been made in our knowledge of the anomalies of the metabolism of glucides. In the beginning, all the research was oriented toward the physiopathology of sugar diabetes and the regulation of glycemia; we know the importance of the secretion of insulin and other glyco-regulatory hormones of the prehypophysis, the adrenal cortex, the adrenal medulla, as well as that of glucagon and the thyroid hormones in explaining the hyperglycemia and glycosuria of sugar diabetes. But sugar diabetes is not the only anomaly of sugar metabolism: an impairment of the renal reabsorption of sugar is the cause of a less serious disorder, characterized by glycosuria without hyperglycemia. This is renal diabetes.

The precision of the methods of analysis of the urinary oses and progress in clinical enzymology have made possible the recent discovery of a new class of metabolic ailments due to congenital intolerance of certain sugars (galactose, fructose and disaccharides especially): dysglycidoses. Formerly, the presence of such sugars in the urine was considered a laboratory curiosity. It was caused by a physiological surcharge (lactosuria of pregnant women) or an alimentary surcharge (saccharosuria of the gourmand, maltosuria of the beer drinker), without any serious pathological consequence. Its greatest problem was that it caused errors in the course of analysis of the sugar in order to diagnose sugar diabetes.

However, it seems that the frequency of dysglycidoses is more significant than we may imagine, and that they now pose, at least in the most serious cases, a significant dietetic problem.

METABOLISM OF FOOD GLUCIDES

First of all, we will survey the general outlines of digestion and the utilization of food sugars; this will help us in determining the seat of dysglycidoses.

The glucidic nourishment of man is composed of a relatively

restricted number of sugars, at least if we only consider those that are significant from a quantitative point of view. Outside of the lactose of milk and the glycogen of meat, plant foods supply the majority of glucides. The glucides are found in plants in the form of starch and disaccharides that can be hydrolysed by the intestinal enzymes. Cellulose, a polysaccharide with a bond resistant to these enzymes, cannot be used and is rejected. Thus, the hydrolysis of the polysaccharides and disaccharides of food into simple oses represents the initial indispensible phase to make it possible for these glucides to pass through the intestinal mucosa and supply the energy elements necessary for cellular function.

A) Digestion

Among the polysaccharides, starch, that is amylose and amylopectin, accumulated in the grains and storage organs of plants, is broken down by the amylase of the saliva and the pancreatic juice into molecules of dextrin, then maltose and isomaltose. The glycogen of animal foods supplies the same disaccharides that join with the natural disaccharides in the intestinal opening. These sugars are formed by the condensation of two molecules of oses: the most important are saccharose (fructose + galactose) and lactose (galactose + glucose) (fig. 1). The action of the intestinal disaccharidases is exercised on these sugars:

— saccharase (or invertase, or maltase Ib) hydrolyzes chiefly saccharose and frees the two components;
— lactase hydrolyzes lactose;
— the maltases (II and III) and isomaltase (or maltase Ia) free two molecules of glucose from maltose and isomaltose.

Thus, the action of the enzymes of the saliva, the stomach acidity, the amylase of the pancreas and the intestinal disaccharides brings about the fact that at the level of the small intestine, the food glucides are normally found in the form of a mixture, soluble in water, of glucose above all, and, in a lesser proportion, of fructose and galactose. Beside these essential sugars, small quantities of mannose, a hexose found in certain fruits and vegetables, and pentose (especially arabinose), abounding in cherries, prunes, apples and onions, are found. Thus, the list of oses, hexoses or pentoses, which have to penetratethrough the intestinal mucosa under normal physiological conditions, is short. However, the mechanisms of this penetration are diverse and thus far not well known.

B) Absorption

The absorption of the oses, after possible hydrolysis, is realized essentially at the level of the small intestine, but not all of them penetrate the intestinal mucosa, a living membrane endowed with its own metabolisms, in the same manner. Therefore, one primary distinction should be made between the sugars. Before being supplied digestively, some of them are found in all cells, including those of the intestine, and their

absorption by the mucosa occurs along a gradient of increasing concentration; that is, utilizing energy stored or manufactured in the intestinal cell itself. Other sugars, on the other hand, are not "physiological"; they diffuse through the mucosa without being modified or utilized. Their absorption is called passive; it is a little slower than that of the oses absorbed actively.

This distinction between sugars absorbed actively and sugars of passive diffusion is based on the fundamental experiments of Cori (1920) and Wilbrandt (1932), confirmed by Cordier (1956). These authors admit that the active transit sugars, i.e. glucose, galactose and, to a lesser degree, fructose, are absorbed more rapidly than the others, because they are phosphorylated. The inhibitors of phosphorylation retard the penetration of these sugars. This biochemical process is extremely limited, so that the speed of absorption, rapid in the case of small concentrations, diminishes clearly if the amount of sugars ingested is increased. In contrast to this, in the case of passive diffusion sugars, the speed of absorption increases with the concentration. Their penetration is regulated by the laws of diffusion and of osmosis.

Of these theories, we still retain today the classification of sugars into substances absorbed by active transport or by passive diffusion, but, as a result of the research of Sols, the hypothesis of the phosphorylation of the sugars has been abandoned. As in the case of all active transport — whether we study the penetration of nutriments in a bacteria or the intestinal absorption of glucose — the enzymatic system that controls these mechanisms uses ATP (adenosine triphosphate), a reserve of cellular energy. Its synthesis depends upon the systems of phosphorylating oxidation, and, in the absence of other energy carriers, it seems that, to counteract the concentration gradient, a part of the active transport sugar is itself consumed by the cell to furnish energy. Thus, its speed of absorption is determined by the rapidity and efficaciousness of its catabolism. Thus we see that in this domain glucose is more energetic than galactose and fructose and is absorbed most rapidly. Moreover, these two latter hexoses are transformed into glucose in the cell of the intestinal mucosa (then in the hepatic cell) and contribute to heightening glycemia. In the course of considerable alimentary surcharge, or in cases of an enzymatic deficiency of the intestinal cells, the active transport sugar becomes a passive penetration sugar, and its diffusion is regulated solely by its solubility. It is thus that the disaccharides, normally hydrolyzed by the intestine, diffuse in the liquids and tissues and are finally eliminated by the natural emunctors without being modified: the cells cannot utilize them and consider them substances foreign to the organism, since the enzymes that are supposed to "recognize" them do not exist or have overflowed.

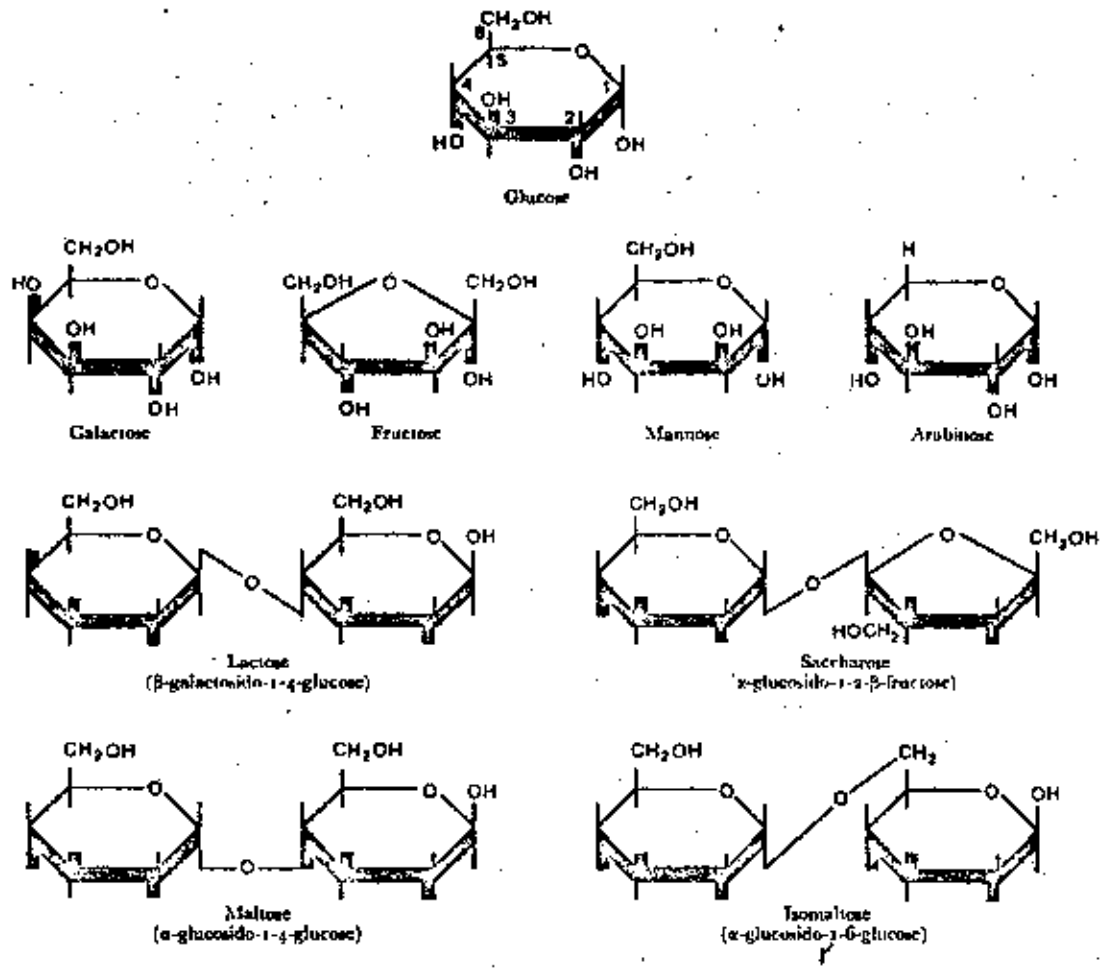


Figure 1. Structure of some food sugars.

C) Utilization

If a part of the sugars can be utilized by the intestinal cell, the majority traverses the mucosa and arrives at the liver through the portal vein. The "utilizable" oses (glucose, galactose, fructose) are transformed into energy (ATP) by means of diminuition in the form of trioses, of pyruvic acid and finally of carbonic gas and water, in the course of a series of chain reactions (decomposition path of Embden-Meyerhof, Krebs tricarboxylic cycle. . .) (fig. 2). Their use as energy can be different, and they are placed in reserve in the form of glycogen, after their conversion into glucose.

The "non-utilizable" oses are those which cannot be transformed into glucose. After having diffused into the liquids and tissues, which neither retain nor accumulate them, they are rejected, eliminated in the urine.

Thus, it is the facility with which the sugar considered is transformed into glucose that will determine its utilization. Thus fructose is supposed to be phosphorylated by a fructokinase into fructose-1-phosphate, then broken down into two trioses by a special aldolase. The research of Hers (1953) and Leuth-

ard (1950) showed that these trioses are then recombined to rejoin the path of glycolysis or development of glycogen. The efficaciousness of this complex metabolism is such that we normally find only very small traces of free fructose in the body liquids (about 40 mg fructose per liter of serum). We also understand the essential role of the fructose-1-phosphate aldolase and of fructokinase in this metabolism.

Figure 2. Diagrams of the metabolism of sugars.

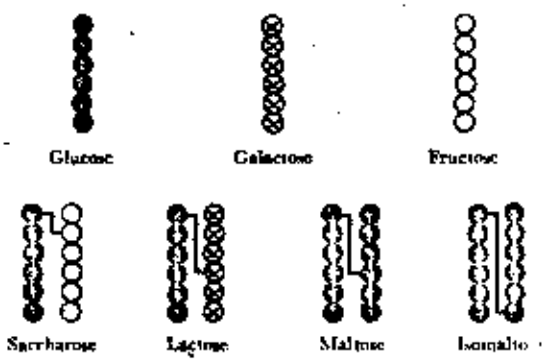


Diagram 1. Schematic formulas of the principal oses and diholosides.

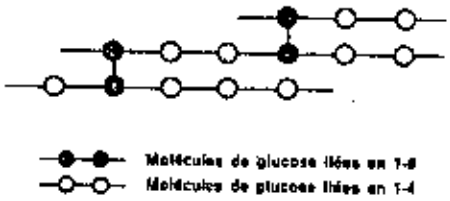
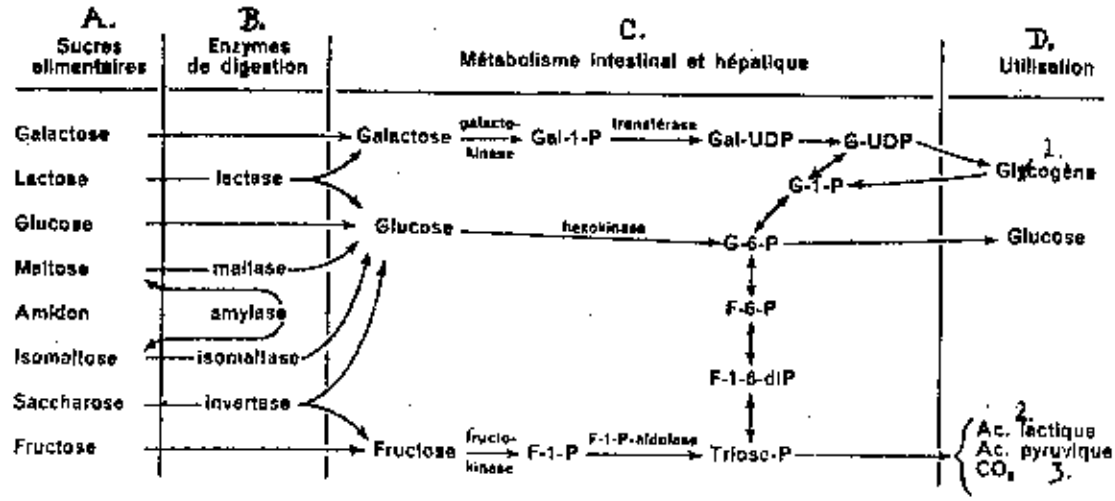


Diagram 2. Starch molecule
A.= Glucose molecules bound in 1-6
B.= Glucose molecules bound in 1-4

Human intestinal disaccharides
(According to Auricchio)

A. Enzymes	B. Activité hydrolysante vis-à-vis de			
	Maltose	Saccharose	Isomaltose	Lactose
Maltase 1 ...	15 %	0	0	0
— 2 ...		0	0	0
— 3 ...	3 –	10 %	0	0
— 4 ...	80 –	90 –	0	0
— 5 ...		0	100 %	0
Lactase	0	0	0	100 %

A.= Enzymes B.= Hydrolyzing activity in relation to:



A.= food sugars B.= enzymes of digestion
C.= intestinal and liver metabolism D.= utilization
1.= glycogen 2.= lactic acid 3.= pyruvic acid

Galactose plays a slightly different metabolic role. It can serve to elaborate polysaccharides and complex lipides that enter into the structure of the cell membranes. It also joins -- and this is the essential metabolic path -- the energy metabolism either directly or through the intermediary of glycogen. Whatever it be, galactose always passes through the same chain reaction: galactose is phosphorylated into galactose-1-phosphate by galactokinase, but this activation of the sugar is not enough. It must further be transformed into uridine-diphosphogalactose by a galactose-1-phosphate-uridyl transferase. It is this "galactose activity" which serves as the springboard for all the transformations of galactose.

