MONOGRAPH

ON

STARCHES

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M O N O G R A P H

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STARCHES

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STARCHES

Summary

Starch, in its native or modified form, is used extensively throughout the food industry as a carbohydrate source, processing aid, thickener, extender, stabilizer, and texture modifier. Although the major commercial source is corn, starches may be obtained from other plants such as wheat, sorghum, rice, potato, cassava, and arrowroot. Manufacturers have evolved various techniques for the modification of starches, enabling them to improve their functional properties (460).

According to Boyd (193), "the basic action of dietary starches is to provide calories in a nontoxic form, at the daily amount needed. The minimal amount of starch necessary to prevent toxicity from proteins or fats appears to be some 50 to 65% of the diet. Amounts slightly in excess of caloric requirements are converted to fat and stored in the body". One gram of starch supplies approximately 4 calories, which is comparable to the energy supplied by sugar (460).

Auricchio et al. (81) determined the absorption coefficient of starch for normal 1-2 year old children to be greater than 99%.

In 1960 Ugolev (1539) proposed that parietal or contact digestion is an important mechanism of breakdown and assimilation of foods such as starch in higher animals. This type of digestion involves the adsorption of enzymes concerned with hydrolysis and absorption of food onto the mucosal surface. Parietal digestion was believed to be best developed in the small intestine. The author suggested that the very first stages in digestion take place chiefly in the lumen of the digestive tract, and subsequently on the surface of the intestine.

Four years later, Jesuitova (663) confirmed Ugolev's hypothesis that the most important hydrolytic activity of starch occurs on the intestinal mucosa. However, Fogel and Gray (426) maintained that the intraluminal process played the primary role in starch digestion and that membrane digestion was of secondary importance. Comparison of starch hydrolysis of intraluminal amylase and amylase adsorbed to the microvillous membrane in man indicated that the available intraluminal hydrolytic capacity was in excess of that needed to account for the observed hydrolytic rates. Fogel and Gray (426) suggested that the different perfusion rates used by the research groups could produce the conflicting results.

Volkheimer (1585) found evidence for the persorption of starch granules in dogs. Persorption is the phenomenon by which solid, undissolved food particles enter the body from the intestinal lumen during normal digestion.

In the first step of digestion, starch is attacked by ptylin, the alpha-amylase in the saliva. In the small intestine pancreatic alpha-amylase also acts on the ingested polysaccharides. Both enzymes attack the 1,4 alpha linkages but neglect the 1,6 alpha linkages, terminal 1,4 alpha linkages, and the 1,4 alpha linkages next to branching points. As a result, the end products of alpha-amylase digestion are maltose, maltotriose, and alpha-limit dextrins (branched polymers containing 8-glucose units). (464).

As suggested previously, the enzymes responsible for further digestion of the starch derivatives are located on the surface of the mucosal cells. Isomaltase hydrolyzes the alpha-limit dextrins while maltases hydrolyze maltotriose and maltose. Most of the glucose formed enters the mucosal cells, yet some reenters the intestinal lumen and is absorbed further along (464).

Based on studies of starch digestion in small children, Auricchio (81) reported that hydrolysis into glucose, maltose, maltotriose, and branched dextrins was probably followed by hydrolysis to glucose through mucosal glucosidases. Maltose and maltotriose comprised 60-70% of the total carbohydrate after a test meal of amylopectin.

Hydroxypropyl maltose was found to be the major metabolite of hydroxypropyl starch both in vitro and in vivo (1680).

When labeled starch adipate was administered to rats, 99.3 and 5.8% of the activity of the free adipic acid was recovered in the respired air and urine, respectively, and none in the feces. The activity of esterified adipic acid was found to be 70.5, 7.2, and 24.5% in the respired air, urine, and feces (1680).

Labeling techniques were used to study the fate of the phosphate radical in starch phosphate. It was thought that this phosphate moiety behaved metabolically like any other ionic phosphate (1680).

According to Booher (180), raw cereal and root starches such as wheat, maize, rice, and cassava are digested efficiently by the human gastrointestinal tract; however, raw potato, arrowroot, and canna starches are not. Further studies involving matched-feeding techniques with rats indicated that oxidized wheat starch was practically indistinguishable from corn and wheat starches as regards weight gain and digestibility.

Yoshida and Morimoto (1707) reported the digestibility coefficients of raw corn, sweet potato, and potato starch in rats to be 99, 97, and 57%, respectively; steaming the starches resulted in almost complete utilization. Borchers (182) assigned digestibility coefficients of 95% and 66-77% for normal corn and high-amylose corn for rats.

An unpublished study by Oser (1680) revealed that acetylated starch, acetylated distarch adipate, and native starch had similar caloric values; however, the weight gain for rats fed acetylated distarch glycerol and distarch glycerols was slightly reduced, an earlier experiment disclosed

no difference in caloric value between distarch phosphate and unmodified starch. Results from an unpublished study by Hixon (1680) confirmed this similarity for distarch phosphate.

One unpublished report by Prier (1680) indicated that hydroxypropyl distarch phosphate and native starch possessed similar coefficients of digestibility. However, Porter (1680) found a slight decrease in caloric utilization with an increasing degree of modification for hydroxypropyl distarch phosphate. Both studies were based on feeding tests with rats.

In vitro experiments with pancreatic alpha-amylase have shown that the digestibility of raw corn starch was not directly associated with amylase content (1275).

The in vitro digestibility by pancreatin and porcine mucosal enzymes of acetylated distarch phosphates, starch acetate, hydroxypropyl distarch glycerol, phosphated distarch phosphate, and hydroxypropyl starch was found to range between 80 and 93% that of native starch (1680). Hydroxypropyl starch and native starch were shown by Kay and Calandra (1680) to be equally digestible by pancreatin. Similar unpublished studies by Shuman and Mertz (669) found oxidized starches to be 10-15% less digestible than native starch.

When less than 0.5% phosphorus oxychloride was used in the production of distarch phosphate, digestibility of pancreatin was unaffected; but, at levels of 0.5 or 1.5% phosphorus oxychloride, the resulting cross-linkage inhibited digestibility in a manner related to the cross-linking agent used (1680). Starch acetates (2.5% acetyl) were shown to be 93.7% as digestible as native starch (1680).

Kohn and Kay's unpublished report (1680) stated that the in vitro digestibility of distarch phosphate and phosphated distarch phosphate by pancreatic amylase was reduced compared to unmodified starch. The digestibility by amyloglucosidase of acetylated distarch adipate, distarch glycerol, and several distarch phosphates was between 96.4 and 98.3%. The digestibility of acetylated distarch glycerol varied with acetyl content, but was not affected by cross-linkage.

Marchis-Moren (913) determine that a starch-rich diet induced a more active synthesis of amylase than a casein-rich diet for rats. A similar study by Abdeljlil (2) concurred. Although amylase is absent in newborn infants, feeding 2% cream of rice to premature infants induced the production of amylase (1016).

When raw sweet potato starch was fed to adult male Wistar rats, the apparent digestibility of dietary protein decreased (1707). Chang (246) determined that rice starch was superior to arrowroot starch but inferior to corn or wheat starch as regards protein utilization in rats.

Kimura and Ashida (733) found liver lipogenesis to be increased in rats by raising the levels of starch ingestion. Farnell and Burns (399)

discovered that dietary starch (20%) lowered liver cholesterol levels in rats fed a cholesterol-free diet; serum cholesterol was raised when soy protein was given, but not when casein was the protein source. Anderson (49) reported that modified starches, cooked or raw, significantly raised serum and hepatic cholesterol levels compared to sucrose in rats. The consumption of potato or corn starch in conjunction with cholesterol by hypercholestermic rabbits enhanced the degree of hypercholesteremia (1161). After measuring fasting serum cholesterol and triglycerides in normal men given a diet containing starch (23% of the total calories), Mann and Truswell (919) found no significant differences between starch and sucrose diets.

Garretson (466) observed decreased iron absorption in rats fed protein deficient starch and sucrose diets. It was suggested that "starch eater's anemia" is caused by a lack of dietary iron and may be complicated by a decreased ability to absorb iron because of protein deprivation.

Numerous short-term studies in which various modified and unmodified starches were fed have been performed using chicks, mice, rats, rabbits, dogs, pigs, and humans. In addition the Joint FAO/WHO Expert Committee on Food Additives has presented a table of Acceptable Daily Intakes (ADI) of modified starches for man, as shown on page 46.

No untoward effects resulted from supplying groups of 40 male chicks with rations containing approximately 54% cornstarch from 5 manufacturers (156).

Groups of 3 mice were fed either a normal diet or one containing 81% sucrose or starch for 6 weeks and then transferred to one of the other diets. Based on collagen and reticulin responses, Zuckerman and MacDonald (1731) reported that excessive dietary carbohydrate led to increased hepatic fibrosis. Seconde (1350) determined that of mice fed 70% raw cornstarch or glucose, the glucose-fed animals had an increase in the occurrence and severity of avillous hyperplasia in the duodenal mucosa.

Liu and Boyd (853) gave rats oral doses of 36-456 g starch per kg daily for up to 49 days; a dose-dependent increase in the weight of the stomach and intestines was observed. Boyd (194) also administered 36, 72, 120, and 168 g starch per kg body weight orally to rats for 2 weeks. Doses of 0.1 (body weight) and above produced growth inhibition; doses of 0.2 (body weight) and above increased susceptibility to pneumonia and bowel obstruction. Hypertrophy of the smooth muscle of the G.I. tract was also observed.

In a feeding study by Reussner (1221), diets containing 66-77% unmodified corn, wheat, rice, tapioca, and amioca starches encouraged normal weight gains and ceca weights in rats. However, corn dextrin and pregelatinized amioca starch were not as well utilized as their unmodified counterparts. Rats fed an unmodified potato starch diet (82.6%) as the sole source of carbohydrate grew poorly and exhibited impaired protein utilization (548).

Growth rates of rats fed 73.6% carbohydrate indicated that raw wheat and corn starches were as well-utilized as sucrose, whereas potato starch was not. Jelinek (662) stated that this was accompanied by intestinal and cecal distention. Cohen et al. (273) found that starch-fed rats gained significantly more weight than rat fed an equal amount of glucose, fructose, or sucrose.

Whistler and Belfort (1630) fed weanling rats 5 g of a basal diet plus 1 or 2 g of cornstarch oxidized by 2.5 or 6% choline, hydroxyethyl corn starch, corn starch phosphate, a very highly oxidized noncommercial starch, or unmodified corn starch. The commercial modified starches produced weight gains similar to that of corn starch after 21 days; weight gain was higher in animals fed 2 g of the various starches than in those fed 1 g. The noncommercial oxidized starch appeared to reduce weight gain and caused a marked dilation of the colon.

Groups of rats were fed 1, 2, or 4 g of three oxidized corn starches in addition to 5 g basal ration for 10 days. White (1631) found no tissue damage to be associated with the diarrhea and cecal enlargements observed in the 2 or 4 g groups; liver, kidney, heart, and spleen weights were normal.

No toxic effects were observed in rats fed a diet containing 70% of an oxidized starch for 10 weeks (1680).

Dialdehyde starches with 0.05 to 100% of their glucose units oxidized were fed by Wilson (1655) to rats as 1 to 20% of the diet for up to 100 days. Animals fed starches with less than 2% of the units oxidized grew normally at all levels of intake. However, rats fed 100% oxidized dialdehyde starch suffered an early death. A 40% oxidized product led to poor growth at the 5 and 10% level and early death at 20% of the diet. Testicular atrophy occurred in rats fed the 100% oxidized starch in levels of 1% or more and in animals fed a 40% oxidized starch in levels of 5% or more.

In an unpublished report by de Groot and Spanjers (1680), the feeding of starch acetate as 50% of the diet induced slight diarrhea in male and female rats over a 7 day period, whereas rats fed 25% starch acetate sustained no ill effects. When hydroxypropyl distarch glycerol was fed at levels of 25 and 50% there was considerable diarrhea at both levels. Hair loss was pronounced at the 50% level in both sexes and slight at the 25% level. When phosphated distarch phosphate was tested, the body weights of test animals were reduced slightly in a dose related manner. Acetylated distarch phosphate was fed to rats at levels of 0, 25, or 50%, resulting in diarrhea as well as hair loss at the higher level.

For 28 days groups of 10 male rats were given diets containing 60% of different starch acetates. In groups receiving starch acetate with 2% or more acetylation, weight gain was reduced and diarrhea occurred (1680).

Feron et al. (1680) reported that male and female rats fed 5, 15 or 45% potato starch acetate for 13 weeks had significantly lowered relative weights of the liver, kidney, adrenal, and pituitary. Male cecal weights were higher than controls; distended ceca were observed at the 15 and 45% levels.

No diarrhea was observed when levels of 25 or 50% starch acetate were consumed by groups of 20 rats for 8 weeks. Cecal weight and cecal enlargement occurred in a dose-related fashion for all groups (1680).

In an unpublished report Kay and Calandra (1680) noted no systemic toxicity from the consumption of diets containing up to 25% hydroxypropyl starch by rats. Mild diarrhea was present at the 25% level, but no abnormalities were uncovered by histological examination of major tissues. In a similar study by Feron et al. (1680), the histological profile of rats fed rations containing 0, 5, 15, and 45% hydroxypropyl starch was normal, however, cecal enlargement was noted at the 45% level.

Over an 8 week period, cecal weights increased as the hydroxypropyl distarch glycerol content of the diet increased for groups of 10 male and 10 female rats, but histological examination of the cecum revealed no abnormalities (1680). Knapp (1680) provided sets of 25 male and 25 female rats with diets containing 1 and 5% hydroxypropyl/distarch glycerol or 5% native starch for 13 weeks. Growth, feed consumption, body-weight hematology, urinalysis, gross and pathological evidence were similar for all groups.

When De Knect-van Eekelen et al. (1680) fed rats 0, 5, 10, or 30% hydroxypropyl distarch glycerol for 90 days, the animals' appearance, behavior, growth, food consumption, hematology, and urinalysis proved normal. While the cecal weights increased for both sexes at the 30% level, none of the rats suffered from diarrhea.

In an unpublished study by Kohn et al. (1680) groups of 20 rats were fed a basal diet plus 10% rising to 35% phosphated distarch phosphate (prepared with trimetaphosphate) for a 60-day-period. Although male liver weights and male and female kidney weights were lower for the test group, these results could not be correlated with any gross or histopathological changes. In a second study groups of 50 rats consumed 0.2, 1.0, and 5.0% trimetaphosphate modified distarch phosphate for 90 days with no untoward effects.

Groups of 10 male and 10 female rats received 0, 5, 15 and 45% of 2 types of distarch phosphate for 90 days. Til et al. (1680) examined the animals for serum, urine, and histological abnormalities and found none.

A dose-related increase in cecal weight occurred in rats fed 0, 25, or 50% of two different types of acetylated distarch phosphate for 8 weeks; de Groot and Spanjers (1680) stated that the cecum was histologically normal. No gross or histological effects were observed from the feeding of 71% distarch glycerol to rats for 90 days (1680).

The growth of male rats fed 50% acetylated distarch glycerol for 90 days was significantly slower than that of control rats fed 50% native starch. Oser (1680) reported the other test parameters to be normal. A similar study indicated that feeding 50% acetylated distarch adipate also reduced the growth of male rats.

Porter (1680) provided groups of 10 male rats with diets containing hydroxypropyl distarch phosphates or native starch. Diarrhea was present whenever 4 g of modified starch was fed; the more highly modified starches produced diarrhea at the 2g/day level as well. Histological examination of the heart, liver, spleen, kidney, and cecum revealed no treatment-related abnormalities.

Growth was reduced when more than 51% hydroxypropyl distarch phosphate was included in the diet of rats for 28 days (1680).

A study of aortic atherosclerosis in rabbits was conducted by Moore (997). When high-starch and high-butterfat diets were compared, over a 42-week-period differences in the proportion of cholesterol, cholesterol esters, and triglycerides in the aortic lipids were detected. The degree of aortic atherosclerosis was similar for both diets. In a second study, a 41.9% starch diet was compared to maize oil, butterfat, and coconut oil diets; no untoward effects were associated with starch (998).

Rabbits fed a cholesterol-free diet with 40% glucose, sucrose, starch, or partially hydrolyzed starch were graded for aortic atheromata. The starch-fed rats exhibited more severe atheromata than other groups (769).

Groups of 4 male and 4 female beagle dogs consumed 1 or 5% hydroxypropyl distarch glycerol or 5% native starch for 13 weeks. According to Knapp (1680), hematological studies, urinalysis, growth, and the organ weights of the thyroid, heart, liver, spleen, kidneys, adrenals and testes showed no test-related changes.

No adverse effects were observed from giving groups of 5 beagles gelatin capsules containing 50, 250, and 1250 mg phosphated distarch phosphate daily for 90 days (1680).

Shillam et al. (1680) gave groups of 8 pigs 0, 35, or 70% acetylated distarch phosphate in their rations for 14.5 weeks. Although growth, food consumption, blood chemistry, urinalysis, opthalmoscopy, organ weight, and gross and histological examinations were normal, 3 pigs on the 70% level died suddenly at various intervals. One pig from each of the test diets developed evidence of some neural malfunction; only the one on the 70% level died.

Swine suffered from excessive cornification of the pars esophaga from the consumption of 48 to 90% pearl starch grits on a 0-12% crude protein diet (1604).

No adverse effects were reported by Pieters et al. (1680) when 12 human subjects ate 60 g starch acetate or hydroxypropyl distarch glycerol for 4 consecutive days. In a similar study 2 different acetylated distarch phosphates were tested; no adverse results were noted.

Excessive intake of laundry starch has been demonstrated to produce parotid enlargement in man (1367). This unusual habit has also led to the development of a starch gastrolith in one young woman (34). Adult male subjects were fed low-fat diets with 500 g raw maize starch, resulting in flatus and borborygmi. Raised serum transaminase levels suggested possible liver damage (891). Iron deficiency has also been correlated with immoderate starch consumption (1610).

Two long-term studies involved the feeding of modified starches to rats. Til et al. (1680) fed groups of 30 male and 30 female rats diets which included 0, 5, 10, and 30% of starch acetate, hydroxypropyl distarch glycerol, and two types of acetylated distarch phosphate for 2 years. In rats fed starch acetate, male cecal weights showed a dose-related increase at 10% and higher levels; female cecal weight was increased at the 30% level. Cecal weight was also increased in a dose-related manner for rats fed hydroxypropyl distarch glycerol but occurred in the females only at a 10% level. A similar trend was observed in animals fed acetylated distarch phosphate. Blood and urine tests were normal for all groups. The histological examination had not been completed.

In their unpublished study, de Knecht-van Eckelen et al. (1680) found the consumption of levels of up to 30% phosphated distarch phosphate in the diets of rats for 2 years to exert no toxic effects. However, the histological examination had not been finished.

Several special studies investigated the effects of feeding various modified starches on cariogenicity, reproduction, carcinogenicity, and sensitivity. Upon maintaining Wistar rats on diets which contained glucose, sucrose, or starch as the sole carbohydrate source for 7 generations, Shaw and Ivimey (1356) found the percentage of caries-free rats to be lower than expected for starch-fed rats. Increased neonatal mortality and retarded growth accompanied these results. Although breeders for each generation were selected on the basis of grossly observable lesions, these results were not considered to result from genetic selection.

Frostell and Baker (449) noted that pregelatinized starches produced more carious areas over a 60-day-period than did various unmodified starches in rats. However, Baer and White (90) reported that dietary pregelatinized tapioca and amioca starches and unmodified arrowroot and potato starches produced significantly less calculus in rats than pregelatinized amioca and wheat starches, and several other unmodified starches. Grenby (508) determined that wheat starch was far less cariogenic when included in the diet as 66% carbohydrate. In a similar study, these results were confirmed by Green and Hartles (503).

Offspring of 2 rats fed a 50% starch diet for 2 years were normal (1632).

A three generation study was undertaken by Til et al. (1680) in which rats were given 10% starch/acetate, hydroxypropyl distarch glycerol, phosphated distarch phosphate, and acetylated distarch phosphate. No untoward effects regarding mortality, growth, fertility, litter size, resorption quotient, or weaning weight were reported. Some of the organ weights differed significantly from those of controls, but the histological examination had not been completed.

Edwards et al. (365) found a higher percentage of stillborn babies to born to women who consumed 15 g to 2 lb of cornstarch daily than to women in the general population.

The injection of arrowroot starch granules into sarcoma 180 appeared to retard tumor growth in mice (243, 244).

While Loveless (865) noted a few cases of hypersensitivity to starches in man, unpublished reports by Majors and Ruben (669) have not confirmed these findings.

The Joint FAO/WHO Expert Committee on Food Additives has required that further studies or reports be submitted on much of the work on modified starches reviewed above. A list of such reports can be found on page 46.

FOOD STARCH, UNMODIFIED

Chemical Information

I. Nomenclature

A. Common Names

1. Starch
2. Amylum
3. Amioca (amylopectin starch from waxy corn)

B. Chemical Name

Starch is a crystalline polymeric compound consisting of about 27% linear polymer (amylose) and 73% branched polymer (amylopectin).

C. Trade Names

1. Maizena (corn starch)
2. Mondamin (corn starch)
3. OK Cerava (corn starch from waxy corn)
4. OK Corn Starch
5. Pearl Corn starch

D. Chemical Abstracts Registry Numbers

1.	Starch	977052177
2.	Starch USP	PM9005258
3.	Starch, Arrowroot	977000091
4.	Starch, Cassava	977002815
5.	Starch, Corn	977050513
6.	Starch, Corn, USP	PM9005258
7.	Starch, Manioca	977002815
8.	Starch, Potato	977000079
9.	Starch, Rice	977000080
10.	Starch, Tapioca	977002815
11.	Starch, Waxy, Maize	977050524
12.	Starch, Wheat	977052268

II. Empirical Formula

$(C_6H_{10}O_5)_n$

III. Structural Formula

Starch is a polymer in which the basic monomeric unit is D-anhydroglucose and the predominant linkage is the 1,4-alpha glucosidic bond. Amylose and amylopectin, the two basic polymers, are both composed

of anyhydroglucose units but differ in the way these units are linked and shaped. Amylose is a linear polymer in which essentially all of these units (200-2,000 units) are linked through 1,4-alpha glucosidic bonds. Each unit contains one primary and two secondary hydroxyl groups, except the terminal unit. At the non-reducing end, the anhydroglucose unit contains 3 secondary and one primary hydroxyl unit. At the reducing end, the anhydroglucose unit contains one primary and two secondary hydroxyls, plus an aldehydic reducing group (inner hemiacetal) (460).

Amylopectin has a highly branched structure. Each branch contains 15-25 anhydroglucose units connected by 1,4-alpha linkages. However, at the branch point, the branches are connected by linkages attaching carbon 1 of the anhydroglucose unit at the beginning of the branch to carbon 6. Amylopectin generally exceeds amylose in size (460).

IV. Molecular Weight

Not Applicable

V. Specifications

None Available

VI. Description

A. General Characteristics

Starch occurs naturally as minute, cold-water insoluble granules. Depending on the plant source, the granules range from 3-100 microns in diameter; the shape is also affected by plant source. The hilum is a characteristic feature in many starch granules. It appears as a tiny spot on the granule and is believed to be the nucleus about which the granule has formed. Striation marks can be detected in some starches as concentric lines around the hilum. Photomicrographs of rice, corn, sorghum, tapioca, wheat, and potato starches are presented in the Handbook of Food Additives (460).

B. Physical Properties

Amylose and amylopectin are associated in a crystal lattice so that starch is practically insoluble in cold water or alcohol. It is soluble in boiling water, yielding a colloidal solution which may form a gel on cooling (460).

The hydroxyl groups in amylose provide the hydrophilic properties of the polymer which lead to an affinity for moisture and dispersibility in water. "However, because the molecules are linear and contain hydroxyls, they have a tendency to become oriented parallel to one another and approach each other closely enough to permit association of one polymer

with another through hydrogen bonding. When this occurs, the affinity of the molecule for water is reduced and the aggregate size increased to the point where precipitation may occur at low concentrations and gelling at higher concentrations, when only partial orientation may occur because of steric factors. The gels are three dimensional networks held together by hydrogen bonding at those sections where the molecules can become closely aligned. This phenomenon of association between amylose molecules is commonly referred to as retrogradation. The tendency of corn starch dispersions to become opaque and to gel on cooling is caused by the retrogradation of amylose molecules present in corn starch. The linearity of amylose molecules contributes toward the formation of strong, flexible films" (460).

Amylose has an affinity for iodine, fatty acids, various surfactants, and other large molecules with hydrophobic and hydrophilic sections. Amylose complexes by forming a helix around these molecules; a blue color occurs when amylose complexes with iodine (460).

"The size and branched nature of amylopectin interferes with the mobility of its molecules and their tendency to become oriented closely enough to permit the extensive hydrogen bonding necessary for retrogradation to occur. As a result, aqueous sols of amylopectin and amylopectin starches are characterized by good clarity and stability, or resistance to gelling or changing with age. Because it is a highly branched molecule, amylopectin does not form films as strong and flexible as those from amylose. It also does not give a blue color complex with iodine. The stability of amylopectin sols is a major factor in the use of modified amylopectin starches; e.g., waxy corn, as thickeners and in the uses where solution stability and freedom from loss in water-holding properties are desired (460)".

Genetic control of corn has led to the development of corn starches such as waxy corn starch (95-100% amylopectin) and a waxy sorghum starch. High amylose corn starches contain 55-70% apparent amylose. These differences in amylose and amylopectin ratios exert a marked effect on starch properties. Waxy corn starch cooks readily to yield solutions characterized by excellent stability or resistance to gelling on cooling or aging. High amylose types are resistant to cooking (460).

C. Stability

Starch granules will absorb water and swell slightly when exposed to high humities.

VII. Analytical Methods

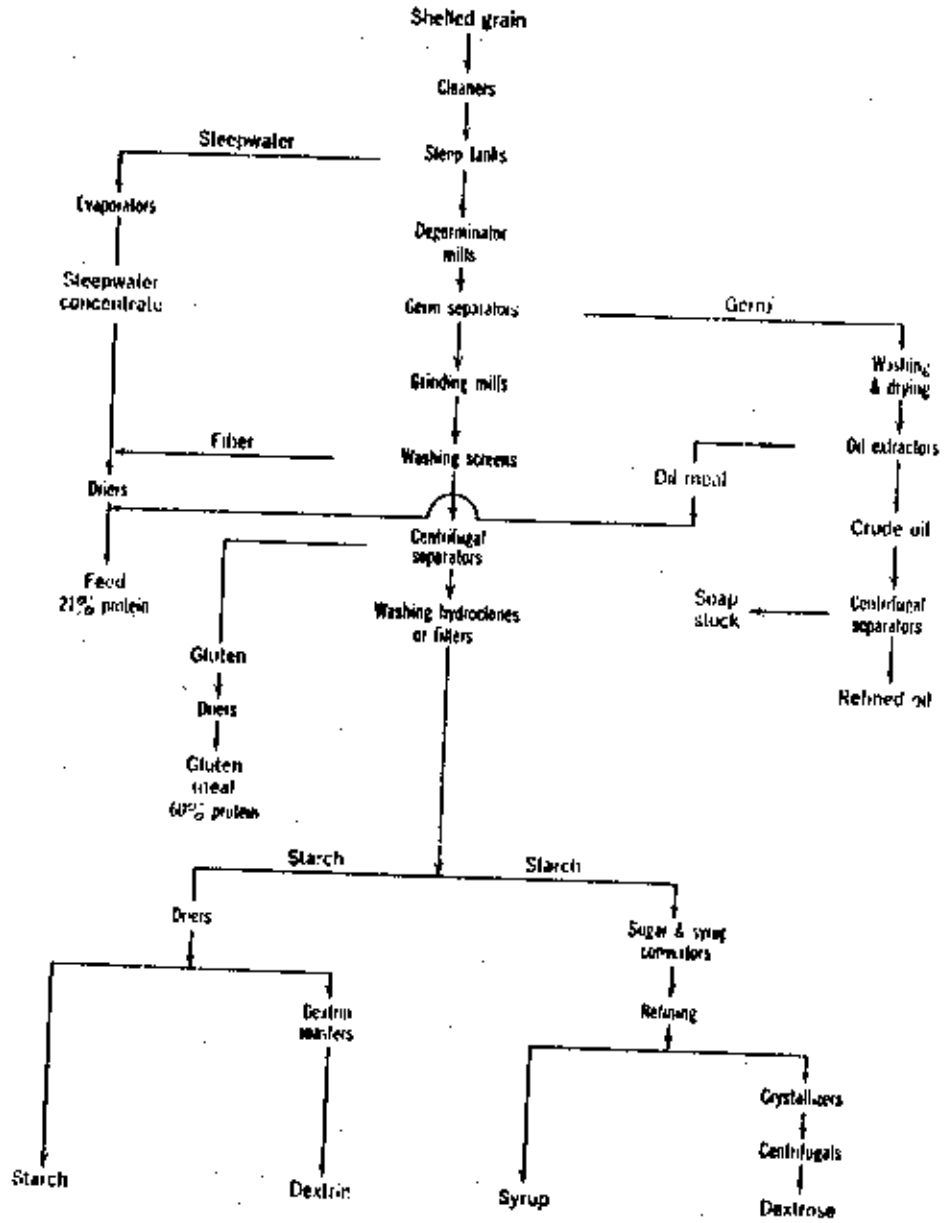
Trop and Grossman developed a method for the determination of starch in foodstuffs. Starch is first hydrolyzed to glucose. The glucose is then selectively oxidized by glucose oxidase, and the rate of oxygen absorption is measured (1519).

Libby reported a method for the analysis of corn, wheat, and potato starch by quantitatives enzymatic hydrolysis. The sample must be dissolved in dimethylsulfoxide (DMSO). Glucose produced by glucoamylose hydrolysis was measured either by an automated colorimetric technique or by a glucose oxidase technique (838).

VIII. Occurrence

Native starches are produced commercially by extraction from the seeds of plants such as corn, wheat, sorghum or rice; the tubers of plants like cassava, potato, or arrowroot; and the pith of the sago palm. The major source of commercial starch is corn (460). Starch is extracted from corn by a wet milling process as shown in figure 1 (736).

Fig. 1



FOOD STARCH, MODIFIED

Chemical Information

I. Nomenclature

A. Common Names

1. Modified food starches
2. Food starch-modified

B. Chemical Name

Modified food starches

C. Trade Name

None Available

D. Chemical Abstracts Registry Number

1.	Starch, Acid Modified	977050740
2.	Starch, Modified	977052188
3.	Starch, Pregelatinized	977050933
4.	Starch, Tapioca, Modified	977052257

II. Empirical Formula

See Occurrence

III. Structural Formula

Modified food starches are products of the treatment of any of several grain- or root-based native starches (corn, sorghum, wheat, potato, sago, etc.) with small amounts of certain chemical agents.

IV. Molecular Weight

The molecular weight of modified starch varies greatly depending on botanical origin and is generally lower than that of the native starch from which it is obtained.

V. Specifications

Food Chemicals Codex

GENERAL SPECIFICATIONS

Crude fat. Not more than 0.15 percent.

Loss on drying. *Cereal starch:* not more than 15 percent; *potato starch:* not more than 21 percent; *sago* and *tapioca starch:* not more than 18 percent.

pH of dispersions. Between 3.0 and 9.0.

Protein. Not more than 0.5 percent, except not more than 1 percent in modified high amylose starches.

Limits of Impurities

Arsenic (as As). Not more than 3 parts per million (0.0003 percent).

Heavy metals (as Pb). Not more than 40 parts per million (0.004 percent).

Lead. Not more than 5 parts per million (0.0005 percent).

Sulfur dioxide. Not more than 80 parts per million (0.008 percent).

ADDITIONAL SPECIFICATIONS. The modified food starches listed below according to method of preparation must meet all of the above *General Specifications* in addition to the specified methods of *Treatment* (the reagent for which, if not specifically limited, should not exceed the amount reasonably required to accomplish the intended modification) and any requirements for *Residuals Limitation.*

Mild oxidation (Bleached starch). The starches resulting from mild oxidation are not altered chemically; in all cases, extraneous color bodies are oxidized, solubilized, and removed by washing and filtration. These treatments may be used in combination with the other forms of treatment listed herein.