The metabolism of the other hexoses or pentoses is less important and generally little known. However, it is possible that certain pentoses can, by complex routes and in small amounts, rejoin the routes of glucose utilization.

D) Elimination

Normally, we find no sugar in the urine, since all the oses are utilized at the level of the tissues and their blood concentration increases relatively little. Only glucose appears in the urine when glycemia surpasses 1.8 g/l, the renal threshold value for this sugar: this is what occurs in the course of sugar diabetes and explains the glycosuria of the diabetic. In the same way, when the capacity of renal reabsorption is lowered, as in the course of renal diabetes of certain renal deficiencies, thus when the threshold is lowered, we observe glycosuria.

The other sugars are eliminated, when the tissue utilization capacity is passed, in the course of massive absorptions or of either congenital or acquired anomalies of the liver or the tissues. The appearance of fructose, galactose, pentose and also

disaccharides in the urine is of great interest as regards the diagnosis of dysglycidoses.

ANOMALIES OF SUGAR METABOLISM

From among the anomalies of sugar metabolism, we exclude those relating to the utilization of glucose. Sugar diabetes is too current and too important an ailment to be treated succinctly in a work of this nature. The same is true of glycogenoses, congenital anomalies of the glycogen metabolism, which have serious consequences for the utilization of the oses and, therefore, for all the metabolic correlations of the most important tissues.

Dysglycidoses are caused by a hereditary enzymatic deficiency, and the discovery of this deficiency was the object of recent research of numerous enzymologists. The absence of an enzyme necessary for digestion or the utilization of an important food sugar is manifested by the accumulation of this sugar or its metabolites in the tissues or the body liquids, by their elimination in the urine, leading to a more or less significant nutritional disequilibrium, and finally, in certain cases, the most serious, by a veritable "intoxication" of the cells where the sugar metabolites accumulate, an intoxication that often brings about the death of the subject with the deficiency.

The most important and most frequent of these anomalies concerns the utilization of galactose -- this is congenital galactosemia.

Hereditary intolerance of fructose is also found in our country. Intolerance of disaccharides which have been described recently provoke serious dyspeptic disorders in children, and study of them is very important to explaining dyspepsia in children.

Finally, there has very recently been described a congenital intolerance of active transfer sugars (Laplane).

All these facts, as well as the electronic microscope study of the structure and physiology of the cell, led R.K. Crane in 1966 to create the concept of "maladies of the brush layer of the intestinal mucosa", since it is in this layer that the enzymes responsible for the hydrolysis of the disaccharides and those necessary for the active transfer of sugars have been identified.

A) Congenital galactosemia

Since 1955, we have known that hereditary intolerance of galactose is a relatively frequent malady that should be known and diagnosed rapidly.

It is due to the absence of an enzyme normally present in the liver and the red blood corpuscles: galactose-1-phosphate-uridyl-transferase. The anomaly is manifested, at least in its most serious form, beginning from the first days of life, in a severe anorexia with vomiting and diarrhea, as well as by hepatic disorders involving a large liver, jaundice and cirrhosis.

A bilateral cataract develops rapidly. Finally, the general condition alters and conducts the small patient into a sort of torpor, a coma that precedes death. Sometimes, in more moderate forms, the development is less rapid, but the milky nourishment, and above all galactose, provoke digestive, liver ocular and general disorders that are not always negligible.

It seems that galactosemia is a genetic malady of autosomic recessive transmission, and the homozygotes have more marked signs of intolerance to sugar than have the heterozygotes. In every sense, the biological diagnosis is easy: the presence of galactosuria, galactosemia, the clinical and biochemical anomalies observed in the course of a surcharge of galactose, confirm the diagnosis. It can be assured by the revelation of an enzymatic deficiency while determining the activity of the transferase in the liver or, more often, in the erythrocytes of the patient.

In order to explain the clinical signs and the histological lesions of congenital galactosemia, it must be made known that they are due to the accumulation of galactose-1-phosphate and galactose in the cells. The child, whose glucidic nourishment is composed almost solely of lactose, is incapable of utilizing the galactose half of this sugar. Moreover, he will utilize energy to activate this galactose, and that will be a dead loss, since the activated galactose will never take part in the catabolic and oxidative mechanisms. Finally, the galactose-1-phosphate that accumulates in the cells provokes disorders on the level of other metabolisms. We have long known how to produce an experimental cataract in the rat by administering a diet rich in galactose. It has also been shown that the phosphorylated sugar inhibited a large number of enzymes in the liver, the crystalline lens, the kidney and the brains. These arguments are sufficient to explain the multiplicity and gravity of the symptoms and to demand that any case of galactosuria of the new-born, even when insufficiently proven, should be submitted to the only effective treatment, the exclusion of galactose from the infant's diet. Only at this price can we hope to affect the development and prognosis of the disease. In fact, in a still limited number of congenital galactosemias treated from birth, the patient has not only survived beyond the first six months of life, but tolerance of galactose has even improved during the course of his existence.

B) Fructose intolerance

The benign anomaly that we call "essential fructosuria", and which is manifested by urinary elimination of fructose, is due to the absence of liver fructokinase. The fructose, being unable the join in the metabolism of the oses, passes through the liver without being modified and is eliminated by the kidneys. This fructosuria is not accompanied by any clinical anomaly.

The same is true of "congenital fructosemia", observed for the first time by Chambers and Bratt in 1956, and since then by Swiss, Belgian and French physicians. As in the case of congenital galactosemia, the anomaly is manifested starting from the absorption of fructose or saccharose, that is, at the time of weaning, by serious dyspeptic disorders with hepatomegalia,

signs of hypoglycemia with pallor, somnolence, profuse sweating attacks, but never by a cataract.

The biological study leads to the discovery of a fructosuria and fructosemia, of varying intensity according to the intake of food fructose. The fructosemia is accompanied by an often severe hypoglucosemia, and this drop in plasma glucose can be induced by the administration of fructose; however, this test is dangerous because of the significance and the duration of the hypoglycemia. Congenital fructosemia is due to the absence of fructose-1-phosphate-aldolase in the liver and the intestine, the absence of whose activity can be ascertained by examination of the biopsy sample.

The interest of the comparison of the two intolerances to fructose lies in demonstrating the modifications produced following the attack of the "enzymatic lesion". In the two cases, the enzymatic deficiency prevents the utilization of fructose by the organism and promotes its urinary elimination. But in the case of lack of fructokinase, the fructose passes through the cells without damaging them. In contrast, the absence of aldolase induces an accumulation of fructose-1-phosphate responsible for the toxic action on the liver and kidneys. The manufacture of this metabolite leads to the exhaustion of the energy reserves of the cells and, thereby, consumption of the glucose. This is the origin of hypoglycemia, and this is all the more important because the fructose-1-phosphate inhibits the enzymes of glycolysis and glycogenolysis. A large number of the clinical manifestations can be related directly to these phenomena of hypoglycemia, which always follow the administration of fructose.

Here again, the sole possible treatment of the anomaly is the suppression of all food fructose supply, either in free form or in a combined form.

C) Intolerance to disaccharides

Intolerance to disaccharides is due to the deficiency of disaccharide activity or activities of the duodenal mucosa. Patients with this disease suffer from malnutrition and chronic diarrhea when the diet contains the "badly tolerated" disaccharide. In this case, there is found in the cells the sugar and its derived metabolic acids, especially lactic acid, which strongly lower the pH of the cells.

A minimal portion of the unhydrolyzed disaccharide penetrates the intestinal mucosa and, since it is not used, is eliminated in the urine. Thus the etiology of the chronic diarrhea of the infant is enlarged: besides the alimentary origins or the purely infectious causes, a certain degree of the diarrhea can be attributed to an intestinal enzymatic anomaly. Durand distinguishes several disaccharide intolerances: it is a phenomenon due either to an enzymatic immaturity of the infant, a definitive absence of one or several enzymes contributing to the digestion of lactose ("hereditary alactasia" of Holzel), or to that of saccharose, maltose and other disaccharides.

It is not doubtful that intolerance of lactose will one day clarify the digestive disorders due to milk. The diarrhea of the

young patient resists dietetic and anti-infectious treatment and disappears when milk is withheld. The fecal acidity, the frequent lactosuria, the absence of hyperglycemia after lactose charge (fig. 3), and finally the lack of lactase activity of intestinal fragments taken out by means of biopsy-aspiration or surgically, make it possible to confirm the diagnosis and suggest an immediate therapy: the suppression of lactose in the food.

Intolerance of lactose is a serious ailment, not only because of the caloric loss due to non-utilization of lactose and the digestive disorders that accompany it, but also because of the toxic action of lactose on the kidney. However, the fact that there has recently been discovered a lactase deficiency in adults intolerant of milk poses the problem of enzymatic adaptation to lactose: the intestine of subjects who never receive lactose loses its lactase activity. Inversely, in certain cases, it is possible to cause, in certain deficient newborn infants, the biosynthesis of the enzyme, by administering lactose progressively.

Intolerance of saccharose was described for the first time in 1960 by Weijers and several cases have been observed by Prader, Delaitre, Francois, Jeune, Launiala, Anderson, etc., about a total of thirty cases. The seriousness of the symptoms is very variable, but they resemble those described in the case of lactose intolerance. However, two points must be underlined: research of urinary saccharose is often negative, while the tests of charge realized with several disaccharides are positive (absence of hyperglycemic curve) for several of them: the absence of invertase would thus be associated with the absence of maltase and sometimes also of isomaltase (tested by its action on palatinose, a sugar related to isomaltose). Sometimes even, this anomaly is accompanied by intolerance to starch.

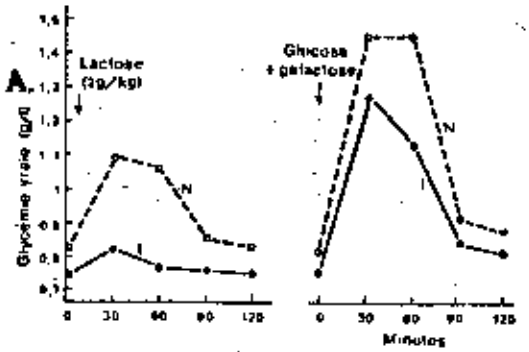


Figure 3. Hyperglycemia following oral surcharge of lactose (2 g/kg) or glucose (1 g/kg) + galactose (1g/kg) to a normal infant (N) and an infant with lactose intolerance (I).
A.= actual glycemia

The revelation of anomalies related to several disaccharides poses an interesting genetic problem and seems to contradict the famous law "one gene — one enzyme", since Dahlquist showed that invertase, maltase and isomaltase are different

enzymes. Prader proposes three possible explanations:

— either we are dealing with a polyvalent inhibitor, determined genetically;
-- or there exists a common site in the disaccharidases, which is lacking;
-- or the mutation is related to a regulatory gene common to these enzymes.

The prognosis of the ailment is less serious than that of lactose intolerance. If saccharose and the other disaccharides involved continue to be administered in the food, a fatal issue can be expected, but most frequently, a slow improvement of the digestive disorders is noted, the disorders amending themselves at around the age of 3 or 4 years. This amelioration can be explained by a retarded enzymatic maturation, as it exists in certain young mammals (fig. 4).

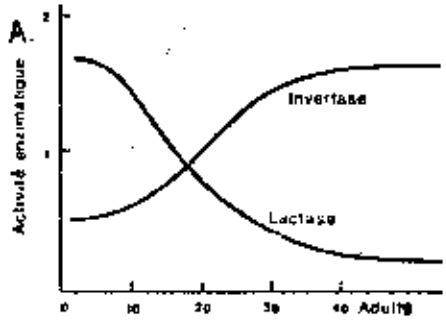


Figure 4. Enzymatic activity of the homogenate of the intestine of a young rat in the course of its development.
(According to R.G. Doell and N. Kretchmer

A.= enzymatic activity

DIETETIC TREATMENT OF DYSGLYCIDOSES

Whether they involve mono or disaccharides, whether they are serious or relatively well tolerated, these congenital intolerances to sugar due to enzymopathy allow only one dietetic therapy, the suppression of all intake of the sugar that is not tolerated. The results thus obtained are always spectacular and supply confirmation of the clinical and biological diagnosis. However, such diets are often very difficult to realize in the case of young, poorly nutritioned children. Besides, each intolerance poses its peculiar problems.

From the diet of the patient with congenital galactosemia we can exclude all galactose, and above all, all lactose, the principal source of galactose in food. The same is true for lactose intolerance. Thus, milk and all milk-based products should be eliminated. Cox and Pugh use a synthetic "milk" with a casein base, cocoanut and peanut oil, saccharose, mineral salts and vitamins. However, we should mistrust such prepara-

tions, for the casein used is impure and often contains lactose. American "Nutramigen" is a milk with a sunflower seed oil and protein hydrolysate base: it is not yet totally free of lactose. Certain people have proposed "soya milk", but it must be remembered that soy meal contains stachyose and raffinose, which are α-galactosides; thus, it can only be used with great caution. English authors prepare a pudding with grain, saccharose and eggs, but still do not succeed in eliminating all impurities containing galactose. Whatever it may be, it seems practically impossible to produce a diet absolutely free of galactose, and we often content ourselves with mixtures containing not more than 20 mg galactose in the amount consumed each day. In any case, it is necessary to insure that the diet chosen furnishes the infant with all the essential nutrients, that the ascertainment of galactose in the diet is nearly negative, and that no clinical or biological aggravation of the intolerance is noted after several days of administration.

The treatment of fructose intolerance consists, beyond symptomatic therapy, in excluding all free or combined fructose intake (especially saccharose), while at the same time supplying the infant with a sufficient and balanced diet. This diet is also difficult to realize, as is the no-lactose diet, since all vegetables contain fructose. Certain foods are well-known for their richness in it: flour, fruits, sugar, roots and tubercles, leguminous plants. In contrast, milk, meat, fish, eggs, potatoes and grains supply glucides in the form of lactose or polysaccharides with starch base, dextrins or maltose and glucose: these foods are therefore allowed. We emphasize that numerous medications or pharmaceutical preparations contain saccharose.

In the course of lactose intolerance, beyond the diets described above, it might be interesting to add lactase to the food. However, this enzyme is destroyed by the pancreatic juice, and this addition poses delicate practical problems. On the other hand, the addition of specialized preparations with an invertase base in cases of saccharose intolerance appears to be more effective, when the enzymes are added to diets poor in disaccharide and rich in glucose or lactose.

CONCLUSION

The discovery and study of dysglycidoses, that is, anomalies of the metabolism of glucides other than glucose, have led to the perfection of our knowledge concerning digestion, absorption, and utilization of sugars. Fructose and galactose are hexoses utilized by the cell to cover its energy needs, and their metabolisms pass through a stage of activation blocked, in cases of intolerance, by an enzyme deficiency: congenital galactosemia and fructose intolerance. The accumulation of their metabolites in the tissues leads to various serious disorders, which must be avoided by eliminating the poorly tolerated sugar from the diet.

Disaccharides must be hydrolyzed in order to be utilizable. This process of intestinal hydrolysis is lacking in case of lactose and saccharose intolerance, and the intestinal accumula-

tion of sugar produces chronic diarrhea in the child with this deficiency. The chapter on these intolerances to disaccharides is not yet closed: numerous problems remain unexplained, and it is probable that their classification is still to be accomplished in the light of new cases that will be described. For these ailments, unknown ten years ago, seem to be more frequent than we might have thought at first. They supply new data on the digestive pathology, and oblige us to develop research on enzymology and nutrition as well as dietetics.

Diet without lactose -- without galactose

Foods	Allowed	Forbidden
animal origin	meat, fish, eggs, Guigoz hyperprotidine	liver, brains, pancreas. quenelles, commercial pates.
milk products	none. synthetic milk without lactose. Nutramigen (Meat Johnson).	female and animal milk. industrial milks. cream cheese.
cereals	breads. non-milky grains. wheat 5 cereals vegelact A. vegelact B. vegelact C. vegelose. Sinlac and Nidine farina	Jacquet bread. Biscuits. Viennese milk bread. milky grains. 1st age wheat. 2nd age wheat.rice.
Fats	oil, margarine (Astra)	butter, cream, "Planta".
vegetables	all vegetables except:	peas, red beets, dried beans, instant purees.
sugar products	all except those containing milk.	candies, caramels, eskimos. instant and milk chocolate. bakery cream. instant fillers, boxed coffee, chewing gum.
condiments and others	all.	commercial mayonnaise. bechamel sauce. soup with milk, medications prepared with lactose, tablets, antibiotics.
beverages	all.	drinks made with milk.

The following remarks refer to the table on page 13:

Careful follow food packaging instructions.
Reject foods containing milk, lactose, casein, lactoalbumin, milk powders and cheeses.

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Sucrosuria with Mental Defect and Hiatus Hernia

by ALAN MONCRIEFF and R. H. WILKINSON

Sir ARCHIBALD GARROD, who was an active member of the staff of The Hospital for Sick Children, Great Ormond Street, from 1892 to 1913, described certain "inborn errors of metabolism". This was the start of an interest in physiological as distinct from anatomical "deformities" in infants and young children. Modern developments in chemical methods such as paper chromatography, flame photometry and spectroscopic analysis have greatly added to the knowledge of the subject. It is now known that not all such deviations from the normal in metabolism are necessarily inborn; some are thought to be transient, the result possibly of a slow maturing of enzyme systems; some can be ameliorated if not cured by certain lines of treatment. It is now the common practice to regard any severely wasted infant who is not making normal progress as possibly suffering from some metabolic error, and undoubtedly as time progresses the elucidation of many hitherto obscure causes of "marasmus" will prove possible. A case of galactosuria had occurred under the care of one of us (A. M.) and as a result it became customary to examine, among other things, for abnormal sugars in the urine in certain babies and young children with obscure wasting. In this rather chance way it was found that a patient was passing sucrose (saccharose, cane sugar) in the urine persistently. Two further examples of this have also been discovered and this communication deals with the clinical findings and some of the difficulties which have been encountered in studying the metabolism of sucrose in young children. The fact that all three children were mentally retarded and had an abnormality at the lower end of the oesophagus associated with hiatus hernia suggests that this may be a hitherto undescribed syndrome.

Methods

The three patients were given diets containing various sugars to study their effects on the urinary sugar excretion. The urine sugars were identified by one-dimensional paper chromatography by Dr. L. I. Woolf of this hospital (Woolf 1951), and their approximate concentrations measured. Blood sucrose was estimated as the difference between the reducing sugar levels before and

after treatment of the whole blood with invertase (see also ELMER KRASOWSKA and PTASZAK 1939). The reducing sugars were estimated by the micro-method of HAGEDORN and JENSEN (1923).

Case Records

Case 1.—M.F., a female, the first and only child of Jewish parents was admitted to The Hospital for Sick Children, Great Ormond Street, on August 11, 1952, at the age of 2½ years. Birth was normal and the baby weighed 5 lb 3 oz (2.35 kg); she was not thought to be premature. At two weeks of age she "fainted" and was put into an oxygen tent. There was some difficulty over breathing and head retraction was present but the cerebrospinal fluid was normal. Feeding was difficult and she was admitted to another hospital for some weeks. Weight was gained slowly and there were occasional vomits which led to a further admission when a barium swallow was said to be normal. Feeding continued to present difficulties and vomiting occurred intermittently. She was said to have held up her head at 3 months and pulled herself up in the perambulator at 5 months. At the age of one year she weighed 11 lb (4.98 kg) and in view of the continued difficulties over feeding she was admitted to a babies' hostel where a diagnosis of "? mental deficiency" was made. Subsequently she vomited blood on two occasions and two blood transfusions were given. She improved after this, began to stand and on admission to Great Ormond Street she had just begun to walk. Now at the age of 2½ years she weighed only 17 lb 11 oz (8.02 kg), was under height, 28½ inches (71.5 cm) instead of 34 inches (85 cm), pale and quiet. She stood with support, recognised her name but clearly was mentally retarded. The head circumference was 18 inches (45 cm). The fontanelle was closed and there was a sharp frontal ridge. The limbs were slightly hypotonic, with poor muscular development and the tendon reflexes were not obtained. Many investigations were carried out. The relevant positive findings were a slight hypochromic anaemia, occult blood in the stools and an X-ray examination of the oesophagus which showed that there was some obstruction to the passage of barium some inches above the diaphragm (Fig. 1). Below this the oesophagus was constricted although it relaxed and dilated from time to time. There was some suspicion of ulceration in this region.

The diagnosis of mental defect was confirmed by observation of the child in the ward. Oesophagoscopy at a later date (6 weeks after the X-ray examination) when the child was obviously better as regards vomiting showed an oesophagus which was rather wide, and full of fluid which was acid to litmus. There was no oesophagitis. The site of the cardia was difficult to determine as the gastric mucosa was very pale. The general appearances were not thought to be those usually associated with hiatus hernia but it was possible that this condition was in fact present being mild and intermittent. The spleen was palpable throughout and this remained unexplained.

Duodenal juice, obtained in a very small quantity, showed no digestion of gelatine at a dilution of 1 in 6.25, digestion of amylase at a dilution of 1 in 1,000 and it was considered that sucrase was possibly absent as no breakdown of sucrose occurred in vitro although very little juice was available for testing. The antithrombin content of the serum was within normal limits and the stools did not suggest fibro-cystic disease of the pancreas.

Paper chromatography of the urine for sugars while the child was on a mixed diet showed sucrose (about 150—[illegible] mg per cent), lactose (about 130—250 mg per cent),

Fig. 1. Case 1. To show oesophageal abnormality.

galactose (about 10 mg per cent), and fructose (about 15 mg per cent).[1] Amino-acid excretion as tested by paper chromatography was normal. Subsequent investigations were mainly concerned with the occurrence of sucrose in the urine. The child was placed on a lactose and sucrose free diet. When the diet was examined by paper chromatography only glucose was detected. To this diet fructose (20 g a day) and then lactose (20 g a day) were added for separate three-day periods and Table 1 shows the urinary sugar excretion. Whilst lactose was being given, vomiting was marked and continuous and the child became collapsed.

Sucrose was given on three occasions as a single test dose whilst the child was taking the sucrose-free diet.

After an 8 g dose was given the blood reducing sugar levels (glucose) at half hour intervals were 70 (fasting), 128, 90, 73, 75 mg per 100 ml. No sucrose was detected in the blood and a 24 hour urine collection (volume 440 ml) contained sucrose 3 mg per cent and fructose 1 mg per cent. After a dose of 12 g of sucrose the blood reducing sugar levels were 72 (fasting), 124, 104, 79, 68 mg per 100 ml, the blood sucrose levels rose to 13 mg per 100 ml, and sucrose 15 mg per cent and fructose 4 mg per cent appeared in the urine. The results of a second dose of 12 g of sucrose on the day following lactose feeding and on a sucrose-free diet are shown in Table 2. Note that lactose was still being excreted in the fasting urine.

[1] For the normal urine sugar levels see page 512.

TABLE 1

The urinary excretion of sugars by Case I (M. F.) on various diets.

	Diet	Urine sugar excretion (mg per 100 ml)					
		Urine collection	Glucose	Fructose	Lactose	Sucrose	Galactose
1	Home diet containing *sucrose* and *lactose* (about 50 g each per day)	After a feed	—	15	120–150	150–250	10
2	Diet containing only *glucose* as the sugar (50 g per day)	After a feed	4	—		—	—
3	Diet as above plus *fructose* (20 g per day for 3 days)	After a feed on the third day	4	2–3		—	—
4	Diet as above plus *lactose* (30 g per day for 3 days)	After a feed on the third day	—	—	20	—	5
	Normal excretion less than		5	7	15	25	15

TABLE 2

The blood and urine sugar levels following a dose of 12 g of sucrose in Case 1 (M. F.) on a sucrose and lactose-free diet. (This was given on the day following the cessation of a three-day diet containing glucose and lactose as the only sugars added and lactose was still being excreted.)

Blood sugars (mg per 100 ml)			Urine sugar excretion (mg per 100 ml)					
Time	Reducing sugar	Sucrose	Time	Glucose	Fructose	Lactose	Sucrose	Galactose
Fasting	74	—	Fasting	—	—	20	—	5
1 hour	133	maximum rise at 1 hour = 16 mg%	4 hours collection (40 ml)	—	4	12	60	6
2 hour	102							
3 hour	[illegible]							
			4th–24th hr. (200 ml)	—	—	4	8–10	—
			Normal less than	5	7	15	25	15

TABLE 3

The urinary sugar excretion of Case 1 (M. F.) when various sugars were added to a basic feed (containing only glucose) for two days. The urines were collected on the second day (in the morning = M and in the afternoon = A).

	Dietary sugars (g per day)				Time of specimen	Urine sugar excretion (mg per 100 ml)				
	Glucose	Fructose	Lactose	Sucrose		Glucose	Fructose	Lactose	Sucrose	Galactose
1	60	-	--	30	M	—	—	—	7	—
					A	-	---	.	1 3	—
2	30	30	--	30	M	-	6		30	—
					A	--	3	.	30	—
3	30	30	30	—	M	—	2–3	10	--	—
					A	—	2 3	10	--	trace
4	30	-	30	30	M	7	2 3	4	12 13	-
					A	10	—	6	10	—
5	30	30	30	30	M	5	2–3	35 40	10-12	2
					A	2-3	1	8-10	6	1
					A	2	1	6	7-8	—
Normal less than						5	7	15	25	15

The daily sugar content of the sucrose and lactose-free diet was then varied as shown in Table 3 for two-day periods, and mid-morning and mid-afternoon specimens of urine were examined on the second day of each period.

On giving a further test dose of lactose there was again a lot of vomiting which led to a collapse. She was found to have a low serum potassium and despite efforts to remedy this by an intravenous infusion she went downhill with hypotonia and abdominal distension and died. There was no post-mortem examination.

A subsequent child (male born to the parents of M. F. at another hospital) was stated to show on the eighth day of life by paper chromatography 100 mg per cent of sucrose in the urine while on a diet of dried milk and cane sugar but at the age of 9 weeks when this was repeated no sucrose was detected and it is therefore unlikely that this baby suffered from the disorder described here.

Case 2. — C. B., a female, was born of a Scottish father and Jugoslav mother, as a result of a third pregnancy. The first child, also a female, was apparently normal. The next pregnancy resulted in a miscarriage at the third month. The present patient was found to suffer from rhesus incompatability and does in fact show evidence of "kernicterus". Her mental retardation may therefore by due to this cause. She was first seen as an out-patient at The Hospital for Sick Children, Great Ormond Street, at the age of one year because of persistent vomiting. Previous history showed that she had been of normal birth weight 7 lb 1 oz (3.2 kg) and that vomiting had been troublesome from birth with remissions of not longer than one week. There was a tendency to excessive head retraction without any stiffness of the neck (Fig. 2). She was admitted and X-ray examination and oesophagoscopy at this time showed some

Fig. 2. Case 1. At age of 1 year.

Fig. 3. Case 2. To show oesophageal abnormality.

narrowing of the oesophagus just above the diaphragm (Fig. 3) and mild general oesophagitis. It was considered that she was probably an example of hiatus hernia and treatment was directed towards relief of symptoms (including propping the child up). At this period her mental retardation was confirmed by observation. She weighed only about 16 lb (7.26 kg) at the age of 14 months and her head circumference was 17 inches (43.5 cm). She went home for a few months and she was readmitted

TABLE 4

The effect on the urine sugar excretion of Case 2 (C. B.) over 6 hours of 20 g doses of various sugars after an 8 hour fast. The diet given otherwise contained lactose and sucrose.

	Test sugar (one dose)	Time of specimen	Urine sugar excretion mg per 100 ml				
			Glucose	Fructose	Lactose	Sucrose	Galactose
1	Sucrose (20 g)	Fasting 6 hours		30	85	100	45
2	Galactose (20 g)	2 hours	for liver function test (normal)				20 g
3	Fructose (20 g)	2½ hours	—	45	trace		
4	Lactose (20 g)	6 hours	—	—	12		25
5	Sucrose (20 g)	Fasting	No ketoses detected				
		1 hour	—		3	7	
		2½ hours		—		25	
		3½ hours		—		10	—
	Normal less than		5	7	15	25	15

at the age of 18 months for a minor chest infection. Vomiting was slightly less. In view of the previous case which was fresh in our memory and because of a suggestion that paper chromatography might throw more light on her liver function, investigations of her urine were made on these lines and showed that on a normal diet (containing lactose and sucrose) she was passing about 170—180 mg of sucrose per cent with fructose 80 mg, galactose 25 mg and lactose 80 mg (see Table 5).

A different plan of testing the effect of sugars in the diet was followed. On admission to the ward a diet of cowsmilk 8 oz, a fine cereal (3 level teaspoons) and sucrose 50 g was given as five meals a day. Glucose water only was allowed after the 10 p.m. feed on the day preceding a test. The test sugar (20 g) was given in the early morning (usually 6 a.m.) just after a specimen of urine was obtained. Thus an eight hour fast preceded each test. A second specimen of urine was collected for six hours after the test dose usually. Six hours cover practically the total excretion of the test dose of sugar.

The urine sugar excretion is shown in Table 4.

The patient was discharged home on a diet (in which glucose was substituted for sucrose) and a single specimen of urine obtained two weeks later contained lactose 25 mg per cent and galactose 8 mg per cent.

On admission to the hospital two months later after a period of vomiting, the sucrose-free diet was continued. Three hours after a feed the urine contained glucose 12 mg per cent, fructose less than 1 mg per cent. A diet containing added sugar as sucrose was given for two days. After a fasting specimen of urine was taken 20 g of sucrose were given. The urinary excretion of sugar is shown in Table 5. After 14 days with a sucrose-free lactose-glucose diet 13 g of sucrose were given as a test dose. Urine and blood sugar levels are shown in Table 6.

TABLE 5

The urinary excretion of sugars by Case 2 (C. B.) on various diets.