Treatment to produce bleached starch:	*Residuals limitation*
Active oxygen obtained from hydrogen peroxide, and/or peracetic acid, not to exceed 0.45% of active oxygen	—
Ammonium persulfate, not to exceed 0.075%, and sulfur dioxide, not to exceed 0.05%	—
Chlorine, as sodium hypochlorite, not to exceed 0.0082 pound (3.72 grams) of chlorine per pound (454 grams) of dry starch	—
Potassium permanganate, not to exceed 0.2%	Not more than 50 ppm (0.005%) of residual manganese (as Mn)
Sodium chlorite, not to exceed 0.5%	—

* * * * *

Moderate oxidation (Oxidized starch). The maximum specified treatment introduces about 1 carboxyl group per 28 anhydroglucose units. The starch is whitened, and its molecular weight and viscosity are reduced.

Treatment to produce oxidized starch:	*Residuals limitation*
Chlorine, as sodium hypochlorite, not to exceed 0.055 pound (25 grams) of chlorine per pound (454 grams) of dry starch	—

* * * * *

Acid depolymerization (Thin-boiling, or acid-modified starch). This treatment results in partial depolymerization, causing a reduction in viscosity. The treatment may be used in combination with the other treatments that follow.

Treatment to produce thin-boiling starch:	*Residuals limitation*
Hydrochloric acid and/or sulfuric acid	—

* * * * *

Monofunctional and/or polyfunctional esterification (starch esters). The starch esters are named individually, depending upon the method of preparation.

Treatment to produce starch acetate:	*Residuals limitation*
Acetic anhydride or vinyl acetate	Not more than 2.5% of acetyl groups introduced into finished product
Treatment to produce acetylated distarch adipate:	
Adipic anhydride, not to exceed 0.12%, and acetic anhydride	Not more than 2.5% of acetyl groups introduced into finished product
Treatment to produce starch phosphate:	
Monosodium orthophosphate	Not more than 0.4% of residual phosphate (calculated as P)
Treatment to produce starch sodium octenyl succinate:	
1-Octenyl succinic anhydride, not to exceed 3%	—
Treatment to produce starch aluminum octenyl succinate:	
1-Octenyl succinic anhydride, not to exceed 2%, and aluminum sulfate, not to exceed 2%	—
Treatment to produce distarch phosphate:	
Phosphorus oxychloride, not to exceed 0.1%	—
Sodium trimetaphosphate	Not more than 0.04% of residual phosphate (calculated as P)
Treatment to produce phosphated distarch phosphate:	
Sodium tripolyphosphate and sodium trimetaphosphate	Not more than 0.4% of residual phosphate (calculated as P)
Treatment to produce starch sodium succinate:	
Succinic anhydride, not to exceed 4%	—

* * * * *

Monofunctional and/or polyfunctional etherification (Starch ethers-hemiacetals, or others). The starch ethers are named individually, depending upon the method of preparation.

Treatment to produce distarchoxy propanol:	*Residuals limitation*
Acrolein, not to exceed 0.6%	—
Treatment to produce distarch glycerol:	
Epichlorohydrin, not to exceed 0.3%	—
Treatment to produce hydroxypropyl distarch glycerol:	
Epichlorohydrin, not to exceed 0.1%, combined with propylene oxide, not to exceed 10%	Not more than 5 ppm (0.0005%) of residual propylene chlorohydrin
Treatment to produce hydroxypropyl starch:	
Propylene oxide, not to exceed 25%	Not more than 5 ppm (0.0005%) of residual propylene chlorohydrin

* * * * *

Etherification and esterification (Starch ether-esters). The starch ether-esters are named individually, depending upon their method of preparation.

Treatment to produce acetylated distarch glycerol:	*Residuals limitation*
Acrolein, not to exceed 0.6%, and vinyl acetate, not to exceed 7.5%	Not more than 2.5% of acetyl groups introduced into finished product
Epichlorohydrin, not to exceed 0.3%, and acetic anhydride	Not more than 2.5% of acetyl groups introduced into finished product
Treatment to produce succinyl distarch glycerol:	
Epichlorohydrin, not to exceed 0.3%, and succinic anhydride, not to exceed 4%	—
Treatment to produce hydroxypropyl distarch phosphate:	
Phosphorus oxychloride, not to exceed 0.1%, and propylene oxide, not to exceed 10%	Not more than 5 ppm (0.0005%) of residual propylene chlorohydrin

* * * * *

Etherification with oxidation (Oxidized starch ethers)

Treatment to produce oxidized hydroxypropyl starch:	*Residuals limitation*
Chlorine, as sodium hypochlorite, not to exceed 0.055 pound (25 grams) of chlorine per pound (454 grams) of dry starch; active oxygen obtained from hydrogen peroxide, not to exceed 0.45%; and propylene oxide, not to exceed 25%	Not more than 5 ppm (0.0005%) of residual propylene chlorohydrin

* * * * *

Alkaline gelatinization (Gelatinized starch)

Treatment to produce gelatinized starch:	*Residuals limitation*
Sodium hydroxide, not to exceed 1%	—

B. World Health Organization

The article of commerce can by specified by the parameter specific for the particular type of modification indicated in Column 3 of Table 1, and further as to loss in weight on drying, to pH and to contents of ash, chloride, protein and fat (168I).

LIMITS OF IMPURITIES

Arsenic:	not more than 3 mg/kg
Lead:	not more than 5 mg/kg
Heavy metals:	not more than 40 mg/kg
Sulphur dioxide:	not more than 80 mg/kg

TABLE I

Column 1	Column 2	Column 3	Column 4
Treatment (may be combined)	Maximum amount of substance reasonably required to accomplish the intended physical or technical effect	Limitation and maximum residual traces in the finished product	Commercial Name
1. Roasted starch with addition of acid	0.15% acid, calculated as hydrochloric acid anhydrous (100%) and based on dry starch	Final pH 2.5 to 7.0	White or yellow dextrin, dextrinised starch
2. Acid treatment in slurry	7% hydrochloric acid, 2.0% sulphuric acid or 7% phosphoric acid	Final pH 4.8 to 7.0	Acid-thinned Starches
3. Alkaline treatment with	Sodium or potassium hydroxide not to exceed 1.0%	Final pH 5.0 to 7.5	Alkali-treated Starches
4. Bleached starch	Sodium hypochlorite, sodium chlorite, hydrogen peroxide, potassium permanganate, peracetic acid, ammonium persulphate, sulphur dioxide in amounts not more than that sufficient to bleach the material	No residues, no carboxyl groups, except 50 mg/kg Mn when permanganate used	Bleached Starches

TABLE I

Column 1	Column 2	Column 3	Column 4
Treatment (may be combined)	Maximum amount of substance reasonably required to accomplish the intended physical or technical effect	Limitation and maximum residual traces in the finished product	Commercial Name
5. Oxidized by treatment with	Chlorine, as sodium hypochlorite, not to exceed 5.5% of chlorine, based on dry starch	0.5% sodium chloride; Carboxyl groups not more than 1% corresponding to 1 carboxyl group per 25 glucose units	Oxidized Starches
6. Esterified by treatment with	Acetic anhydride, not to exceed 10% ; Vinyl acetate, not to exceed 7.5% ; Phosphorus oxychloride, not to exceed 0.1% ;	Acetyl groups, not to exceed 2.5 % ;	Starch Acetate; Acetylated Distarch phosphate ($POCl_3$); Acetylated Distarch glycerol; Acetylated Distarch Adipate.
	Sodium trimetaphosphate	residue phosphate in starch treated with sodium trimetaphosphate not to exceed 0.04% calculated as phosphorus	
	Adipic anhydride, not to exceed 0.12%		Acetylated Distarch Adipate
	Sodium tripolyphosphate and/or sodium trimetaphosphate and/or orthophosphoric acid and/or sodium or potassium salts thereof	Residual phosphate, not to exceed 0.4%, calculated as as phosphorus	Mono-Starch Phosphate; Distarch Phosphate (sodium trimetaphosphate) Phosphated Distarch Phosphate

TABLE I

Treatment (may be combined)	Maximum amount of substance reasonably required to accomplish the intended physical or technical effect	Limitation and maximum residual traces in the finished product	Commercial Name
7. Etherified by treatment with	Propylene oxide, not to exceed 10%	Propylene oxide, 5 mg/kg, Propylene chlorohydrin, 5 mg/kg.	Hydroxypropyl Starch; Hydroxypropyl Distarch Phosphate* ($POCl_3$ or sodium trimetaphosphate)
	Succinic anhydride, not to exceed 4%		Starch sodium succinate
	Phosphorus oxychloride, not to exceed 0.1%	Residual phosphate not to exceed 0.04% calculated as phosphorus	Distarch phosphate (phosphorus oxychloride)
	Epichlorohydrin, not to exceed 0.3%	Epichlorohydrin, 5 mg/kg Glycerol monochlorohydrin, 5 mg/kg Glycerol dichlorohydrin 5 mg/kg	Distarch glycerol; Acetylated Distarch glycerol; Hydroxypropyl Distarch glycerol

Identification tests for the various treatments are given in the Annex

* Tentative

VI. Description

A. General Characteristics

Modified food starches are usually produced as white or nearly white, tasteless, odorless powders, as intact granules, and, if pregelatinized (subjected to heat in the presence of water), as flakes, amorphous powders, or coarse particles.

B. Physical Properties

Modified food starches are insoluble in alcohol, ether, and in chloroform. If not pregelatinized, they are practically insoluble in cold water. Upon heating in water, the granules begin to swell at 45-80 degrees, depending on botanical origin and degree of modification. They gelatinize completely at higher temperatures. Pregelatinized starches hydrate in cold water. A more detailed account of physical properties may be found in the Handbook of Food Additives (460).

C. Stability

None Available

VII. Analytical Methods

Hypochlorite-oxidized starch can be distinguished from native and acid modified starch of the same botanical origin. Because of the anionic properties of hypochlorite-oxidized starch, the starch can be dyed with cationic dyes such as methylene blue. Microscopic inspection will reveal the blue coloring (1681).

Saponification of acetylated starch liberates acetate which is converted to acetone by heating with calcium hydroxide. The acetone produced stains blue with o-nitro-benzaldehyde (1681).

Ester groups present in modified starch are detected through the presence of an absorption band at 1720 cm-1 in the infrared spectrum (1681).

Phosphorus in modified and unmodified corn starches can be determined quantitatively. The sample is ignited in the presence of an added electrolyte in order to convert phosphorous to a stable form. The residual phosphate is taken up in acid and determined spectrophotometrically as the molybdivanadophosphoric acid. Phosphorus may be measured by a second method which applies to both modified and unmodified starches. The sample is digested with a mixture of nitric and sulfuric acids. Phosphorus content is determined spectrophotometrically by the metol method (1681).

VIII. Occurrence

Modified food starches are products of the treatment of any of several

grain- or root-based native starches.

"Starch is chemically modified by mild degradation reactions or by reactions between the hydroxyl groups of the native starch and the reactant selected. One or more of the following processes are used: mild oxidation (bleaching), moderate oxidation, acid depolymerization, monofunctional esterification, polyfunctional esterification (cross-linking), monofunctional etherification, polyfunctional etherification (cross-linking), alkaline gelatinization, and certain combinations of these treatments. These methods of preparation can be used as a basis for classifying the starches thus produced. Generally, however, the products are called modified food starches, or food starch-modified (427)."

The treatments involved in the manufacture of various modified starches are outlined below (669).

Acetylated distarch adipate - Modification is carried out by the use of five per cent acetic anhydride and a maximum of 0.12 per cent adipic acid, the latter acting as cross-linking agent. The maximum number of acetyl groups introduced is 2.5 per cent. The number of adipic cross-links does not exceed more than one in about 1000 anhydroglucose units, or not more than 0.09 per cent adipyl groups introduced on the starch.

Acetylated distarch glycerol - Modification is carried out with epichlorhydrin to a maximum of 0.3 per cent and acetic anhydride to a maximum of five percent. The amount of acetyl groups introduced does not exceed 2.5 per cent. Further treatment, such as bleaching, is often carried out.

Acetylated distarch phosphate - Modification is usually performed by the use of up to 0.1 per cent of phosphorus oxychloride and five per cent acetic anhydride. Vinyl acetate may be used as an alternative acetylating agent. Maximum acetylation amounts usually to 2.5 per cent acetyl groups.

Alkali-treated starches - Modification is carried out by treatment with a maximum of 1 per cent alkali hydroxide. No chemical transformation except depolymerization seems to occur. If any amylose appears in crystalline form it may not be accessible to enzymic degradation.

Distarch glycerol - Treatment with epichlorhydrin under alkaline conditions up to 0.3 per cent tends to produce cross-linking at a rate of one glycerol diether linkage per 220 anhydroglucose units.

Distarch phosphate (prepared using phosphorus oxychloride) - Cross-linking of starch chains may be produced also by the use of phosphorus oxychloride. Although theoretically likely, there is no relevant chemical evidence available to show that modification by phosphorus oxychloride in fact produces distarch phosphate. The maximum number of phosphate bridges could reach one per 100 glucopyranose units.

Distarch phosphate (prepared using sodium trimetaphosphate) - Native starches are known to contain phosphoric acid esters equivalent to 0.004 per cent P and some potato starches up to 0.1 per cent. Distarch phosphate is made by the use of sodium trimetaphosphate which cross-links starch chains at an approximate rate of one phosphate link per 620 glucopyranose units. The amounts of phosphate introduced are at the most 0.04 per cent.

Hydroxypropyl distarch glycerol - Modification is performed by the use of 0.1 per cent epichlorhydrin and 10 per cent propylene oxide. Cross-linkage would be no greater than expected on modification with 0.1 per cent epichlorhydrin. Substitution by treatment with 10 per cent propylene oxide would probably add no more than 15-20 ether linkages per 100 glucopyranose units.

Hydroxypropyl distarch phosphate - Modification is carried out with 0.1 per cent phosphorus oxychloride and 8-10 per cent of propylene oxide. Cross-linkage would be no greater than is experienced on modification with phosphorus oxychloride alone and ether linkages would probably not exceed 20 per 100 anhydroglucose units.

Hydroxypropyl starch - Modification is carried out by propylene oxide at levels up to 25 per cent and the resultant starch is usually lightly oxidized, bleached or acid modified after etherification. Substitution may amount to a maximum of 4 ether linkages per 10 glucopyranose units if 25 per cent propylene oxide is used and 4-6 ether linkages per 100 glucopyranose units if 5 per cent propylene oxide is used.

Oxidized starches - Whatever oxidizing agent is used only minor modifications of the starch molecule are achieved in normal manufacturing practice. These are equivalent to the introduction of 1 per cent w/w of carboxyl (-COOH) or 0.5 per cent w/w of keto (-CO) groups or, 3.6 carboxyl and 2.9 carbonyl groups per 100 glucopyranose units. Some depolymerization takes place by rupture of the bonds between glucose units. No chlorine is introduced into the molecule and the final products usually contain only residues of sodium chloride, sodium sulfate and sulfur dioxide.

Phosphated distarch phosphate - These modified starches are prepared by the combined use of sodium tripolyphosphate and sodium trimetaphosphate which results in cross-linking and esterification of starch chains. The overall extent of modification is still small, the residual phosphate being of the order of 0.4 per cent P.

Starch Acetate - Esterification is achieved by using either acetic anhydride (up to 8 per cent) or vinyl acetate (up to 7.5 per cent). A maximum of 2.5 per cent acetyl groups is introduced corresponding to a maximum degree of substitution of 0.1.

White and yellow dextrins - White dextrins are prepared by heating dry starch in the presence of an acid at a temperature generally below 150 degrees C. White dextrins may also be obtained by further continuing the acid process for making thin boiling starches. Because of the nature of the application, as well as their flavour, their use in food is restricted. Dextrins are a stage in the normal digestion of starch occurring in the human gastro-intestinal tract. They represent a broad range of products with considerably smaller molecular size than native starch.

Yellow dextrins are prepared in a similar manner but at a higher temperature and using less acid. Apart from depolymerization, a good deal of internal re-arrangement occurs with formation of highly branched molecules. These materials are used in foods in limited quantities as adjuvants in flavor encapsulation and similar minor uses.

Biological Data

I. Acute Toxicity

Substance	Animal	Sex & No.	Route	Dosage (mg/kg Body Weight)	Measurement	Ref.
Distarch Phosphate	Mice	Female	Oral	> 24,000	LD_{50}	669
Distarch Phosphate	Mice	Female	Oral	> 19,000	LD_{50}	669
Distarch Phosphate	Rats	Female	Oral	>20,000	LD_{50}	669
Distarch Phosphate	Rats	Female	Oral	>35,000	LD_{50}	669
Distarch Phosphate	Guinea Pigs	--	Oral	>8,800	LD_{50}	669
Distarch Phosphate	Guinea Pigs	--	Oral	>18,000	LD_{50}	669
Distarch Phosphate	Rabbits	--	Oral	>7,000	LD_{50}	669
Distarch Phosphate	Rabbits	--	Oral	>10,000	LD_{50}	669
Distarch Phosphate	Cats	--	Oral	>6,800	LD_{50}	669
Distarch Phosphate	Cats	--	Oral	>9,000	LD_{50}	669
Hydroxypropyl Starch	Rats	--	Oral	>10,000	LD_{50}	669

Wilson tested DAS-100, a cornstarch in which 100% of the glucose units are oxidized to the dialdehyde, for acute toxicity in rats. Seven young adult rats were given this material at 1 g/kg. One male died after 11 days with multiple lung abscesses, presumably not due to DAS-100; the others remained healthy for the 2-week observation period (1655).

II. Short-Term Studies

A. Unmodified Starches

Chicks

Blamberg provided 5-day-old male chicks with rations containing approximately 54% cornstarch from five different manufacturers for 20 days. Quadruplicate groups of 8 chicks were alloted to each treatment and fed ad libitum; weight gain and caloric efficiency were determined. Results were similar for all five sources, and no untoward effects were reported (156).

Mice

Zuckerman and MacDonald found that excessive dietary carbohydrate led to increased hepatic fibrosis in mice. Male albino mice weighing 15 g were fed a 12% casein diet plus 81% sucrose or starch; others consumed a normal diet. After 6 weeks on each diet, the groups of 3 mice were either switched to one of the other diets or maintained on the original diet for a total of 17-24 weeks. After sacrifice, the amounts of collagen and reticulin present in the liver was determined. The collagen response was greater in animals fed sucrose than in those fed starch, but all three diets led to a similar reticulin response. The liver lipid of all test groups was higher than for the control group (1731).

Upon feeding 61 and 57 weanling Princeton mice a basal diet containing either 70% raw cornstarch or glucose, respectively, for 4 weeks, Seronde determined that cornstarch-fed animals had fewer lesions of the duodenal mucosa. This lesion, avillous hyperplasia, is a focal thickening, composed of tubules derived from the crypts of Lieberkuehn; the villi are absent (1350).

Rats

Liu and Boyd gave male albino rats a 60% starch suspension in distilled water by intragastric cannula in increasing doses of from 36 to 456 g starch/kg/day. Each dose was given for 14-49 days to 12-40 rats with controls given (a) the same volume (60-760 ml/kg/day) of distilled water and (b) cannula insertion only. Autopsy at 14 days revealed a marked, dose-dependent, increase in weight of the stomach and intestines. Control rats given distilled water suffered an enlargement of the cardiac stomach. The pyloric stomach was decreased in weight and there was a stress reaction in the adrenal and thymus glands and the spleen. Histologically there were minor degenerative changes in the liver and minor capillary-venous congestion of several organs, due solely to the large amount of water administered (853).

Doses of 36, 72, 120, and 168 g of starch per kg body weight were administered by stomach tube to male albino CBL rats weighing approximately 140 g daily for 2 weeks. In animals which did not succomb to gastric rupture, doses were gradually increased to 204, 240, and 288 g/kg; doses were divided into 4 equal portions and administered at 2.5 hour intervals.

The starch was prepared by digesting potato starch and treating it so as to yield a soluble starch of low amylose content, and made up to a 60% solution or paste. Experimental groups consisted of 12-40 rats, thereby insuring 10 survivors per group at 14 days. Volume controls were given the same volumes of distilled water per kg (60-480 ml/kg), using 10 animals per level. A second set of 10 controls received only gastric tube insertions, 4 times per day. A third group received no treatment. Weight gain, food and water consumption, colonic temperature, urinary volume, glucose, and protein were monitored. Weights and water levels of the cardiac and pyloric stomachs, small bowel, cecum, colon, adrenals, brain, heart, kidneys, liver, lungs, muscle (ventral abdominal wall), skin, spleen, submaxillary salivary glands, testicles, thymus gland, and residual carcass were determined after 2 weeks (194).

Increasing daily doses of starch produced increasing growth inhibition and reduced food consumption; this was attributed partially to the large volume of water given. Starch caused irritability, drowsiness, pallor, hyporeflexia, epistaxis, and abdominal bloating on the first 2 days of administration; symptoms were associated with gastric distention. Gastric rupture appeared in 50-75% of the animals given 168 g starch/kg or more. Five percent of the survivors of gastric rupture died from pneumonia and 20% from bowel obstruction during the two week period. Autopsy indicated that rats adapted to administration of large volumes of starch paste by enlargement of the gastrointestinal tissues. Water was absorbed from the paste in the stomach and upper bowel, and the starch converted to a calculus. Hypertrophy of the smooth muscle of the G.I. tract followed. This was thought to result from the extra work required to pass the calculi. Doses of one-tenth body weight and above produced some growth inhibition; doses of one-fifth body weight and above increased susceptibility to pneumonia and bowel obstruction. Boyd stated that it was unlikely that enough starch could by readily taken by man to be toxic (194).

Groups of 10 male Wistar rats weighing 50-55 g were fed diets containing unmodified corn, potato, wheat, arrowroot, rice, or tapioca starch: pregelatinized potato, arrowroot, tapioca, or amioca starch: or corn dextrin, potato amylose, or potato amylopectin for 28 days. Each starch was fed in conjunction with 6 and 15% casein; most were also given with 6 and 15% wheat gluten. At 6, 9, and 15% dietary protein, the diets contained 77, 73, and 66% carbohydrate. Unmodified potato starch and potato amylose resulted in increases in body weight gains and enlarged ceca when either 6% casein or 9 and 15% wheat gluten was fed (1221).

"These 2 sources of carbohydrate gave relatively poor weight gains and protein efficiency values when the casein level of the diet was increased to 15% protein. Unmodified corn, wheat, rice, tapioca, and amioca starches, as well as maltose, corn amylopectin, and potato amylopectin gave normal body weight gain values, protein efficiency ratios and ceca weights at both the high and low dietary protein levels. Unmodified arrowroot starch gave inferior body weight gain and protein efficiency value. The prevalance of diarrhea and an enlargement of the ceca gave evidence that commercial samples of corn dextrin and pregelatinized amioca starch were not as well utilized as unmodified corn starch or unmodified amioca starch (1221)."

In a study by Harper at al., rats fed an unmodified potato starch diet as the sole source of carbohydrate grew poorly and demonstrated impaired digestion of casein. Groups of 5-6 male weanling Wistar rats were given diets containing 18% casein plus either 82.6% unmodified potato starch, autoclaved potato starch, sucrose, glucose or dextrin as the carbohydrate source for 4-6 weeks. All groups grew similarly except for the unmodified potato starch group which gained substantially less weight. Bulky, white feces were excreted and found to consist largely of undigested starch granules. Raw potato starch produced a significant loss of dietary protein, whereas dietary protein was almost completely digested and absorbed when either sucrose or autoclaved potato starch was consumed. Fecal excretion of lysine, valine, histidine, and methionine was much higher for diets supplying either form of potato starch than on those containing sucrose or glucose (548).

Groups of 5 weanling male Wistar rats were fed diets containing 73.6% carbohydrate. Carbohydrates tested were potato starch (raw), potato starch (ground), wheat starch, corn starch, and sucrose. Growth rates indicated that raw wheat and corn starches were as well utilized as sucrose, whereas potato starch was poorly utilized. Ingestion of unmodified potato starch caused distention of the cecum and intestines; accumulation of food and the consequent production of gas by fermentation were thought responsible (662).

For 40 days groups of 4 male albino rats were fed diets containing 79% glucose, fructose, sucrose, or starch with 11% casein. Food consumption was restricted to the amount eaten by the rat with the smallest intake. Starch-fed rats gained significantly more weight than the other animals (273).

Two groups of 12 male 6-month-old New Zealand white rabbits were fed either a high-butterfat diet or a high-starch diet (wheat starch 48.1%). Experimental diets gradually replaced a commercial diet over a 4-week period and were fed for 38 weeks. After sacrifice, the aorta was removed. The degree of aortic atherosis was the same for both diets; lesions were confined to the intima. Extensive amounts of lipids were deposited between the internal elastic membrane and the endothelial surface. Examination of lipids from the aortic lesions revealed that the lesion cholesterol esters contained higher concentrations of oleic acid and lower concentrations of linoleic acid than did the plasma cholesterol esters. In rabbits fed the high-butterfat diet, the proportion of cholesterol and cholesterol esters were higher and the proportion of triglycerides lower than in the aortic lipids of rabbits fed the high-starch diet (997).

Eight male New Zealand White rabbits (6 months old) were fed a diet containing 41.9% starch for 9 months. Other groups were fed high levels of maize oil, butter, butterfat, or hydrogenated coconut oil. Six of the eight rabbits survived; no untoward effects were reported (998).

Male Dutch-belted rabbits were fed semi-synthetic, cholesterol-free diets containing 40% glucose, sucrose, starch, or partially hydrolyzed starch for a period of 33 weeks. A fifth group of rabbits were fed the

common atherogenic regimen of rabbit chow, augmented with 2% cholesterol and 6% corn oil. Rabbits were sacrificed and the livers and aortas removed. Data was included on 6 normal rabbits who were sacrificed at the same time, but who had not been maintained in the author's colony for the duration of the experiment. Aortic atheromata were graded visually. None of the carbohydrate-fed rabbits gained the weight expected over an 8-month period. Animals fed starch exhibited more severe atheromata than animals fed the other carbohydrates. Liver cholesterol levels were elevated in all 4 carbohydrate groups while aortic cholesterol levels were high in every experimental group (769).

Swine

Four groups of 7-8 weanling pigs were fed diets containing 0, 5, 10, and 16% crude protein plus approximately 90, 77, 64, and 48% pearl starch grits, respectively, for 80 days. When the pigs on the 16% protein diet reached 45 kg, they were switched to a 12% protein plus 59% pearl starch grits ration. Pearl starch grits was described as a granular, raw cornstarch product containing 7 to 10% gelatinized cornstarch. After sacrifice stomachs were examined histologically for ulcers. Results are tabulated below:

	% dietary protein			
	0	5	10	16-12
No. Pigs	7	8	8	8
Total Ulcers	6	8	6	1
Deaths from ulcers	4	4	I	1
Excess cornification	4	8	8	8
Ulcers in survivors	2	4	5	0

Excessive cornification of the pars esophaga occurred in 28 pigs' stomachs. In a second study raw cornstarch and partially heated (gelatinized) cornstarch were equally ulcerogenic. Wallin et al. stated that "although a 10% protein ration is inadequate for young swine, it is unlikely that protein deficiency alone accounted for the ulcers observed in these experiments (1604)."

Man

Silverman et al. presented a case history of a 29-year-old Negro woman with bilateral, nontender parotid gland enlargement and a history of starch ingestion (half pound daily). Swelling was thought to be due to parotid gland work hypertrophy due to chronic starch ingestion (1367).

Non-inflammatory parotid enlargement was reported in a 36-year-old Negro housewife who had consumed a full box of laundry starch daily for one year. Her diet was supplemented only by an occasional sandwich or piece of meat. Eight months prior to admission, painless bilateral swelling appeared at the angles of the jaw, accompanied by excessive salivation. The patient showed no symptoms of malnutrition except for a possible iron-deficiency anemia. It was suggested that a daily diet of laundry starch could have initiated considerable stimulus to the parotid glands, with enlargement on the basis of "work hypertrophy (962)."

A starch gastrolith resulted from excessive consumption of laundry starch in an 18-year-old female (34).

Seven adult male subjects were fed low-fat diets containing 500 g raw maize starch for a 25 day period. Flatus and borborygmi were reported by the subjects. Serum lipids fell; raised serum transaminase on the starch diet suggested possible liver damage (891).

In a study by Warshauer, consumption of Argo Gloss Starch, a cornstarch used for laundry, resulted in severe hypochromic anemia due to iron deficiency (1610).

B. Modified Starches

I. Oxidized Starches

Rats

Male and female weanling rats were fed 5 g of a balanced diet daily plus 1 or 2 g of the following starches: corn starch oxidized by 2.5 or 6% chlorine, hydroxyethyl corn starch, corn starch phosphate (0.5-0.9 degree of substitution), or corn starch. One group was given a very highly oxidized noncommercial starch. The diets were arranged in a randomized incomplete block design; each was replicated 4 times. Equal numbers of male and female weanling rats were grouped (3 litter mates per block, 3 blocks per replication) and fed 5.0 g of the basal diet for a week. They were then transferred to their appointed experimental rations. Weights were taken after 3, 7, 14, and 21 days of supplementation. All commercial starches produced weight gain similar to corn starch. Growth was significantly higher in rats supplemented with 2 g of these starches than in animals given 1 g. Animals fed the noncommercial oxidized starch grew poorly and had a marked dilation of the colon. The other rats appeared normal (1630).

Three groups of 3 rats were given corn starch oxidized with 3.9, 4.5, or 5.5% hypochlorite calculated as chlorine. "To 5 g basal diet were added 1, 2 or 4 g modified or control starch and this diet was fed to rats for 10 days. Comparison of digestibilities showed an apparent decrease with increasing oxidation but no effect on caloric values. No tissue damage was associated with the diarrhoea and caecal enlargements observed in groups receiving 2 g or 4 g starch in their feed. Liver, kidney, heart and spleen weights were normal (1631)."

Oxidized starch "treated at a level of 0.375 per cent chlorine was fed to weanling albino rats at 70 per cent of their diet for 10 weeks with corn starch as control. Feeding was either unrestricted or by paired-feeding technique. No toxic effects were noted. No details of this work, carried out in 1944-5, were available (1680)."

Wilson examined the effects of feeding oxidized cornstarches to young rats. Four dialdehyde starches with 0.05, 2, 40, or 100% of the glucose units oxidized (DAS-0.5, -2, -40, and -100) and a dicarboxyl starch with 5% of the glucose units oxidized (DCS-5) were fed to groups of 4-6 thirty-day-old male rats for 90-100 days. Products were added to the diet in concentrations of 1 to 20% for the DAS substances and 0.2 to 20% for DCS-5. The basal diet contained 51.5% corn meal and 20% regular cornstarch. In the DAS-0.05 test, female rats were used; the study of rats given 20% DAS-0.05 was terminated at 41 days because of an inadequate supply of the diet (1655).

"Continued feeding of diets containing 10 and 20% of DAS-100 led to early death. Concentrations of 5 and 2%, and probably 1%, inhibited growth. DAS-40 permitted normal growth at dietary level of 1%, inhibited growth at 5%, very poor growth at 10%, and early death at 20%. Feeding of DAS-2 and -0.05 permitted normal growth at all levels including 20%. Low food intake accompanied poor growth; in no case was excessive food intake noted. Growth and appearance were normal with DCS-5, but food intake seemed excessive (1655)."

"No gross abnormalities which could be attributed to the diets, were seen at autopsy. Histopathological examination of tissues showed testicular atrophy in rats receiving DAS-100 and -40. In spite of finding testicular atrophy in one control, it appears very probable that DAS-100 produces this atrophy in sexually mature rats in concentrations of 1% or more of the diet. There was an unusual multinucleated cell formation within testicular tubules. Diets containing DAS-40 produced various degrees of testicular atrophy in sexually mature rats in concentrations of 5% or more. Formation of multinucleated cells was not as conspicuous in this group. No significant microscopic abnormalities were detected in any of the DCS-5 animals (1655)."

"No toxicity occurred when 2% or less of the units were oxidized, even at levels of 20% of diet. Starch further oxidized to contain 5% of glucose units in the dicarboxyl form, produced no toxic symptoms with as much as 20% of diet consisting of this modified starch, but food intake was increased and utilization decreased, perhaps due to faulty digestion of the dicarboxyl starch (1655)."