	Diet	Urine sugar excretion (mg per 100 ml)					
		Urine collection	Glucose	Fructose	Lactose	Sucrose	Galactose
1	Home diet containing *sucrose* and *lactose* (about 50 g each per day) before first admission	After a feed	—	80	80	180	25
2	Home diet containing *no sucrose* (after discharge). Lactose and glucose about 50 mg per day	After a feed	—	—	25	—	8
3	2 months later on admission to hospital. Lactose and glucose about 50 mg per day	2 hours after a feed	12	Less than 1	—	·	—
4	After 2 days on diet containing all added sugar as sucrose (and milk lactose)	Fasting	—	trace	50	40	7
	Then 1 dose of sucrose 20 g given	1st hour	10	25	—	400	trace
		1st–3rd hour	10	40	—	400	trace
		3rd–4th hour	—	15	—	100	—
	Normal less than		5	7	15	25	15

A glucose tolerance test (12 g dose) showed at half hour intervals blood levels of 115 (fasting), 160, 144, 130, 111, 96 mg per cent.

A specimen of stool digested gelatine at a dilution of 1 in 200 and appeared normal on microscopical examination. The antithrombin titre of the serum was normal. Various secretions were tested for sucrase activity. No activity was found in the gastric juice, and in the stool. Saliva digested 0.5 per cent of sucrose after 5 minutes at 37° (normals — 3.5—4 per cent).

In view of the development of cataract in galactosemia the eyes were examined by Mr. J. H. Doggart, F.R.C.S. who reported that there were no crystalline or other deposits in either cornea, the ocular media were not obstructed and the fundi were normal. Other findings in this child were the development of laryngitis with spasm (? related to low spasticity). There was no splenomegaly. She continued to vomit occasionally with stages of blood from time to time in the vomited material. Sucrose was eliminated from the diet. Physical progress was slow.

TABLE 6

The blood and urine sugar levels following a dose of 13 g of [illegible] after taking a diet containing lactose and glucose [illegible] no sucrose

Blood sugars (mg per 100 ml)			Urine sugar excretion (mg per [illegible])					
Time	Reducing sugar	Sucrose	*Time*	Glucose	Fructose	Lactose	Sucrose	Glucose
Fasting	77	0	Fasting	[illegible]	[illegible]	trace		
½ hour	81	12	1 hour	1[illegible]	6	trace	[illegible]	
1 hour	90	0						
1½ hours	83	8						
2 hours	78	0	1–3 hours	20	30	trace	200–250	5
			3–6 hours		[illegible]	[illegible]	60	4
Normal less than				5	7	15	25	15

Case 3. — J. M., a male, the first child of Irish parents with no consanguinity and no history of illnesses on either side, was born after a normal pregnancy and normal labour at 36 weeks weighing 4 lb 13 oz (2.18 kg). There was no difficulty over breathing, no cyanotic attacks or fits. Breast feeding was established and the baby left the maternity hospital at the age of 3 weeks weighing 5 lb (2.27 kg). Breast feeding continued for another 3 weeks and then the mother had an acute pyelitis necessitating admission to hospital. Lactation was stopped at once and the baby was given half cream dried milk with added cane sugar. Later the milk was changed to full cream and the cane sugar addition (1 teaspoonful to each feed) continued. Solids were introduced at 5 months and the usual weaning foods followed. Cane sugar was used in the normal way for sweetening. Vomiting occurred as early as the third day of life and continued intermittently ever since, usually immediately after feeds, occasionally projectile and sometimes with bright or altered blood. Constipation with hard infrequent stools was also noted early in life by the mother. From the age of four months to ten months the baby was in Eire with his grandmother and he is stated to have had pneumonia there at the age of eight months. At the age of ten months he was brought to the out-patient department of The Hospital for Sick Children, Great Ormond Street, where he was seen by our colleague Dr. R. E. BONHAM CARTER who has kindly allowed us to quote his case records and to make certain special observations on the patient. At this time (age of 10 months) the baby weighed only 10 lb 14½ oz (4.95 kg), was failing to thrive with the persistence of vomiting and constipation. He was admitted the same day to University College Hospital as a possible example of fibrocystic disease of the pancreas. Tryptic activity of the stools was normal, however. Other observations at that time showed that he had signs of inflammation in both lungs with clubbing of the fingers, pallor and inability to lift up his head or sit up, indicating definite mental retardation.

Fig. 4. Case 3. Oesophageal abnormality.

X-ray examination showed some stenosis of the oesophagus with appearances suggestive of oesophagitis, hiatus hernia of the stomach and some regurgitation of gastric contents (Fig. 4). Oesophagoscopy (by Professor R. S. PITCHER) confirmed these findings and repair of the hiatus hernia was performed by Professor PITCHER one month later just before the child's first birthday. Feeding which had been difficult before operation continued to be a problem; vomiting recurred; fever and bronchitis interfered with progress. He was sent to a special convalescent home where he was kept in the sitting position because of his continued vomiting. After returning home he had severe vomiting attacks with a lot of blood in the vomit and evidence of pulmonary consolidation, possibly from aspiration. Retention of urine, owing to severe constipation, yielded to rectal washouts. Examination of the urine at this time indicated the presence of a reducing substance to Benedict's solution. Paper chromatography showed the presence of sucrose and lactose. He was transferred to The Hospital for Sick Children, Great Ormond Street, for further investigation of his sucrose metabolism. On admission the following relevant points were noted: he weighed only 15 lb (6.8 kg) at the age of 18 months. There was definite finger clubbing. Extensive signs in the chest of patchy consolidation and bronchitis persisted. He was still very constipated, difficult to feed and tended to vomit easily despite being kept in an upright position. He could not sit up unaided and despite a brightness at times there seemed no doubt of his mental retardation (Fig. 5). Examination of the eyes showed no abnormality of the corneae, media or fundi.

He continued on a diet of cows milk 42 oz, glucose 2 oz, a fine cereal 2 oz, and 1 egg as seven feeds a day, i.e. a sucrose-free, lactose and glucose containing diet (60 g

Fig. 5. Case 3 at age of 20 months.

each per day). Urine collected from two to three hours after a feed contained no ketones. This diet had been taken for seven weeks. The effect of sucrose added to a normal feeding routine which (*a*) contained lactose and glucose and (*b*) contained lactose and sucrose, was investigated. A single dose of 15 g of sucrose was given as a feed, viz. cows milk 6 oz, sucrose ½ oz (15 g). Urine was collected before the feed and for three two-hour periods afterwards, and the results are shown in Table 7. As the child was being fed three-hourly, the next feed given in the middle of the second to fourth hour urine collection contained lactose and glucose 8 g each, but no sucrose. After two days on a sucrose and lactose containing diet (60 g per day each) a similar feed was given once which contained sucrose 15 g and lactose (8 g). Urine was collected before the feed and for three two-hour periods afterwards. A feed given in the middle of the second to fourth hour urine collection contained sucrose [illegible] g and lactose 8 g. Blood specimens were taken before and at half-hourly inter-

TABLE 7

Showing effect of 15 g of sucrose in a feed (without glucose) on urinary sugar excretion of Case 3 (J. Mc.) whilst on sucrose free, lactose-glucose containing diet (60 g each per day).

Urine sugar excretion (mg per 100 ml)					
Time	Glucose	Fructose	Lactose	Sucrose	Galactose
Before feed	–	–	25		10
2 hours		—	15	a trace	—
2nd–4th hour*	15	10	70	80	20
4th–6th hour	15	10	10	15	20
Normal less than	5	7	15	25	15

* Feed containing lactose (8 g) and glucose (8 g) given at 3rd hour.

TABLE 8

Showing the effect of 15 g sucrose in a feed on blood and urinary sugar levels of Case 3 (J. Mc.) whilst on sucrose and lactose containing diet (60 g per day each) started 2 days before.

Blood sugars (mg per 100 ml)			Urine sugar excretion (mg per 100 ml)					
Time	Reducing sugar	Sucrose	*Time*	Glucose	Fructose	Lactose	Sucrose	Galactose
Fasting	75	0	Before feed	10	8	35	90	10
½ hour	126	29	2 hours	10	20	45	150	15
1 hour	100	(2)						
1½ hours	100	(3)						
2 hours	91	(2)						
			2nd–4th hour*	8	12	25	90	10
			4th–6th hour	—	10	40	120	15
			Normals less than	5	7	15	25	15

* Feed containing lactose (8 g) and sucrose (8 g) given at 3rd hour.

vals for two hours after the first feed and analysed for reducing sugars and sucrose—Table 8. The child's diet was then changed back to cows milk and glucose, i.e. a sucrose-free diet.

An attempt was made to pass a tube well down the duodenum to obtain specimens of intestinal contents and succus entericus to test for sucrase activity. As in other patients this was not successful. He was transferred to a convalescent home and two days later died of a fulminating chest infection. Specimens of the intestinal mucosa at the duodeno-jejunal junction were obtained at autopsy. These showed marked activity, splitting sucrose and lactose.

A summary of the *autopsy findings* has been supplied by the Pathologist, Dr. L. L. R. WHITE:—

Externally: The body was wasted, and weighed only 15½ lb (7 kg). The skull was of normal size and shape, but the anterior fontanelle was widely patent. There was flaring of the costal margins, and a Harrison's sulcus was present. The fingers and toes showed a moderate degree of clubbing.

Internally: The brain was of normal size and general configuration but showed a number of developmental abnormalities, including severe hypoplasia of the corpus callosum, failure of normal convolutional folding of the cingulate gyri, partial interlocking of the medial gyri of the frontal lobes across the longitudinal fissure, absence of the septum pellucidum and reduction in the white matter of the centrum ovale.

An extensive lipoid pneumonia was present in both lungs. There was no recurrence of the hiatus hernia, but a chronic oesophagitis was present in the terminal 3 cm of the oesophagus. The liver and kidneys were congested and reduced in weight. The gastro-intestinal tract was normal to the naked eye.

Microscopy: Preliminary studies of frozen sections of brain show no demyelinisation, but a narrow zone of subependymal gliosis is present in relation to the lateral ventricle. There is a mild degree of fatty change in the liver, and of nephrocalcinosis in the kidneys, but neither in these organs nor in the gastro-intestinal tract is any specific histological change apparent. Histochemical investigations proved useless on account of the time elapsing between death and autopsy. The nature of the fat in the lungs is still under investigation.

The Results of Feeding Sugars

Tables 1, 3 and 5, show the urinary sugar excretion whilst common sugars in various combinations as ordinary feeds were given to the patients. Tables 2, 4, 5, 6, 7, and 8 show the urinary sugar excretion and blood sugar levels of reducing sugar and sucrose when single doses of sugars were added to various diets. The data is summarised in Table 9.

1. When a normal milk feed (i.e. a lactose and sucrose containing diet) was given, fructose, lactose and sucrose were excreted in abnormal amounts in the urine. The urinary glucose concentration was raised occasionally. A single dose of sucrose added to this diet increased these effects. When sucrose was removed from the diet and glucose substituted the excretion of sugars was practically normal though glucose and lactose excretion was slightly raised occasionally. A single dose of sucrose added to the sucrose-free diet (i.e. lactose, glucose diet) again caused the abnormal excretion of sugars.

2. Various combinations of the dietary sugars glucose, fructose, lactose, sucrose and galactose were fed as diets and as single doses superimposed on diets to try and demonstrate interactions between the sugars. Care has to be exercised when comparing the various results that the conditions of feeding were otherwise identical. On diets not containing lactose and sucrose normal urine sugar excretion was found. When glucose and fructose were

TABLE 9

Summary of the effect of dietary sugars on the urinary sugar excretion of three cases of aminoaciduria, hiatus hernia, and mental deficiency.

Dietary sugars	Urine sugar excretion (N = Normal)				
	Glucose	Fructose	Lactose	Sucrose	Galactose
Lactose sucrose diet	N–2 N	2 N–10 N	2 N–10 N	4 N–10 N	N–2 N
Lactose glucose diet	N–2 N	N	N–2 N	N	N
Glucose diet	N	N	N	N	N
Glucose sucrose diet*	N	N	N	N	N
Glucose fructose diet*	N	N	N	N	N
Glucose fructose sucrose diet*	N	N	N	N	N
Glucose fructose lactose diet*	N	N	N	N	N
Lactose sucrose glucose diet*	2 N	N	N	N	N
Lactose sucrose glucose fructose diet*	N	N	3 N	N	N
Dietary sugar / **Sugars added in single dose**					
Lactose sucrose diet / Sucrose	N–2 N	3–6 N	2–3 N	4–16 N	N
Lactose glucose diet / Sucrose	3–4 N	N–4 N	N–3 N	3–12 N	N+
Lactose sucrose diet / Fructose	N	5 N	N	N	N
Lactose sucrose diet / Lactose	N	N	N	N	2 N
Lactose sucrose diet / Galactose	Normal liver function 2 g				

* These diets were given for 2 days only.

fed no sucrose was excreted. A sucrose glucose and fructose diet caused only a slight increase in sucrose excretion. A lactose glucose fructose diet gave normal results.

3. When both lactose and sucrose were fed, the addition of glucose caused the excretion of glucose, a dose of fructose caused the excretion of fructose, a dose of lactose merely raised the excretion of galactose, and the addition of glucose and fructose caused a raised lactose excretion. It would appear therefore when lactose and sucrose occur together in the diet of these patients that other sugars appeared more easily in the urine. Galactose added as a single dose to the lactose sucrose diet was excreted in amounts in the urine which were normal for a galactose tolerance test of liver function.

When conditions approximating to normal feeds were analysed the effect of lactose and sucrose together was more complicated. It took several days of feeding to cause a markedly raised fasting excretion of lactose and sucrose

(together with a variable amount of glucose, fructose and galactose). A dose of sucrose added to a feed (lactose-sucrose diet—see Table 8) caused a rise in fructose, lactose and sucrose excretion and when a further feed was given without added sucrose three hours later a second rise in these urinary sugars occurred.

When the same patient J. Mc. (Table 7) was fed a lactose and glucose diet a similar dose of sucrose produced an increase of fructose, lactose and sucrose over the level seen just before the feed. The increases above the level before a feed in each case with and without sucrose in the diet were approximately the same.

Two series of blood levels of reducing sugars and sucrose after a dose of sucrose whilst on lactose-glucose diet showed a rise and fall of sucrose in the two hours. One reducing sugar curve rose markedly and one did not. On a lactose-sucrose diet a dose of sucrose produced a sharp peak in the blood sucrose level, and a marked rise in the blood reducing sugar levels.

These observations represent the broad outline of the results in Tables 1 to 8. The three cases can be seen to vary in reaction to the different sugars fed (the excretion of lactose varied when sucrose was fed to different cases). Therefore no more exhaustive analyses of the records would yield useful results.

Previous Literature

There is remarkably little in the literature about the occurrence of sucrose in the urine. Various writers on carbohydrate metabolism mention that in cases of hysteria or in men seeking to avoid military service cane sugar may be added to the urine but since this does not produce any reduction in the usual copper test solutions it is an ineffective procedure! The high specific gravity of the urine might be noted and after hydrolysis glucose could be detected. This procedure however is not often carried out and this probably explains why so little has been written about sucrosuria which may, in fact, be commoner than is realized.

REUSS (1910) describes the finding of various sugars in the urine of infants severely ill with gastro-enteritis and he found twelve with what he terms saccharosuria. He explains this by suggesting an increased permeability of the bowel wall rather than by decreased or absent enzyme activity. There does not seem to have been any follow-up of these infants in convalescence to see if the saccharosuria disappeared. JOLLES (1913) discussing tests for saccharose in the urine mentions a child of two years with "severe intestinal catarrh" who was treated with very sweet tea and passed saccharose in the urine. BENCRI and FALKIEWICZ (1934) describe a case of saccharosuria after a pancreatic stone in a woman of 34 years. They think this was probably

of endogenous origin. HOESCH (1934) reports a case, at a clinical meeting, of a patient aged 22 years who for several months after a pancreatitis had a glycosuria. Carbohydrate restriction led to a decrease in this but the specific gravity of the urine remained high and sucrose was demonstrated. He considered that the sucrosuria was of endogenous origin. REINER and WEINER (1939) describe a boy of seven months, admitted with fever, who was found to have a reducing substance in the urine. He was treated as a diabetic. More detailed studies of the urine showed that saccharose was present in the urine and that the amount present varied with the amount of cane sugar in the diet. When the child recovered from the febrile illness and was well he seemed to be free from saccharosuria. It is said that this child only passed saccharose when this was given with fructose. REINER and WEINER suggest that saccharosuria in their patient could be of alimentary origin (increased permeability of intestinal wall or decrease in intestinal enzymes). They favour increased permeability. Relevant to the cases described in the present communication is the fact that the boy was found to be mentally defective at the age of eighteen months. ELMER, KRASOWSKA and PTASZAK (1939) discuss the literature and physiology of sucrosuria. They think that normally a very small amount evading hydrolysis is absorbed through the wall of the alimentary tract and appears in the urine. They divide examples of the condition into exogenous and endogenous, and produce cases in adults clearly of exogenous origin due, they consider, to absence of enzyme ("sucrolytic") activity in the gastro-intestinal tract. RABINOWITCH and MOUNTFORD (1947) give a valuable review of the metabolism of sucrose but as this deals with adults it does not altogether solve some of the difficulties revealed by the clinical findings and investigation in young children. They stress the very ready availability of sucrose given by mouth, as for example in insulin hypoglycaemia when it restores the blood glucose level even more quickly than glucose given by mouth despite the need for preliminary hydrolysis before absorption. Gastric contents removed at one minute intervals after giving sucrose by mouth show the rapid appearance of dextrose and laevulose and also substances of "as yet unknown composition". It is clear that the normal mechanism for dealing with sucrose acts rapidly and efficiently in adults and any degree of sucrosuria must represent a serious breakdown. FOX (1950) investigating sucrose absorption in sprue found that the rise of blood sugar levels (glucose) after a test dose of sucrose was lower than in normal subjects suggesting that absorption was impaired or delayed or that hydrolysis was faulty, but he makes no comment on examination of the urine for sucrose. WILMER (1944) discussing the mechanism of sucrose damage in the kidney tubules concludes that in rabbits sucrose given intravenously in large doses produces severe tubular degeneration.

In summary the literature reveals examples of increased absorption of sucrose from the alimentary tract due either to increased permeability of the wall or decreased enzyme activity and hence decreased hydrolysis. The [illegible] said to be of endogenous origin are difficult to understand and do not appear relevant to the present communication.

Physiology

The sugars of major interest in children's metabolism are the hexoses—glucose, fructose, galactose and mannose; the pentoses—xylose and arabinose; and the disaccharides—sucrose (glucose + fructose), lactose (glucose + galactose) and maltose (glucose + glucose). All these sugars except sucrose reduce alkaline copper reagents. Sucrose has no reducing properties as the glucose and fructose moieties are joined by their respective aldehydic and ketonic groups.

The sugars normally are absorbed from the small intestine as the monosaccharides. The disaccharides are hydrolysed into their constituent monosaccharides partly perhaps by the acid gastric contents and certainly by digestive enzymes found mainly in the succus entericus. The speed of absorption of the monosaccharides across the wall of the small intestine is in the decreasing order:— galactose: glucose: fructose: mannose and the pentoses. The rate of absorption of one sugar is depressed by the presence of other sugars, e.g. galactose absorption is depressed when glucose is being absorbed simultaneously and still more so when lactose and glucose are present.

Glucose, fructose and galactose are utilised by the body. They are carried across the intestinal wall by an active mechanism which is probably a phosphorylating one, and this is active against a considerable concentration gradient.

The other monosaccharides and the disaccharides (which are all little used by the body) are also transferred across the wall of the small intestine. When they are fed to normal individuals in large enough doses they can be detected in the blood and in the urine.

The blood levels attained after ingestion of the sugars depend on the quantity ingested, the rates of intestinal hydrolysis and absorption, of diffusion into the tissues fluid spaces, of utilisation by the liver and tissues and the manner of renal excretion. These factors vary for each sugar. Further, only glucose and a small fraction of the fructose entering the blood from the intestine can be utilised to any extent by the body tissues. The other monosaccharides have to be converted to glycogen by the liver. The sugar not used by the liver enters the systemic circulation and diffuses through the tissue fluids.

When galactose and fructose are absorbed, the liver rapidly removes them

from the blood, and the levels of these sugars and of glucose do not vary greatly. The kidney tubules have a very limited capacity for absorbing galactose and fructose and these sugars appear in the urine, when their blood levels are comparatively low. Normally the absorption and utilisation of these two sugars are so closely balanced that their urinary excretion is minimal.

Glucose metabolism need not be discussed except to remark that it occupies the opposite end of the scale of renal excretion. The renal tubules have a large capacity for reabsorbing glucose so that little appears in the urine until the blood level is high and the quantity delivered in the glomerular filtrate to the tubule for reabsorption is great.

Sucrose, if it escapes intestinal hydrolysis and is absorbed into the blood stream, is not metabolised by the liver. It diffuses through the extracellular fluid space, is not metabolised by the tissues and passes in the glomerular filtrate to the tubules. Sucrose, as well as the other sugars which are little or never used by the tissues (e.g. lactose), are only reabsorbed by the normal tubules to a limited extent (10 per cent) and appear in the urine in raised amounts. Sucrose behaves like a foreign substance when it is present in the tissue fluids. It has been used as a measure of the extracellular fluid space, and the glomerular filtration rate. When given in hypertonic solution it can be used to increase the extracellular fluid space; to promote diuresis and to withdraw fluid from the subarachnoid space. The kidneys of animals may be damaged after such infusions. This brief summary is based essentially upon statements in various pages of PETERS and VAN SLYKE (1946).

The application of paper chromatographic analysis to urinary sugar excretion in infants and children shows, because of its specificity and sensitivity, that most sugars are excreted in varying degrees. Dr. L. I. WOOLF (of this hospital) who kindly examined the urines of the patients under discussion by this means communicated the following levels found in post-prandial urine specimens from a large series of normal infants and children. The first figure indicates the highest level of the urinary sugar found in the majority of patients, whilst the second figure in brackets shows the highest figure found.

Sucrose not more than 25 mg per 100 ml (occasionally 30 mg per 100 ml). Infants excrete less.

Glucose not more than 5 mg per 100 ml (occasionally 10 mg per 100 ml). Infants excrete less.

Fructose not more than 7 mg per 100 ml (occasionally 10 mg per 100 ml). Most cases excrete very little.

Lactose not more than 15 mg per 100 ml (occasionally 30 mg per 100 ml). Neonates and older infants excrete up to 40 or occasionally 60 mg per 100 ml.

Galactose not more than 10 mg per 100 ml (occasionally 15 mg per 100 ml). Infants not more than 10 mg per 100 ml (occasionally 15 mg per 100 ml).

Arabinose occasionally up to 7 mg per 100 ml. Neonates and infants excrete up to 7 mg per 100 ml.

l-Xylose not more than 3 mg per 100 ml. Neonates and infants excrete up to 7 mg per 100 ml.

Discussion

Although the results obtained in the investigation of these three children leave no doubt of a serious disorder in the metabolism of certain sugars it is difficult to take the matter much further as regards the problem of failure of digestion or abnormal absorption. It has not been possible to investigate adequately the intestinal sucrase in vivo. Specimens of duodenal juice obtained from children thought to be suffering from fibrocystic disease of the pancreas have given varying results in relation to the digestion of sucrose. Considerable splitting must be taking place in the cases here described since glucose and fructose appear in the urine after a test-dose of sucrose but it would appear that rapid absorption of sucrose is occurring before the usual digestive process — which is certainly rapid in adults—has time to be totally effective. Increased or more rapid absorption does not appear to have anything to do with the state of the oesophagus since in children with a hiatus hernia who were investigated for the presence of sucrose in the urine completely negative results were obtained. It is possible however that the fact of an abnormal condition of one part of the alimentary tract would permit of the assumption that lower down there may also be disordered function. It would appear that sucrosuria in the cases here reported is mainly due to increased permeability of the intestinal wall, thereby resembling the temporary condition noted in infants recovering from gastro-enteritis.

The occurrence of mental defect may represent a genetic association but on the other hand the presence of excess sucrose in the blood might have been held responsible for producing a lesion in brain cells. In galactosaemia the abnormal presence of another sugar is thought to be responsible for the mental retardation and certainly improvement tends to occur when lactose is eliminated from the diet. The serious effects of sucrose on the renal epithelium in experimental animals already referred to indicates the possibilities of similar toxic action on other cells of the body. To this extent—and clearly there is an element of speculation—the three "lesions", viz. the metabolic error, the oesophageal anomaly and the mental retardation could be linked. In view of the autopsy findings in case 3, of a brain which showed gross changes of a developmental nature, this hypothesis, however, must clearly be discarded until more examples of the syndrome have been studied.

The urinary sugar excretion of these three patients was not qualitatively or quantitatively constant in relation to various dietary sugars. The excretion tended to rise to a maximum over several days and short-term changes of

due produced small effects, limiting the scope of trials (see Table 3). Each patient reacted differently to a lactose and sucrose diet in the amount of sucrose and lactose excreted. This variability has made it difficult to summarise some of the details of the effects of feeding sugars on the urinary sugar excretion of the three patients, but the following observations are thought to be valid.

If milk (i.e. lactose) was fed, the presence or absence of dietary sucrose was paralleled by the presence in abnormal amounts or absence of sucrose, lactose and fructose in the urine. If lactose and sucrose were present in the diet and other sugars were eaten, each of them tended to appear in varied amounts in the urine. If lactose and sucrose were not fed, sugar excretion was normal. The results place the emphasis on the conjunction of lactose and sucrose in the diet of these cases of sucrosuria. REINER and WEINER (1939) found that fructose and sucrose given to their case were followed by sucrosuria.

It has not been possible to obtain sufficient evidence to explain the error in sugar metabolism. Intestinal hydrolysis, absorption, liver and tissue metabolism and renal excretion may be at fault. No sucrose was excreted unless sucrose was fed, so the sucrosuria was not of the endogenous type. The intestinal splitting of disaccharides certainly occurred and was probably efficient. ELMER *et al.* (1939) were unsuccessful (as we were) in obtaining succus entericus during life. They felt that exogenous sucrosuria was probably due to the loss of sucrolytic activity of the duodenum so that ingested sucrose left the body as an unusable substance. They did not mention the excretion of other sugars in abnormal amounts in the urine such as were found in the present series. The succus entericus of case 3 obtained at autopsy was able to split sucrose and lactose actively. When lactose and sucrose were fed, their constituent monosaccharides appeared in the urine and it may be presumed that the splitting of sucrose and lactose occurred in the intestine. REINER and WEINER (1939) found no saccharase in the blood of either their patient or controls. Sucrose is not metabolised in any practical quantity by the tissues of normal individuals. Liver and tissue metabolism of the patients was not exhaustively examined. One patient metabolised normal quantities of galactose whilst on a lactose and sucrose containing diet. Case 3 in particular showed a satisfactory weight gain when sucrose was excluded from his diet. The renal loads at which the various sugars appeared in the urine was not examined in these patients. It was felt unlikely that the renal tubular absorption was affected only when lactose and sucrose were fed.

By a process of tentative exclusion it is suggested that the error in the metabolism of sugars is one of absorption when lactose and sucrose are fed together. Sugar absorption was otherwise normal—normal reducing sugar levels in the

blood were found after the ingestion of glucose. When lactose and sucrose were fed, hydrolysis occurred in the intestine producing an equimolecular mixture of disaccharides (lactose and sucrose) and monosaccharides (glucose, fructose and galactose). Though absorption of disaccharides is poor normally, it is suggested that it occurred unusually rapidly in these cases. Lactose fed alone appeared in abnormal quantities in the urine. When sucrose was present too, fructose, lactose and sucrose were rapidly absorbed. When fructose is absorbed rapidly it may exceed utilisation and the blood level rise to unusual levels. Raised lactose and sucrose blood levels followed the sugars rapid absorption. All these sugars have low renal tubular reabsorptions and so appeared in abnormal quantities in the urine.

A great many interesting possibilities of abnormal sugar metabolism are raised by these cases. As further instances are recognised, it should be possible to examine the parts played by hydrolysis, absorption, utilisation and excretion in the metabolic error.

Summary

Three instances are described of the association in early childhood of sucrosuria, mental retardation and an anomaly of the lower end of the oesophagus, thus producing the three types of congenital abnormality in the one subject—inborn metabolic error, mental defect and an anatomical abnormality. Autopsy findings in one case revealed a congenital defect in the brain, thus ruling out any hypothesis that the mental retardation might be associated with a sucrosaemia. It is concluded that the sucrosuria resulted essentially from rapid absorption of the unsplit disaccharide but technical difficulties made it impossible to investigate further the failure of splitting or its degree. The previous, scanty, literature mostly deals with similar instances, as far as the sucrosuria is concerned, of exogenous origin. Careful study of the sugars fed and subsequently passed in the urine revealed a more complicated story than that of excessive alimentary absorption of sucrose and urinary excretion. Provisional "normal" values for urinary sugars obtained by chromatography are suggested. The close association of lactose and sucrose in the diet in relation to the occurrence of sucrose in the urine seems definitely established in the cases reported. Glucose metabolism was unaffected.