2. Starch Acetate

Rats

"Groups of 10 male and 10 female rats were given 25 per cent and 50 per cent of starch acetate (acetylated to 1.98 per cent) in a low residue diet for 7 days. Thereafter 4 per cent cellulose was added for a further

3 days. Body-weights were slightly reduced in both sexes at the 50 per cent level after 7 days. Faecal dry matter was increased in all test groups but not in a dose-related manner. Slight diarrhea occurred only at the 50 per cent level in both sexes and this was unaffected by the feeding of additional cellulose in the diet." No loss of hair was noted by de Groot and Spanjers (unpublished report) (1680).

"Groups of 10 male rats were fed for 28 days diets containing 60 percent of various starch acetates (the degree of acetylation varied from 0, 1.24 per cent, 2 per cent, 2.56 per cent to 3.25 per cent). Weight gain was reduced in groups receiving starch acetates with more than 2 per cent acetylation but feed efficiency remained unaffected. Diarrhea occurred at 2 per cent and higher degrees of acetylation and there was noticeable caecal enlargement at the same levels. No tissue damage or inflammation were noted in association with the diarrhea (669)."

In an unpublished report by Feron et al. "potato starch acetates (acetylated to 1.36 per cent) were fed for 13 weeks to groups of 10 male and 10 female rats at levels of 5 per cent, 15 per cent and 45 per cent of the diet. The 5 per cent level was fed for only 4 weeks. No animal died. Growth rates and haematological findings were not significantly affected. The relative weights of liver, kidney, adrenal, pituitary and thyroid showed some significant differences compared with controls, being generally lower except for male thyroids. Male caecal weights were higher than controls and distended caeca were seen at the 15 per cent and 45 per cent dietary levels. No other histopathological changes due to starch acetate were seen (669)."

In a further experiment by de Groot and Spanjers (unpublished report), "starch acetate (acetylated to 1.98 per cent) was fed to groups of 10 male and 10 female rats for 8 weeks at dietary levels of 25 per cent and 50 per cent. No effects were noted on growth and body-weight. Water content of faeces and faecal production, as measured by dry matter content, showed no consistent effects but there was a tendency towards increased faecal dry matter at the 50 per cent dietary level in both sexes. No diarrhea was observed at any dietary level. Caecal weight and caecal enlargement occurred in a dose-related manner in all treatment groups. However, histological examination revealed no abnormality of the caeca examined (669)."

The Joint FAO/WHO Expert Committee on Food Additives stated that 2500 mg starch acetate per kg body weight in the diet caused no toxicological effects in the rat (669).

Man

"Twelve volunteers consumed on each of 4 consecutive days 60 g starch acetate with 1.98 per cent acetyl content. No effect was noted on frequency and amount of faeces, faecal water or lactic acid content. No other adverse effects were noted by Pieters et al. (unpublished report) (669)."

3. Hydroxypropyl Starch

Rats

"Groups of 10 male and 10 female rats were fed for 90 days diets containing 0, 2, 5, 10 and 25 per cent of hydroxypropyl starch (25 per cent propylene oxide) and 25 per cent unmodified starch. No systemic toxicity was noted. There were no adverse effects regarding mortality, urinalysis or haematology at any level. There was slight reduction in growth rate at the highest dietary level with lower food utilization and without an equivalent increase in food consumption. Mild diarrhea occurred at 25 per cent dietary level. No adverse effects occurred at any other level. At autopsy there were no significant differences in the organ weights of liver, kidney, spleen, gonad, heart or brain. The observed increased ratios at 25 per cent dietary level for liver/body-weight and kidney/body-weight were due to the relatively lower body-weight."

Kay and Calandra further noted in their unpublished report that "gross and histological examination of all major tissues revealed no abnormalities due to the feeding of highly modified starch (1680).

Feron et al. (unpublished report) provided groups of 10 male and 10 female rats with diets "containing 0, 5, 15 and 45 per cent of low modified starch (5 per cent propylene oxide). Haematological findings at 12 weeks were comparable for all groups. Body-weights did not differ significantly from controls but were consistently lower in male rats only. Feed efficiency was similar in all groups. Caecal enlargement was seen at the 45 per cent and very slightly at the 15 per cent level. No histological abnormalities were detected in any major organs, which were due to the test substance. The enlarged caeca showed no evidence of inflammation or changes in the muscular coat (1680)."

4. Hydroxypropyl Distarch Glycerol

Rats

Groups of 10 male and 10 female rats were fed for 8 weeks on a diet containing 25 or 50 per cent modified starch. Although the body-weights of males at the higher level tested were slightly lower than those of the controls, the differences were not statistically significant. The faecal dry matter content was increased at both test levels in both sexes and there was considerable diarrhea at the 50 per cent level in both sexes. Slight diarrhea occurred at the 25 per cent level in both sexes. Caecal weights were increased in a dose-related manner at all levels in all test groups but no histological abnormalities could be detected in the caeca by de Groot and Spanjers (unpublished report) (1680).

Groups of 25 male and 25 female rats were fed for 13 weeks on a diet containing 1 and 5 per cent of modified starch or 5 per cent of native starch. The general appearance and behavior of test rats was comparable with the controls. One male and one female rat died at the

lower test level. Growth, feed consumption, body-weight, haematology at one and three months, urinalysis at one and three months, gross and histopathological findings were comparable in all groups according to Knapp's unpublished report (1680).

De Knect-van Eekelen et al. (unpublished report) gave groups of 10 male and 10 female rats diets containing 0, 5, 10, or 30% hydroxypropyl distarch glycerol for 90 days. "No differences between test and control animals were noted with regard to appearance, behavior, growth, food consumption, haematology, serum chemistry urinalysis. No definite diarrhea occurred at any test level. The amounts of dry matter in the faeces did not vary significantly between controls and treated animals. Only at the 30 per cent level were the caecal weights increased in both sexes. Gross and histopathology were otherwise unremarkable in all groups. Histopathology of the enlarged caeca showed no recognizable abnormality (1680)."

"Groups of 10 male and 10 female rats were fed for 7 days on a low-residue diet containing 25 or 50 per cent modified starch. Thereafter, they were fed an additional 4 per cent cellulose. Body-weight was slightly reduced at the 50 per cent test level in both sexes and faecal dry matter was increased in all groups compared with controls. There was considerable diarrhea in all test groups which was unaffected by the additional dietary cellulose. Hair loss was pronounced at the 50 per cent test level in both sexes and was slight at 25 per cent (1680)."

Dogs

"Three groups of 4 male and 4 female beagle dogs were given" by Knapp (unpublished report) "in their diet 1 per cent and 5 per cent of modified starch or 5 per cent of native starch for 13 weeks. Haematological studies, blood sugar, blood urea, serum GPT and alkaline phosphatase as well as urinalysis were comparable for all groups. Appearance and behaviour were normal. Body-weight changes were unrelated to the administration of the test substance. Gross and histopathology showed no consistent abnormalities. Organ weights of thyroid, heart, liver, spleen, kidneys, adrenals and testes showed no test-related changes (1680)."

Man

In an unpublished study by Pieters et al., "twelve volunteers consumed 60 g modified starch on each of 4 successive days. No adverse effects were noticed and no abnormalities occurred as regards frequency and amount of faeces or faecal water and lactic acid content (1680)."

5. Distarch Phosphate

Rats

In an unpublished study by Kohn et al., "groups of 10 male and 10 female rats were fed on a diet containing 10 per cent rising to 35 per

cent of phosphated distarch phosphate (trimetaphosphate) for a total of 60 days. Female rats showed a consistent reduced rate of weight gain throughout the test. Although 4 test and 2 control animals died during the test these incidents were regarded as unrelated to the test substance. All animals behaved normally. Haematological examination and urinalysis were normal and comparable in the various groups. The liver weights of male rats were lower for the test group than for controls and the kidney weights were lower for both sexes but these findings were not associated with any gross or histopathological changes (1680)."

"Groups of 25 male and 25 female rats were fed diets containing 0.2, 1.0 and 5.0 per cent trimetaphosphate modified or unmodified starch for 90 days. Eleven controls and 3 test animals died from intercurrent disease. There were no obvious gross or histopathological changes attributable to the test substance. Organ weights and haematological examination (days 45 and 90) were normal in both groups. Pooled urinalysis was comparable for all groups (1680)."

"Groups of 10 male and 10 female rats received 0, 5, 15, and 45 per cent of two types of distarch phosphate (0.085 per cent esterified and 0.128 per cent esterified phosphate) in their diet for 90 days. No abnormalities compared with controls were seen as regards general appearance, behavior, mortality, food consumption, haematology, serum chemistry and urinalysis which could be ascribed to the action of either of the test substances. No diarrhea or increased caecal weights were observed. Gross and histopathology revealed no abnormalities attributable to the test substances (1680)."

6. Phosphated Distarch Phosphate

Rats

"Groups of 10 male and 10 female rats were given 0, 25 or 50 per cent modified starch in a low residue diet for 7 days. This was followed by 3 further days on a diet containing additional 4 per cent cellulose. The body-weights of test animals were slightly reduced in both sexes in a dose-related manner but the actual changes were small. No diarrhea was noted and faecal dry matter was somewhat higher in test animals compared with controls. The addition of cellulose to the diet had no adverse effect. No histological abnormality of the enlarged caeca was noted (1680)."

"Groups of 10 male and 10 female rats received in their diet 0, 25 and 50 per cent of modified starch (0.3 per cent P) for 8 weeks. There were no detectable adverse effects on body-weight. Faecal water content appeared to be higher in animals fed the 50 per cent starch level but the results were too variable to allow any definite conclusions. Production of faeces appeared to be unaffected by this modification when compared with controls. No diarrhoea occurred at any test level. Caecal weight was only slightly increased at the 25 per cent level in male rats but there was no consistent effect on females at any level tested (1680)."

Dog

"Groups of 3 male and 3 female beagles were given daily for 90 days gelatine capsules containing 50, 250 and 1250 mg modified starch/kg body weight. No adverse effects were observed as judged by behaviour, body-weight changes, mortality, haematological studies, blood chemistry, urinalysis, liver function tests, organ weights, gross and histopathological findings (1680)."

Man

"Twelve volunteers consumed on each of 4 successive days 60 g of a phosphated distarch phosphate with 0.35 per cent introduced P. No adverse effects were noticed. No changes occurred as regards frequency and amount of faeces or faecal water and lactic acid content (1680)."

7. Acetylated Distarch Phosphate

Rats

"Groups of 10 male and 10 female rats were fed 0, 25 or 50 per cent of two different modified starches (acetic anhydride and vinylacetate modified) in a low residue diet for 7 days followed by the additional feeding of 4 per cent cellulose for 3 days. The body-weights of all animals fed 50 per cent modified starch were somewhat lower than those of the controls. Production of faecal dry matter was increased in the higher groups and less so at the 25 per cent level. Diarrhoea occurred at the higher level with both modified starches. The addition of 4 per cent cellulose had no effect on the severity or frequency of the diarrhoea. At the higher level there was also some loss of hair in both sexes (1680."

"Groups of 10 male and 10 female rats were fed 0, 25 and 50 per cent of two different modified starches (acetic anhydride and vinylacetate modified) in their diet for 8 weeks. Body-weights of animals on the higher test level were somewhat lower than at the 25 per cent level and also lower than controls in both sexes. However, the differences were not statistically significant. The faecal water content was variable and could not be related to dietary dosage levels. Production of faecal dry matter was increased in both sexes at the higher level tested and slightly so at the 25 per cent dietary level. The incidence of diarrhoea was insignificant. A dose-related increase in caecal weight occurred in both sexes. No histological abnormality of the enlarged caeca was noted" by de Groot and Spanjers in their unpublished report (1680).

Pigs

An unpublished study in pigs was performed by Shillam et al. "Groups of 4 male and 4 female pigs were given 0, 35 or 70 per cent of modified starch in their diet over 14-½ weeks. Growth rate and food consumption were satisfactory. Haematology, blood chemistry and urinalysis revealed no treatment-related abnormalities. Ophthalmoscopy showed no abnormalities associated with the test substance. Organ weight, gross and histopathology revealed no abnormalities in test or control groups. Three pigs in the

higher test group died suddenly at various intervals during the test without any evidence pointing to the cause of their death. One pig in the higher test group and another in the 35 per cent group showed evidence of neurological malfunction. The animal on 70 per cent test substance died, that on the lower dietary level recovered. No histological evidence of nervous system involvement was noted in these two nor in any other animal" (1680).

Man

"Twelve volunteers consumed on each of 4 successive days, 60 g acetylated distarch phosphates of either 1.6 per cent or 2.33 per cent acetyl content. No abnormalities were observed as regards frequency and amount of faeces as well as faecal water and lactic acid content." No other adverse effects were noticed by Pieters et al. (unpublished study) (1680).

8. Distarch Glycerol

Rats

Three groups of 10 male and 10 female rats were fed for 90 days unmodified starch or starch treated with 0.07 per cent or 0.50 per cent epichlorhydrin at 71 per cent of their diet to produce distarch glycerol. "Growth rate, appearance, behaviour and food intake were similar in all 3 groups. Haematological investigations showed no abnormalities related to the feeding of modified starches. Non-protein nitrogen blood levels and blood sugar levels remained normal in all groups. The weights of all major organs were normal and no abnormal gross or histopathological changes related to treatment were seen (1680)."

9. Acetylated Distarch Glycerol

Rats

Groups of 15 male and 15 female rats were fed by Oser (unpublished report) for "90 days on diets containing 50 per cent either native or modified starch (0.3 per cent epichlorhydrin + 10.5 per cent acetic anhydride). The growth rate of male rats was significantly lower compared with controls on unmodified starch. The full and empty caecal weights of both male and female rats in the test groups were significantly greater than in controls. Haematology, blood chemistry, urinalysis, organ weights and gross as well as histological examination were normal (1680)."

10. Acetylated Distarch Adipate

Rats

A 90-day feeding study was carried out in groups of 15 male and 10 female rats at a dietary level of 50 per cent modified or unmodified starch. The growth rate of males was significantly lower in the test group and the full and empty caecal weights of both sexes were signi-

ficantly greater in rats fed the treated starch. All rats survived the test period and no differences were observed between the groups in respect of liver and kidney weights, haematology, blood chemistry, urinalysis, gross and histopathology. The modified starch used had been treated with 0.12 per cent adipic acid and 10.5 per cent acetic anhydride resulting in 3.1 per cent of acetyl groups being present (1680).

11. Hydroxypropyl Distarch Phosphate

Rats

"Groups of 10 male rats were fed" by Porter et al. (unpublished study) "for 10 days either unmodified starch or starch modified with 3 per cent, 6 per cent or 8 per cent propylene oxide as well as 0.0123 per cent phosphorus oxychloride equivalent to a degree of substitution ranging from 0.085 to 0.23." "Diarrhoea occurred whenever 4 g modified starch was included in the daily ration but also at the 2 g/day level with the more highly modified starches. The relative organ weight of the empty caecum was always increased at the 4 g/day level but also at the 2 g/day level with the 2 highly modified starches. Histological examination of the heart, liver, spleen, kidney and caecum revealed no obvious abnormalities related to treatment. Similar data were obtained in a series of tests using starches modified in the intermediate range of degree of substitution by the use of propylene oxide and phosphorus oxychloride (1680)."

In a second study, "groups of 10 male rats were fed diets containing 17, 34, 51, or 68 per cent modified starch for 28 days. Growth and bodyweights were reduced compared with controls at the two highest levels tested. The relative organ weights of empty caeca were raised at all levels tested and the extent of the increase was dose-related. No histological abnormalities were seen in heart, liver, spleen, kidney and caecum (1680)."

III. Long-Term Studies

A. Unmodified Starches

No Information Available

B. Modified Starches

1. Starch Acetate

Rats

"Groups of 30 male and 30 female weanling rats were fed on diets containing 0, 5, 10 and 30 per cent of starch acetate (acetylated to 1.98 per cent) for 2 years. No significant differences were observed with respect to behaviour, general health and mortality. Growth and food consumption were essentially similar to those of the controls. Production

of faeces during weeks 11 and 12 showed no dose-related differences among the various groups. Haematology, serum chemistry and serum enzymes as well as urinalysis showed no effects related to the administration of the test material. Among organ weights only the caecal weight of male rats showed a dose-related increase at 10 per cent and higher levels and the caecal weight of female rats was increased at the 30 per cent level compared with controls. No other significant changes were noted which could be ascribed to the test substance. The histological examination is still incomplete" according to the unpublished report of Til et al. (1680).

2. Hydroxypropyl Distarch Glycerol

Rats

Til et al. (unpublished report) fed "groups of 30 male and 30 female rats for 104 weeks modified starch at 0, 5, 10 and 30 per cent of their diet. No differences were noted between test and control animals with regard to appearance, behaviour, food consumption, haematology, serum chemistry and urinalysis. The mortality of females at the 30 per cent level was slightly higher. The growth rate was slightly reduced for both sexes at the 30 per cent level when compared with controls. Caecal weight was increased in a dose-related manner but occurred only in females at the 10 per cent level. The gross pathology has revealed no undue differences nor was there any obvious evidence of a carcinogenic effect. The histopathology has not yet been completed (1680)."

3. Phosphated Distarch Phosphate

Rats

Groups of 30 male and 30 female rats were fed a modified starch (phosphated distarch phosphate) at dietary levels of 0, 5, 10 and 30 per cent for 104 weeks. "No adverse effects were noted on general appearance, behaviour, mortality experience or food intake. Growth rate and food efficiency were similar to controls. Haematology, serum chemistry and urinalysis reveal no consistent changes related to the administration of the test substance. Relative organ weights were comparable with controls except for significantly decreased spleen weight in males and significantly increased spleen and kidney weights in females at the highest levels fed. These changes were not associated with any gross pathological findings. Caecal weights were normal at all test levels. There was no obvious evidence of any carcinogenic effect. The histopathological examination has not yet been completed" by de Knecht-van Eckelen et al. (unpublished report) (1680).

4. Acetylated Distarch Phosphate

Rats

"Groups of 30 male and 30 female rats were fed" by Til et al. (unpublished report) "two different modified starches (acetic anhydride and vinylacetate modified) at levels of 0, 5, 10 and 30 per cent in their

diet for 104 weeks. Appearance, behaviour, food consumption and mortality were not adversely affected nor was diarrhoea observed at any level. Growth was slightly depressed at the highest dietary level with both modified starches. Haematology, serum chemistry and urinalysis revealed no effects related to treatment in any group. There was a dose-related increase in the caecal weight in both sexes at the 30 per cent level but in males only at the 10 per cent level. All other organ weights showed no treatment-related changes. No gross or microscopic pathological abnormalities were noted which could be related to the test substances or which would point to any carcinogenic effects or to any compound related effects. The caeca appeared remarkably normal (1680)."

IV. Special Studies

Cariogenicity - Rats

Shaw and Ivimey examined the effect of dietary carbohydrate on caries production in rats. Wistar rats were fed diet 2718, Harvard cariogenic diet 2700 in which all sucrose had been replaced by corn starch. Breeders were selected from each generation on the basis of grossly observable carious lesions. The remaining rats (groups of 5-47) were sacrificed at 84 days for the first four generations and at 150 for the fifth through seventh generations. Control rats were maintained for 4 generations on rations with glucose or sucrose as the sole carbohydrate. Offspring of the starch-fed rats grew slower and suffered higher neonatal mortality than control rats. Throughout the seven generations the percentage of caries-free rats was lower and the extent of carious lesions higher than expected by the authors. This was not considered to be a result of genetic selection (1356).

Frostell and Baer provided groups of 60 weanling Sprague-Dawley rats of both sexes with a diet containing 66% starch, amylose, or amylopectin. Unmodified wheat, rice, arrowroot, tapioca, amioca, and potato starches and pregelatinized arrowroot, potato, amioca, tapioca, and wheat starches, and regular cornstarch were tested. Diets were administered for 30 or 60 days; caries and calculus were scored. No significant difference in the number of carious enamel areas between animals fed the cornstarch control diet and those given unmodified wheat, rice, arrowroot or tapioca starch diets was found. However, animals fed the unmodified potato starch and unmodified amioca had a tendency to have more carious areas. The pregelatinized starches produced several times more carious areas than cornstarch (449).

Baer and White studied the effect of dietary starches on calculus formation in the rat. Diets containing 32% skim milk plus 66% of unmodified wheat, rice, arrowroot, tapioca, amioca, potato, cornstarch; or, pregelatinized potato, tapioca, amioca, or wheat starch were fed to groups of 60 weanling Sprague-Dawley rats of both sexes. Animals were sacrificed at 30, 60, and 90 days and the molar teeth scored for calculus. None of the starches produced significantly more calculus than cornstarch,

but pregelatinized tapioca and amioca and unmodified arrowroot and potato starch produced significantly less (90).

In a second study, groups of 40 female weanling Sprague-Dawley rats were fed diets containing 66% regular cornstarch, high-amylose cornstarch, corn amylose, or corn amylopectin for 30 days. Rats fed either amylopectin or regular cornstarch formed significantly more calculus than rats on the other two diets (90).

Grenby investigated the effects of cooking on the cariogenicity of wheat starch. Two experiments were performed in which 47 and 42 weanling Wistar rats (male and female) of a cariogenic strain were randomly assigned to diets containing 66% sucrose, 66% cooked wheat starch, or 66% uncooked wheat starch. After 8 weeks, the incidence of caries was far lower for both starch groups than for the sucrose group. In the first experiment the caries score was significantly higher on the cooked starch diet than the raw starch diet, yet in the second experiment, no significant difference could be determined. Rats fed cooked starch gained 1.7 g/day compared to 1.3 g/day for the rats fed raw starch. In vitro decalcification tests revealed that mixtures of cooked wheat starch and saliva brought about significantly more dissolution of ground rat dental enamel than raw wheat starch mixtures (508).

Green and Hartles provided groups of 16 weanling albino rats with the following diets: 72% sucrose, 72% uncooked wheat starch, 72% roll-dried wheat starch, 36% sucrose plus 36% uncooked starch, and 36% sucrose plus 36% roll-dried starch. After 20 days, animals were sacrificed and assessed for dental caries. Sucrose rats had significantly more caries than starch rats; however, roll-dried wheat starch produced significantly more caries than raw starch. All animals remained healthy throughout the experiment (503).

Carcinogenicity - Mice

Chambers et al. reported that the injection of starch granules into mouse tumors resulted in a marked accumulation of polymorphonuclear leukocytes in and around the tumor. The accumulation inhibited tumor growth and often caused a complete regression of the tumor through a process of aseptic necrosis and subsequent resorption of the necrotic material. Arrowroot starch (0.2-0.3 ml of a 15% solution) was injected into 50 mice with sarcoma 180 in one or both axillary regions. Control mice were injected with Ringer's solution. Survival was highest in the treated group (243).

In a similar study, Chambers et al. again found that the injection of starch into tumors often induced an accumulation of granulocytes and a necrosis of the entire tumor. Rapidly growing transplantable tumors (sarcoma 180 and carcinoma 63) regressed completely 40% of the time; the percentage of regressions was a little higher for the slowly growing spontaneous and methylcholanthrene-induced tumors (244).

Reproduction

Unmodified Starch - Rats

Rats fed a ration containing 50% starch reproduced normally and had normal ovarian structures. Rations were fed to many groups of rats over a two year period. Two of the starch-fed rats were bred and then switched to a regular stock ration. Offspring were normal, as were vaginal smears of other starch-fed animals (1632).

Man

The diet, blood, and excreta of a group of pregnant women who consumed 15 g to 2 lb of cornstarch daily were analyzed by Edwards et al. Serum hemoglobin, protein, calcium, and glucose were determined, as well as urinary calcium, phosphorous, total nitrogen, and total solids. Fecal total nitrogen, calcium, and iron were measured. Patients were interviewed concerning their diets three times over a 5-6 month period. The condition of infants at birth was also rated. Protein intake was low compared to a nonpregnant control group, as were calcium and iron intakes. Only 33% of the infants delivered to cornstarch eaters were rated as "good" (delayed respiration for a few seconds, borderline weight, and average physical abilities) compared to 79% of the control infants. While only 11% of the control infants were rated as "poor" or "very poor" (babies rated as such usually died within 2-4 days), 33% of the cornstarch infants fell into this category. A higher percentage of the cornstarch infants were stillborn. The quality of the mothers diet generally paralled her offspring's rating (365).

Modified Starch - Rats

1. Starch Acetate

"A three generation study was performed by Til et al. (unpublished report) using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations by mating at week 12 and 20 after weaning. The F_{3b} generation was kept for 3 weeks after weanling and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material (starch acetate), fed at 10 per cent of the diet consists of starch modified with 5 per cent acetic anhydride (degree of substitutions 0.079). No adverse effects were noted regarding health, behavior, mortality, body-weights, fertility, litter size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were increased. Gross pathology of the F_{3b} generation revealed that kidney weights in females differed in a statistically significant way from controls but histopathology is still underway (1680)."

2. Hydroxypropyl Distarch Glycerol

"A three generation study was performed using 5 males and 10 females

for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10% of the diet consisted of starch modified with 5% phosphorus oxide and 0.1% epichlorhydrin (D.S. of 0.05). No abnormalities were noted by Til et al. (unpublished report) regarding health, behaviour, mortality, body-weights, fertility, little size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were increased in both sexes. Similarly kidney and thyroid weights in the females differed in a statistically significant way from the controls. There were no pathological changes on gross examination at autopsy in the F_{3b} generation but the histopathology is still outstanding (1680)."

In a second study, "groups each of 10 male and 20 female rats were fed the modified starch at a level of 10% in their diet for a period of 3 generations. Rats were mated (P_1, F_1 and F_2) at week 12 and 20 after weaning. Each generation was mated twice and the litter from the second generation used to produce the next generation. No adverse effects were noted on fertility, number of pups per litter, bodyweight of young, mortality experience or resorption quotient that could be ascribed to treatment. The average relative cecum weight (filled) of females of the P and F_2 generation was greater than controls, but empty cecum weight did not differ from controls. The relative cecum weight (filled and empty) of males of the F_1 generation did not differ from controls. Growth of the F_{3b} generation maintained on test diet for 3 weeks was normal. Relative organ weights were comparable to control, with the exception of increased thyroid weight and distinctly increased cecal weight (both filled and empty) in male pups, and increased empty cecal weight in female pups. Gross examination of organs at autopsy did not show any compound related changes. Histopathological studies of organs have not been completed (1680)."

3. Phosphated Distarch Phosphate

A three generation study was performed by Til et al. (unpublished report)" using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations by mating at week 12 and 20 after weaning. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10 per cent of the diet consisted of starch modified with sodium trimetaphosphate up to 0.04 per cent P and sodium tripolyphosphate up to a total of 0.35 per cent P. No adverse effects were noted regarding appearance, behaviour, mortality, body-weights, fertility, litter size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were increased. In the F_{3b} generation spleen weights in females fed this starch differed in a statistically significant way from controls. Gross examination at autopsy did not reveal pathological changes attributable to ingestion of this starch but histopathology is still outstanding (1680)."

4. Acetylated Distarch Phosphate

"A three generation study was performed using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations by mating at week 12 and 20 after weaning. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10 per cent of the diet consisted of a starch modified with 5 per cent acetic anhydride and 0.02 per cent phosphorus oxychloride (D.S. of 0.093) and another starch modified with 4.5 per cent vinylacetate and 1.2 per cent phosphorus oxychloride (degree of substitution of 0.064, 0.04 per cent P introduced). No adverse effects were noted in respect of health, behaviour, mortality, growth, fertility, litter size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were only increased in tests using starch modified with acetic anhydrides. Gross pathology of the F_{3b} generation revealed a significant difference in the average thyroid weights. Gross examination at autopsy did not reveal any pathological changes attributable to the ingestion of the starch but histopathology is still outstanding (1680)."

Sensitivity - Man

Loveless reported a few cases of hypersensitivity to the ingestion of corn, tapioca, and arrowroot starches among 25 subjects with histories suggestive of corn allergy (865).

Unpublished reports by Majors and Ruben Koenig and by Ruben Koenig have revealed no evidence of sensitization to powdered high and low modified starch in man (669).

Table 1 presents the acceptable daily intakes (ADI) for man of modified starches as set by the Joint FAO/WHO Expert Committee on Food Additives in their thirteenth report (669).

ACCEPTABLE DAILY INTAKES (ADI) FOR MAN OF MODIFIED STARCHES

Substance[a]	ADI (mg/kg body weight)
Amylose, amylopectin	No limit[2]
Enzyme-treated starches	No limit[2]
White and yellow dextrins	No limit[2]
Acid-treated starches	No limit[2]
Bleached starches	No limit[2]
Oxidized starches	No limit[1,2]
Alkali-treated starches	No limit[2]
Mono starch phosphate	No limit[2,4]
Starch acetate	0 - 12.5[1]
Starch sodium succinate	No ADI
Hydroxypropyl starch	0 - 25[1]
Distarch phosphate (phosphorous oxychloride)	No ADI
Distarch phosphate (sodium-trimetaphosphate)	0 - 25[3,4]
Phosphated distarch phosphate	0 - 25[3,4]
Acetylated distarch phosphate	No ADI
Hydroxypropyl distarch phosphate	No ADI
Distarch glycerol	No ADI
Acetylated distarch glycerol	No ADI
Hydroxypropyl distarch glycerol	0 - 12.5[1]
Acetylated distarch adipate	0 - 12.5[1]

[a] Specifications will be published for these compounds (see Annex 1, Ref. 10) except for the first three items. The specifications are tentative for those items which have not been given an ADI.

[1] Temporary acceptable daily intakes - details on further information required are given in the respective monographs.

[2] Not limited except by good manufacturing practice.

[3] As distarch phosphate (sodium-trimetaphosphate) or phosphated distarch phosphate or the sum of both.

[4] Subject to limits of phosphorous load (see Annex 1, Ref. 7, p. 22).

Note: No monographs were prepared for certain other modified starches (see Annex 1, Ref. 10, section 3.2.1).

Listed below are the further studies or reports required by the Joint FAO/WHO Expert Committee on Food Additives on Food Additives by 1973 (1600).

Oxidized starches - Histological report to complete the two-year rat study and the reproduction study.

Starch acetate - Same as above.

Hydroxypropyl starch - Histological report to complete the two-year rat study and the reproduction study.

Hydroxypropyl distarch glycerol - Histological report to complete the long-term rat study and reproduction study.

Distarch phosphate - Histological reports to complete the long-term and reproduction studies in the rat.

Hydroxypropyl distarch phosphate - Appropriate 90-day studies in 2 species.

Phosphated distarch phosphate - Histological reports to complete the long-term and reproduction studies in the rat.

Acetylated distarch phosphate - Histological report to complete the reproduction study in the rat.

Distarch glycerol - Histological report to complete the 2-year rat study and the reproduction study on hydroxypropyl distarch glycerol.

Acetylated distarch glycerol - Histological /eports to complete the 2-year and reproduction rat studies on starch acetate. Histological reports to complete the 2-year and reproduction rat studies on hydroxylpropyl distarch glycerol.

Acetylated distarch adipate - Histological report to complete the long-term and reproduction studies in the rat on starch acetate. Histological report to complete the reproduction study in the rat on acetylated distarch phosphate.

Biochemical Data

I. Breakdown

During baking, starch is gelatinized. The amylose is irreversibly retrograded or crystallized, and provides a major portion of the structural framework of the bread. The retrogradation involves the parallelization of the polymers and consequent association through hydrogen bonding. The amylopectin fraction "begins to crystallize slowly after the cooling of the bread, and this crystallization or coacervation continues for several days after the bread is baked. Coacervation of the amylopectin causes a gradual increase in the firmness of the bread with the passage of time and is the most important of the three factors involved in staling: 1) a firming of the crumb; 2) loss of flavor; and 3) loss of crispness in the crust with resultant leatheriness. The addition of a small quantity of specific surfactants, 0.25-0.5%, based on the flour weight will retard the firming of the crumb and loss of crispness in the crust (460)."

Davies and Wilmhurst destructively distilled commercial starch at atmospheric pressure and a temperature of 370-390 degrees C, a temperature likely to be attained during cooking. The charred residue contained 0.2 mcg of benzopyrene per 100 g of char (306).