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Note

Synthesis of sucrose and of α-D-glucopyranosyl α-D-fructofuranoside through the use of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose

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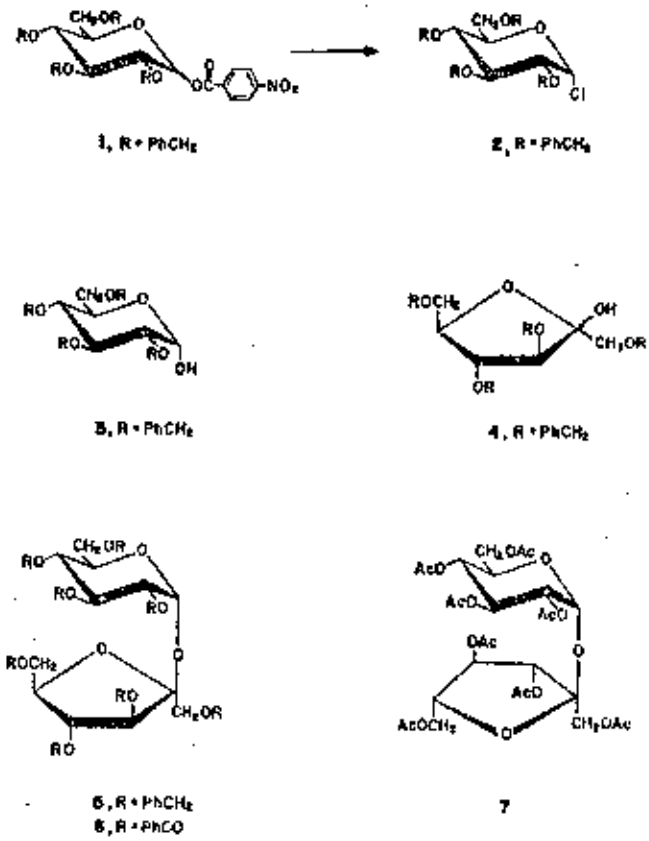
In a recent paper[1], we described the preparation of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (4) in crystalline form. The accessibility of this compound, together with the well-recognized tendency of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (2) and bromide to yield α-D-glucopyranose derivatives on nucleophilic attack[2-4], suggested that interaction of 2 with 4 might lead to the formation of octa-*O*-benzylsucrose (5), although it must be conceded that the anomeric configuration of the crystalline form of 4 is as yet undetermined. We now report two experiments in which the use of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (4) has led to the formation of mixtures from which α-D-glucopyranosyl D-fructofuranosides could be isolated.

Chittenden[3] has recently reported that the condensation of 2 with 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (3) in the presence of silver carbonate, Drierite, and silver perchlorate affords (after debenzylation) α-D-glucopyranosyl α-D-glucopyranoside (α,α-trehalose) and α-D-glucopyranosyl β-D-glucopyranoside (α,β-trehalose). A similar condensation in which 3 was replaced by 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (4) was found in the course of the present research to give, after repeated chromatography, a crude syrup (in 36% yield) which showed an optical rotation not far from that[1,5,6] of octa-*O*-benzylsucrose (5). As 5 has not as yet been obtained in crystalline form, the crude material was debenzylated and then benzoylated. The known solvate of octa-*O*-benzoylsucrose (6) with carbon tetrachloride[7] was isolated in crystalline form in an overall yield of 4.5%, based on the crystalline 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-D-glucopyranose (1) that had been used for the preparation of 2. The yield of 6 closely approximates that of octa-*O*-acetylsucrose (5.5%) that Lemieux and Huber[8] obtained subsequent to the condensation of 3,4,6-tri-*O*-acetyl-1,2-anhydro-α-D-glucopyranose with 1,3,4,6-tetra-*O*-acetyl-D-fructofuranose; it is, however, significantly less than the yield of octa-*O*-methylsucrose (6–8%) that Klemer and Dietzel[9] attained through the condensation of 2,3,4,6-tetra-*O*-methyl-D-glucopyranose with 1,3,4,6-tetra-*O*-methyl-D-fructofuranose in the presence of anhydrous zinc chloride. However, the parameters affecting such condensations are numerous and, in the example reported here, no attempt was made to maximize the yield. The reader will have noted that the yield of crude product obtained from the

condensation of 2 with 4 was substantially greater than that of the octa-*O*-benzoylsucrose isolated. In a separate experiment, a sample of the crude product was catalytically debenzylated, and two major products were then separated chromatographically. One of these was sucrose, constituting ~35% of the mixture, and the other (~45%) showed a specific rotation close to that reported[10] for α-D-glucopyranosyl α-D-fructofuranoside; the properties of a crystalline acetate (7), prepared from the material, confirmed this identification.



Klemer and Kutz[11] have shown that 2,3,4,6-tetra-*O*-methyl-D-glucopyranose undergoes self-condensation when treated in toluene solution with Drierite and perchloric acid. We have carried out a similar reaction with an equimolar mixture of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (3) and 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (4). Chromatography showed that the mixture produced was complex. It was found possible, however, to separate from it those components that were formed when either 3 or 4, alone, was subjected to the condensation conditions. Catalytic debenzylation of the remaining mixture, followed by acetylation and further chroma-

tography, led to the isolation of the octaacetate of α-D-glucopyranosyl α-D-fructofuranoside (7) in 6% yield. Although other D-glucopyranosyl D-fructofuranosides had quite probably been formed, the quantities involved were too small for isolation under the conditions employed.

EXPERIMENTAL

General methods. — Melting points are equivalent to corrected values. Qualitative t.l.c. was conducted on Silica Gel GF (250 μm, Analtech, Inc., Wilmington, Del.) with the solvent systems specified. Components were detected with a Gelman–Camag "universal" u.v. lamp, Model 51402, and also by spraying with 10% sulfuric acid and heating. Preparative t.l.c. was performed on plates (20 × 20 × 0.2 cm) of Silica Gel No. 3370 (E. Merck, Darmstadt). Column chromatography was carried out with Silica Gel No. 7734 (0.05–0.20 mm; E. Merck). The composition of solvent mixtures used in chromatography is indicated in terms of volume, except for the aqueous phenol which was measured by weight.

Condensation of 1,3,4,6-tetra-O-benzyl-D-fructofuranose (4) with 2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl chloride (2). — A suspension of molecular sieve (type 4A, 4 g) and silver carbonate (2 g, 7.3 mmoles) in a solution of 1,3,4,6-tetra-*O*-benzyl-D-fructofuranose (4, 2.00 g, 3.7 mmoles) in dry benzene (4 ml) was stirred in the dark for several minutes. Dry benzene was evaporated from silver perchlorate (0.2 g, 1 mmole), and the salt was added to the mixture, stirring being continued. A solution of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl chloride (2) was prepared in the following way: a mixture of the anomers of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*p*-nitrobenzoyl)-D-glucopyranose[2,5] (1, 2.00 g, 2.9 mmoles) in dry dichloromethane (20 ml) was saturated at room temperature with hydrogen chloride and, when precipitation was complete, the *p*-nitrobenzoic acid was removed by filtration and the filtrate was evaporated to dryness *in vacuo*. Dichloromethane and benzene were successively added to and distilled from the residual syrup, which was then dissolved in dry benzene (20 ml); the solution was added during 10 min to the stirred solution of 4, already described. After 4 h, t.l.c. (11:1 benzene–ether) revealed the presence of a new product; 4 h later, t.l.c. showed no further change. After 48 h, the reaction mixture was placed on a column of Silica Gel (425 g), prepacked in dichloromethane and then treated (immediately prior to use) with benzene (25 ml). The column was eluted with benzene (50 ml) and then with 60:1 dichloromethane–methanol (3.3 liters), 23.5-ml fractions of eluate being collected. Fractions 141–152 contained the condensation product (2.53 g); each fraction (~0.25 g) was subjected to preparative t.l.c., first with 80:1 dichloromethane–methanol and then, after drying of the plates, with a 60:1 mixture of these solvents. The major component, which migrated at a rate slightly greater than that of 4, was eluted with ether; like fractions thus obtained were pooled, and dried *in vacuo* to give 1.12 g (36%, based on 1) of syrupy product that gave a positive color test for a fructose derivative when t.l.c. spots were sprayed with an orcinol–hydrochloric acid reagent. In chloroform (*c* 1.62), the material showed

$[\alpha]_D^{20}$ +45.0°; values of +38.6° (refs. 1 and 6) and +31.6° (ref. 5) have been recorded for the specific rotation of octa-*O*-benzylsucrose (5) in this solvent.

A portion (502 mg) of the syrupy material was dissolved in warm methanol (10 ml), and well-washed palladium catalyst (freshly made by the reduction of 1.0 g of palladium chloride in methanolic suspension) was added. The suspension was shaken with hydrogen for 24 h, and then examined by t.l.c. with 3:1:1 propyl alcohol–ethyl acetate–water; no material visible under u.v. light was detected. The catalyst was removed from the reaction mixture, and successively washed thoroughly with methanol and water. Concentration of the combined filtrate and washings afforded a syrup, wt. 167 mg (103%). T.l.c. with 3:1:1 propyl alcohol–ethyl acetate–water and with 5:1:4 butyl alcohol–ethanol–water revealed the presence of two major and two minor components; one of the major components was chromatographically indistinguishable from sucrose. The whole material (167 mg) was treated overnight with a mixture of benzoyl chloride (0.5 ml) and pyridine (2 ml). After the usual processing, the crude, syrupy product (453 mg) was subjected to preparative t.l.c with 80:1 dichloromethane–methanol. After the solvent front had advanced, the plate was dried and further developed with 60:1 dichloromethane–methanol. Three clearly separated bands were discernible; the intermediate band was extracted with ether to give a syrup (158 mg) which, from its solution in carbon tetrachloride–pentane, gave 88 mg (69 mg on a solvent-free basis, 4.5% of the theoretical from 1) of crystalline material, m.p. 61–64°, undepressed on admixture with an authentic sample of the carbon tetrachloride solvate of octa-*O*-benzoylsucrose[7]. Dried at 57°/0.15 torr, the product lost 21.7% of its weight, leaving a residue that showed $[\alpha]_D^{20}$ +40.0° (*c* 2.09, chloroform). Solvent-free octa-*O*-benzoylsucrose, prepared through its crystalline solvate with carbon tetrachloride, has been reported[7] to have $[\alpha]_D^{20}$ +40.6° (*c* 1.55, chloroform).

Another sample (499 mg) of the once-chromatographed product from the condensation was catalytically debenzylated as described earlier, to give 179 mg of a syrup. G.l.c. of the per(trimethylsilyl) derivative of this material on 1% SE-30 (on Gas-Chrom P) at 211° failed to effect a resolution. Parallel experiments with authentic specimens of sucrose and isosucrose (β-D-glucopyranosyl α-D-fructofuranoside) showed that their trimethylsilyl derivatives were not separable under these conditions, suggesting that this chromatographic technique is of doubtful value for the investigation of isomers of sucrose. The remainder of the syrupy material (177 mg) was chromatographed on a column of silica gel (100 g) with 16:3:1 ethyl acetate–methanol–water and collection of 8-ml fractions of eluate. One major component appeared in fractions 69–93 (79.9 mg, 45%) and the other major component was found in fractions 124–174 (61.2 mg, 35%). The material in the second group of fractions was chromatographically homogeneous, and indistinguishable from sucrose on t.l.c. in 16:3:1 ethyl acetate–methanol–water and in 4:1 phenol–water. On hydrolysis with 0.05 M sulfuric acid at 60°, both major components afforded glucose and fructose, as shown by t.l.c. with 4:1 phenol–water. The faster-moving major component (fractions 69–93, 79.9 mg) was slightly contaminated with sucrose,

as shown by t.l.c. in phenol–water. In water (after clarification of the solution with decolorizing carbon and Filter-Cel), the material showed $[\alpha]_D^{20}$ +109.5° (*c* 3.22); Klemer, Gaupp, and Buhe[10] reported $[\alpha]_D^{20}$ +118.3° (*c* 0.8, water) for α-D-glucopyranosyl α-D-fructofuranoside. Acetylation of the sample (64.5 mg) used for the determination of the optical rotation gave a crystalline acetate: 26.3 mg (3.7%, based on 1), m.p. 111–112°, $[\alpha]_D^{20}$ +83.0°, $[\alpha]_{546}^{20}$ +97.4°, $[\alpha]_{436}^{20}$ +162°, $[\alpha]_{365}^{20}$ +252° (all *c* 0.8, chloroform); lit.[10] for α-D-glucopyranosyl α-D-fructofuranoside octaacetate (7), m.p. 110–112°, $[\alpha]_D^{20}$ +83.5° (*c* 1, chloroform).

The condensation of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (3) *with 1,3,4,6-tetra-O-benzyl-D-fructofuranose* (4) *in the presence of perchloric acid.* — A mixture of 3 (999 mg, 1.85 mmoles) and 4 (998 mg, 1.85 mmoles) was added to dry benzene (40 ml), and the suspension was warmed slightly to effect dissolution. The solution was stirred while molecular sieve (type 4A, 1 g) and 70% perchloric acid (54 μl, 0.6 mmole) were added; stirring was continued and, after 67 h, the mixture was concentrated *in vacuo* and then placed on a column of silica gel (270 g) prepacked in benzene. The column was successively eluted with benzene (15 ml) and 11:1 benzene–ether (1.5 liters), 10-ml fractions of eluate being collected. Fractions 81–95 were examined by t.l.c. (11:1 benzene–ether) and were found to contain three or four components that did not appear to be present in the products from parallel experiments involving 3 or 4 alone. Of these components, that which moved most slowly in t.l.c. greatly preponderated; it was isolated from these fractions by preparative t.l.c. with 11:1 benzene–ether. The syrupy product (587 mg, 30%) contained a small amount of a slightly faster-moving component; a sample (300 mg) in methanol solution was catalytically debenzylated with hydrogen in the presence of well-washed palladium black (freshly prepared by the reduction of 1.0 g of palladium chloride). After removal of the catalyst, and concentration of the solution, the product was precipitated by the addition of ether—a procedure which was found (t.l.c., 4:1 phenol–water) to remove a faster-moving impurity. The precipitate was acetylated at room temperature with acetic anhydride–pyridine to yield a two-component mixture (t.l.c., ether) which was preparatively chromatographed (t.l.c., ether). The slower-moving component crystallized from its solution in ethanol–pentane, and proved to be chromatographically indistinguishable from the octaacetate of α-D-glucopyranosyl α-D-fructofuranoside (7): 47 mg (6%, based on 3), m.p. 111–112°, $[\alpha]_D^{20}$ +82.0° (*c* 1.58, chloroform).

The faster-moving band of acetylated material was obtained as a mixture of two crystalline forms from its solution in ethanol–ether. Analytical data and the p.m.r. spectrum of this material showed it to be a mixture of the anomeric D-glucopyranose pentaacetates.

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Sucrose, the arch criminal of dental caries

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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 (Zita, Mc. Donald and Andrews 1959, Ludwig, Denby and Struthers 1960, Bradford and Crabb 1961, Mansbridge 1960, Winter, Hamilton and James 1966, Goose 1967). 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 (Gustafsson *et al.* 1954) and Hopewood House (Goldsworthy *et al.* 1953, Sullivan and Harris 1958, Harris 1963) where the human diet has been carefully controlled. These latter have clearly established 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 further shown 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 (1960), Toverud (1964) and Sognnaes (1948) provide further circumstantial evidence showing a corretation between sucrose consumption and human caries prevalence.

The above clinical studies have been reviewed elsewhere (Bibby 1961, Hartles 1967) and shall not be considered further. There has recently emerged 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 certain oral 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 organisms 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 can synthesize dextrans, polymers of glucose. Other organisms form levans, polymers of fructose (Fig. 1). Biosynthesis of these polysaccharides occurs by the agency of enzymes which for the most part are extracellular or bound to the cell surface and 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 (Fig. 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 (Bernfeld 1963).