Marshall and Wasik determined that the starch in spagetti gelatinized slowly during cooking (922).

II. Absorption and Distribution

The absorption coefficient of starch for normal children 1-2 years old was found to be greater than 99% (81).

Rats have been found to absorb 23.2 ± 9.17 g starch per kg daily or about 90 kcal/kg (193).

Based on in vitro studies in which segments of rat intestine were incubated with pancreatic amylase and starch, Ugolev reported that parietal or contact digestion is an important mechanism of breakdown and assimilation of food in higher animals. This type of digestion involves the adsorption of enzymes onto the mucosal surface; these enzymes are concerned with both the hydrolysis and absorption of food. Results indicated that the large surface of the small intestine performs an important function - that of reconstituting a living porous adsorbant. Under normal conditions, part of the digestive enzymes remain in solution, and bring about digestion in the lumen, while the remaining part of the enzymes are adsorbed on to the surface of the mucosa where they bring about contact or parietal digestion. Parietal digestion was believed to be best developed in the small intestine (1539).

Ugolev "compared the rates of breakdown of soluble (partially hydrolyzed) amylose and insoluble amylose by the enzyme in solution and the enzyme adsorbed onto intestine. It was found that the larger amylose molecules are more intensely attacked by the dissolved enzyme, while the smaller molecules (previously partially hydrolyzed) on the contrary were more rapidly broken down in the parietal layer by the adsorbed enzyme (1539)."

The author suggested that the very first stages in digestion take place chiefly in the lumen of the digestive tract, and subsequently on the surface of the intestine. It was noted that the arrangement whereby parietal hydrolysis represents the chief supply of absorbable products and takes place directly on the surface of the villi constitutes extremely favorable conditions for absorption (1539).

Jesuitova confirmed Ugolev's hypothesis that starch hydrolysis in the gut is not merely an intraluminal process and that the most important hydrolytic activity occurs on the mucosa itself. Pancreatic amylose (alpha-1,4-glucan-4-gluconohydrolase, EC 3.2.1.1) was selectively adsorbed on the intestinal mucosa. In vivo experiments with rats indicated that the adsorption of amylase occurred in the proximal parts of the gut and seemed to decrease in areas where the villi were shorter (663).

However, Fogel and Gray maintained that the intraluminal process played the primary role in starch digestion and that membrane digestion was of secondary importance. Jejunal perfusion and test meal experiments were performed in normal as well as pancreatic-insufficient humans to compare starch hydrolysis of intraluminal amylase and amylase adsorbed to the microvillous membrane. The available intraluminal hydrolytic capacity was found to be in excess of that needed to account for observed rates of hydrolysis. In contrast to Ugolev (539), in vitro rates of starch hydrolysis were always found to be greater than in vivo rats for both perfusion and test meal studies. Fogel and Gray indicated that the different perfusion rates used by the two research groups could be involved. The authors stated that "it would seem very likely that a rapid rate would wash most of the pancreatic amylase out of the lumen, thereby producing low levels of intraluminal amylase after the initial few minutes. Since pancreatic amylase is very difficult to wash off the intestinal mucosa (1), an artificial preparation was created in which little intraluminal amylase was present in the small intestine, but in which pancreatic amylase was adherent to the mucosa. Thus, a membrane digestion of starch was uncovered by greatly reducing the intraluminal amylase activity (426)."

Volkheimer et al. have studied the persorption of starch granules in dogs. Persorption is the phenomenon by which solid, undissolved food particles enter the body from the intestinal lumen during normal digestion, as opposed to absorption, by which dissolved substances of molecular magnitude enter the epithelial cell of the small intestine. Particles

suitable for persorption enter the space between epithelial cells and are transported in a basal direction in the subepithelial region. Persorption is possible wherever a single-layer-epithelium covers the mucosa of the intestinal tract and is most often observed in the region of desquamation zones of the epithelium. Primary transport from the intestinal wall occurred via the portal system as well as the chylus. After dogs were fed 250 g potato starch, the portal vein blood contained more starch granules than the arterial blood, supporting the theory of primary portal transport of persorbed starch granules. According to the author, primary transport of persorbed starch granules via the chylus has already been proven by histological examination (1585).

III. Metabolism and Excretion

"Starch is attacked by ptyalin, the alpha-amylase in the saliva. However, the optimal pH for this enzyme is 6.7 and its action is inhibited by the acid gastric juice when food enters the stomach. In the small intestine, the potent pancreatic alpha-amylase also acts on the ingested polysaccharides. Both the salivary and the pancreatic alpha-amylases hydrolyze 1,4 alpha linkages but spare 1,6 alpha linkages, terminal 1,4 alpha linkages, and the 1,4 alpha linkages next to branching points. Consequently, the end products of alpha-amylase digestion are the disaccharide maltose, the trisaccharide maltotriose, and alpha-limit dextrins, branched polymers containing an average of about 8 glucose molecules", as shown in figure 1 (464).

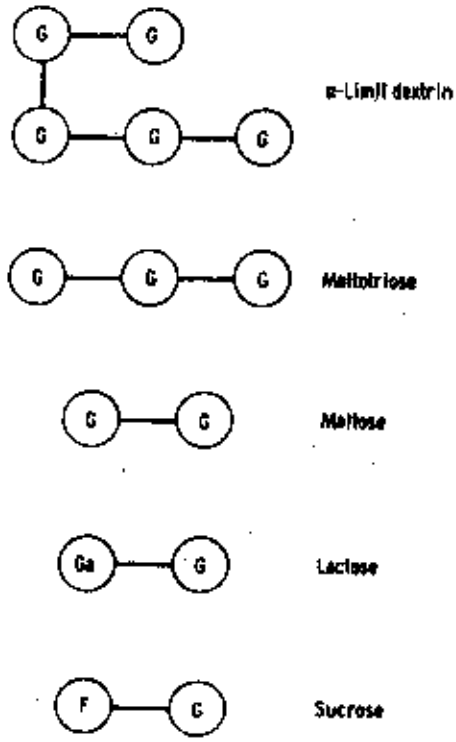
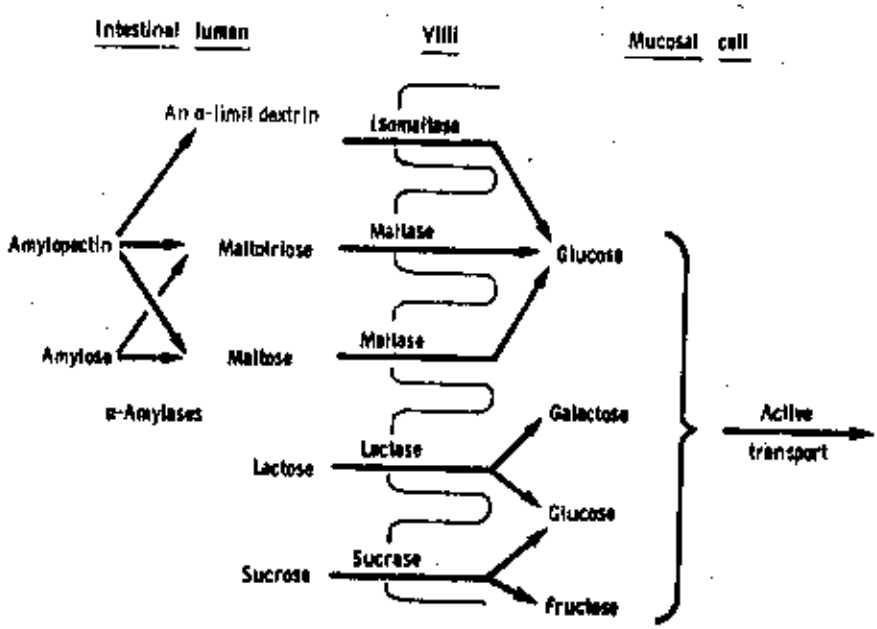


FIG 1. Principal end products of carbohydrate digestion in the intestinal lumen. Each circle represents a hexose molecule. G, glucose; F, fructose; Ga, galactose.

"The enzymes responsible for the further digestion of the starch derivatives are located on the surface of the mucosal cells, principally in the ileum. Isomaltase hydrolyzes the alpha-limit dextrins, and maltases hydrolyze maltotriose and maltose. Most of the glucose molecules that are formed enter the mucosal cells, although some reenter the intestinal lumen and are absorbed further along. Ingested disaccharides are hydrolyzed by lactase or sucrase on the luminal surface of mucosal cells (Figure 2). Deficiency of one or more of these disaccharidases leads to diarrhea, bloating, and flatulence after ingestion of sugar (464)."

Fig. 1



Outline of carbohydrate digestion and absorption.

Auricchio et al. investigated the digestion of starch in infants and small children after a meal of amylopectin. In infants at the end of the first year of life and in small children, the alpha-amylase activity of duodenal juice is high and the in vivo intestinal starch hydrolysis is rapid. Amylopectin, a 5,000-glucose-unit polysaccharide, is digested at the end of the duodenum into carbohydrates with an average degree of polymerization of 3. Hydrolysis into glucose, maltose, maltotriose, and branched dextrins is probably followed by hydrolysis to glucose through mucosal glucosidases. Maltose and maltotriose comprise 60-70% of the total carbohydrates, and were considered the limit compounds of in vivo alpha-amylosis in man. Maltotetraose represented 1-3% of the total carbohydrates; further hydrolysis of maltotetraose was thought to arise from salivary and pancreatic amylase. Digestion in adults proceeded at similar rates (81).

In infants 6 months old or younger, the intestinal hydrolysis of amylopectin is incomplete, resulting in large amounts of 30-glucose-unit dextrins, a decreased glucose and maltose content, and an increased maltotetraose content. These infants have a lower level of human saliva alpha-amylase activity which would decrease hydrolysis of maltotetraose into glucose and maltose (81).

"Corn starch treated with propylene oxide-2-C^{14} to produce hydroxypropyl starch (degree of substitution 0.12) was given to a male rat by gavage. Ninety-two per cent of the radioactivity was excreted in the faeces and 3.6 per cent in the urine over the next 50 hours. The urinary activity was probably derived from propyleneglycol in the test material. Further investigation of the degradation products in the faeces revealed hydroxypropyl maltose to be the major metabolite in vitro and in vivo (1680)."

"The metabolic fate of adipate-modified starches was investigated in male rats in vivo using adipic acid labelled at C-1 and C-6 with C^{14}. The rate of appearance of $^{14}CO_2$ was compared between starch modified with 1,6 - C^{14} adipic acid. Starch adipate is absorbed and/or metabolized more slowly as evidenced by the delayed appearance of $^{14}CO_2$ in the respired air. 99.3 per cent of the C^{14} activity of the free adipic acid was recovered in the respired air; 5.8 per cent appeared in the urine while none was detected in the faeces, in the gastro-intestinal tract or in the carcass. 70.5 per cent of the C^{14} activity of the esterified adipic acid appeared in the respired air and 7.2 per cent in the urine; 24.5 per cent was found in the faeces. No activity appeared in the carcass (1680)."

"In vitro enzymatic hydrolysis of mono starch phosphate by wheat alpha-amylase was compared with unmodified wheat starch. No difference was noted in the rate of production of reducing substances. The metabolic behaviour of the phosphate radical in starch phosphate was studied in vivo by comparing the distribution of P^{32}, after oral administration of labelled starch phosphate to rats, with that from either labelled orthophosphate or pyrophosphate. The percentage activity excreted in urine and faeces over 48 hours as well as the percentage activity retained in liver, kidney, blood plasma and bone showed no significant difference for the 3 types of phosphate examined. The phosphate moiety of starch phosphate probably behaves metabolically like any other ionic phosphate (669)."

The human gastrointestinal tract digests raw cereal and root starches such as wheat, maize, rice, and cassava very efficiently; however, raw potato, arrowroot, and canna starches are not (180).

The digestibility of oxidized wheat starch (conditions not stated) was examined in rats by matched-feeding techniques using the modified starch as the sole source of carbohydrate at a level of 63.7 per cent (dry basis) of the diet. The degree of assimilation by and the general effects on groups of 6 rats over a feeding period of 28 days were assessed from consideration of body weight changes, faecal residues, digestibility coefficients for starch and post-mortem appearance of the animals and their gastrointestinal tracts. The digestibility coefficients

were calculated from the starch content of ingested food and residues found in faeces and post-mortem gastrointestinal contents. Body weight gain and digestibility coefficients were practically indistinguishable from those obtained for wheat starch or corn starch. Nothing abnormal was noted on post-mortem examination (180).

Table 1 presents the coefficient of digestibility of various starches for rats (180).

Table 1

Comparisons of relative utilizations of various unmodified and modified starches as determined by ad libitum feeding of diets containing 83.7% (anhydrous basis) of the starch products specified. (Animals 45–60 gm in initial weight; feeding periods, 28 days unless otherwise specified in column 1)

STARCH COMPONENT OF DIET	EXPT'L. ANIMALS [1]	TOTAL DRY FOOD EATEN	NET BODY WEIGHT GAIN [2]		AVERAGE FINAL BODY LENGTH	DRY FECES PER GRAM DRY FOOD EATEN	STARCH, COEFF. OF DIGESTIB.
			Total	Per gram dry food			
	no.	gm S.D.	gm S.D.	gm S.D.	cm	gm	%
Wheat 1	6	283 ± 21	127 ± 11.7	0.45 ± 0.022	19.9	0.05	98.4
Wheat 1	6	310 ± 4	126 ± 9.9	0.41 ± 0.022	20.1	0.05	97.8
Wheat 2 acid-modified	6	302 ± 23	131 ± 7.7	0.43 ± 0.021	20.4	0.05	98.3
Wheat 3 oxidized	6	304 ± 14	126 ± 6.8	0.41 ± 0.011	20.2	0.05	98.4
Maize 1	6	313 ± 17	121 ± 18.4	0.39 ± 0.043	19.8	0.05	97.7
Maize, waxy, 1	6	312 ± 11	130 ± 5.2	0.42 ± 0.006	19.9	0.05	98.3
Rice 1	6	322 ± 20	130 ± 11.5	0.40 ± 0.015	20.2	0.05	97.5
Cassava 1	6	307 ± 40	110 ± 27.2	0.36 ± 0.059	19.6	0.05	98.0
Cassava 2	6	326 ± 38	135 ± 15.5	0.42 ± 0.021	20.4	0.05	97.7
Sweet potato 1	6	301 ± 20	121 ± 14.7	0.40 ± 0.025	19.9	0.07	96.6
Sweet potato 2	6	281 ± 35	97 ± 6.4	0.35 ± 0.025	19.3	0.09	95.4
Arrowroot 1	6	293 ± 31	91 ± 7.2	0.31 ± 0.021	19.1	0.18	79.9
Sago palm 1	8	364 ± 36	114 ± 5.5	0.31 ± 0.016	20.0	. .	. .
Sago palm 1	6	342 ± 59	109 ± 21.6	0.32 ± 0.013	19.8	0.26	65.3
Sago palm 1 ball-milled 2 hr.	6	349 ± 81	135 ± 18.2	0.39 ± 0.026	20.0	0.09	96.1
Sago palm 1 ball-milled 40 hr.	6	334 ± 20	127 ± 10.8	0.38 ± 0.024	19.9	0.06	96.5
White potato 1	5(1d)	337 ± 28	106 ± 13.0	0.31 ± 0.028	19.7	. . .	
White potato 2, lab. product, no chemical treatment	4	350 ± 88	95 ± 20.6	0.27 ± 0.021	19.5	0.35 [3]	50.9 [3]
White potato 1 ball-milled 40 hr.	6	278 ± 28	111 ± 11.7	0.40 ± 0.014	19.5	0.06	98.2
White potato 3, Lintner's soluble	8	384 ± 44	107 ± 13.8	0.28 ± 0.027	19.5	0.37	51.0
White potato 4; 7-day feeding period	12	48 ± 10	9 ± 4.8	0.17 ± 0.076	14.4	0.43	42.7
White potato 4; 14-day feeding period	12	123 ± 24	35 ± 9.0	0.28 ± 0.041	16.5	0.38	49.8
White potato 4; 21-day feeding period	12	221 ± 29	72 ± 8.5	0.32 ± 0.020	18.4	0.35	53.8
White potato 4; 28-day feeding period	9(3d)	328 ± 55	100 ± 12.6	0.31 ± 0.021	19.7	0.33	59.2

[1] Parenthetical figures in this column indicate number of animals that did not survive the experimental period, all records on which were excluded in computation of the data presented in columns 3 to 8 inclusive.

[2] Net gain = final gross live weight (posthumous contents of G.I. tract + initial gross live weight).

[3] The posthumous contents of the G.I. tracts were not collected for this group of animals, hence not taken into account in this computation.

Based on tests with adult male Wistar rats, Yoshida and Morimoto determined the coefficients of digestibility for raw corn, sweet potato, and potato starch to be 99, 97, and 57% respectively. When the diets were steamed, all three starches were almost completely utilized (1707).

Borchers gave normal corn a digestibility coefficient of 95%, and high-amylose corn, one of 66-77% for rats (182).

In an unpublished report, Oser determined the caloric value of acetylated distarch adipate, acetylated distarch glycerol, and acetylated starch in rats. Groups of 10 male rats were fed either 1.5 or 3.0 g of acetylated distarch adipate or acetylated distarch glycerol for 28 days; 3 or 4.5 g of acetylated (1.8% acetyl) or native starch were fed in a similar fashion. Caloric values were determined from a dose-response curve obtained by feeding groups of rats 0, 0.75, 1.5, 3.0, and 4.5 g of sucrose supplements equivalent to 0, 3, 6, 12, and 18 calories per day, except for acetylated starch which was compared to rats fed 0, 1.5, 3.0, 4.5, and 6.0 g dextrose (equivalent to 0, 6, 12, 12, and 24 calories). Acetylated distarch adipate had been treated with 0.2% adipic anhydride and 5.5% acetic anhydride. Acetylated distarch glycerol had been modified by the use of 0.1% or 0.3% epichlorhydrin plus 5.5% acetic anhydride. While there was no difference in caloric value between native starch, acetylated starch, and acetylated distarch adipate, weight gain on acetylated distarch glycerol was slightly reduced (1680).

In a similar study distarch glycerols were found to be only slightly lower in caloric value than unmodified starch, but the differences were not significant. These modified starches were produced by treatment with 0.07 and 0.50% epichlorhydrin (1680).

An earlier experiment by Oser had disclosed no difference in caloric value between distarch phosphate and unmodified starch. As stated in his unpublished report, groups of 6 male and 6 female rats received 52% distarch phosphate for 6 weeks as the sole dietary carbohydrate. The product had been treated with 0.06% phosphorous oxychloride (1680).

"Caloric value and digestibility of a distarch phosphate using trimetaphosphate were tested in groups of 10 rats fed for seven days on 4 g basal diet with either 0.9 g or 3.6 g starch supplement by observing the gain in body weight and the organ weights of liver, kidney, heart and spleen after the feeding period. No significant differences were noted between the modified and the unmodified starches (1680)."

Prier (unpublished study) tested for digestibility in groups of 5 rats fed a basal ration plus 0, 1, or 3 g hydroxypropyl distarch phosphate or control starch. No difference in weight gain was observed (1680).

In an unpublished caloric utilization study, groups of 10 male rats were fed for 10 days either unmodified starch or hydroxypropyl distarch phosphate modified with 3, 6, or 8% propylene oxide as well as 0.0123% phosphorus oxychloride equivalent to a degree of substitution ranging from 0.085 to 0.23. Porter found a slight decrease in caloric utilization with increasing degree of modification (1680).

Sandstedt et al. reported that the digestibility of raw corn starch was not directly associated with amylose content, based on in vitro tests with pancreatic alpha-amylase. A 40%-amylose cornstarch and a 5% amylose waxy cornstarch were the most susceptible to enzyme action. Wrinkled-pea starch with 65% amylose was intermediate in digestibility. A 61%-amylose cornstarch was highly resistant, but less so than a 22%-amylose potato starch (1275).

The in vitro digestibility of several modified starches by pancreatic and porcine mucosal enzymes was compared to that of native starch in an unpublished paper by Leegwater. Acetylated distarch phosphates, modified to 1.6 and 2.3% acetyl content were 93 and 81%, respectively, as digestible as native starch. Starch acetate (containing 1.98% acetyl) and hydroxypropyl distarch glycerol (degree of substitution 0.04) were found to be 90 and 86% as digestible. A similar trend was noted for phosphated distarch phosphate. Leegwater determined that the digestibility of hydroxypropyl starch decreased as the degree of substitution increased. At 0.04 degree, the digestibility was estimated at 80% that of starch (1680).

Kay and Calandra (unpublished report) estimated the in vitro digestibility by pancreatin of hydroxypropyl starch by comparing the amount of reducing material liberated with that formed by native starch. No significant difference could be detected between low (1 in 10) and high (1 in 4) substituted starches, and unmodified starch (1680).

A similar study by Shuman and Mertz (unpublished report) indicated that oxidized starch was 10-15% less digestible by pancreatin than native starch. However, no difference in salivary digestion was observed (669).

In vitro digestibility by pancreatin of corn or potato starch modified with 0.05 or 0.1% phosphorus oxychloride (distarch phosphate) was found to be similar to the unmodified starch. When 0.5 or 1.5% of phosphorus oxychloride was used the resulting cross-linkage considerably inhibited digestibility in vitro in a manner related to the concentration of cross-linking agent used (1680).

Starch acetates containing 2.5% acetyl groups are only 93.7% as digestible as native starch, based on in vitro studies (1680).

Kohn and Kay's unpublished report stated that the in vitro digestibility of distarch phosphate (modified by trimetaphosphate) and phosphated distarch phosphate by pancreatic amylase was reduced compared to unmodified starch. Digestibility was also examined in groups of 10 male rats fed 5 g of a basal ration plus 1, 2, or 4 g unmodified or trimetaphosphate modified starch (distarch phosphate) for 10 days. Weight gains were similar for all groups and no unusual behavioral reactions were observed (1680).

The in vitro digestibility by amyloglucosidase of various modified starches was discussed in recent unpublished study by Kruger. Acetylated distarch adipate, distarch glycerol (0.3% epichlorhydrin), and several distarch phosphates (0.035, 0.07, or 0.1% phosphorous oxychloride) were shown to have digestibilities of 98.3%, 98.3%, and between 96.4 and 98.3%, respectively. The in vitro digestibility of acetylated distarch glycerol (0.3% epichlorohydrin, 1.2 or 2.5% acetyl content) varied with acetyl content but was not affected by cross linkage. At 1.2% acetylation, approximately 82% of the modified starch was digestible, but at 2.5% only 68.5% was hydrolyzed enzymatically (1680).

IV. Effects on Enzymes and Other Biochemical Parameters

Abdeljlil et al. found diet composition to influence the amount of amylase, chymotrypsinogen, and trypsinogen excreted by the rat pancreas. Adult male Wistar rats were fed either a starch-rich diet (75% starch-15% casein) or a casein-rich diet (20% starch - 70% casein); after 20 days, pancreatic juice was tested for enzyme activity. Amylase activity was 2-3 times higher for the starch-fed group, and chymotrypsinogen and trypsinogen were 2-3 and 1.5-1.7 times higher, respectively, for the casein-fed group. Chromatographic studies indicated that the amount rather than the activity of the enzymes was altered (2).

Marchis-Moren indicated that a starch-rich diet induces a more active biosynthesis of amylase than a casein-rich diet in rats (913).

Feeding 2% cream of rice induced the production of amylase in 10 out of 12 premature infants aged 11-40 days. This enzyme is absent in newborns, but occurs to a greater extent in premature infants, generally within 2 months. Weight gain increased 40% over that of control infants fed only the cow's milk formula; no digestive disturbances were present (1016).

When raw sweet potato starch was fed to adult male Wistar rats, the apparent digestibility of dietary protein decreased conspicuously; animals also suffered from diarrhea. The diet contained approximately 16% protein and 51% starch. Diarrhea was thought to result from some component of the sweet potato which could be removed by washing (1707).

Upon investigating the effect of starches on protein utilization and liver lipid composition, Chang et al. determined that rice starch was superior to arrowroot starch but inferior to wheat and corn starches as regards protein utilization. Four sets of 12 four-week-old rats were fed basal rations containing 61% rice, arrowroot, corn or wheat starch for 9 weeks. Then, a protein-deficient diet was given for 5 days, and finally, the rats returned to their respective starch rations. At the end of 12 weeks, animals were sacrificed and liver lipids measured. In a second series, four groups of rats were treated similarly except that 0.7% L-lysine was added to the diets. Growth was much lower for arrowroot rats and slightly lower for rice rats than for the corn and wheat rats. Without lysine supplementation protein efficiency paralleled growth; with the extra lysine, protein efficiency was similar for rats fed rice, corn, and wheat, but low for rats fed arrowroot. In both series, rats fed arrowroot showed distention of the cecum and diarrhea. Without lysine supplementation, rats fed arrowroot had the lowest liver lipid concentration, but there were no significant differences with such addition (246).

Kimura and Ashida found liver lipogenesis to be stimulated by increased levels of starch ingestion. Rats were given either 5 g of a starch-casein mixture or 6 g of a starch-corn oil mixture both containing 25, 50, 75, or 95% starch (733).

Weanling male Sprague-Dawley rats were maintained for 21 days on

cholesterol-free diets containing either soybean protein or casein, with and without cornstarch. The addition of 20% cornstarch (at the expense of cereloss) significantly lowered liver cholesterol levels. In the soy diet, starch increased serum cholesterol; however, no effect was noted in the starch-casein diet. No mention was made of methionine supplementation in the soy protein diet. Weight gain was depressed in both soy diets. Farnell and Burns concluded that "dietary starch lowers liver cholesterol but may raise serum levels, probably by increasing the mobilization and excretion of cholesterol (399).

Anderson reported that modified starches, cooked or raw, significantly raised serum and hepatic cholesterol levels compared to sucrose in rats. Rations fed included sucrose, corn starch, a modified corn starch, a modified tapioca starch, and an experimental modified white milo starch. Carbohydrates were fed at the 67.8% level, cooked and raw, to groups of 10 male weanling Harlan-Wistar rats for four weeks. Animals fed modified food starches had higher serum and hepatic cholesterol levels and total hepatic lipid than sucrose-fed rats, regardless of whether the starch was cooked or raw. However, these levels were higher when raw starch was given. Serum and hepatic cholesterol levels were similar in rats fed sucrose and unmodified cornstarch, except that feeding raw cornstarch resulted in an elevated hepatic cholesterol level. Anderson noted that modified starches from the same source treated by different chemical processes could easily produce varied physiological effects (49).

The consumption of potato or corn starch in conjunction with cholesterol by hypercholesteremic rabbits enhanced the degree of hypercholesteremia. Twenty grams of either dextrose, maltose, sucrose, rice, corn starch, or potato starch plus 2 g of cholesterol was administered orally to groups of 10 New Zealand bucks for a 2 week period. Animals fed starches gained more weight, had heavier livers, higher and more persistent serum cholesterol levels, and more aortic plaques than did rabbits fed rice or hexoses. Normocholesteremic rabbits were unaffected as regards serum cholesterol (1161).

Mann and Truswell measured fasting serum cholesterol and triglycerides, as well as postprandial insulin secretion and lipemia in healthy normal-lipemic men, who received an ordinary diet in which sucrose was isocalorically replaced by starch at 23% total calories. No significant differences in serum lipid concentration or insulin response were ascertained either after breakfast or fasting. The fatty acid patterns of serum lipids were similar for both diets (910).

Garretson observed decreased iron absorption in rats fed protein deficient starch and sucrose diets. This was attributed to both a retarded growth rate and a relative protein deficiency for hemoglobin synthesis, rather than a direct intraluminal effect. It was suggested that "starch eater's anemia" is caused by a lack of dietary iron and may be complicated by a decreased capability to absorb iron because of protein deprivation and decreased erythropoiesis (466).

V. Drug Interaction

No Information Available

VI. Consumer Exposure Information

Dry granular starch is used as a diluent, bulking agent, fluidifying agent, and mould and moisture adsorbing agents. Native starches are used primarily as thickening or gelling agents and processing aids. However, many of the modified starches have properties which provide superior thickening power and a variety of textures. A detailed discussion of the functional properties of modified starches can be found in the Handbook of Food Additives (460).

The food industry consumed over 300,000,000 pounds of starch or modified starches annually. While the bulk of this is corn and waxy corn starch, sorghum, waxy sorghum, and wheat starch are also used. In the United States, limited amounts of potato, tapioca, and arrowroot starches are used (460).

The following tables were compiled from data submitted by user firms. Food consumption values for each food category were derived from the Market Research Corporation of America (MRCA) data on frequency of eating and from the USDA data on mean portion size of foods in each food category. The food consumption values thus derived were coupled with the usage level data obtained in the surveys to calculate the daily intake of each substance.

Tables 2 and 3 report the usage of modified starches in foods; Table 11 reports the annual poundage data. Table 13 shows possible daily intake per food category and total dietary based on food consumption by total sample.

COMPREHENSIVE GRAS SURVEY -- NAS/NRC 1972 10/03/72

-- ANNUAL POUNDAGE DATA FOR NAS APPENDIX A SUBSTANCES (GROUPS I & II)

SUBSTANCE NAME (SURVEY NO.)	# REPORTS TO NAS 1969/1970	POUNDAGE REPORTED TO NAS (MATCHING REPORTS FOR BOTH YEARS) 1969	1970	TOTAL 1970 POUNDAGE REPORTED TO NAS	# REPORTS TO FDA	POUNDAGE REPORTED TO FDA 1970 ONLY	TOTAL 1970 POUNDAGE NAS + FDA
STARCH, MODIFIED NAS 0325	79/ 96	33,395,972	61,625,337	70,375,465		-	70,375,465

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, %
STARCH, MODIFIED NAS 0325	01	BAKED GOODS(R)	19	1.67814	2.71310
	03	OTHER GRAIN(R)	7	7.46901	7.83138
	04	FATS OILS(R)	9	1.76774	3.29535
	05	MILK PRODS(R)	*	.01000	.01000
	06	CHEESE(R)	*	1.00000	1.23000
	08	PROCSD FRUT(R)	7	2.19726	4.25296
	10	MEAT PRODS(R)	18	1.49765	2.20514
	11	POULTRY(R)	12	1.45741	1.77029
	12	EGG PRODS(R)	4	3.49151	5.18000
	13	FISH PRODS(R)	8	2.73243	5.15160
	14	PROCSD VEGS(R)	18	1.08671	1.49383
	15	ECHOP RELSH(R)	5	.93569	.93726
	16	SOFT CANDY(R)	10	8.28755	11.92733
	17	COMP FROST(R)	6	2.35404	9.82434
	20	GELATIN PUD(R)	17	3.02336	5.10641
	21	SOUPS(R)	7	1.22036	1.46545
	22	SNACK FOODS(R)	5	1.81493	3.67052
	25	NUT PRODS(R)	*	1.20000	1.20000
	26	RECONST VEG(R)	*	1.36000	2.50000
	27	BEVERS(R)	21	1.70349	2.85260
	28	DRY DAIRY(R)	4	3.91162	4.02686
	31	CHEWING GUM(R)	10	.42582	.45975
	48	SEAS FLAVRS(R)	*	.07300	.07800

TABLE 3 — USAGE LEVELS REPORTED FOR NAS APPENDIX A SUBSTANCES (GROUP II) USED IN INFANT FORMULA PRODUCTS & BABY FOODS(2)

NAS SURVEY NO.	SUBSTANCE NAME	FDLD CATEGORY NO.	NAME	# FIRMS REPORTING	*** USUAL USE *** WTD MEAN, %	*** MAXIMUM USE *** WTD MEAN, %
0325	STARCH, MODIFIED	84	PROCSD FRUIT(B)	*	4.18493	5.25412
		89	PROCSD VEGS(B)	*	4.31502	5.25184
		90	PUDDINGS(B)	*	4.53120	6.11468
		91	SOUPS,MIXES(B)	*	3.25000	4.74000
		92	MEAT DINNER(B)	*	3.15944	4.41270
		93	CORN DINNER(B)	*	3.00159	4.93213

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
STARCH, MODIFIED NAS 0325	79/ 98	33,395,972	61,625,337	70,375,685			70,375,685

TABLE 11, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & III), 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
STARCH, MODIFIED NAS 0325	01 BAKED GOODS(R)	19	0-5 MO.	55.016760	72.816300	92.245400
			6-11 MO.	411.007560	838.195520	689.127400
			12-23 MO.	881.666300	1453.089720	1478.679500
			2-65+ YR.	2220.065030	3297.769320	3722.373200
STARCH, MODIFIED NAS 0325	03 OTHER GRAIN(R)	7	0-5 MO.	37.345050	126.973170	39.156900
			6-11 MO.	724.453970	2136.136860	759.643850
			12-23 MO.	1224.917690	2850.754790	1284.346320
			2-65+ YR.	2076.204700	4589.972140	2177.123640
STARCH, MODIFIED NAS 0325	04 FATS OILS(R)	9	0-5 MO.	8.838700	8.838700	16.476750
			6-11 MO.	49.496720	132.588500	92.269900
			12-23 MO.	111.367620	212.128800	207.607050
			2-65+ YR.	305.354500	558.605340	576.686250
STARCH, MODIFIED NAS 0325	05 MILK PRODS(R)	*	0-5 MO.	.540000	.400000	.540000
			6-11 MO.	6.240000	30.030000	6.240000
			12-23 MO.	5.450000	17.440000	5.450000
			2-65+ YR.	3.950000	12.050000	3.950000
STARCH, MODIFIED NAS 0325	06 CHEESE(R)	*	0-5 MO.	**********	1.000000	**********
			6-11 MO.	27.000000	97.000000	33.210000
			12-23 MO.	78.000000	222.000000	95.940000
			2-65+ YR.	94.000000	236.000000	115.620000
STARCH, MODIFIED NAS 0325	03 PROCSD FRUT(R)	7	0-5 MO.	103.272140	276.857280	200.359120
			6-11 MO.	1138.191040	2834.491200	2208.213260
			12-23 MO.	2210.462680	4387.968160	4268.537760
			2-65+ YR.	2595.382240	5506.383680	5043.081680

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	********** POSSIBLE DAILY INTAKE, MG. ********** (AGE)	AVERAGE	HIGH A	HIGH B
STARCH, MODIFIED NAS 0325	10 MEAT PRODS(R)	12	0-5 MO.	16.474150	43.431850	24.256540
			6-11 MO.	310.013550	835.688700	456.453980
			12-23 MO.	452.210360	777.280350	665.452280
			2-65+ YR.	1174.157800	1540.442650	1728.839760
STARCH, MODIFIED NAS 0325	11 POULTRY(R)	12	0-5 MO.	7.207050	33.520430	8.091450
			6-11 MO.	56.018990	192.378120	69.041310
			12-23 MO.	96.189060	266.163440	116.139140
			2-65+ YR.	188.005850	476.030480	228.367410
STARCH, MODIFIED NAS 0325	12 EGG PRODS(R)	*	0-5 MO.	**********	34.915100	**********
			6-11 MO.	17.457550	62.047180	25.900000
			12-23 MO.	31.423590	136.160890	46.620000
			2-65+ YR.	66.338690	240.914190	98.420000
STARCH, MODIFIED NAS 0325	13 FISH PRODS(R)	8	0-5 MO.	2.732430	8.193290	5.151600
			6-11 MO.	35.521590	133.889070	68.570800
			12-23 MO.	147.551220	368.878050	275.186400
			2-65+ YR.	338.821320	844.320870	638.798400
STARCH, MODIFIED NAS 0325	14 PROCSD VEGS(R)	18	0-5 MO.	15.213940	45.641820	20.914320
			6-11 MO.	260.810400	608.557600	358.581200
			12-23 MO.	482.816900	709.631630	902.012200
			2-65+ YR.	923.708580	1556.168720	1249.798000
STARCH, MODIFIED NAS 0325	15 CONCN RELSH(R)	5	0-5 MO.	**********	.935690	**********
			6-11 MO.	7.425520	20.565180	7.496060
			12-23 MO.	26.199320	71.112440	26.243280
			2-65+ YR.	82.340720	198.366250	82.478880
STARCH, MODIFIED NAS 0325	16 SOFT CANDY(R)	10	0-5 MO.	16.575100	165.751000	23.854660
			6-11 MO.	182.326100	563.553400	262.401260
			12-23 MO.	290.066250	770.742150	417.456550
			2-65+ YR.	480.677900	1458.500000	691.745140
STARCH, MODIFIED NAS 0325	17 CONF FROST(R)	6	0-5 MO.	**********	2.384640	**********
			6-11 MO.	2.384640	4.769280	9.824340
			12-23 MO.	4.769280	16.692480	19.648650
			2-65+ YR.	7.153920	19.077120	29.473020
STARCH, MODIFIED NAS 0325	20 GELATIN PUD(R)	17	0-5 MO.	60.467200	81.630720	102.128200
			6-11 MO.	386.990080	1173.068480	653.620480
			12-23 MO.	417.223680	1815.848960	704.684580
			2-65+ YR.	614.765440	1587.264000	1041.707640

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
STARCH, MODIFIED NAS 0325	21	SOUPS(R)	7	0-5 MO.	2.440720	18.305400	2.930900
				6-11 MO.	284.343880	887.201720	341.449850
				12-23 MO.	424.885290	1172.765960	509.976600
				2-65+ YR.	386.854120	1031.284200	464.547850
STARCH, MODIFIED NAS 0325	22	SNACK FOODS(R)	5	0-5 MO.	**********	1.814930	**********
				6-11 MO.	7.259720	19.964230	14.682080
				12-23 MO.	19.964230	56.262830	40.375720
				2-65+ YR.	23.594090	67.153410	47.716760
STARCH, MODIFIED NAS 0325	25	NUT PRODS(R)	*	0-5 MO.	**********	2.400000	**********
				6-11 MO.	44.400000	160.800000	44.400000
				12-23 MO.	32.400000	100.000000	32.400000
				2-65+ YR.	62.400000	186.000000	62.400000
STARCH, MODIFIED NAS 0325	26	RECONST VEC(R)	*	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	.000000	.000000	.000000
				12-23 MO.	.000000	.000000	.000000
				2-65+ YR.	2.720000	8.160000	5.000000
STARCH, MODIFIED NAS 0325	27	GRAVIES(R)	21	0-5 MO.	1.703490	5.110470	2.452600
				6-11 MO.	23.846360	66.436110	37.136400
				12-23 MO.	61.325640	173.755980	95.493600
				2-65+ YR.	141.385670	362.843370	220.165800
STARCH, MODIFIED NAS 0325	29	IMIT DAIRY(R)	4	0-5 MO.	.000000	.000000	.000000
				6-11 MO.	54.763660	89.967260	56.376040
				12-23 MO.	31.292460	132.995080	32.214860
				2-65+ YR.	35.204580	58.674300	36.241740
STARCH, MODIFIED NAS 0325	31	CHEWING GUM(R)	10	0-5 MO.	**********	**********	**********
				6-11 MO.	.425520	.425520	.459750
				12-23 MO.	.425520	1.276560	.459750
				2-65+ YR.	.851040	1.702080	.919500
STARCH, MODIFIED NAS 0325	48	SEAS FLAVES(R)	*	0-5 MO.	**********	**********	**********
				6-11 MO.	**********	.007300	**********
				12-23 MO.	**********	.014600	**********
				2-65+ YR.	.007300	.036500	.007800
STARCH, MODIFIED NAS 0325	84	PROCSD FRUT(B)	*	0-5 MO.	2709.253800	6452.949960	3469.039200
				6-11 MO.	2783.142540	8764.025550	3563.649360
				12-23 MO.	689.628240	2885.765790	883.028160

TABLE 13, PART A -- POSSIBLE DAILY INTAKES OF NAS APPENDIX A SUBSTANCES (GROUPS I & III), 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	POSSIBLE DAILY INTAKE, MG. (AGE)	AVERAGE	HIGH A	HIGH B
STARCH, MODIFIED NAS 0325	89 PREPD VEGS(B)	*	0-5 MO.	1156.438780	2843.631130	1407.278720
			6-11 MO.	832.828810	2718.494100	1013.490720
			12-23 MO.	50.616470	151.027450	110.271860
STARCH, MODIFIED NAS 0325	90 PUDDINGS(B)	*	0-5 MO.	357.964800	1209.516800	483.059720
			6-11 MO.	439.524400	1626.770800	593.123550
			12-23 MO.	122.342400	140.467200	165.059360
STARCH, MODIFIED NAS 0325	91 SOUPS,MIXES(B)	*	0-5 MO.	48.750000	195.000000	71.400000
			6-11 MO.	100.750000	266.500000	147.560000
			12-23 MO.	45.500000	149.500000	66.640000
STARCH, MODIFIED NAS 0325	92 MEAT DINNER(B)	*	0-5 MO.	31.594400	126.372400	44.287600
			6-11 MO.	230.640500	916.243400	323.300940
			12-23 MO.	18.956760	60.629740	36.573680
STARCH, MODIFIED NAS 0325	93 CORN DINNER(B)	*	0-5 MO.	890.365700	2353.241560	1134.389900
			6-11 MO.	1534.007720	4271.262870	2509.822040
			12-23 MO.	465.248450	1770.938100	764.480150
STARCH, MODIFIED NAS 0325	ALL CATEGORIES **********	99	0-5 MO.	5322.274410	14211.647650	7148.973780
	**********		6-11 MO.	9542.574120	29451.775850	14340.086930
	**********		12-23 MO.	8403.996790	20060.689150	12945.774480
	**********		2-65+ YR.	11834.145360	24243.755550	18205.442270

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ADAPTATION OF THE EXOCRINE SECRETION OF RAT PANCREAS TO THE COMPOSITION OF THE DIET

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We reinvestigated last year (Desnuelle, Reboud and Ben Abdeljlil, 1962 ; Reboud, Ben Abdeljlil and Desnuelle, 1962) by more reliable techniques the early postulations of Grossman et al. (1943, 1944) according to which the composition of the diet induces statistically significant variations of the specific activities of several exocrine enzymes in rat pancreas. As a matter of fact, specific activities in fresh pancreas homogenates were found high for amylase when the animals received a starch-rich diet, and high for chymotrypsinogen when a casein-rich diet was given. Lipase behaved differently. It was low on the casein-rich diet and high on the others.

The case of amylase, chymotrypsinogen and other proteolytic enzymes of pancreas seems to represent a very interesting example of enzyme adaptation in mammals. It has now been investigated further by statistic and kinetic methods on rat pancreas homogenates and pancreatic juices.

In a first series of experiments, two groups of male, adult rats of the Wistar strain were fed ad libitum, respectively, on a starch-rich diet (starch, 75 % ; casein, 15 % ; corn oil, 4 % ; Osborne's salts, 4 % ; cellulose, 2 % by weight) and a casein-rich diet (starch, 20 % ; casein, 70 % ; oil, 4 % ; salts, 4 % ; cellulose, 2 %). Both diets were supplemented with the necessary vitamins. At the end of the experiments, all animals had an heal

ny appearance and their weights were normal. On an average, the weight of pancreas and protein output per hour in pancreatic juice were the same in both groups.

After about 25 days, a Bollmann's fistula w placed on each animal. After an initial period of about 12-15 h., during which the secretion started again, bile was washed out of the cannula and the composition of the juice became constant, samples of pure pancreatic juice were collected in the cold for about 20 h. Samples showing turbidity or containing free trypsin activity were discarded. In the others (at least 10 for each group), specific activities of amylase, chymotrypsinogen and trypsinogen were determined by published techniques (Reboud et al., 1962). The average value for amylase activity was found to be about 2-3 times higher in the first group than in the second. The values for chymotrypsinogen and trypsinogen were, respectively, 2-3 times and 1.5-1.7 times higher in the second group than in the first. The differences were fully significant at the $p \leq 0.01$ level.

The close parallelism between results obtained with homogenates and juices confirms in the first place that the diet exerts a significant influence on the composition of pancreas exocrine secretion. It further shows that the diet does not affect the relative rates of excretion of the enzymes into the juice, but actually governs the activity or number of enzyme molecules in pancreatic tissue and juice. Chromatography of rat juice in diethyl-amino-ethyl and carboxy-methyl cellulose columns demonstrates that the amount of amylase and chymotrypsinogen, rather than their activity, is modified by the diet (Marchis-Mouren, Paséro and Desnuelle, unpublished experiments).

The kinetic aspects of the variations were investigated in a second series of experiments when groups of rats fed for about a month on a given diet (starch-rich or casein-rich) were suddenly

fed on the other. After periods ranging from 1 to 30 days, the specific activities of amylase, chymotrypsinogen, trypsinogen and procarboxypeptidase B were determined in mixtures of five pancreas or three juices. Several pancreas or juices were used each time in order to lower the fluctuations around the average. Juices were mixed in proportions inversely proportional to their protein content. Fig. 1 shows the results of a typical experiment on juices. Results obtained with homogenates are quite similar.

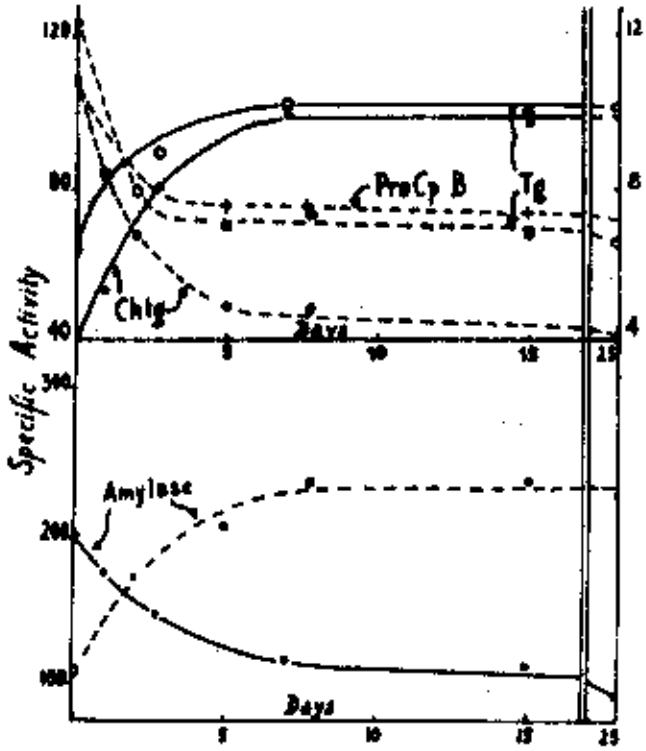

Fig. 1. Kinetics of enzyme adaptation in rat pancreas juice.

Specific activities (μ-equivalents/min./mg total protein) of four enzymes (chymotrypsinogen (ChTg) and amylase, on the left ; procarboxypeptidase (Pro Cp B) and trypsinogen (Tg), on the right are determined as a function of time in pancreatic juices of rats stabilized on a given diet (starch or casein-rich) and suddenly fed on the other at the start of the experiment. Solid and dashed lines indicate, respectively, the changes occuring when the starch rich diet is replaced by the casein-rich or, on the contrary, when the casein-rich diet is replaced by the starch-rich.

The following comments can be made concerning Fig. 1 : (1) The adaptation to the new diet appears to start immediately or almost immediately. After one day, significant variations of the enzymatic levels are already detectable. (2) These variations occur for the main part within 3-5 days and become almost negligible after about 8 days. Similar kinetics have been found for the adaptation of urea cycle enzymes in rat liver when the protein content of the diet is varied (Schimke, 1962). (3) The new levels attained by the four pancreatic enzymes at the end of the adaptation period are quite stable for several weeks and reproducible within the limits of individual fluctuations and experimental errors. When experiments are performed in a given direction (for example, from a starch-rich to a casein-rich diet), the final levels reached by the four enzymes studied are very close to the initial levels found in the other direction.

Grossman et al. (1962) had pointed out that starch could be replaced by dextrose in a "balanced" diet without disturbing the enzymatic pattern which corresponds in pancreas to this kind of diet. We found that the kinetic curves obtained for amylase, chymotrypsinogen and trypsinogen by substituting to the casein-rich diet, either the starch-rich diet or a diet containing an equivalent amount of dextrose (75 % by weight) are very similar. This fact suggests that blood dextrose may be at least one of the factors determining the amount of amylase in pancreas and pancreatic juice.

Experiments designed for confirming this hypothesis and establishing a possible correlation between enzymatic levels in pancreas and rates of biosynthesis of the corresponding enzymes are in progress.

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STARCH GASTROLITH*

Report of a Case of Obstruction

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THE term "bezoar" was derived from either an ancient Arabian or Persian word meaning "antidote." Concretions from the alimentary canals of animals were thought to have medicinal properties, and they were frequently used as an antidote for poison and protection from pestilential diseases. When worn as charms, they supposedly warded off evil spirits and were thus more precious than gold even up to the eighteenth century. Syrian and Persian goat stomachs were the main source of the oriental bezoar, which consisted of cholesterol, bile pigments and salts. The less highly prized occidental types, composed of calcium and magnesium phosphate, came from vicuña sheep.[1] They were still being sold in the United States in the 1920's under the colorful name of "mad stone."[2]

Bezoars are best defined as concretions built up in the stomach from repeated swallowing of an indigestible material that fails to pass the pylorus in a reasonable length of time. These are a rare occurrence in man, and less than 500 cases have been reported in the medical literature. The first such case, described by Baudamant[3] in 1779, was a trichophytobezoar. They are generally classified into three groups, depending upon their principal constituent: *trichobezoar* (hairball); *phytobezoar* (ball of plant fibers); and *gastroliths* (concretions of various chemical substances). A survey by De Bakey and Ochsner[3] found them to occur in the following frequency: trichobezoars, 56 per cent; phytobezoars, 40 per cent; and gastroliths (concretions), 4 per cent. Occasionally, there are combinations.

Gastroliths have been found in association with or in the skeletons of dinosaur fossils, primarily the cretaceous marine plesiosaurs of the Western States.[4] It is not known whether these reptiles had a gizzard like that of present-day birds, which use swallowed stones to aid in trituration of food. Instead, they may have had a powerful digestive juice, similar to that of the crocodile, capable of dissolving even the bones and other resistant parts of their prey.[5,6]

In man the occurrence of gastroliths, like that of other bezoars, is the result of a bizarre habit. The most frequently reported cases have been due to shellac, found in the stomachs of painters and employees in furniture factories who have taken furniture polish (a strong alcoholic solution of shellac) as a stimulant.[4] Pyloric obstruction due to a mass of asphalt has also been reported.[7] A 40-gm. gastrolith of pure bismuth carbonate was found at operation twenty-three days after its use for roentgenographic study,[8] and other cases of bismuth concretions have been reported as a rare complication of Sippy's method for ulcer treatment.[9] Recently, Wolf and Bruce[10] reported a bezoar due to the excessive ingestion of lactose in an eighteen-day-old infant.

Starch eaters are found chiefly in rural Southern communities among Negro females and may be a version of pica (dirt eating). The slaves, when depressed from the rigorous treatment of their masters or other causes, acquired the habit of dirt eating. Betten, in 1687, and Craigin, in 1835, reported pica as an obsession permitting the mind no rest until it is satisfied.[11] Postell[12] reviewed the anxiety of planters whose slaves, addicted to dirt eating, showed utmost craftiness and determination in pursuing the habit in spite of punishment and restraint. Edwards et al.,[13] in 1959, presented a dietary study of 42 clay eaters and 23 starch eaters, among whom they found a

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significantly lowered intake of calcium, iron, thiamine and niacin as compared to a control group. The starch consumption in their series ranged from 15 to 907 gm. (2 pounds) a day, usually of a preferred brand. Since meals were frequently omitted, the caloric intake was lower.

In 1950 Ferguson and Keaton[13] reported a study of the geography and amylography in 391 pregnant females in rural Mississippi. Their propensity to dirt eating paralleled that to ingestion of starch, for 25 per cent were clay eaters and 39 per cent ate laundry starch, beginning with the onset of their pregnancy. Merkatz[14] recently described parotid enlargement from the ingestion of a full box of starch daily over the period of a year. A study by Sage[15] has shown that the ingestion of one or more boxes of starch daily results in a hypochromic, microcytic type of anemia suggesting an iron deficiency.

Case Report

G.G., an 18-year-old housemaid, was admitted to the Noble Hospital in Westfield, Massachusetts, because of an acute condition of the abdomen. She had been in excellent health except for a previous episode of acute abdominal pain 3 months previously while living in North Carolina. At that time her physician diagnosed the condition as "female trouble." Subsequently, she remained well until the morning of admission, when severe, crampy pain developed across the upper and middle quadrants of the abdomen. This continued during the day and became associated with nausea and vomiting. She reported that her bowel movements had been regular until the morning of admission, when the stools were described as "watery." There was no history of previous abdominal surgery or disease.

Physical examination in the emergency room showed a well developed and well nourished woman who was writhing about on a stretcher and moaning while holding her abdomen during the abdominal "cramps." The bowel sounds were infrequent and somewhat high pitched, occurring as rushes about 3 times per minute. She was very frightened, and full co-operation was difficult to obtain. The abdomen was not distended, and palpation revealed generalized tenderness, with some localization to the middle quadrant, without rebound. No masses, glandular enlargement, hernia or previous surgical scars were noted.

The temperature was 98.8°F., the pulse 100, and the respirations 24. The blood pressure was 140/90.

A plain film of the abdomen revealed a large, irregular, radiopaque density in the left upper quadrant that filled a portion of the stomach (Fig. 1). No abnormal distention of the small or large bowel was noted. The patient was then asked whether or not she had ingested anything that might have contained barium or bismuth, and she gave a history of eating 3 or 4 boxes of laundry starch daily for the past year. She had decided to stop eating the starch 1 week before this acute episode because her employer was beginning to wonder why all the starch was disappearing. Careful questioning revealed only that she liked the starch better than ice cream and that many other women in her area of the South also ate it. She was asked whether or not she thought that the starch might make her lighter skinned, but she denied this as a reason.

The hemoglobin was 10.5 gm. per 100 ml., and the hematocrit 38.7 per cent. The red cells were hypochromic, and the platelets appeared increased. The white-cell count was 7300, with 53 per cent neutrophils, 2 per cent eosinophils and 41 per cent lymphocytes. Urinalysis showed a ++ test for glucose and a +++ test for protein, with a trace of acetone; microscopical examination revealed 0 to 1 red cells and 4 to 6 white cells per high-power field, with an occasional fine granular cast, many bacteria and a few amorphous urates. Subsequent urinalysis failed to reveal a positive glucose test, and the protein was reduced to a trace. The fasting blood sugar was 115 mg. per 100 ml. The serum amylase on the day after admission was 18 Bodansky units. The nonprotein nitrogen was 19 mg. per 100 ml., and a sickle-cell preparation was negative. The stools gave negative tests for ova and parasites, but contained an enormous amount of starch granules.

The patient was treated with intravenous infusions of fluids and sedation for the 1st 36 hours, when liquids were allowed. There was gradual improvement in her condition after the first 24 hours, but she continued to have intermittent abdominal pain. Forty-eight hours after admission most of the starch had moved out of the stomach, and a small amount of barium was administered under fluoroscopy. The esophagus showed no delay or obstruction. There was a considerable lack of distensibility of the distal half of the stomach. The small bowel at this time showed segments that were somewhat dilated. Eight days after admission a plain film revealed only a minute amount of the radiopaque substance in the pelvic region, without evidence of any obstruction of the large or small bowel. Clinically, the patient took 5 days to become free of abdominal symptoms.

The final impression was gastric obstruction due to a starch gastrolith and subsequent partial small-bowel obstruction. There was an incidental finding of hypochromic anemia.

One year after discharge from the hospital the patient had been able to break the habit of eating starch. She had had no other abdominal symptoms, and the hemoglobin had risen to 14.5 gm. per 100 ml. without any specific treatment. Urinalysis continued to show a trace of protein. After this episode a sister still living in the South was reported to have cut her intake of starch down to a small amount.

Discussion

The granules of laundry starch are not significantly metabolized in the human digestive system and are insoluble, which readily explains the formation of a thick, pasty starch "cake" when starch is taken in excessive amounts. The outer surface of the "cake" is relatively impermeable to fluid and would slow

Figure 1. *Plain Film of the Abdomen Taken on Admission.*

hydrolysis by the gastric juice. The surprising and apparently unknown fact that such a "cake" is radiopaque is more difficult to ascertain. However, according to the manufacturer, since laundry starch does form into a dense, amorphous mass, it will take on a crystalline nature, causing a certain amount of scattering and refracting of the x-ray beam.

The bizarre habit of starch eating has not been fully explained and at present can only be regarded as a "craving" among certain ethnic groups and largely in certain areas. There is apparently a relation to pica, from the Latin word for the magpie, which collects assorted and useless objects. The manufacturer of the starch ingested by the present patient has stated that a lump of starch on the tongue gives a slightly sweet sensation as it disintegrates and that this is due to the small amount of solubles and low molecular weight of the fractions present. Cases have been reported of starch eaters in whom an intestinal flora subsequently developed that produced alcohol, causing a continuous drunken state for the patient.

The treatment of bezoars has been both surgical and expectant, depending on the type of bezoar and the probability of its disintegrating spontaneously in the stomach, as in the case presented above. Newer therapy includes the use of enzymes on the phytobezoars, and one can speculate whether or not lavaging the stomach with a weak hydrochloric acid solution could have hastened the treatment in this case.

Summary

An unusual case of gastric obstruction due to the excessive ingestion of laundry starch is presented, and the literature concerning this curious custom has been reviewed. It is suggested that the treatment of a gastrolith (concretion) should depend on the type of material of which it is made.

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Effect of Carbohydrate Source on Serum and Hepatic Cholesterol Levels in the Cholesterol-Fed Rat (33679)

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Modified food starches are used widely in the food industry because of their special cold stability and resistance to syneresis. This stability is achieved through the selection of starches high in amylopectin content (usually waxy maize or sorghum) which are then chemically treated to cross-link the starch polymer.

Several review articles emphasized that the consumption of complex carbohydrates in both man and experimental animals is usually accompanied by a slight but statistically significant decrease in the concentrations of cholesterol and lipid in the blood whereas a high intake of simple sugars has the opposite effect (1–3). There is not complete agreement, however, on the comparative effects of starch vs sucrose feeding since there are reports that starch feeding is not followed by reductions in serum cholesterol in man (4) and that starch-fed rats actually had higher serum and hepatic cholesterol levels than sucrose-fed controls (5). No published information is available on the influence of modified food starches on serum or hepatic lipid levels in man or experimental animals. The purpose of the present study was to evaluate the comparative effects of sucrose and complex carbohydrates in the form of modified food starches on serum and hepatic lipids in the cholesterol-fed rat.

Methods. Six groups of 10 male weanling rats each of the Harlan-Wistar strain were fed the basal ration shown in Table I. The carbohydrate portion of the various rations was supplied from the following sources:

TABLE I. Basal Ration.

Item	(%)
Carbohydrate	67.8
Vitamin-free casein	18.0
Soybean oil	7.0
Salt Mixture USP XIV	4.0
Vitamin mix*	2.2
Cholesterol USP	1.0
Total	100.0

* Vitamin diet fortification mixture, Nutritional Biochemicals Corporation.

Ration 1, sucrose; Ration 2, corn starch; Ration 3, a modified waxy maize starch;[1] Ration 4, a modified corn starch;[2] Ration 5, a modified tapioca starch;[3] and Ration 6, an experimental modified white milo starch.[4]

Two separate experiments were conducted, the first fed cooked starches and the second fed starch in the raw form. The cooked starches were prepared by making a 10% slurry of raw starch and water, then steam-injecting this suspension at 208°F. The gelatinized starch was drum dried at 245°F and 20 lb of steam pressure to an average moisture content of approximately 5%. The cooked, dried starches were milled through an 0.033-in. screen to obtain a particle size comparable to sucrose.

The rats were individually caged and fed the assigned rations *ad libitum* for 4 weeks. At the end of the 28-day feeding period all rats were quickly killed in random order with a Harvard model 130RM decapitator. Blood serum and livers were collected for further analysis. Total serum and hepatic cholesterol were determined according to the method of Mann (6). Total hepatic lipid was determined gravimetrically by evaporating a 2:1 chloroform–methanol extract of liver tissue to dryness after Folch washing the extract (7). The data were subjected to an analysis of variance as outlined by Snedecor (8).

Results and Discussion. Every effort was made to standardize as closely as possible the conditions under which the two experiments were conducted. The various treatments were assigned the same cage positions and feeding, lighting, watering, and temperature conditions were identical in each experiment.

The effect of carbohydrate source on serum and hepatic lipids is presented in Table II. It was postulated before this study was undertaken that modified food starches being cross-linked polymers might be less easily absorbed than unmodified starch from the same source, therefore might act similarly to mucilaginous polysaccharides by inhibiting cholesterol absorption (10). The data show that the feeding of complex carbohydrates did not result in a lowered serum or hepatic cholesterol level. The findings reported here are in close agreement with those of Guggenheim *et al.* (5) who observed increases in serum and hepatic cholesterol levels in starch-fed rats compared with sucrose-fed control animals.

When the data from Expt. I were analyzed and it was found that serum and hepatic cholesterol were significantly elevated by the feeding of complex carbohydrates in the form

TABLE II. Effect of Carbohydrate Sources on Serum and Hepatic Lipids in the Cholesterol-Fed Rat.

	Serum cholesterol (mg/100 ml)		Total hepatic lipid (%)		Hepatic cholesterol (mg/g)	
Ration	I[e]	II[f]	I	II	I	II
1	83[ab]	80[a]	7.2	7.8	11.9[a]	10.3[a]
2	84[ab]	126[abc]	9.2	12.0	16.0[a]	24.5[b]
3	94[bc]	165[c]	8.2	12.7	28.6[bc]	41.4[cd]
4	104[bc]	144[bc]	9.3	15.0	23.3[bc]	41.7[d]
5	114[c]	103[bc]	9.5	13.6	28.7[c]	39.2[cd]
6	101[bc]	156[bc]	9.3	15.1	22.5[bc]	38.5[cd]
Mean	97	140	8.8	12.7	21.8	32.6
SE of mean	±7.0	±13.2			±3.24	±4.10

[e] Expt. I. Steam-injected, drum-dried starch.
[f] Expt. II. Raw starch.
[a] Vertical values not followed by the same letter are significantly different ($p < 0.01$) using Duncan's Multiple Range Test (9).

[1] National Starch and Chemical Corporation, Plainfield, New Jersey.
[2] A. E. Staley Co., Decatur, Illinois.
[3] National Starch and Chemical Corporation, Plainfield, New Jersey.
[4] Corn Products Company, Argo, Illinois.

of cooked, modified food starches, the second experiment was undertaken to establish whether or not the anomalous results of the first experiment could be explained as due to the physical form in which the various starches were fed. This possibility was suggested by Lees (4) who found that the response of plasma cholesterol concentrations to high carbohydrate diets was identical whether his patients were fed sugar or cooked starch of wheat or rice origin. The results of the second experiment do not support the theory advanced by Lees (4) that reports showing a cholesterol lowering effect with starch may be due to the fact that the starch used was uncooked and therefore poorly absorbed.

Under the specific experimental conditions described herein, it was demonstrated that feeding complex carbohydrates in the form of modified food starches, whether raw or cooked, results in a statistically significant elevation in serum and hepatic cholesterol levels, compared either with the sucrose-fed controls or rats fed unmodified corn starch. Serum cholesterol levels of rats fed unmodified corn starch were not significantly different from the sucrose-fed controls in either experiment although the elevation in serum cholesterol noted in rats fed Ration 2 closely approached statistical significance in the second experiment.

Total hepatic lipid concentrations were also markedly increased in all the starch-fed rats but statistical significance could not be assigned to this observation since these determinations were made on pooled liver samples from each treatment group.

The increases in serum and hepatic cholesterol and total hepatic lipid in the rats fed complex carbohydrates were much more pronounced when the ration was composed of raw starch. The reason for this finding is not clear. If raw modified starches are not digested as easily as cooked starches one would assume that this might in turn have an inhibitory effect on cholesterol absorption. Changes in intestinal flora or pH could have a bearing on the different levels of hepatic or serum cholesterol noted between Expts. I and II.