Table 1. *Energies of hydrolysis of glycosyl donors and some polysaccharides*

Compound	Standard free energy of hydrolysis ΔF_H (cal/mole)
Glucose-1-phosphate	—5000
UDP-glucose	—7600
Sucrose	—6600
Maltose	—3000
Lactose	—3000
[illegible]	—4300
[illegible]	—2000
Levan	—4600

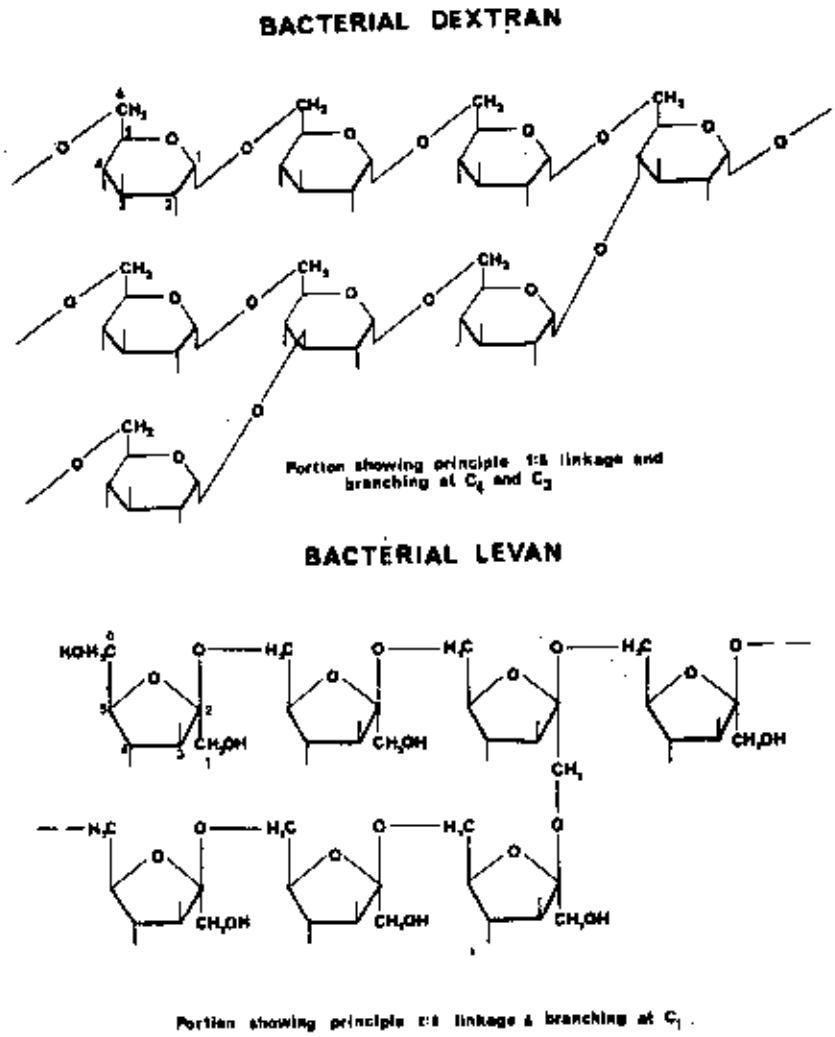


Fig. 1. Chemical structure of a portion of the bacterial dextran and levan molecules. Hydroxyl groups are represented by a straight line and hydrogens have been omitted. The dextran is a homologous polymer of D-glucopyranose with predominantly α-(1→6) linkages. The levan is a homologous polymer of D-fructofuranose with predominantly β-(2→6) linkages. The frequency of branching and overall size of these polysaccharides varies with the organism from which it is obtained.

The enzymes involved in the synthesis, dextransucrase and levansucrase or more correctly α-1,6 glucan: D-fructose 2 glycosyltransferase (EC 2.4.1.5) and β—2, 6 fructan: D-glucose 6 fructosyltransferase (EC 2.4.1.10) have been partially purified (Hestrin, Feingold and

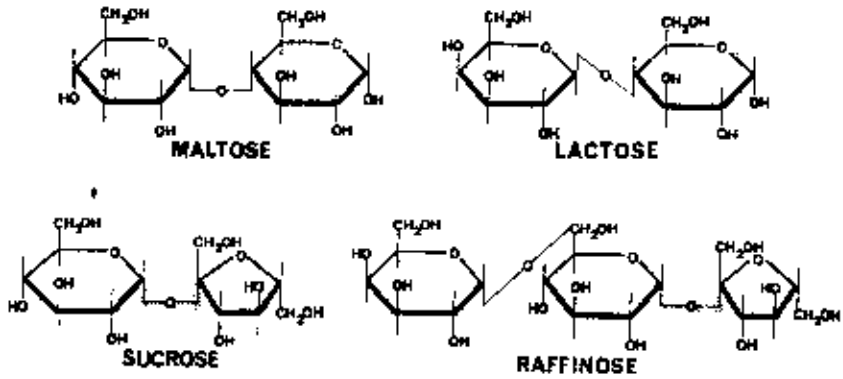


Fig. 2. Chemical structure of maltose, lactose, sucrose and raffinose. The potentially high-energy dihemiacetal linkages between the aldehyde and ketone carbons of glucose and fructose respectively in sucrose and raffinose are shown by stippling. In maltose, lactose, and between glucose and galactose in raffinose the glycosidic link is a hemiacetal between an aldehyde and hydroxyl carbon and is of much lower energy

Avigad 1956, Hestrin and Avigad 1958) from some of the organisms mentioned. Levansucrase is 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, xylsucrose and galsucrose. Compounds with the same group linked to a carbinol carbon (i.e. one with a hydroxyl group) proved ineffective glycosyl donors. The dextransucrase is the more highly specific and will not utilize any substrate other than sucrose (Feingold, Avigad and Hestrin 1957).

MICROBIOLOGICAL EVIDENCE

Certain strains of oral streptococci can be shown to be cariogenic following inoculation into gnotobiotic (Fitzgerald, Jordan and Stanley 1960, Gibbons *et al.* 1966, Gibbons and Banghart 1967 b, Shklair *et al.* 1967), quasignotobiotic (Guggenheim *et al.* 1966) or conventional rodents (Fitzgerald and Keyes 1960, Zinner *et al.* 1965, Krasse 1966). A consistent feature of these streptococci has been their ability to form extracellular polysaccharide. Significantly more polysaccharide is formed from sucrose than from an equivalent amount of glucose, maltose, lactose or fructose (Gibbons *et al.* 1966) or glucose and fructose combined (Gibbons and Banghart 1967 a). The polysaccharides formed by the oral streptococci have, as yet, been only partially

characterized. Most of the streptococcal strains studied so far form dextrans which on hydrolysis yield glucose or glucose and a minor amount of fructose (Guggenheim *et al.* 1966, Wood and Critchley 1966, Dahlqvist *et al.* 1967). However at least one cariogenic streptococal strain produces extracellular levan (Gibbons and Banghart 1967 b). *Streptococcus salivarius* synthesizes a levan of extremely high molecular weight, approximately 23×10^6, which although soluble could diffuse only slowly from the dental plaque (Newbrun and Baker 1967).

Sucrose has been further incriminated in the caries process by some important animal studies (Krasse 1965 a and b). 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 these "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 (Krasse *et al.* 1967).

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 (Hartles 1967, Krasse 1965 b, Shafer 1949, Gustafsson *et al.* 1955, Grenby 1963, Grenby 1966, König and Grenby 1965, Guggenheim *et al.* 1966). 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 or 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 (Stephan 1966).

It should also be pointed out 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 (Fitzgerald, Jordan and Archard 1966).

EVIDENCE FROM STUDIES ON PLAQUE FORMATION

There can be little doubt that the dental plaque plays an integral role 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 (Jordan and Keyes 1965, Barry 1966). 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 (Carlsson and Egelberg 1965). 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. In a subsequent study Carlsson (1967) was able to show quantitatively that the amounts of plaque recovered from the teeth surfaces were significantly greater following a sucrose diet than following an equivalent glucose diet period. Furthermore, the total number of cultivable organisms in the plaque was higher after the sucrose rinse.

Chemical analyses of the water soluble polysaccharides obtained from scrapings of plaque indicate that dextrans compose 7—10 % and levans 1—2 % of the total plaque (Critchley *et al.* 1967). The plaque scrapings were able to rapidly incorporate ^{14}C-sucrose into these extracellular polysaccharides confirming that they were formed from the dietary sugar, sucrose.

HEREDITARY FRUCTOSE INTOLERANCE

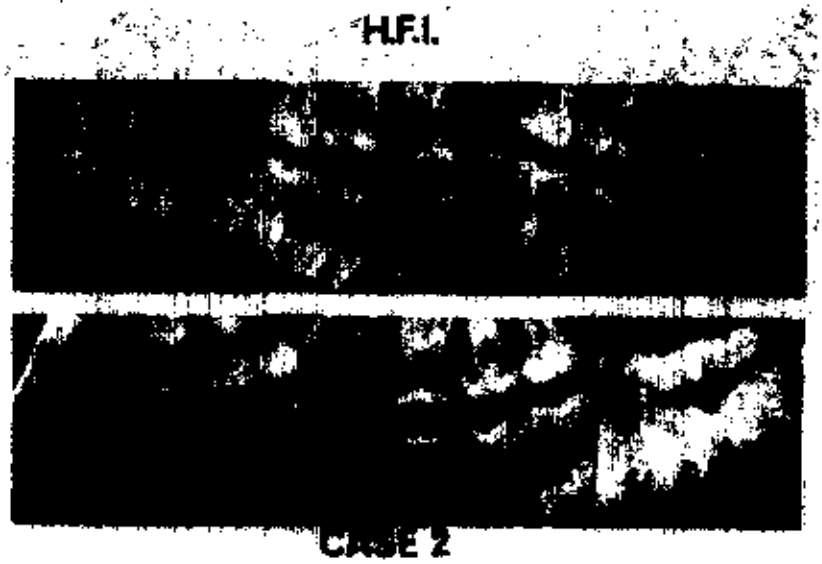
Hereditary fructose intolerance (H.F.I.) is an uncommon metabolic disorder believed to be due to an absence of hepatic fructose-1-phosphate aldolase, the enzyme which splits fructose-1-phosphate into two three carbon fragments which are subsequently metabolized. H.F.I.

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 strain in groove of 2nd primary molar	
DFC	15	M	0	0	5	5	deep development grooves prophylactic fillings?	Cornblath *et al.* 1963
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 suprisingly intact	
EP	33	M	0	0	1	1	no active caries	Froesch, Wolf and Baitsch 1963
F	>33	M	0	0	1	1	no active caries	
	39	M	0	0	2	2	very good dental health	Hubschmann and Cobet 1964
IG	18	F	?	?	?		excellent dental health	
FS	54	F	?	?	?		good dental health	
OS	63	M	?	6	?		teeth in good condition	Linden and Nisell 1964
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

is characterized by symptoms of nausea, vomiting, malaise, excessive sweating, tremor, coma and convulsions following the ingestion of foods containing fructose (Cornblath *et al.* 1963). 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 (Cornblath *et al.* 1963, Froesch,



NO CARIES

Fig. 3. Roentgenograms of Case 2. Total absence of caries in 32 year old patient with hereditary fructose intolerance.

Wolf and Baitsch 1963, Hubschmann and Cobet 1964). Linden and Nisell (1964) have commented on the "excellent" or "good dental health" of these patients. Table 2 summarizes the caries prevalence of 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 (Rovelstad 1967). Surprisingly, several recent reviews concerning the influence of genetic factors on dental caries (Goodman 1962, Witkop 1964, Finn 1965, 1966) failed to mention H.F.I., the one genetic disease where an unequivocal effect on the caries prevalence can be demonstrated. 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 restorations. 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. 3). 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 proven frustratingly elusive, Perhaps one of the reasons has been the indiscriminate attempt to drastically limit all dietary carbohydrate consumption (Becks, Jensen and Millarr 1944, Jay 1947, Templeman 1964).

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 predilection for sweet foods. The burgeoning market in sugar substitutes, saccharin, cyclamate and sorbitol (Gray 1965) reflects some progress in this direction. Another possible alternative is the development of phosphorylated sucroses, although experiments with these are sparse (Lilienthal *et al.* 1966, Napper and Smythe 1966, Harris *et al.* 1967) and their routine use would be premature.

Certain strains of oral microorganisms have the ability to store carbohydrates as intracellular iodine-staining polysaccharide (Gibbons and Socransky 1962, Gibbons and Kapsimalis 1963, Gibbons 1964, van Houte 1964, Berman and Gibbons 1966, Walker 1966, Loesche and Henry 1967). These intracellular polysaccharides are polymers of glucose, linked by predominantly α 1—4 bonds as evidenced by their susceptibility to cleavage by α-1, 4-glucan hydrolase (α amylase) (Gibbons and Kapsimalis 1963). They resemble the starchs of plants and the glycogen of mammals. It has been proposed that the capacity of microorganism to form intracellular polysaccharides 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 hypothesis, 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 (Berman and Gibbons 1966). 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.

Furthermore the intracellular polysaccharide could not contribute 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 pellicle bound to the enamel surface and apparently derived from the saliva (Leach *et al.* 1967). However, the bulk of the plaque consists of bacteria embedded in a homogenous matrix, which is not salivary in origin but formed by the bacteria themselves. Not only are those extracellular polysaccharides important in enabling cariogenic organisms to colonize on the teeth, but they may also play a 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 (Jordan and Keyes 1964). 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 (Howell and Jordan 1967).

SUMMARY

Certain cariogenic strains of microorganisms possess the ability to synthesize extracellular levans and dextrans from sucrose. Evidence supporting the hypothesis that this is the most important property determing cariogenicity has been presented. Extracellular polysaccharides, thus formed, function both as a reservoir of fermentable carbohydrate for continous acid production and as structural components of the dental plaque "gluing" the bacteria to the teeth. Biochemical and microbiological evidence as well as studies on plaque growth and experimental animal caries support this hypothesis. Hereditary fructose intolerance, a human genetic defect, illustrates the claim that elimination of specific carbohydrates, especially sucrose, can prevent caries. The dental condition of two patients with this defect have been reported. Of all the cast of carbohydrate characters sucrose stands indicted as the arch criminal of dental caries.

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THE DISCOVERY OF SUGAR AS A MEDICINE

Knowledge of its Medicinal Properties a Development of Recent Times . . . Some of the Many Ways in Which it Has Proved its Value

By Edward Podolsky, M.D.

IN the days when man was struggling for reason and searching for the truth, the weird and the unusual were the elements most endowed with desirable attributes. In medicine this was even truer than elsewhere. The primitive doctor disdained the everyday for the out-of-the-way in his search for curative agents. Somehow it was his belief that the search for medicines would not be interesting if these medicines were readily available. The medical man of former times saw medicinal virtues in crab shells, snake eyes and tiger brains, but such common, everyday articles as salt and sugar did not interest him.