The observed elevation in serum and hepatic cholesterol levels associated with starch feeding is markedly at variance with the studies of Portman *et al.* (11) who fed 250-g rats rations containing (in percent) casein, 20; corn oil, 8; carbohydrate, 56; cholesterol, 5; salts, 4; cholic acid, 1.5; and vitamins and trace minerals. No body weight gain data were included in Portman's paper, but rations containing these high levels of cholesterol and cholic acid, in our experience, have had a very depressing effect on performance and for this reason are not considered to be justified when evaluating the physiological significance of a particular ration ingredient.

Staub and Thiessen (12) recently reported that rats the same age as those used by Portman *et al.* (11) and fed diets containing 1% cholesterol, 0.5% cholic acid, and pregelatinized starch as the carbohydrate source had serum cholesterol levels significantly lower than the sucrose-fed control animals. No hepatic lipid or cholesterol data were included in their paper. These authors also fed a basal ration with 24% hydrogenated vegetable oil, considerably higher than the 7% level used in the modified starch diets.

Guggenheim *et al.* (5) fed 50-g rats rations which were very similar to those chosen for the study reported here (in percent) casein, 18; carbohydrate, 73; soya oil, 5; salt mixture, 4; and vitamins. One and one-half percent cholesterol and/or 0.5% cholic acid were added to this basal ration. Serum and hepatic cholesterol values were reported for each treatment group. The addition of cholic acid to the ration was necessary before even a moderate depression in serum cholesterol could be demonstrated with starch feeding. Hepatic cholesterol levels were consistently higher in the starch-fed rats regardless of whether cholesterol or cholic acid were fed alone or in combination. Total hepatic lipid was also significantly elevated in all starch-fed animals.

It is apparent that the experimental design will have a considerable influence on the comparative effects of sucrose and complex

carbohydrates on serum and hepatic lipid levels. The age, species, and particular strain of experimental animal chosen, whether or not the ration contains cholesterol and/or cholic acid, the level and physical form of carbohydrate in the ration, and even the duration of the feeding study as brought out by Fillios *et al.* (13) can all have a bearing on the experimental results.

It is suggested that the addition of cholic acid to diets used in evaluating the comparative effects of simple vs complex carbohydrates on cholesterol metabolism places an unwarranted physiological stress on the experimental animal which makes extrapolation of the data to the "normal" situation particularly hazardous. Even the addition of 1% cholesterol to the diet represents stress of unknown magnitude. Certainly no one would advocate that human subjects consume a diet consisting, in part, of 5% cholesterol and 1.5% cholic acid to evaluate the effect of carbohydrate source on lipid metabolism, yet many animal feeding studies include variables which make it difficult to ascertain the practical significance of the data obtained. Hence, the generally accepted opinion that starch per se has a cholesterol-lowering effect in rats without first defining carefully the experimental protocol under which this effect was observed.

Under the specific experimental conditions imposed in this study, a marked increase in serum and hepatic cholesterol levels was observed in rats fed modified starches. This effect was more pronounced when these starches were fed in raw form. Obviously complex carbohydrates which have been modified by various chemical processes are not metabolized in a manner identical to their unmodified counterparts. It also follows that two starches from the same source (i.e., waxy maize) which have been modified by different chemical treatments may have markedly differing physiological effects upon lipid metabolism. It is not possible to make any inferences from this study as to what effect modified food starches would have on cholesterol metabolism in man but the question is an intriguing one.

Summary. The comparative effect of sucrose and complex carbohydrates in the form of corn starch and four different modified food starches was studied in the cholesterol-fed rat. Two separate experiments were conducted, one in which the starch was fed in the cooked form, the second in which the starch was fed raw. Sucrose-fed rats had significantly lower serum and hepatic cholesterol levels ($p<0.01$) and total hepatic lipid than any of the animals fed modified food starches. This finding held true regardless of whether the starch was cooked or raw; however, cholesterol and lipid levels were markedly higher when raw starch was fed. Feeding unmodified corn starch resulted in serum and hepatic cholesterol levels which were significantly lower than those observed with modified food starch, but not significantly higher than the sucrose-fed controls with the exception of hepatic cholesterol which was significantly elevated when raw corn starch was fed.

This feeding study was conducted at the Bushy Run facilities of Mellon Institute. The author is indebted to Mr. Carrol S. Weil, Senior Fellow, for randomizing the treatment groups and to Murray Woodside for care of the animals. A special note of thanks is due Dr. Raymond D. Bowman for performing the chemical analyses.

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STUDIES ON INTESTINAL DIGESTION OF STARCH IN MAN. II. INTESTINAL HYDROLYSIS OF AMYLOPECTIN IN INFANTS AND CHILDREN

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STARCH is a mixture of two different polyglucoses, amylose and amylopectin. Amylose has a linear structure and is composed of glucose units which are linked by 1,4α glucosidic bonds (maltose units). Amylopectin has a branched structure similar to glycogen; in amylopectin the majority of the glucose units are connected by 1,4α glucosidic bonds (maltose units), but there are also 1,6α glucosidic bonds at the branch points (isomaltose units).

Starch is digested into glucose by salivary and pancreatic amylases and by the glucosidases of intestinal mucosa.

In vitro the α amylase of human saliva hydrolyzes amylose into maltose and maltotriose rapidly,[1] and maltotriose into glucose and maltose very slowly.[2-4] *In vitro* the same enzyme splits amylopectin into glucose, maltose, maltotriose, and a mixture of branched dextrins,[5-8] the smallest being a tetraglucose.[9]

In the human intestinal mucosa five maltases (one of which also has isomaltase activity)[10-12] and one glucoamylase[13] were identified; the further hydrolysis into glucose of the products of the α-amylolysis of starch *in vivo* is probably due to the activity of these mucosal glucosidases.

There is only one study on intestinal digestion of starch in man *in vivo*. After a test meal containing soluble starch the average number of glucose units of intestinal carbohydrates was measured in three adults and found to range between 2.1 and 2.4.[14]

The present report describes studies on the carbohydrates which accumulate in the duodenum of normal infants and small children after a test meal containing amylopectin.

MATERIAL AND METHODS

Subjects

Nine normal infants and four normal small children between 2½ and 28 months were studied. The infants were artificially fed with powdered milk to which dextri-maltose and, from the beginning of the fourth month, 4 gm of flour per kg of body weight were added. Starchy foods were given from the end of the fourth month.

A 26-month-old child, D.F., affected by exudative enteropathy of unknown etiology and successfully treated the last 3 months by prednisolone, was also examined.

Composition of the Test Meal

The test meal contained amylopectin (about 9 gm/100 ml), casein (4 gm/100 ml), and vegetable oil (4 gm/100 ml) and is prepared by dissolving, without heat, 50 gm of amylopectin in 500 ml of water by shaking for 60 minutes. The amylopectin solution is centrifuged and the sediment discarded. The supernatant contained about 90 mg/ml of carbohydrate, measured as glucose after acid hydrolysis. Casein was dissolved in the amylopectin solution by shaking for 90 minutes. Just before administration vegetable oil was added slowly to the test meal under homogenization with the Ultra Turrax; then the meal was further homogenized for 30 minutes.

In the present paper the carbohydrate content is expressed as glucose, which was obtained after hydrolysis of the polysaccharide or oligosaccharides.

Intubation Technique and Sampling

The test meal (500 ml/m²) was given by a tube situated in the stomach, when the

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subjects had fasted for 10 to 12 hours; during this time water was given *ad libitum*. The duodenal juice was continuously sampled for 2 to 4 hours after the test meal by a rubber tube (internal diameter 2 mm) situated at the end of the duodenum; the position of the tube was checked by radiography.

In order to prevent further hydrolysis of the carbohydrate during the collection, the juice was directly collected into a vessel maintained in a freezing solution of dry ice and acetone.

The duodenal juice was then rapidly homogenized at 4°C. After withdrawing a sample for assay of amylase activity, the rest was heated at 100°C for 5 minutes to inactivate the amylase.

Assay of Amylase Activity

The amylase activity was determined according to the method of Dahlqvist[15]—incubation time at 37°C:30 minutes. One unit of amylase activity liberates reducing groups corresponding to 1 μmole of maltose/minute.

Determination of Average Degree of Polymerization

The duodenal juice after heating was used for: (1) determination of the total carbohydrates after hydrolysis of dextrins into glucose (expressed as mole of glucose), (2) determination of the reducing power before hydrolysis, compared with a standard maltose curve (expressed as mole of maltose). The ratio between a and b is the average degree of polymerization (average DP), of the intestinal carbohydrates.[16]

Determination of the Total Carbohydrates as Glucose Liberated by Hydrolysis

The heated juice was deproteinized in either of two ways:

1. By tungstic acid[17]—1 ml of duodenal juice was added with 1 ml of 10% sodium tungstate and 8 ml of $\frac{1}{12}$N sulfuric acid. After 30 minutes at 4°C, the mixture was centrifuged at 27,000 g for 10 minutes. The sediment was washed with 8 ml of $\frac{1}{12}$N sulfuric acid and centrifuged. The supernatants were combined, neutralized with NaOH (phenolphthalein),[18] and diluted with water to 25 ml.

2. By chloroform[19]—1 ml of duodenal juice was added with 0.25 ml of chloroform and 0.1 ml of amyl alcohol and the mixture was shaken at 4°C for 30 minutes, then centrifuged at 3,000 g for 60 minutes. The chloroform layer containing the protein was washed twice with 1 ml of water; then the aqueous layers were combined and once more deproteinized with chloroform. The small amounts of chloroform present in the aqueous solution were eliminated by evaporation under diminished pressure at 60°C after neutralization with NaOH (phenolphthalein). The deproteinized solution was then diluted with water to 25 ml.

After deproteinization by tungstic acid or chloroform the carbohydrates were hydrolyzed into glucose by sulfuric acid,[16] for 2 hours. The liberated glucose was measured as reducing power by the titrimetric methods of Somogyi[20] or Shaffer and Hartmann;[21] in the latter method glucose (60 mg/l) was added to the reagent.[22]

In some experiments the total carbohydrates were also determined by two other methods, in which the duodenal juice was deproteinized after enzymatic hydrolysis of the carbohydrates. In one method the oligosaccharides were first directly hydrolyzed into glucose by the glucoamylase of Rhizopus Delemar, and then the glucose was determined with the glucose oxidase reagent after deproteinization by $Zn(OH)_2$.[23] In the other method the oligosaccharides were first hydrolyzed by pancreatic amylase, then deproteinized by phosphotungstic acid and hydrolyzed into glucose by hydrochloric acid; the glucose was determined with the glucose oxidase reagent.[23,24]

Determination of the Reducing Power of Intestinal Carbohydrates Before Hydrolysis

After deproteinization of the duodenal juice by tungstic acid[17] or chloroform[19] the reducing power of the carbohydrates was

determined by titrimetric methods[20,22] and compared with a standard maltose curve.[14] The heating time with the reagent was 60 minutes in order to develop full reducing power.[6]

In some experiments the reducing power was measured without deproteinization with the 3:5-dinitrosalicylate reagent.[14]

Fractionation of the Carbohydrates in Duodenal Juice

The carbohydrates of duodenal juice were first fractionated by gel filtration on Sephadex G 50;[8] the oligosaccharides with an average DP less than 7 were further separated by chromatography on charcoal celite column.[23]

Gel Filtration on Sephadex G 50: The duodenal juice was deproteinized first by tungstic acid[17] and then by chloroform[18]. The neutralized solution, which contains about 1.5–2.5 gm of total carbohydrates, was concentrated to 25 ml under diminished pressure at 60°C. After centrifugation the sample was applied to a Sephadex G 50 column (3 x 125 cm) and the chromatogram developed with water. Flow rate was about 60 ml/hour. Volume of each fraction was 5 ml.

In order to determine the average DP of the carbohydrates in the chromatographic fractions total carbohydrates were measured by phenol-sulfuric acid[20] and reducing power by the colorimetric reagent of Somogyi-Nelson (heating time 60 minutes; maltose standard curve).[27]

The fractions containing carbohydrates with an average DP less than 7 were combined, concentrated to 20 to 25 ml under diminished pressure at 60°C and then utilized for charcoal chromatography.

Charcoal-Celite Chromatography: The charcoal-celite column (4.2 x 25 cm) was prepared according to Whistler and BeMiller.[23] Glucose was eluted with water and the oligosaccharides (DP from 2 to 5) with a gradient water-ethanol 30% (mixing chamber 1 liter); the column was then washed with 50% ethanol in order to elute larger dextrins.[6] Volume of each fraction was 20 ml. The rate of flow through the column was kept at about 80 ml/hour by applying air pressure with a micropump.

The separated oligosaccharides were identified by the following methods:

1. Measurement of the DP.[16] Acid hydrolysis for 120 minutes was found to be sufficient to give complete hydrolysis of oligosaccharides into glucose.

2. Co-chromatography with known materials, on Whatman no 1. paper, in the following solvents (descending technique): ethylacetate-pyridine-water (10:4:3 by volume) and butanol-pyridine-water (6:4:3 by volume). Silver nitrate-sodium hydroxide reagent was used for location of oligosaccharides.[28]

3. Measurement of the partition function z^1 by using the multiple descending paper chromatography (solvents as above).[29]

The maltotetraose was also identified by α-amylolysis with human saliva.[2]

Reagents

Amylopectin powder (M.W. over 1,000,000) and celite 535 were obtained from Koch-Light Lab. Casein white soluble, for nutritional experiments, activated charcoal powder for decolorizing purposes, and glucose (micro-analytical reagent) were obtained from British Drug Houses. Maltose monohydrate puriss and pancreas α-Amylase (Pancreatin) were obtained from Fluka.

Maltotriose, maltotetraose, maltopentaose, and maltohexaose were a gift of Prof. W. J. Whelan (London); isomaltose, isomaltotriose, and panose were a gift of Dr. A. Jeanes (Peoria, Illinois) and of Dr. S. C. Pan (New Brunswick, New Jersey). Glucoamylase from Rhizopus delemar (Sumizyme; Shim Nihon Chemical Co., Japan) was a gift of Equitra (Brussels).

Sephadex G 50 was obtained from Pharmacia.

RESULTS

The amount of the duodenal juice obtained after the test meal during a 2 to 4-hour period ranged between 60 and 280 ml (av-

TABLE I

AVERAGE DP OF CARBOHYDRATES OF DUODENAL JUICE AFTER A TEST MEAL CONTAINING AMYLOPECTIN

After Deproteinization by Tungstic Acid	*After Deproteinization by Chloroform*
11.15	11.05
9	8.95
8.4	8.45
6.17	6.3
6	6
5	5
3.5	3.6
3.38	3.30
2.92	2.98

erage value 140 ml). The recovery of the carbohydrates of the test meal from the duodenum ranged between 20 and 77% (average value 51%).

The deproteinization of intestinal juice by tungstic acid or chloroform produced no significant loss of oligosaccharides or polysaccharides, as demonstrated from the following observations:

1. The recovery of amylopectin from the meal is about 90% with the two deproteinization methods.

2. The recovery from the meal and from the duodenal juice of oligosaccharides with an average DP equal to 5 (prepared *in vitro* by α-amylolysis of amylopectin) is about 95% with both deproteinization methods.

3. The average DP of intestinal contents is the same if measured after tungstic acid or chloroform deproteinization (see Table I).

4. The total carbohydrate content of duodenal juice is about the same if the hydrolysis of dextrins into glucose is done before or after deproteinization (see Table II).

5. The reducing power of intestinal carbohydrates with an average DP = 3 is the same if measured without deproteinization by the 3:5-dinitrosalycilate reagent[14] or after deproteinization with tungstic acid or chloroform by titrimetric methods.

The duodenal juice collected before the test meal and deproteinized by tungstic acid or chloroform contains only trace amounts of reducing substances, both before and after acid hydrolysis by sulfuric acid.

The α-amylase activity and the average degree of polymerization of the carbohydrates of duodenal juice after the test meal containing amylopectin in infants and small children are reported in Table III. In very small infants (between 2½ and 6 months of age) the average DP is high and the α-amylase activity is low; only in case No. 5 in there an average DP equal to 3.15; in the other subjects the average DP ranges between 5 and 9. In all older normal infants and in small children (between 10 and 28 months of age) the α-amylase activity is high and the average DP is correspondingly low, ranging between 2.6 and 3.6. In the 28-month-old child with exudative enteropathy treated with prednisolone the α-amylase activity is very high, 366 U/ml, and the average DP is very low (only 2.2).

In order to identify the carbohydrate which are present in the intestinal juice the duodenal dextrins were fractionated by gel filtration and then by charcoal chromatography in 5 subjects, with varying velocities

TABLE II

TOTAL CARBOHYDRATES OF DUODENAL JUICE AFTER A TEST MEAL CONTAINING AMYLOPECTIN IN 4 SUBJECTS—MEASUREMENT BY DIFFERENT METHODS

Method	*mg glucose/ml*			
	Case No. 1	*Case No. 2*	*Case No. 6*	*Case No.* [illegible]
1	68.2	42.8	54	[illegible]
2	65	42	53	[illegible]
3	68	47	50	[illegible]
4	61.5	44	54.4	67.2

Method 1—deproteinization by tungstic acid + sulfuric acid hydrolysis + titrimetric measurement of glucose.

Method 2—deproteinization by chloroform + sulfuric acid hydrolysis + titrimetric measurement of glucose.

Method 3—α-amylolysis + deproteinization by phosphotungstic acid + hydrochloric acid hydrolysis + glucose oxidase method.

Method 4—Glucoamylolysis + deproteinization by $Zn(OH)_2$ + glucose oxidase method.

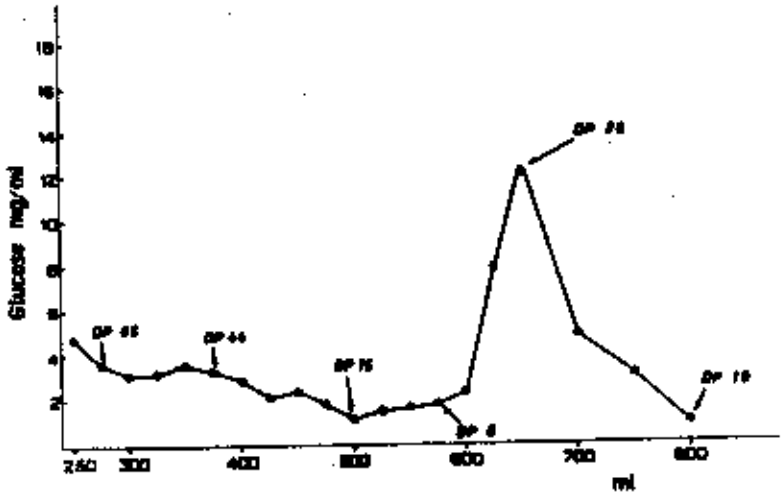

FIG. 1. Separation by gel filtration on Sephadex G 50 of the carbohydrates present in duodenal juice after a test meal containing amylopectin—No. 4, age 5 months. The average DP of some fractions is also reported.

of α-amylolysis. The recovery of the carbohydrates from the Sephadex and charcoal columns is satisfactory and the average DP of the recovered carbohydrates is practically unchanged (see Tables IV and V); therefore, the results are given in percentage of recovered material.

The gel filtration on Sephadex G 50 separated dextrins with an average DP larger than 7 from dextrins with an average DP smaller than 7 (Fig. 1). The former were subdivided into six groups of decreasing DP (see Table IV). In the subject with the lowest average DP of total carbohydrates in duodenal juice (No. 14) there are no dextrins larger than 30 glucose units. There are trace amounts of dextrins larger than 15 glucose units, and large amounts of smaller carbohydrates. In the other subjects with higher average DP of total carbohydrates in duodenal juice, there are correspondingly larger amounts of large dextrins and smaller amounts of oligosaccharides; in the 3-month-old infant (No. 3) with an average DP of total carbohydrates as high as 8.2, 39% of the dextrins are larger than 100 glucose units and only 34% of the dextrins are smaller than 7 glucose units.

In four subjects the oligosaccharides with a DP lower than 7 were further separated by charcoal chromatography (Fig. 2). By this method a good separation of glucose and linear oligosaccharides (maltose to maltopentaose) was obtained. Other dextrins,

TABLE III

TOTAL CARBOHYDRATES, THEIR AVERAGE DEGREE OF POLYMERIZATION AND AMYLASE ACTIVITY OF DUODENAL JUICE OF INFANTS AND SMALL CHILDREN AFTER A TEST MEAL CONTAINING AMYLOPECTIN

Case Number	Age (mo)	Total Carbohydrates (mg/ml)	Average DP	Amylase Activity (U/ml)
1	2½	68	6.7	7.65
2	3	44	8.95	5.3
3	3	47	8.2	3.10
4	5	63	5	6.2
5	6	71	3.15	38
6	6	52	9	8.2
7	6	79	6.0	18.2
8	10	68	3.6	48
9	12	60	2.6	87
10	19	34	3.32	53
11	21	74	2.92	84
12	25	45	3.05	72.3
13	28	56	2.80	—
14 (D.F.)	28	34	2.2	366

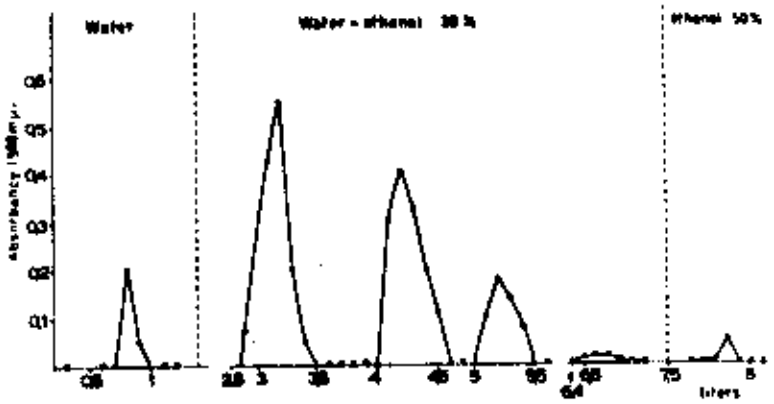

FIG. 2. Separation by charcoal chromatography of glucose and oligosaccharides present in duodenal juice after a test meal containing amylopectin —No. 4, age 5 months. The glucose was first eluted with water; then, the linear oligosaccharides from maltose to maltopentaose were eluted with gradient water-ethanol 30%. The column was then washed with 50% ethanol in order to elute low Rf oligosaccharides.

which are probably branched (DP between 4 and 7), were not separated from each other.

The pattern of small oligosaccharides differs according to the value of average DP of total carbohydrates in duodenal juice. In the subject with a very low average DP (No. 14) there were small amounts of glucose; maltose and maltotriose together make up as much as 70% of total carbohydrates, with a molar ratio maltose: maltotriose equal to 3.5:1. In the other subjects with less complete hydrolysis of amylopectin, there were correspondingly smaller amounts of glucose and maltose and larger amounts of maltotetraose; maltopentaose is also present in No. 3 and No. 4. (see Table V).

In case No. 14, with a very low average DP of total carbohydrates of intestinal juice, about 5% of the dextrins have an average DP equal to 4.13. These dextrins are resolved by paper chromatography in two different spots, with a low Rf (lower than the Rf of the maltohexaose); these are probably branched dextrins formed by 4 and 5 glucose units. These compounds are not detectable in the other subjects with less complete digestion of amylopectin. In all cases small amounts of oligosaccharides with a very low Rf on paper and average DP ranging from 5.5 to 7.2 are eluted from charcoal by 50% ethanol; these are probably large branched dextrins.

COMMENT

In infants at the end of the first year of life and in small children the α-amylase activity of duodenal juice after a test meal containing amylopectin is high and the intestinal hydrolysis of starch *in vivo* is very rapid. The amylopectin, composed of more than 5,000 glucose units, is digested at the end of the duodenum into carbohydrates with an average DP equal to about 3. In adults we found intestinal digestion of the amylopectin to proceed at about the same velocity.

Sixty to seventy percent of the total carbohydrates are represented by maltose and maltotriose; the human saliva α-amylase which has no maltase activity,[30] can hydrolyze maltotriose very slowly *in vitro*.[31] Therefore, both maltose and maltotriose have to be considered limit compounds of the α-amylolysis *in vivo*. In pigs and rats the intestinal hydrolysis of maltotriose is probably due to mucosal glucosidases.[32]

Only 1 to 3% of the total carbohydrates are represented in duodenal juice by malto

TABLE IV

DEXTRINS OF DIFFERENT DP PRESENT IN DUODENAL JUICE AFTER A TEST MEAL CONTAINING AMYLOPECTIN AND SEPARATED BY GEL FILTRATION ON SEPHADEX G 50

Dextrins of Different DP	Case No. 3		Case No. 7		Case No. 4		Case No. 10		Case No. 15 (D.F.)	
	%*	DP†	%	DP	%	DP	%	DP	%	DP
>100	20	130	19	117	8.7	108	0		0	
100–40	10	66	21.5	60.5	15.1	71.5	0		0	
40–30	7.45	34	2.3	33.5	12.7	38	0.3	37	0	
30–20	11.74	24.5	7.7	25.6	5.8	22.4	6.5	--	1.15	
20–15	2.45	17.1	1.0	17.1	0		12.3	18.7	1.9	15.5
15–7	4.43	10.4			6.5	12	3.06	8.1	9.8	8.31
<7	34	2.06	48.5	3.08	31.5	2.88	71.5	2.32	87.5	2.01
DP of total carbohydrates of duodenal juice	8.2		6		5		3.3		2.2	
DP of recovered carbohydrates	7.83		5.95		5		3.3		2.2	
Recovery from the column	92.5%		97.7%		98.7%		92%		94.2%	

* Results expressed for each group of dextrins as percentages of recovered carbohydrates.
† DP = average DP of each group of dextrin.

TABLE V

OLIGOSACCHARIDES AND GLUCOSE PRESENT IN DUODENAL JUICE AFTER A TEST MEAL CONTAINING AMYLOPECTIN AND SEPARATED BY CHARCOAL CHROMATOGRAPHY*

Dextrins	Case No. 3		Case No. 4		Case No. 10		Case No. 14 (D.F.)	
	%†	DP	%	DP	%	DP	%	DP
Glucose	0.007		1.07		2.46		6.75	
Maltose	8.7		16.6		39.8		50.2	
Maltotriose	10.5	2.85	16.9	2.94	22.2	2.94	21.3	3
Maltotetraose	10.7	3.97	11.7	4.17	3.27	3.94	1.32	3.94
Maltopentaose	1.47	4.85	1.1	4.9	0		0	
Lin. III Oligosaccharides‡							5.12	4.13
Lin. III Oligosaccharides§	2.13	7.2	3.67	7.2	2.46	6	2.38	5.55
DP of total carbohydrates*	2.06		2.86		2.32		2.04	
DP of recovered carbohydrates	2.96		2.81		2.25		2.15	
Recovered from the column	86%		94.5%		88.7%		80%	

* These oligosaccharides were separated by gel filtration as a group of dextrins with a DP<7 (see Table IV).
† Results expressed as percentage of recovered carbohydrates from the Sephadex column.
‡ These oligosaccharides were eluted by water-ethanol 50% like the linear oligosaccharides from maltose to maltopentaose.
§ These oligosaccharides were eluted by ethanol 80%.

tetraose; larger amounts of this oligosaccharide have been found in smaller infants, who have lower levels of α-amylase activity. This suggests that the *in vivo* hydrolysis of maltotetraose is probably due to salivary and pancreatic amylase.

The α-amylolysis of amylose, which is random in the early stages of hydrolysis, becomes more selective in the further digestion of the smaller linear liberated oligosaccharides, in consequence of the greater resistance to enzymatic attack of the two bonds nearer to the non-reducing end and of the bond near the reducing end.[2,3,30,32-34] *In vitro* human saliva α-amylase hydrolyzes maltotetraose more rapidly than maltotriose and more slowly than larger linear oligosaccharides.[30,32] This would explain the presence of large amounts of maltotetraose in the duodenum of infants with lower levels of α-amylase activity; at higher levels of α-amylase this enzyme splits maltotetraose into maltose and small amounts of glucose and maltotriose.[2,33]

Isomaltose was not found in duodenal juice after a test meal containing amylopectin; the smallest oligosaccharide containing one 1, 6 bond liberated *in vitro* from amylopectin by human salivary α-amylase is not isomaltose,[5] but a tetraglucose.[9] This and other larger branched dextrins[5-8] are probably the *in vivo* substrates of the mucosal "isomaltase" and glucoamylase activities.

In most infants 6 months old or younger the intestinal hydrolysis of amylopectin in the duodenum is incomplete, with large amounts of dextrins composed of more than 30 glucose units and, in the small oligosaccharides group, a decrease in the glucose and maltose content as well as an increase in the maltotetraose content.

The incomplete digestion of amylopectin is a consequence of the low levels of α-amylase activity in duodenal juice after the meal; on the contrary, the mucosal α-disaccharidases, with the single exception of maltase 1, are already present at adult levels in the newborn.[35]

It is to be expected that the incomplete α-amylolysis of starch in the duodenum would give a relatively high frequency of starch malabsorption in the first year of life, because in this age group the intestine probably has little reserve capacity for the digestion of starch.

The results of this study can also help elucidate the pathogenesis of starch malabsorption in congenital sucrase and isomaltase deficiency.[36] In normal conditions the mucosal "disaccharidases" have to hydrolyze in the digestion of starch not only maltose, but also maltotriose and branched dextrins. In pigs and rats maltotriose is hydrolyzed by mucosal glucosidases more slowly than maltose.[31] In congenital sucrase and isomaltase deficiency the remaining "maltase" activity can be sufficient to hydrolyze maltose, but not maltotriose. One can suppose that in this disease starch malabsorption is probably due to malabsorption of maltotriose and branched dextrins.

SUMMARY

In 14 infants and small children the α-amylase activity and the carbohydrates of duodenal juice were studied after a test meal containing amylopectin.

In infants at the end of the first year of life and in small children the amylopectin is very rapidly hydrolyzed into glucose, maltose, maltotriose, and branched dextrins; these compounds are probably hydrolyzed into glucose by the action of mucosal glucosidases.

In infants 6 months old or younger the intestinal hydrolysis of amylopectin in the intestine is incomplete, with large amounts of dextrins containing more than 30 glucose units and, in the small oligosaccharides group, a decrease in the glucose and maltose content as well as an increase in the maltotetraose content.

The analysis of carbohydrates which accumulate in intestinal lumen during digestion of starch will be of value in studying pathogenesis of starch malabsorption.

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Studies on Experimental Calculus Formation in the Rat. X. The Effect of Various Starches

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DIETS CONTAINING large quantities of cornstarch have been found to enhance the formation of dental calculus when fed to weanling Sprague-Dawley rats.[1] The purpose of the present study was twofold: (1) to determine the effectiveness of other starches, and (2) to determine whether amylopectin, the insoluble fraction of starch, or amylose, the soluble fraction, played the more important role in calculus formation.

PART I. EFFECT OF VARIOUS STARCHES

Materials and Methods. Eleven groups of weanling Sprague-Dawley rats of both sexes, containing 60 animals per group, were fed a diet containing 32% skim milk, 2% liver powder and 66% of one of the following commercially available starches: unmodified wheat, rice, arrowroot, tapioca, amioca, potato, cornstarch; or, pregelatinized potato, tapioca, amioca or wheat, Table 1.

The animals were housed two per wire bottom cage, and given distilled water and food ad libitum. Body weights were recorded at the beginning and at the end of the experimental periods.

Approximately 24 animals in each group were sacrificed after a 30-day experimental period, an additional 24 per group were sacrificed after 60 days, and the remainder at the end of 90 days. The animals were then decapitated, defleshed by the use of Dermestid beetles and the molar teeth scored for calculus.[1]

Results. None of the starches tested formed significantly more calculus than the cornstarch, Tables 2, 3, 4 and Chart 1. Four of the starches, pregelatinized tapioca and amioca, and unmodified arrowroot and potato formed less calculus, $p<.001$, Tables 2, 3, 4 and Chart 1. While there was some correlation between the weight gained and the calculus scores at the 30-day period, no correlation existed by the end of the 90-day experimental period ($r = .02$). See Chart 2.

PART II. EFFECT OF AMYLOSE VS. AMYLOPECTIN

Materials and Methods. Four groups of weanling Sprague-Dawley female rats, containing 40 animals per group, were fed for an experimental period of 30 days, one of the four diets shown in Table 5. All other experimental conditions were the same as in Part I.

Results. Rats fed the diets containing 66% amylopectin or regular cornstarch formed significantly more calculus than

TABLE 1
Diets*

Unmodified Starch (66%)	*Pregelatinized Starch (66%)*
Wheat (a)	Wheat (a)
Rice (b)	Potato (c)
Arrowroot (b)	Tapioca (d)
Tapioca (c)	Amioca (d)
Amioca (d)	
Potato (c)	
Cornstarch (e)	

*In addition, all diets contained 32% skim milk and 2% liver powder.

Sources: (a) Hercules Powder Co., New York; (b) Morningstar Paisley, Inc., Clifton, N.J.; (c) Stein Hall Co., New York; (d) National Starch & Chemical Co., New York; (e) Clinton Corn Processing Co., New York.

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**U. S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, National Institute of Dental Research, Epidemiology and Biometry Branch, Bethesda, Md.

TABLE 2

Effect of Various Starches on Calculus Formation—30-Day Experimental Period

Diet No.	Diet Regimen (Starch)*	N	Combined Mean Calculus Scores ± S.E.**	Combined Mean Weight Gained ± S.E.**
S - 1	Unmodified wheat	24	3.13 ± .519	87.3 ± 3.17
Control	Cornstarch, regular	24	3.67 ± .428	89.6 ± 4.54
S - 3	Pregelatinized potato	24	1.63 ± .300	73.9 ± 4.72
S - 4	Unmodified rice	23	2.65 ± .473	83.6 ± 4.97
S - 5	Unmodified arrowroot	23	1.57 ± .402	72.9 ± 3.68
S - 6	Unmodified tapioca	23	2.70 ± .442	82.7 ± 3.96
S - 7	Pregelatinized amioca	22	0.77 ± .130	61.9 ± 4.01
S - 8	Pregelatinized tapioca	23	1.61 ± .265	67.5 ± 4.82
S - 9	Unmodified amioca	24	3.38 ± .496	83.0 ± 2.70
S -10	Pregelatinized wheat	23	1.78 ± .398	63.9 ± 3.09
S -11	Unmodified potato	15	0.47 ± .369	55.7 ± 3.67

*In addition to 66% starch, each diet contained 32% skim milk and 2% liver powder.
**Standard Error.

TABLE 3

Effect of Various Starches on Calculus Formation—60-Day Experimental Period

Diet No.	Diet Regimen (Starch)*	N	Combined Calculus Scores ± S.E.**	Combined Mean Weight Gained ± S.E.**
S - 1	Unmodified wheat	24	4.79 ± .478	141.9 ± 1.09
Control	Cornstarch, regular	23	4.57 ± .555	137.5 ± 4.43
S - 3	Pregelatinized potato	23	2.48 ± .338	117.3 ± 3.65
S - 4	Unmodified rice	24	4.67 ± .476	115.2 ± 4.94
S - 5	Unmodified arrowroot	23	2.17 ± .528	116.7 ± 2.74
S - 6	Unmodified tapioca	21	3.71 ± .594	130.9 ± 1.21
S - 7	Pregelatinized amioca	22	1.77 ± .436	132.3 ± 4.05
S - 8	Pregelatinized tapioca	24	2.67 ± .238	127.9 ± 1.22
S - 9	Unmodified amioca	24	4.63 ± .339	126.9 ± 4.69
S -10	Pregelatinized wheat	24	2.83 ± .280	106.7 ± 4.00
S -11	Unmodified potato	19	0.37 ± .114	95.5 ± 5.22

*In addition to 66% starch, each diet contained 32% skim milk and 2% liver powder.
**Standard Error.

TABLE 4

Effect of Various Starches on Calculus Formation—90-Day Experimental Period

Diet No.	Diet Regimen (Starch)*	N	Combined Calculus Scores ± S.E.**	Combined Mean Weight Gained ± S.E.**
S - 1	Unmodified wheat	10	4.90 ± .809	140.4 ± 10.38
Control	Cornstarch, regular	12	5.08 ± .570	155.1 ± 9.34
S - 3	Pregelatinized potato	8	3.75 ± .527	121.3 ± 3.45
S - 4	Unmodified rice	12	4.58 ± .743	118.4 ± 13.39
S - 5	Unmodified arrowroot	12	1.08 ± .451	147.6 ± 12.11
S - 6	Unmodified tapioca	11	4.18 ± .615	153.7 ± 15.14
S - 7	Pregelatinized amioca	12	1.92 ± .259	123.8 ± 7.66
S - 8	Pregelatinized tapioca	11	2.73 ± .304	131.4 ± 7.98
S - 9	Unmodified amioca	11	6.27 ± .662	120.2 ± 8.57
S -10	Pregelatinized wheat	11	4.45 ± .718	113.5 ± 5.60
S -11	Unmodified potato	9	0.00 ± .000	122.1 ± 10.35

*In addition to 66% starch, each diet contained 32% skim milk and 2% liver powder.
**Standard Error.

those on either of the two high amylose diets, $p<.01$, Table 6. There was no consistent relationship between the calculus scores and the weight gained.

DISCUSSION

The discussion below concerning the chemical properties of starch is based on the work of Schoch,[1] and that concerning its physiologic reactions in the rat is based on the work of Reussner.[2]

Starch is a natural polymer of the sugar D-glucose, and is formed in plants by the chemical interlinking of hundreds or thousands of glucose units. Starch granules obtained from various plant materials differ in many ways, such as in their crystalline structure, rates of digestibility, and in their effect on the utilization of protein. Which of these properties or combinations of properties exerts its effect on the deposition of dental calculus is unknown. However, it is known that if one feeds cornstarch to rats it produces not only a heavy deposition of dental calculus, but results in good growth, good protein-efficiency values, and does not cause enlarged ceca. On the other hand, unmodified potato starch, a poor calculus former, results in enlarged ceca, poorer growth and less efficient utilization of protein. Potato starch also differs from cornstarch in that it gelatinizes more readily, swells more freely and is more soluble. These properties reflect a weaker bonding within the potato starch granule than is present within the granules of corn and other cereal starches.

Similarly, when one compares corn amylose, a poor former of dental calculus, with corn amylopectin, a good former, chemical and physiologic differences are noted. Amylose is the soluble and amylopectin the insoluble constituent of starch. Amylose is formed by the interlinking of 500 to 2,000 glucose units in a linear chain, while the amylopectin molecule is formed of branches. Each of these latter branches is, in turn, composed of 20 to 30 glucose units,

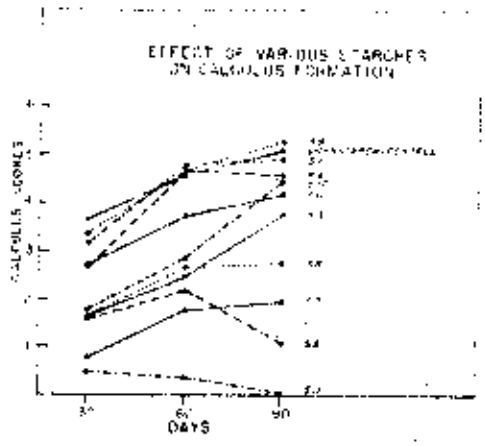

Chart 1

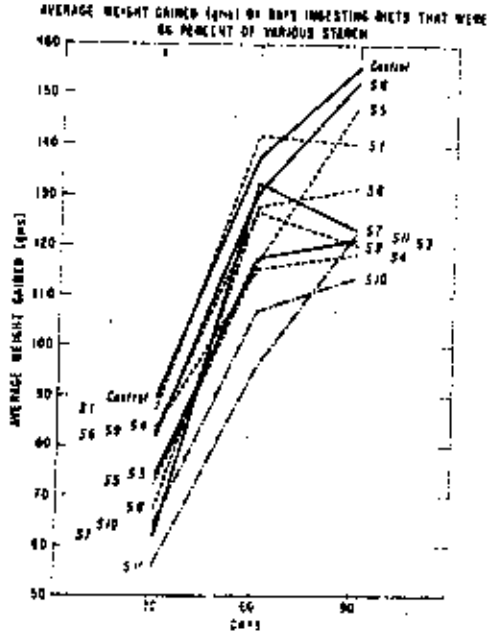

Chart 2

S 1 = Unmodified wheat starch
Cornstarch is control starch
S 3 = Pregelatinized potato starch
S 4 = Unmodified rice starch
S 5 = Unmodified arrowroot starch
S 6 = Unmodified tapioca starch
S 7 = Pregelatinized sagoo starch
S 8 = Pregelatinized tapioca starch
S 9 = Unmodified sagoo starch
S 10 = Pregelatinized wheat starch
S 11 = Unmodified potato starch

TABLE 5

Diets*

*Diet** (55%)	*Percent Amylose or Amylopectin Contained in Each Starch Fraction*	
	Amylose	*Amylopectin*
a. Regular cornstarch (1)	28	72
b. High amylose cornstarch (2)	70	30
c. Corn amylose (3)	100	—
d. Corn amylopectin (3)	—	100

*In addition, each diet contained 32% skim milk and 2% liver powder.

Sources: (1) Clinton Corn Processing Co., New York; (2) National Starch & Chemical Co., New York; (3) A. E. Staley Mfg. Co., Decatur, Ill.

TABLE 6

Effect of Amylose vs. Amylopectin on Calculus Scores

Diet	*N*	*Calculus Scores Mean ± S.E.**	*Weight Gained Mean ± S.E.**
Corn starch, regular	40	4.48 ± .41	58.20 ± 1.79
High amylose cornstarch	40	1.45 ± .30	24.70 ± 3.08
Corn amylose	40	0.65 ± .18	41.97 ± 1.62
Corn amylopectin	37	2.89 ± .37	30.94 ± 2.06

*Standard Error

and the entire molecule contains hundreds of these branches. Amylose and amylopectin also have different physiologic effects when fed to rats. For instance, corn amylose results in enlarged ceca and poor utilization of protein, while corn amylopectin results in normal protein-efficiency values and normal cecal sizes.

It is hypothesized, on the basis of these experiments, that the differences in the chemical and physiologic properties of the various starches directly effects, in some manner, the chemical or physical properties of the saliva, or the bacterial flora of the oral cavity. These differences, subsequently, are reflected in the calculus scores. This hypothesis is tenable with our previous observations that in the rat dental calculus cannot form in the absence of the major salivary glands.[4] It is also not contrary to any multifactorial theory of calculus formation. However, as we have previously stated: "While nutritional and endocrine factors play an important role in influencing the amount of dental calculus which is formed, the importance of the local factor should not be lightly dismissed, as its importance as an etiologic factor may even be greater than most of the systemic factors."[5]

SUMMARY AND CONCLUSIONS

1. Eleven starches were tested as to their ability to form calculus on the molar teeth of Sprague-Dawley rats. None were more effective than cornstarch.

2. In an additional experiment, it was shown that amylopectin, the insoluble constituent of starch, was more important for the formation of dental calculus than amylose, the soluble constituent.

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EFFECT OF CORN STARCH SOURCE ON GROWTH RATE AND CALORIC EFFICIENCY OF CHICKS FED A SEMI-PURIFIED DIET

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Progressive reduction in chick growth has been observed when increments of glucose were substituted for corn starch in a crystalline amino acid diet (Scott *et al.*, 1966); glucose-containing diets showed evidence of the "browning reaction." Liener (1958) has reviewed the implication of reducing sugars in the Maillard reaction. Scott *et al.* (1966) also reported that samples of corn starch, from two manufacturers, differed by as much as 30% in growth-promoting ability when used as the source of carbohydrate in the crystalline amino acid diet. The present investigation was undertaken to determine the effect on chick growth and caloric efficiency of a series of corn starch samples produced by several manufacturers.

EXPERIMENTAL

Two identical experiments were conducted utilizing male broiler-type chicks hatched from eggs produced by the University of Maine flock. The chicks were placed in electrically-heated starting batteries (Petersime) and fed a practical corn-soy starting ration for a preliminary period of four days. At the conclusion of the preliminary period, the chicks were distributed into experimental groups in such a manner that variation within groups was minimized and initial average weights of all groups were similar. The chicks were then fed the experimental diet shown in Table 1. This diet is calculated to contain 23.6% crude protein and 3,474 metabolizable Kcal./kg. Corn starches from five manufacturers were employed in these studies. Quadruplicate groups of eight chicks were allotted to each experimental treatment and feed and water were provided *ad libitum*. After an experimental period of 20 days, weight gains and caloric efficiencies were determined.

TABLE 1.—*Composition of experimental diet*

Ingredients	g./kg.
Corn starch (source-variable)	535.5
Isolated soybean protein[a]	175.0
Soybean meal, 50% protein	175.0
Corn oil, refined	47.0
$Ca_3(PO_4)_2$	29.0
Cellulose[b]	13.0
Choline chloride, 25% mix	5.4
Microingredient mix[c]	5.0
pt-Methionine	4.5
Salt, iodized	3.8
Unidentified factor supplement[d]	2.5
Trace mineral mix[e]	2.0
Glycine	1.6
$MgSO_4$	0.7

[a] ADM Assay Protein C-1, Archer Daniels Midland Co., Minneapolis, Minn.

[b] Avicel, FMC Corp., Newark, Del.

[c] Provides per kilogram of complete diet: vitamin A, 6,000 I.U.; vitamin D_3, 1,428 I.C.U.; vitamin E, 22 I.U.; vitamin K, 6.4 mg.; thiamine HCl, 6.4 mg.; riboflavin, 8.8 mg.; niacin, 44 mg.; d-calcium pantothenate, 22 mg.; pyridoxine HCl, 6.6 mg.; folacin, 4 mg.; biotin, 0.28 mg.; vitamin B_{12}, 35 mcg.; ascorbic acid, 22 mg.; procaine penicillin, 8.5 mg. and ethoxyquin, 125 mg.

[d] Fermacto 500, The Borden Co., New York, N.Y.

[e] Z-2 Delamix, Limestone Products Corp. of America, Newton, N. J. When used at level indicated, provides the following (mg./kg. of complete diet): Fe, 69.0; Cu, 4.0; Zn, 40.0; Mn, 120.0; Co, 0.4 and I, 2.4.

RESULTS AND DISCUSSION

A summary of the results of these experiments is presented in Table 2. Statistical analyses of these data failed to show any significant differences among the various corn starches in either rate of growth or caloric efficiency.

It is difficult to compare these results with those of Scott *et al.* (1966). It is not known, for example, whether or not any of the corn starch samples included in the present studies were produced by the manufacturers which produced the starches investigated by the latter workers. Also, one must take into consideration the nature of the experimental diets employed. The diet used by Scott *et al.* (1966) was based on crystalline amino acids whereas the diet employed in the present studies contained relatively small quantities of crystalline amino acids. The latter diet was chosen because its amino acid pattern meets the minimum levels suggested by Combs (1967) and contains minimal levels of amino acids in excess of those suggested levels. Such a diet should be suitable for subsequent studies relating to amino acid balance.

TABLE 2.—*Effect of corn starch source on growth rate and caloric efficiency*

Starch source	4–30 day weight gain, g.			Caloric efficiency, metabolizable Kcal. consumed/g. gain		
	Exp. 1	Exp. 2	Ave.	Exp. 1	Exp. 2	Ave.
A	399	425	412	5.15	5.06	5.10
B	423	411	417	4.97	4.79	4.88
C	406	423	414	4.93	4.96	4.94
D	402	402	402	5.01	4.87	4.94
E	400	407	404	5.00	5.08	5.04

CONCLUSION

It is concluded that comparable growth and caloric efficiency could be expected if any of the corn starches investigated in these studies were to be incorporated into semi-purified diets of the general type described in this report.

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The unidentified factor supplement, Fermacto 500, was provided by the Borden Co., New York, N.Y.

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BIOLOGIC UTILIZATIONS OF UNMODIFIED AND MODIFIED FOOD STARCHES

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TEN FIGURES

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Despite long-established recognition of differences in the histologic features of starch granules obtained from various plant materials, and more recent knowledge of the chemical heterogeneity of their starch-components, rather scant attention has been directed toward studies of the relative utilizations of starches as food for man and animals. The paucity of information on the relative food values of unmodified starches presumably stems from the facts that (1) most of the starch in human dietaries is consumed as modified starch, gelatinized or dextrinized; and (2) the widespread availability of cereal grains has largely allayed search for, or consideration of, other starch-rich feedstuffs for domestic livestock.

Scattered reports have appeared in the scientific literature of the past half-century with clear evidence that the human body manifests much greater efficiency in the digestion of certain unmodified starches — including those isolated from the cereal grains, cassava and taro — than in the case of others, such as those from the potato, arrowroot (genus *Maranta*) and canna (Fofanow, '11; Langworthy and Deuel, '20, '22; Langworthy and Merrill, '24). The starch in raw potato, in contrast to that in cooked potato, has also been

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noted to be poorly utilized by various species of livestock (Kennard, '36; Klooster and Card, '36; Gutteridge, '37; Halnan and Fermor, '42; Halnan, '44). Levy and Jacquot ('48) have reported unmodified potato starch to be poorly digested by normal young rats as compared with potato starch which has been ball-milled for three days, an observation which these workers interpret as indicating that the *in vivo* digestibility of raw starch depends upon the degree of polymerization of the starch molecules.

The investigations reported here were designed to throw further light upon (1) the nature of the physiologic mechanisms which limit the utilization of various unmodified starches while permitting almost complete utilization of others; and (2) certain basic attributes of starch granules associated with their different degrees of physiologic utilization. In pursuit of these objectives we have (1) investigated the physiologic utilizations of a variety of isolated starches with and without modification under strictly comparable conditions; (2) varied the management of the experimental animals (rats) in order to observe feeding conditions which would alter the degree of utilization of certain starches; and (3) conducted various *in vitro* experiments on starches which we believe may suggest some rational explanation of the divergences among unmodified starch granules from different sources as provisioners of food energy.

The sources of the starches investigated included wheat *(Triticum vulgare)*, rice *(Oryza sativa)*, maize *(Zea mays)*, cassava *(Manihot utilissima)*, arrowroot *(Maranta arundinacea)*, sago palm (an imported product from the Straits Settlements of Borneo, probably *Metroxylon rumphii* or *M. laeve)*, white potato *(Solanum tuberosum)*, and sweet potato *(Impomoea batatas)*.

EXPERIMENTAL

Descriptions of starches and starch products

Wheat starches included: (1) 4 commercial preparations made without use of chemicals except for the addition of

enough alkali to make the starch slurry neutral in reaction; (2) a commercial preparation, hydrochloric acid-modified to make a thin-boiling product; (3) a commercial preparation, oxidized with hypochlorite to achieve a thin-boiling product; (4) dry dextrinized wheat starch (torrefaction dextrins) prepared by heating thin layers of wheat starch in a rotary (2 r.p.m.) shelf-oven at 191°C. for 95 minutes with frequent stirring, which treatment was attended with loss, or very rapid fading, of the blue coloration on addition of iodine; (5) autoclaved wheat starch prepared by heating the starch moistened with 0.1% citric acid for 6 hours at 15 lb. steam pressure; (6) a gelatinized product prepared by heating an aqueous 20% starch slurry for 10 minutes at 15 lb. steam pressure, after which the product was spread in thin layers, dried overnight in a stream of air (at room temperature) and ground in a Wiley mill; and (7) a laboratory preparation made without the use of any chemicals.

Maize starches included: (1) a commercial preparation purchased in small retail packages; and (2) an unmodified commercial lot of waxy maize starch.

Rice and arrowroot starches included in each case one sample of an unmodified commercial product.

Sago starch included: (1) two lots of ordinary commercial type; (2) one of the commercial samples subsequent to ball-milling for two hours and 40 hours, respectively, wherein 125-gm batches along with 5 kg of one-half inch porcelain balls were added to a one-gallon container and rotated at 70 r.p.m.; (3) fecal residues from ingested commercial sago starch after partial purification to remove the major part of the non-starch fecal materials; and (4) commercial sago starch subsequent to one-hour treatments at room temperature of 20% slurries in which the aqueous phases consisted of 0.12, 0.16 and 0.20 M NaOH, respectively. The two commercial samples of this starch were associated with a small amount of brownish pigment along with other non-starch material from the pith or woody parts of the sago palm. The samples

of this starch contained on the anhydrous basis approximately 1.3% nitrogen, 0.15% fat, 0.42% fiber, 0.45% ash and 91% starch (by difference).

Cassava or "tapioca" starch included two lots of the usual commercial type prepared from cassava roots, no heat treatment being applied in the process.

Sweet potato starch included two lots of unmodified commercial products.

White potato starches included: (1) three lots of isolated commercial products; (2) a laboratory product prepared entirely without use of chemicals; (3) "Lintner's soluble" potato starch (Lintner, 1886) taken from a commercial lot manufactured for use in measurements of amylase activities; (4) a dry dextrinized product prepared by the same means described for torrefaction dextrins from wheat starch; (5) a vacuum-dried product (2.6% moisture) following 10 hours' refluxing with absolute alcohol and 44 hours' extraction with ether; (6) a sample of a commercial product ball-milled for two hours without prior treatment and a portion of the same sample ball-milled for two hours in the presence of absolute alcohol subsequent to 10 days' agitation and 6 months' standing, in the presence of absolute alcohol, at room temperature; (7) sample lots of an unmodified product separated by means of a laboratory air-classifier into a fraction concentrated in smaller granules (0.5% of the granules larger than 50 μ, 11.9% between 20 and 50 μ and 87.6% of 20-μ size or smaller), and a fraction concentrated in larger granules (15% of the granules 50 μ or more, 50% between 20 and 50 μ and 35% smaller than 20 μ): (8) a commercial product ball-milled for 40 hours as described for ball-milled sago starch; (9) gelatinized starch prepared by treating an aqueous 20% starch slurry precisely in the manner described for gelatinized wheat starch: (10) fecal residues from commercial potato starch, one with and one without partial purification to remove the major portion of the non-starch fecal materials, and (11) samples of a commercial product following one-hour treat-

ments at room temperature of 20% slurries with increasing concentration of alkali in the aqueous phases analogous to the products described under sago starch.

The physical characteristics of the granules in each of the above-mentioned starches, herein referred to as unmodified starches, were readily identifiable by microscopic examination, using both ordinary and polarized light; and, with the exception of the sago starch, very few of the starch samples revealed more than about 5% of disrupted or injured granules. The sago starch samples were estimated to consist of 20 to 25% of grossly and slightly injured granules and, as is characteristic of this kind of starch, many more granules showed a transverse fissure or a three-armed fissure radiating from the hilum. The sample of waxy maize starch yielded the characteristic reddish-brown coloration of amylopectin with iodine, while granules of all of the other unmodified starches stained a deep blue with the same reagent.

Animal-feeding techniques

All *in vivo* experimentation reported here, except as otherwise indicated, was initiated with weanling male rats of 45 to 60 gm body weight.

Each animal was housed in an individual cage having an elevated one-half inch mesh screen bottom in a laboratory maintained at a temperature of 23° to 25°C. and a relative humidity of between 40 and 60%. Each animal was weighed at least once each week and daily food records were kept for each. Continuing records were also kept of the development and progress of any outward signs of physiologic abnormalities among the animals.

Depending upon the specific objective in different instances, the feeding techniques employed were ad libitum feeding; equalized feeding of matched groups, in which the quantity of rations consumed daily was controlled by the individual animal in a given group that ate the least; or controlled, arbitrarily-restricted feeding of matched groups. Except for

variations described subsequently in the text, all of the diets used were constituted, in terms of dry weights of the ingredients, as follows:

	%		%
Starch	63.7	Salt mixture, modified, U.S.P. 1	4.0
Casein (commercial)	18.8	Cod liver oil, U.S.P. grade	1.0
Roughage material ("Ruffex")	2.0	Wheat germ oil	0.5
Cottonseed oil	9.4	Liver concentrate powder (1:20)	0.6

Daily vitamin supplement per rat per day: thiamine hydrochloride 33 μg, riboflavin 67 μg, pyridoxine hydrochloride 20 μg, calcium pantothenate 100 μg, inositol 10 μg, p-aminobenzoic acid 500 μg, folic acid 20 μg, nicotinic acid 20 μg, biotin 0.2 μg and choline chloride 1 mg.

The 63.7% of anhydrous starch included in the above ration refers in the case of every kind of starch used, except sago starch, to the isolated product which contained, according to actual analyses, 98% or more of pure starch. The sago starch samples incorporated in this ration carried approximately 9% of non-starch (mostly nitrogenous) material.

In preliminary studies of the over-all or gross responses to different starches incorporated in the above ration at the 63.7% level, the ad libitum feeding technique was employed, thus allowing the animals to make such adjustments in food intakes as were physiologically induced or occasioned by variations in the utilization of the respective starches. For more quantitative comparisons of the relative utilizations of various unmodified and modified starches, one or the other of the matched-feeding techniques was employed. The relative biologic utilizations of the various unmodified and modified starches were assessed in terms of the following criteria: (1) their relative capacities to promote body weight increments in young rats when comprising the sole source of carbohydrate in the ration; (2) their respective coefficients of digestibility; (3) the respective average weights of dry feces excreted per gram of dry food ingested; and (4) notation of any aberrations in the gross appearance of the animals or their gastrointestinal tracts when they were sacrificed. Because of the massive accumulations of undigested starch in the ceca and colons of the rats when certain of

the starches investigated were consumed in the type-ration, the "net body weight gain" was computed for each animal by subtracting the initial gross live weight from the final gross live weight minus the weight of the posthumous contents of the gastrointestinal tract.

The digestibility coefficients for the various starches and starch products were determined in the usual manner from analyses of the food ingesta and fecal excretions (plus the posthumous contents of the gastrointestinal tracts) for moisture, ash, protein (N × 5.7, 5.95, 6.25 or 6.38, whichever was judged to be most applicable for all or part of the dietary constituents), fat and crude fiber — the starch contents being computed "by difference." The fecal collections and records of food ingesta, except where specifically indicated, covered the entire span of the experimental periods.

RESULTS

As is evidenced by the data in table 1, the various unmodified starches investigated may be grouped roughly into three categories as regards their relative utilizations; viz., those approaching almost complete (98%) digestibility and in the highest degree contributive to the support of body weight gains — wheat, maize, rice and cassava; those showing decidedly low coefficients of digestibility and limited by reason thereof as regards support of the body weight increments of young animals — arrowroot, sago and white potato; and sweet potato starch, intermediate as regards digestibility and assimilability. No outward signs of physical abnormality were noted among any of the animals on unmodified cereal or cassava starches. The acid-modified and the oxidized wheat starches elicited responses indistinguishable from those obtained with unmodified wheat starch. It is obvious, on inspection of the data in table 1, that some groups of rats on the type-ration containing unmodified potato and sago starches, respectively, consumed rather extraordinary amounts of food as if, teleologically speaking, to compensate for the decidedly poor digestibilities of these two starches.

TABLE 1

Comparisons of relative utilizations of various unmodified and modified starches as determined by ad libitum feeding of diets containing 63.7% (anhydrous basis) of the starch products specified. (Animals 45–60 gm in initial weight; feeding periods, 28 days unless otherwise specified in column 1)

STARCH COMPONENT OF DIET	EXPT'L. ANIMALS [1]	TOTAL DRY FOOD EATEN	NET BODY WEIGHT GAIN [2]				AVERAGE FINAL BODY LENGTH	DRY FECES PER GRAM DRY FOOD EATEN	STARCH, COEFF. OF DIGESTIB.
			Total		Per gram dry food				
	no.	gm S.D.	gm	S.D.	gm	S.D.	cm	gm	%
Wheat 1	6	283 ± 21	127 ±	11.7	0.45 ±	0.022	19.9	0.05	98.4
Wheat 1	6	310 ± 4	126 ±	9.9	0.41 ±	0.022	20.1	0.05	97.8
Wheat 2 acid-modified	6	302 ± 23	121 ±	7.7	0.43 ±	0.021	20.4	0.05	98.3
Wheat 3 oxidized	6	304 ± 14	126 ±	6.8	0.41 ±	0.011	20.2	0.05	96.4
Maize 1	6	313 ± 17	121 ±	18.4	0.39 ±	0.043	19.8	0.06	97.7
Maize, waxy, 1	6	312 ± 11	130 ±	5.2	0.42 ±	0.006	19.9	0.05	98.3
Rice 1	6	322 ± 20	130 ±	11.5	0.40 ±	0.015	20.2	0.06	97.5
Cassava 1	6	307 ± 40	110 ±	27.2	0.36 ±	0.050	19.6	0.05	96.0
Cassava 2	6	326 ± 33	135 ±	16.5	0.42 ±	0.021	20.4	0.05	97.7
Sweet potato 1	6	301 ± 20	121 ±	14.7	0.40 ±	0.025	19.9	0.07	96.6
Sweet potato 2	6	281 ± 25	97 ±	6.4	0.35 ±	0.025	19.3	0.09	95.4
Arrowroot 1	6	293 ± 31	91 ±	7.2	0.31 ±	0.021	19.1	0.18	79.9
Sago palm 1	8	364 ± 36	114 ±	5.5	0.31 ±	0.016	20.0	..	.
Sago palm 1	6	342 ± 59	109 ±	21.6	0.32 ±	0.013	19.8	0.26	45.3
Sago palm 1 ball-milled 2 hr.	6	349 ± 31	135 ±	18.2	0.39 ±	0.025	20.0	0.09	85.1
Sago palm 1 ball-milled 40 hr.	6	324 ± 20	127 ±	10.8	0.39 ±	0.024	19.9	0.06	96.5
White potato 1	5(1d)	337 ± 28	106 ±	13.0	0.31 ±	0.028	19.7	.	...
White potato 2, lab. product, no chemical treatment	4	350 ± 88	95 ±	20.6	0.27 ±	0.021	19.5	0.35 [3]	50.9 [3]
White potato 1 ball-milled 40 hr.	6	278 ± 28	111 ±	11.7	0.40 ±	0.014	19.5	0.05	98.2
White potato 3, Lintner's soluble	8	384 ± 44	107 ±	13.8	0.28 ±	0.027	19.5	0.37	51.0
White potato 4; 7-day feeding period	12	48 ± 10	9 ±	4.5	0.17 ±	0.076	14.4	0.43	48.7
White potato 4; 14-day feeding period	12	123 ± 24	35 ±	9.0	0.28 ±	0.041	16.5	0.38	49.8
White potato 4; 21-day feeding period	12	221 ± 29	72 ±	8.5	0.32 ±	0.020	18.4	0.35	53.8
White potato 4; 28-day feeding period	9(3d)	328 ± 55	100 ±	12.6	0.31 ±	0.021	19.7	0.32	53.2

[1] Parenthetical figures in this column indicate number of animals that did not survive the experimental period, all records on which were excluded in computation of the data presented in columns 3 to 8 inclusive.

[2] Net gain = final gross live weight (posthumous contents of G.I. tract + initial gross live weight).

[3] The posthumous contents of the G.I. tracts were not collected for this group of animals, hence not taken into account in this computation.

The data assembled in the last grouping of table 1 show that, with prolongation of the feeding period, young animals appeared to exhibit a gradually and continuously increasing coefficient of digestibility for unmodified potato starch. However, as is shown in several groupings of data in tables 1 and 2, it is obvious that animals subsisting on diets containing 63.7% of unmodified potato starch frequently did not survive relatively short feeding periods. Moreover, it was found that the gradual increase in digestibility coefficients for unmodified potato starch, noted among young animals with prolongation of the feeding period, were not to be explained on the basis of increasing age or stage of development. Two groups of 6 male animals each from our stock colony, when placed at 90 days of age on the type-ration carrying 63.7% of unmodified potato starch, were observed, under ad libitum feeding conditions, to digest on the average 55.6% of the starch ingested (average, 161 gm per rat) over a period of two weeks and 73.5% of that consumed (average, 302 gm per rat) over a period of 4 weeks. Similar groups comprised of 6 adult male animals, one year of age and weighing over 400 gm each, when placed on the type-ration containing 63.7% of unmodified potato starch, were observed to digest on the average 58.0% of the starch ingested (average, 144 gm per rat) over a period of two weeks and 64.6% of that consumed (average, 293 gm per rat) over a 4-week period.