Only within very recent times has sugar come to the fore as a valuable medicine. In the year 1885, Dr. Goule, a Swiss surgeon thought of using a solution of sugar instead of the popular solution of salt as a means of replacing lost tissue elements. He had some good reasons for making this substitution: sugar has definite nutritive properties, it is stimulating, and it does not have a tendency to form blood clots in the veins. In 1905 Drs. Friedrich and Lamander further popularized the use of sugar solutions in medicine, and two years later Dr. Fleig published an essay in which he pointed out the full advantages of using sugar in preference to any other stimulating fluid. Interest in sugar as a medicine now became world wide.

Among the most ancient of diseases is seasickness, and unfortunately, not very much is known about it, even at this time. Many theories have been advanced; many remedies have been tried. When chemical methods of determining the substances found in the blood reached a high stage of development, it was found that in seasickness the blood contained a great many acid bodies, which gave rise to a state known as acidosis. This discovery proved of particular interest to Dr. G. H. Oriel, of the Canadian Pacific Steamship Line, for to him seasickness was a highly important problem. For many years it had been known that sugar had a favorable influence on acidosis. For this reason Dr. Oriel determined to try sugar as a remedy in his cases of seasickness, and he had many such during the year. In his first thousand cases he gave sugar by mouth without any other medicines. To his surprise and delight he noted that under the sugar treatment there was a distinct improvement in all his patients. The severer the seasickness, the more dramatic was the result. The bothersome symptoms, such as headache and vomiting, ceased almost at once. The sugar was absorbed without delay, as sugar always is. The acid bodies in the blood were neutralized. The urge to vomit was controlled and the patient felt in high spirits once more. Dr. Oriel found that those patients who had plenty of food, particularly food containing large amounts of sugar, were free from dizziness and vomiting spells. Other medicines which had been used previously were much less effective.

There is not much difference between one type of vomiting and another; the vomiting of seasickness is akin to the vomiting of pregnancy. There is an underlying sugar deficiency in each. This was realized by Drs. Underhill, Rand and Ewing as far back as 1910. It interested Dr. Paul Titus years later even more. He made up solutions of sugar in water, sterilized and purified to the highest degree. Dr. Titus, an obstetrician, was concerned, as is every obstetrician, with seeing that the mother went through her period of child-bearing with as much ease as possible. His great bugbear was constant devitalizing vomiting which affects some women. Many hundreds of remedies have been tried, but it was sugar which finally proved to be the desired medicine. Dr. Titus had a great many cases of which to test the action of sugar in controlling vomiting: 328 of them. In each of these cases he had his solution of sugar ready for injection into the veins as soon as signs of vomiting became manifest. In all 328 cases the administration of sugar abolished the vomiting at once. The reason for giving sugar in the vomiting of pregnancy is the lack of sugar in the mother's body. The lack occurs because the growing child in the mother's womb requires great amounts of sugar for growth and development. This constant drain without subsequent replenishment soon brings on a whole train of unpleasant symptoms, the most serious of which is vomiting. The injection of sugar directly into the blood stream restores the sugar level at once to its proper height.

Not many years ago, Dr. Fagley of the Veterans' Administration in St. Louis, had a great many cases of sleeping sickness to take care of. It was the sleeping sickness which followed severe attacks of influenza, and it left in its wake a strange sort of tremor of the arms and legs. Dr. Fagley recalled that sugar injections had been used with good results in influenza, and as sleeping sickness was a sequel to many influenza cases, he thought that sugar might prove of value. He had some really desperate cases in his wards, and a great many remedies had been tried without much success. There is still no medicine which will cure sleeping sickness, but sugar proved to be a remedy which helped the patient to a certain extent. It at least made life more worthwhile. Dr. Leland B. Alford, a nerve specialist of St. Louis, became interested in the results that Dr. Fagley had obtained with sugar and decided that it deserved a little more study. He selected forty cases of sleeping sickness at the very height of helplessness and began to inject sugar into their veins. Twenty-two of his cases were relieved in a short time of all their undesirable symptoms, with sugar as the only medicine. There were eight cases of delirium which it seemed as if nothing could help, but sugar relieved five of them, while three of the most desperately hopeless received partial relief. Convulsion was another terrible condition, but this yielded readily to sugar. Headaches and general aches and pains were abolished in almost all of the victims of this disease.

Dr. Alford found that the sooner the sugar was given, the better were the results. In the acute cases the benefits obtained were most gratifying. In the older cases, particularly where the tremor of the hands had already set in, the results were not very good. However, Dr. Alford established the fact that sugar, when given by vein, was a good medicine in that particular form of sleeping-sickness which sometimes follows a severe attack of influenza.

The first doctor to advocate the use of sugar in diseases of the liver was Dr. A. P. Beddard. Several years later Dr. Alford, who had always been interested in the medicinal properties of sugar, together with Dr. Ople, began to study the effects of sugar treatment on the livers of animals suffering from the effects of chloroform and phosphorus. They found that if large amounts of sugar were fed to these animals there was a considerable reduction in the liver damage when the chloroform or phosphorus was given later. Since then many doctors have become interested in sugar as a means of preventing liver damage and as a medicine for diseases of that organ. How does sugar prevent liver damage? First, it has the property of burning away poisonous substances, and in this way converting them into harmless products. Second, it may take these poisonous substances and by uniting them with other substances produce non-poisonous com-

pounds. In this way it prevents the harmful action on the liver of such poisons as chloroform and phosphorus.

Another use of sugar was found by Drs. Havdin, Riegel and Morrison. In jaundice, in which the skin becomes yellow, due to the leakage of bile into the blood, the liver is often diseased. The blood is also in an abnormal condition, particularly in that it fails to clot in the normal time. Giving sugar to such patients not only improves the disagreeable, itching jaundice, but what is more important, it brings the coagulation time of the blood up to normal. It has been found a very good idea give all patients suffering from liver diseases a great amount of sugar. This saves the liver much work in producing glycogen, or animal sugar, and gives it an opportunity to rest and recover. It also, in this manner, assists the diseased portions of the liver to regenerate themselves. In the milder cases an abundance of sugar in the diet is helpful, but in the severer cases the sugar must also be given by vein.

A patient with a wound in the stomach wall does not have much desire to eat food, because the ulcer is constantly irritated by the rough food particles. And yet the patient must eat to live. This was a problem that particularly concerned Dr. George Recht. He began to experiment with sugar and found that it was a very desirable food for patients with stomach ulcer, not only because it furnished the required energy, but also because it did not irritate the stomach wound. Dr. Recht, in the course of his investigation with sugar, also found that when the blood was supersaturated with sugar the pain of the stomach ulcer was considerably lessened. This was due to a reduction of the acidity of the stomach secretions and a consequent minimization of the irritative media within the stomach. Another remarkable thing he discovered was that sugar controlled the hunger contractions of the stomach as soon as it was given. As hunger contraction results in a constant churning motion, with subsequent excruciating pain, the administration of sugar to patients suffering from stomach ulcers proved very desirable.

Shock is one of the most damaging things that can occur to a human being. For years doctors have been seeking means to combat it. Drs. Erlanger and Woodyatt produced a state of shock in dogs and ascertained the value of sugar as a means of reducing it. They injected large quantities of sugar into the blood stream and found that the lowered blood pressure promptly rose to its normal level. The tissues hungrily took up the readily available food, and the pulse became fuller and stronger. Dr. R. Matas, whose brilliant surgery with blood vessels won him world renown, was particularly concerned with the dangers of shock, and he was among the first to use sugar to combat it in human beings. The sugar was so quickly absorbed and utilized by the sick human tissues that its benefits were evident in a short time. Severe crises in patients may be brought on by a variety of conditions: long exposure to cold or privation, extreme fatigue, wasting diseases, exhaustion after child bearing. In such cases an injection of sugar solution directly into the blood stream is often the means of saving life.

Some years ago Dr. Lawrence Litchfield had a wonderful experience with sugar given by vein in cases of pneumonia. He had always had faith in sugar as a remedy of value in other conditions, and here he found an opportunity to see what it would do in pneumonia. He found that when he gave sugar to pneumonia sufferers, the body was able to maintain its proper quota of fluids, devitalizing nausea was abolished, and strength was imparted to the starving body. Under its benign influence the general appearance of the pneumonia victim began to improve at once. The features lost their pinched look. The patient became bright. The pulse became slower, fuller and stronger; the blood pressure rose. The tongue became moist. The patient began to ask for food and water. The kidneys and bowels became active. If the patient had been restless or delirious, he became quiet and went to sleep. Since then many other doctors have found that sugar, while it has no direct effect on the diseased lung itself, is nevertheless of paramount value in furnishing food and water to the dangerously starved body tissues. It gives the patient the necessary strength, assists him to ward off the devitalizing effects of the disease, and helps him to acquire a new lease on life.

About thirty years ago Dr. Muller became very much interested in heart action. He removed the heart from a cat and kept it beating by artificial means to study its action at close range. He was particularly interested in ascertaining the effects of different drugs on the action of the beating heart. He found that some drugs slowed and weakened the heart action; others strengthened it. Dr. Muller then added a solution of sugar when the heart was tired out by too strenuous activity; he observed that the heart hungrily sopped up the sugar. The sugar solution acted as a reviving tonic to the exhausted heart. It added strength to its weakened contractions, and gave it more vigor and energy. Some years later, Dr. Stewart repeated Dr. Muller's experiment, but in greater detail. A little later he took this experiment to the clinic for further study. Other scientists repeated these experiments and confirmed the original observations. Among the most important discoveries in this connection was that of Drs. Locke and Rosenheim, that there was a definite relationship between the amount of sugar the heart consumed and the length of time the heart kept beating.

As time went on other interesting things were discovered. Drs. Patterson and Starling found that when the heart rate was increased by an injection of adrenalin there was also an increase in the consumption of sugar. The effect of adding insulin to the sugar solution was another interesting phenomenon which demanded careful study. It was Dr. Goulston who found that when he fed sugar to patients with weak hearts they experienced a marked improvement. Other doctors soon became interested in the good results which Dr. Goulston had obtained with sugar feedings. It was found that the addition of four ounces of sugar to the daily diet was of pronounced benefit to the disease-exhausted heart.

Two years after Dr. Goulston had established the value of sugar as a strength-giver to weak hearts, Dr. Theodore Budingen demonstrated the very important role that sugar played in influencing heart action. He found that the intake of large amounts of sugar caused the surplus to be stored in the muscles and in the heart and that storage was most valuable as a reserve to be used in time of need. He also found that sugar was a valuable supplement to digitalis. Dr. Budingen recommended the injection of carefully prepared sugar solutions into the veins in cases of heart exhaustion of which the origin was unknown. Sugar was also found to be valuable in that very painful heart condition, angina pectoris. Among the first to use sugar as a heart calmer were Drs. Nonnebruch, Klewitz and Kirchsein, who did much to make sugar popular as a heart medicine.

A few years ago Drs. Edwards and Page, two heart specialists, became very much interested in nding out what sugar would do in cases of disturbed heart rhythm. They carried out a large number of experiments on animals with this form of heart disease and were amazed with the good results they obtained. The next step was the clinic, where Drs. Gomez, Lemaire, Mougeot, and others found that in some cases of disordered heart beat, sugar acted as a wonderfully effective regularizer. Not only did sugar act to regularize the crazy rhythm but it also increased the value of other drugs used in this disease.

As the years went by, even more startling properties of sugar were discovered. It was found to be one of the greatest poison fighters known, particularly the poisons secreted by germs which seemed to have a peculiar affinity for the heart. Drs. Edmunds and Cooper infected a group of small animals with diphtheria toxin, perhaps the most deadly of all heart poisons. In those animals in which the resulting collapse was extreme and in which the

usual heart stimulants had very little effect, the injection of a ten per cent solution of sugar by vein was followed by marked improvement in the heart and an increase in the blood pressure. Drs. Edmunds and Cooper regarded the injection of sugar as a life-saving measure in many instances. It was found by these investigators that they were able to save the animal's heart if sugar was injected into the vein before the heart stopped. These results were attributed to the increased amounts of energy provided by the sugar. So interested was Dr. Toomey in these animal experiments that he determined to use sugar as a medicine in cases of human beings whose hearts had been crippled by diphtheria. He found sugar to be one of the most valuable of all medicines in aiding the heart over an exhausting crisis.

Sugar has also been found to be of distinct value in the heart poisoned by the toxins of pneumonia. Dr. Nassau, who became especially interested in this problem, found that sugar was a life-saver in supporting the heart muscle weakened by the toxins of the pneumonia germ. Injections of a clear solution of sugar into the blood stream furnished a constant supply of sugar for the sorely laboring heart.

Still another form of heart poisoning attracted the attention of two other physicians, Drs. Moore and Stewart, who found that uremic poisoning often killed the patient by poisoning the impulse-conducting mechanism of the heart muscle. They found that when a fifty per cent solution of sugar was injected into the vein, there was an immediate change for the better in the patient's condition, almost as soon as the sugar entered the vein. On the average, it was found that after three days of constantly feeding sugar to the heart, the patient passed the crisis safely and lived.

It is, however, in that form of crippling heart disease known as angina pectoris that sugar has been found to be most useful. Dr. Hasenecamp, who was among the first to become interested in this problem, ascertained that sugar was of value in angina pectoris by supplying an excess of readily available sugar to the heart muscle, and what was even more important, by relaxing the spasm of the smaller blood vessels which convey nourishment to the heart.

It was in 1922 that insulin was discovered, and two years later Dr. Pick saw great healing possibilities in combining insulin with sugar in the treatment of heart disease. He was so enthusiastic about these possibilities that he published a detailed account of what this combination treatment did in patients who suffered from failing heart. This enthusiasm soon spread to other heart specialists, who also reported remarkable results with this type of treatment after having evaluated its effectiveness in large groups of patients. It was found that under the regimen of sugar and insulin, the amount of waterlogging was reduced, the heart sounds became stronger, the pulse fuller. When combined with the regular heart medicines, the results are in some instances even more startling. While sugar was found to be of undoubted value in certain types of heart pain, a combination of sugar with insulin was found to be of even greater value in the same cases. When this problem is analyzed in detail it is found that heart pain is most frequently due to a lowered nutrition of the heart muscle. The furnishing of sugar in readily available form at once supplies the deficient element and the pain is abolished.

So valuable has sugar proved as a medicine that doctors have looked about for new conditions in which it might be tried. Dr. Sansum found that sugar was of value in reducing the terrific eye tension in glaucoma, a terrible eye disease. Dr. Guthrie found that when sugar was injected into the veins it raised the lowered blood pressure resulting from gangrene of the limbs. Drs. Shultz and Richter found that grape sugar was particularly valuable in curing acute inflammatory diseases of the skin, drug rashes, and a very uncomfortbale, blistering condition of the skin known as pemphigus. Drs. Remenotsky and Kantor found that concentrated solutions of sugar were effective in closing up varicose veins and improving the general circulation of the leg. Dr. Turrettini found that when sugar was injected into the blood stream it had a favorable influence on cases of mercury poisoning.

Sugar is the fuel which fires life's engines. It is the means by which the body is run. It does not require much conversion to reduce it to a form readily available for use by the cells of the body. It is the most quickly utilized of all foods. It is the only food which can be injected directly into the veins without setting up a train of unpleasant reactions. It spares cell destruction in starved conditions, frees the tissues of undesirable excesses of water and other fluids, and bolsters up bodily energy quickly and without much effort.

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