In the light of the much higher food consumption of older animals it is obvious that they digested larger amounts of unmodified potato starch than did animals only two to 4 weeks beyond weanling age. It would seem, then, that whatever factor was involved in limiting the digestibility of unmodified potato starch is concerned with a digestive capacity more or less corresponding to normal over-all requirements for food energy.

In the case of animals subsisting on the type-ration containing unmodified potato, sago and arrowroot starches, the question arose of whether the coefficients of digestibility observed for these starches were inordinately depressed by

reason of the accompanying large bulk of undigested material's having hastened the passage of food through the small intestines, and thus having created something of a vicious circle. Groups of 7 animals on stock ration until 33 days of age were starved overnight and then transferred to carmine-marked type-rations containing 63.7% of unmodified potato, sweet potato and wheat starches, respectively, and were observed to begin excretion of the marker in 5.82 ± 0.94 (S.D.) hours, 16.67 ± 4.58 hours and 15.31 ± 5.67 hours, respectively. After one week or 6 weeks on these same type-rations, added carmine marker began to be excreted in 10 to 12 hours, irrespective of the kind of starch in the ration. Obviously, then, any accelerated rate of passage of unmodified potato starch relative to that of unmodified sweet potato or wheat starch was limited to the first few days of the experimental feeding period. We observed no significant differences in average daily water intakes or in percentages of moisture in the cecal contents among comparable groups of animals on the type-ration containing 63.7% of unmodified potato and wheat starches.

On the basis of the experiments just described, the possibility suggests itself that what appeared to be a gradually increasing coefficient of digestibility for unmodified potato starch with prolongation of the feeding period may have involved nothing more than an averaging out of the effects of the abnormally low coefficient of digestibility for the first few days. As something of a corollary to this proposition, it was observed that a group of 12 weanling rats transferred immediately to the type-ration containing 63.7% of unmodified potato starch, and three other groups of 12 each which were transferred to this same ration after one, two and three weeks on our stock ration, showed at the end of one week on the type-ration average digestibility coefficients for unmodified potato starch no. 4 of 43.0, 44.1, 50.3 and 46.7%, respectively. In the light of these two complementary lines of observation we are inclined to regard any *seeming* increases in digestive capacity for unmodified potato starch,

subsequent to the first few days on the type-ration containing 63.7% of this starch, as more likely to be an artifact (inherent in the usual method of computation) than a reality.

Langworthy and Deuel ('22) noted in their studies with human subjects that those unmodified starches found by them to be of relatively low digestibility were also the ones composed of granules of relatively large size, such as potato, arrowroot and canna starches. These authors questioned whether this was accidental or was occasioned by the larger surface areas or attributable to the thicker "cellulose" covering of the larger starch granules. As we have already pointed out, unmodified sweet potato starch, whose range in granule size is included within the limits of the sizes of wheat, maize and cassava starch granules, was repeatedly observed by us to be significantly less digestible than the unmodified cereal or cassava starches. In addition, entirely comparable feeding tests conducted on potato starch granules from a single lot of starch (see first grouping of data in table 2) showed no significant differences among the digestibilities of the fraction concentrated in smaller granules, the fraction concentrated in larger granules, and a sample of the original assortment of unfractionated granules. Photomicrographs of the original sample of potato starch and of the smaller granule fraction, respectively, used in these experiments are shown in plate 1 along with photomicrographs of fecal residues obtained from animals fed these two products at the 63.7% level in the type-ration.

Since a portion of the granules in every lot of unmodified potato, sago and arrowroot starches was digested, while another portion was excreted in large measure as intact granules in the feces, it might be supposed that some considerable lack of homogeneity existed among the granules from each of these sources as regards susceptibility to *in vivo* digestion. Inspection of the second grouping of data in table 2 suggests that the undigested potato starch granules excreted by one set of animals and incorporated as dried fecal material, in

TABLE 2

Comparative physiologic responses to diets containing 63.7% of various unmodified starches and equivalent levels of treated or recovered starch products, as determined by controlled feeding techniques. (Animals used were young male rats, 45–60 gm initial weight)

CARBOHYDRATE COMPONENT OF DIET	EXPT'L. ANIMALS [1]	EXPT'L. PERIOD	TOTAL DRY FOOD EATEN	NET BODY WEIGHT GAIN [2]				AVERAGE FINAL BODY LENGTH	DRY FECES PER GRAM DRY FOOD EATEN		CARBOHYDRATE COEFFICIENT OF DIGESTIBILITY	
				Total		Per gram of dry food						
	no.	*days*	*gm*	*gm*	*S.D.*	*gm*	*S.D.*	*cm*	*gm*	*S.D.*	%	*S.D.*
White potato starch 1, unfractionated	4	14	125	36 ±	1.5	0.29 ±	0.019	16.3	0.40 ±	0.034	46.2 ±	5.90
White potato starch 1, large gran. fraction	4	14	128	37 ±	7.6	0.29 ±	0.037	16.3	0.35 ±	0.037	53.2 ±	6.99
White potato starch 1, small gran. fraction	4	14	128	37 ±	5.8	0.29 ±	0.030	16.4	0.39 ±	0.017	47.0 ±	2.90
White potato starch 4	5(1d)	10	68 [3]	14 ±	9.0	0.20 ±	0.109	14.0	0.38	....	50.5	....
White potato starch 4, fed as feces	5(1e)	10	68 [3]	10 ±	4.8	0.14 ±	0.071	13.9	0.32	....	65.4	....
White potato starch 4	6	7	51 [3]	11 ±	5.6	0.20 ±	0.098	14.1	0.45 ±	0.021	38.9 ±	3.48
White potato starch 4, recovered from feces	6	7	51 [3]	10 ±	3.4	0.19 ±	0.050	14.4	0.42 ±	0.062	44.7 ±	10.51
White potato starch 1, ball-milled 2 hr.	4	21	200	77 ±	2.8	0.39 ±	0.015	18.2	0.12 ±	0.012	90.5 ±	2.07
White potato starch 1, alcohol-extracted and ball-milled 2 hr.	4	21	200	74 ±	4.4	0.37 ±	0.027	18.2	0.14 ±	0.014	87.0 ±	2.41
Wheat starch 1	8	21	212	93 ±	9.3	0.44 ±	0.022	18.9	0.05 ±	0.003	98.0 ±	0.24
Wheat starch 1 dextrinized (dry heat)	8	21	212	88 ±	8.8	0.42 ±	0.017	18.7	0.05 ±	0.003	97.7 ±	0.21
Wheat starch 1 autoclaved (acid-medium)	8	21	212	91 ±	7.4	0.43 ±	0.012	18.9	0.09 ±	0.005	94.3 ±	2.10
White potato starch 1	8	28	296	89 ±	13.2	0.30 ±	0.039	18.9	0.29	....	68.5	....
White potato starch 1 dextrinized (dry heat)	8	28	299	109 ±	9.5	0.36 ±	0.024	19.9	0.11	....	91.4	....
Wheat starch 6 gelatinized	10	7	42	17 ±	2.3	0.41 ±	0.055	14.4	0.07	....	95.8	....
White potato starch 5 gelatinized	10	7	42	17 ±	1.5	0.39 ±	0.036	14.5	0.07	....	95.5	....
White potato starch 5	8	7	44	5 ±	5.2	0.12 ±	0.118	14.3	0.40 ±	0.036	45.6	....
White potato starch 5 (0.12 M NaOH-treated)	5(3e)	7	44	7 ±	3.4	0.15 ±	0.078	14.7	0.40 ±	0.044	45.3	....
White potato starch 5 (0.16 M NaOH-treated)	7(1e)	7	44	7 ±	6.5	0.17 ±	0.127	14.6	0.36 ±	0.024	52.9	....
White potato starch 5 (0.20 M NaOH-treated)	6(2e)	7	44	16 ±	4.2	0.36 ±	0.096	15.1	0.28 ±	0.004	73.5	....
Sago palm starch 2	7(1e)	7	44	12 ±	4.5	0.28 ±	0.103	14.9	0.24 ±	0.018	68.7	....
Sago palm starch 2 (0.12 M NaOH-treated)	8	7	44	12 ±	3.8	0.27 ±	0.086	14.8	0.25 ±	0.021	67.6	....
Sago palm starch 2 (0.16 M NaOH-treated)	7(1e)	7	44	13 ±	4.8	0.29 ±	0.104	14.9	0.19 ±	0.009	76.7	....
Sago palm starch 2 (0.20 M NaOH-treated)	6(2e)	7	44	18 ±	3.4	0.42 ±	0.078	15.2	0.10 ±	0.010	91.9	....

[1] Parenthetical figures in this column followed by "d" indicate numbers of animals which died prior to conclusion of test-period; those followed by "e" indicate numbers of animals which did not consume prescribed amounts of the diet. Only those animals not included in parentheses were included in averages shown in other columns of data.

[2] Net gain = final gross live-weight (posthumous contents of G.I. tract + initial gross live-weight).

[3] In order that this diet should provide the same level of protein, starch and fiber as the diet with which it was pair-fed, it was necessary to make it a slight modification of the type-ration. This necessity arose from the fact that undigested protein and fiber were associated with undigested starch in the feces.

the amount required to provide 63.7% of dry starch in the ration of another set of animals, may have been slightly more digestible than the original potato starch fed in identical quantity. However, microscopic examination of the feces from rats on a diet containing 63.7% of unmodified potato starch, as is indicated in figure 2, plate 1, revealed that an appreciable proportion of undigested starch granules had been disrupted or eroded during passage through the digestive tract. Removal of most of these injured granules and much of the non-starch food residue through partial purification of the fecal residues yielded a product possessing a digestibility coefficient not significantly different from that of the original, unmodified granules, as evidenced by data presented in the third grouping of data in table 2. Analogous sets of data (not recorded here) were obtained in feeding trials with sago starch recovered from the feces. It may be concluded, therefore, that lack of homogeneity among granules of a given sample of potato or sago starch offers no possibility of explaining the low assimilabilities of these starches.

Having at this stage been led to consider that the prime physiologic factor which limits the utilization of various unmodified, non-cereal, starches may well be the lack of a sufficiently powerful digestive mechanism, it became of interest to investigate the nature of the modifications of these starches which enhanced their physiologic utilization.

As is evidenced by data presented in table 2, gelatinization and dextrinization of potato starch are attended with marked increases in digestibility and assimilability of the modified products, whereas in the case of wheat starch these treatments are of very little consequence. Guerrant and Dutcher ('34), Guerrant et al. ('37) and Mannering et al. ('44) reported observations indicative of some slight lowering in the absorption of the end products of digestion of dextrinized cornstarch and commercial dextrins as compared with isolated raw cornstarch. An analogous situation was found here for aqueous acid-dextrinized wheat starch, but the digesti-

bility and assimilability of dry-dextrinized wheat starch were not found to be significantly different (statistically) from those of untreated wheat starch. In contrast to this, the torrefaction dextrins from potato starch, prepared under conditions identical with those prevailing with respect to the dextrins of wheat starch, were found to be only about 91% digestible. In all likelihood the digestibilities of both aqueous, acid-dextrinized and dry-dextrinized starches vary with the source of starch and with the detailed conditions of their preparation, especially if these conditions invoke some "browning" or carmelization. As is shown by data in table 2, completely gelatinized wheat and potato starches are equally digestible and equally effective and efficient in supporting growth in young animals.

On the basis of data not included here because of space limitations it was shown that a step-wise dilution of unmodified potato starch in the type-ration with unmodified wheat starch or with dextrose, while the potential heat of combustion in the food consumed was held constant, was attended with consistent step-wise increases in all criteria indicative of higher assimilability of the potato starch components of the diets. However, in terms of the absolute quantities of potato starch digested, there was a step-wise decrease as the proportion of potato starch in the ration was reduced by dilution with unmodified wheat starch or dextrose. These observations on animals not only confirm but extend the less comprehensive observations noted in studies on human adults by Langworthy and Deuel ('20, '22) and Langworthy and Merrill ('24).

Prolonged refluxing of potato starch (previously dried to a moisture content of 2.6%) with absolute alcohol, followed by extraction with ether, was entirely without effect upon the utilization of the resultant product. As is shown by data in table 2, prolonged refluxing of dehydrated potato starch (2.6% moisture) with absolute alcohol followed by two hours of ball-milling in the presence of absolute alcohol did not in the least enhance the digestibility of the product over that

of a sample from the same lot which was ball-milled for two hours without any prior treatment.

In the 8th and 9th sets of data in table 2 are shown various physiologic criteria indicative of the relative degrees of assimilation of potato and sago starch granules subsequent to their treatment with different concentrations of aqueous NaOH. Application of the "t" test to these two sets of data did not establish statistically significant improvement in assimilability by reason of the alkali treatments except when the concentration of alkali used was 0.20 M. The marked swelling or hydration of the granules in 0.20 M NaOH, just as in the case of injury of the physical structures of a considerable proportion of the granules with two or more hours of ball-milling, is attended with a significant increase in digestibility of those starches originally of low digestibility. It is also obvious that the digestibilty of sago starch granules treated with 0.20 M NaOH approached that of the untreated cereal starches, while potato starch (originally more poorly digested than the sago starch) treated in the same manner attained a digestibility of only about 74%. In view of these experiments, the normal alkaline gradients of the digestive juices of the small intestine would appear not to be concerned at all with the digestibility coefficients observed for unmodified potato and sago starches.

Inspection of the data in table 1 relative to ball-milled sago starch and of the data in tables 1 and 2 relative to ball-milled potato starch will show clearly that ball-milling for as short a period as two hours — which is, according to Lampitt et al. ('47, '48), far short of the time required to depolymerize most of the molecules of potato starch — sufficed to raise the digestibilities of these modified starches to values only slightly less than did 40 hours of ball-milling, and only slightly less than the digestibilities observed for cereal grain and cassava starches.

In vitro digestibility experiments employing two-hour incubation periods at 38°C. of mixtures comprised of 2 gm U.S.P. pancreatin, and 0.5 gm NaCl, along with different

amounts of raw, solid starch suspended in enough phosphate buffer (pH 7.0) to provide a total aqueous phase of 50 ml, have shown: (1) that the structural organizations of the intact wheat starch granules are attacked by enzymatically active pancreatin (see fig. 10, plate 2), with practically all of the granules shattered when 1% of unmodified wheat starch is included in the mixture, and markedly decreasing proportions shattered as the percentage of the same starch in the mixture is increased from 1 to 5 and 10%; (2) that the production of reducing sugars in the mixtures increased (maltose equivalent to 525, 1,210 and 1,490 mg after subtraction of appropriate blanks for reducing powers of the pancreatin and of the starch substrates in the absence of pancreatin) as the percentages of the unmodified wheat starch therein were increased from 1 to 5 and 10%; (3) that both wheat and potato starch granules which had been ball-milled for two hours yielded increasing amounts of total reducing sugars (and graded decreases in maltose equivalents *per gram* of starch substrate) as the percentages of these starch substrates in the mixtures were increased from 1 to 5 and 10%; (4) that wheat and potato starches modified by two hours of ball-milling yielded very similar quantities of reducing sugars from identical amounts of these respective substrates included in the digestion mixtures; while (5) so few unmodified potato starch granules were affected by digestion with pancreatin as to make it doubtful whether any granules were attacked which had not been subject to prior injury (see fig. 8, plate 2).

We have also noted repeatedly that the addition of 0.25 to 1 gm of whole powdered ox-gall to our pancreatin digest mixtures which included unmodified wheat and potato starch, respectively, appeared to increase the proportion of granules showing evidence of disrupted physical structures. The unmodified wheat and potato starches employed in our *in vitro* digestion experiments were laboratory preparations, estimated by microscopic counts to carry less than 0.5% of injured granules. These *in vitro* experiments, therefore, proved indeter-

minate with regard to the question of whether the outermost layers of unmodified wheat and potato starch granules consist of modifications of starch substance or of integuments of non-starch material; but, whichever they may be, those of wheat are far more susceptible to structural disorganization and formation of reducing sugars by action of U.S.P. pancreatin than are intact potato starch granules. Resolution of this question could presumably be achieved only by experimentation with more highly purified enzyme preparations than were available to us.

Our experimental work considered *in toto* suggests that the relatively low digestibilities of such unmodified starches as those from the potato, sago palm and arrowroot are entirely attributable to some firmer degree of hydrogen-bonding of the molecules (or some other inherent characteristic) of the outermost layer or layers of the granules than is the case with starch granules from the cereal grains, cassava root and, in lesser degree, from the sweet potato, which makes the former granules more resistant to attack by amylase. From a physiologic standpoint the low digestibilities of unmodified potato, sago and arrowroot starches relative to cereal and cassava starches must, seemingly, be due to insufficient concentration of amylase in the digestive juices, or insufficiency of some other agent in the digestive medium which is required to enhance the activity of amylase or to modify these starches.

As a matter of some general interest, it was also noted throughout the experiments herein reported, whether the food intakes of the animals were restricted or unrestricted, that the apparent digestibility coefficients for fat were consistently 97% or greater, while the apparent digestibility coefficients for protein (from around 80 to 97%) varied directly with the digestibility of the starch component.

DISCUSSION

In general there would appear to be some correlation between the relatively low digestibility coefficients observed

for various unmodified non-cereal starches (sweet potato, arrowroot, sago and potato) and the continuous storage of these granules in plant organs possessing high water content (roughly 70% or higher) until time of harvest or until the full maturation of the plant organ in which the starch is stored. Could the outermost layers of starch granules represent gradations in hydrogen-bonding corresponding roughly with the moisture content in which the granules were continuously or finally stored in the intact plant organs? A search of the literature for data on the moisture content of thoroughly fresh but mature samples of plant organs carrying the different kinds of non-cereal starches investigated by us would suggest this possibility, as revealed in reports by Adriano et al. ('32), Raymond et al. ('41), Yoshimura and Sagava ('18) and Chatfield and Adams ('40) although data in this last reference undoubtedly include, in the averages, products analyzed at considerable intervals after harvesting. Starch deposited in cereal grains, of course, begins in a high moisture environment which steadily decreases until a moisture content normally less than 15% is achieved in the fully-ripened grains.

SUMMARY

Investigations of unmodified starches, including those from cereal grains (wheat, rice, maize and waxy maize), from aerial stems (sago palm) and from plant roots and subterranean stems (arrowroot, cassava, sweet potato and white potato) have shown these, under thoroughly comparable conditions of feeding, to possess wide differences in physiologic assimilability.

Unmodified starches isolated from cereal grains and cassava roots evidenced very high degrees of assimilability coincident with roughly 98% digestibility; those from arrowroot, white potato and sago palm evidenced decidedly low degrees of digestibility which varied inversely with the intake or dietary level of these starches; and that from the sweet potato occupied something of an intermediate position.

Viewed from the standpoint of physiologic processes, the data herein presented suggest that the relatively low digestibilities of unmodified sweet potato, potato, arrowroot and sago starches were occasioned by an insufficient amount or concentration of amylase, or of some agent in the digestive juices which is required to modify these starches prior to attack by amylase.

Viewed from the standpoint of the digestion-resistant properties of unmodified sweet potato, potato, arrowroot and sago starches, it would appear that this property resides consistently and exclusively in the outermost layers of the organized granules and, therefore, according to modern concepts, is associated with the hydrogen-bonding of these structures or with a granule integument of non-starch substance. Conditions which increase the digestibility of these starches include various modifications which produce obvious hydration of the granules, distinct changes in chemical nature, or disruption of the organized granule structures. Despite the *frequent* association between large-sized starch granules and digestion-resistant properties, the evidence presented here suggests that this is merely coincidental and *not exclusive*. It has also been shown that lack of homogeneity as regards digestion-resistant properties among granules of such unmodified starches as those of the potato or the sago palm offers no basis for explaining the low digestibility coefficients of these starches. There is some suggestion that, teleologically speaking, the digestion-resistant properties of starch granules from certain sources exist as a protective factor during storage *in situ* under conditions of high moisture.

According to the observations made here, unmodified wheat starch granules which show no signs of injury are quite susceptible to digestion *in vitro* by U.S.P. pancreatin preparations, and very likely the same is true of cereal starches in general.

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A NOTE ON THE DIGESTIBILITY OF THE STARCH OF HIGH-AMYLOSE CORN BY RATS[1]

RAYMOND BORCHERS

During the course of studies on various starches in this laboratory, Sandstedt *et al.* (6) observed that the starch of high-amylose corn was resistant to amylolytic enzymes. The following study was, therefore, undertaken to determine whether these observations applied in the animal, the rat being used as a matter of convenience.

The *in vivo* digestibility of a number of starches has been investigated by Booher *et al.* (2), who have reviewed the prior literature. In their studies, isolated corn starch was reported to have a digestibility of 98% by the rat.

The starch and amylose contents of the corn samples used in the following study are listed in Table I. Starch was determined on the corn and feces samples by an acid-hydrolysis method (1), modified as follows. The initial wash with water was omitted, the sample being refluxed directly with hydrochloric acid, neutralized, diluted to volume, and filtered. Another sample was refluxed with water only. Reducing sugar was determined on a suitably diluted aliquot of the filtrate by the Folin-Wu method (4). Glucose value from the acid-hydrolyzed sample minus the water-refluxed sample times 0.9 gave the starch value.

The rations contained the following in g. per 100 g.: corn, 75; casein, 8; salt mixture (5), 2; cerelose vitamin supplement (3), 5; and hydrogenated fat, 10. The rations were fed to weanling rats of the Holtzman strain. The animals were individually housed in screen-bottomed cages with food and water available *ad libitum*. Each ration was fed to 8 animals. Fecal collections were made during a 10-day period. The feces were collected on filter paper, retrieved daily, and dried at 100°C. The 10-day excretion of each animal was composited and analyzed for starch.

The starch of normal corn was found to have a digestibility of 95%, of high-amylose corn from 66 to 77%. The several digestibility values are listed in Table I. On the basis of the limited number of samples, there does not appear to be a relationship between the percent of amylose in the high-amylose corn and the *in vivo* digestibility of the starch. These results on the *in vivo* digestibility of high-amylose corn starch confirm those of Sandstedt *et al.* (6), who first observed the

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TABLE I

DIGESTIBILITY OF THE STARCH OF NORMAL AND HIGH-AMYLOSE CORN BY RATS

Corn	Starch	Amylose[a]	Digestibility[b]
	%	%	% ± S.E.
Normal	72	25	95.1 ± 0.3
High-amylose	64	50	71.3 ± 1.0
	67	63	76.9 ± 1.5
	64	77	88.9 ± 0.9

[a]Amylose content as percent of the corn starch. Values supplied by Charles L. Ford, American Maize-Products Co., Roby, Indiana.

[b]Digestibility of starch = (starch consumed − fecal starch) / starch consumed × 100.

resistant character of such starch to amylolytic enzymes. The nature of this resistance remains obscure. Presumably, the genetic selection for high-amylose content has resulted in simultaneous selection for starch having altered properties with respect to susceptibility to amylolytic attack.

Acknowledgment

Samples of high-amylose corn were graciously made available by Charles L. Ford of the American Maize-Products Co., Roby, Indiana.

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Toxicity of Starch Administered by Mouth

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IN view of a recent report in *Time* entitled "An Urge for Argo",[1] studies in our laboratory on the toxicity of starch given orally to albino rats are of interest. Argo refers to Argo Gloss Starch, a form of cornstarch, which is eaten in large amounts by Negro women and especially pregnant women living in the United States of America; these women are described as "starch addicts".

The habit of eating huge quantities of starch appears to have evolved from clay-eating, a form of pica. In the folklore of the 16th to 19th centuries, eating clay was believed to be of value against several diseases; to produce beautiful children when eaten by pregnant women, and to give mental relief from the cares and worries of life. Among the Negroes of the southern U.S.A., laundry starch replaced clay and the habit passed from one generation to the next. Satisfaction from eating starch is said to be akin to that derived from chewing gum or smoking tobacco, and the amount eaten may vary from less than one pound per week to over four pounds per day. The remaining diet may be deficient in essential nutrients, and anemia is common. Further information may be found in recent reviews.[2-6]

Starch addiction is confined almost exclusively to adults, and must be distinguished from the much more serious condition which results from feeding weanlings a diet high in starch and correspondingly low in protein which produces stunted growth and death. The latter condition was believed by Czerny and Keller[7] in 1909 to be caused by excess starch intake, but it is now ascribed to deficient protein intake and is commonly called kwashiorkor. This protein deficiency syndrome can be reproduced in animals.[8] On the other hand, we have found in preliminary studies that doses of starch up to one-third of body weight rarely produce death in young adult albino rats.[9]

METHODS

The experiments were performed upon CBL male albino rats weighing 140 ± 15 (mean ± S.D.) g., which were fed Purina laboratory chow checkers and water *ad libitum*. The animals were segregated, one to a metabolism cage, before the administration of starch. Starch was used in the form of Starch, Soluble, Fisher Certified A.C.S., prepared by digesting potato starch in boiling water, filtering and evaporating the filtrate to yield soluble starch of low amylose content.[10] This is a fine white powder which contains 18% water, less than 0.3% ash and a negligible amount of pulp and fibre. It was given to the animals in the form of a starch paste containing 60 g. of starch mixed with distilled water to a final volume of 100 ml. and kept at body temperature and in suspension on a Fisher Thermix Stirring Hot Plate until ready for use. Concentrations greater than 60% (w/v) gradually solidified and could not be forced through the intragastric cannula.

No deaths resulted from the intragastric administration of the 60% starch paste in volumes from 50 to 100 ml. per kg. body weight, but larger doses produced gastric rupture. Volumes of from 50 to 100 ml. per kg. were then given in pilot tests at intervals of from one to five hours. It was found that a dose of 50 ml. per kg. every 2½ hours for four administrations (a total of 120 g. per kg. of starch) did not produce gastric rupture, while a dose of 70 ml. per kg. every 2½ hours for four administrations (a total of 168 g. per kg. of starch) produced gastric rupture in half of the animals during the first day of administration. It was also found that animals which did not have gastric rupture after the first day on the larger dose of starch could be given increasing volumes per kg. of 60% starch paste on subsequent days.

Based upon these preliminary trials, starch was given daily for 14 consecutive days in total daily oral doses of 36, 72, 120 and 168 g. per kg. and in animals which did not succumb to gastric rupture in doses gradually increasing to 204, 240 and 288 g. per kg. Each dose was divided into four equal parts and each part was given at intervals of 2½ hours. Each dose was given to 12 to 40 rats so that there were at least 10 survivors per dosage group at 14 days. Volume controls were given the same volume per kg. of distilled water, i.e., from 60 to 480 ml. per kg. per day for 14 days, 10 animals per volume group. A second set of 10 controls received gastric tube insertions only, four times a day at 2½-hour intervals for 14 days, and the third group of 10 controls received no treatment

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TABLE I. WET WEIGHT† AND WATER CONTENT‡ OF THE GASTROINTESTINAL ORGANS OF ALBINO RATS FOLLOWING INTRAGASTRIC ADMINISTRATION OF 60% STARCH PASTE OR DISTILLED WATER (DW) DAILY FOR 14 DAYS§

Organ and measurement	Group	Daily dose of starch (1st subheading line, g. per kg.) or distilled water (2nd subheading line, ml. per kg.)			
		38	72 - 120	168 - 207	230 - 288
		80	120 - 200	280 - 340	300 - 380
Cardiac stomach, weight	Starch	− 2.6	+ 5.6**	−40.6**	+ 87.7**
Cardiac stomach, weight	D.W.	+ 1.0	+22.5††	−95.1††	+ 135.1††
Cardiac stomach, water	Starch	− 5.0	+ 1.6	+ 5.2	+ 13.0
Cardiac stomach, water	D.W.	− 2.4	+ 0.4	+ 7.2††	+ 13.1††
Pyloric stomach, weight	Starch	−26.9*	−19.1**	−18.7**	− 8.0**
Pyloric stomach, weight	D.W.	−16.8††	− 7.2†	− 4.7	+ 12.2††
Pyloric stomach, water	Starch	− 3.3	+ 0.1*	+ 4.2	+ 6.0
Pyloric stomach, water	D.W.	− 1.2	− 4.5*	− 0.2	+ 8.5††
Small bowel, weight	Starch	+ 5.5	−46.9**	−68.2**	+ 78.8**
Small bowel, weight	D.W.	− 0.8	12.9†	−19.2††	+ 21.4††
Small bowel, water	Starch	− 6.2	+ 0.6*	− 4.7**	+ 5.9*
Small bowel, water	D.W.	+ 2.5	− 4.1†	− 5.3†	+ 1.1
Cecum, weight	Starch	− 0.5	−10.1**	−51.5**	− 72.3**
Cecum, weight	D.W.	− 7.9	− 3.4	− 5.2	− 1.7
Cecum, water	Starch	+11.2*	+ 9.8*	−17.1**	+ 22.0*
Cecum, water	D.W.	− 1.3	− 1.7	− 1.6	+ 10.3††
Colon, weight	Starch	− 3.3*	+ 8.3**	+12.9**	+ 31.7**
Colon, weight	D.W.	−20.0††	− 2.0	−11.5†	− 7.0
Colon, water	Starch	+ 5.0	− 2.1**	+ 5.6**	+ 5.2**
Colon, water	D.W.	− 4.0	− 7.0†	− 0.2†	− 4.1

†Wet weight was measured in g.

‡Water content was measured as g. water per 100 g. dry weight of tissue.

§The results are expressed as mean per cent change from controls given daily gastric tube insertions only, specifically as $((\bar{X}_d - \bar{X}_c)/\bar{X}_c) \times 100$ where $\bar{X}_d$ is the mean in rats given starch or distilled water and $\bar{X}_c$ is the mean in controls receiving only gastric tube insertions. In rats given starch, * indicates that the mean per cent change was significantly different from that in rats given distilled water at $P = 0.05$ to 0.02 and ** at $P = 0.01$ or less. In rats given distilled water, † indicates that $\bar{X}_d - \bar{X}_c$ was significant at $P = 0.05$ to 0.02 and †† at $P = 0.01$ or less.

except for measurements in metabolism cages. To determine an ultimate cause of death other than gastric rupture or regurgitation asphyxia, 41 rats were given gradually increasing daily dosages of starch until death occurred, after which autopsies were performed; blood hemoglobin was determined at intervals.

Clinical measurements were recorded daily for 14 days and included body weight in g., food consumption in g. chow per kg. body weight per 24 hours, water intake in ml. per kg. per 24 hours, colonic temperature in degrees Fahrenheit, urinary volume in ml. per kg. per 24 hours, urinary glucose and protein output in mg. per kg. per 24 hours, urinary pH on 24-hour samples, and other clinical signs as they appeared, and were graded as 1+ to 4+. Colonic temperature was recorded by a Thermistemp Telethermometer and urinalyses were performed using Ames Combistix Reagent strips.

Gross pathology was recorded on all animals that died. Weights and water levels of organs listed in Tables I and III were measured on survivors and controls at 14 days. The sample of skeletal muscle was the right half of the muscle layer of the ventral abdominal wall. The contents of the lumen of the gastrointestinal tract were removed before weighing. Water levels were measured on aliquots dried to constant weight at 95° C. in a Fisher Forced-Draft Isotemp oven and were calculated as g. per 100 g. dry weight of tissue. The sample of skin for water analysis was taken from the dorsolumbar region. After removal of the organs listed in Tables I and III, the residual carcass was cut into small pieces and homogenized in a Waring blender, and a sample was used for water analysis. Histopathological examinations were made upon blocks of tissue fixed in Lillie's buffered formalin, and sections were stained with hematoxylin-phloxine-saffron. Statistical methods were those of Croxton.[11]

RESULTS

Data on clinical measurements are summarized in Table II. Values in rats given starch or distilled water have been compared with corresponding values in rats given gastric tube insertions. Insertion of the intragastric cannula four times a day for 14 days produced a slight but significant inhibition of growth and a decrease in water intake, urinary volume and urinary pH when compared with rats that were given no treatment. The animals became accustomed to gastric tube insertion and gradually offered little or no resistance, so that the measured effects of

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TABLE II.—DAILY CLINICAL MEASUREMENTS AND OBSERVATIONS IN ALBINO RATS GIVEN STARCH AS A 60% PASTE BY STOMACH TUBE AND IN CONTROLS GIVEN DISTILLED WATER DAILY FOR 14 DAYS†

Starch: g. per kg. per day / Water: ml. per kg. per day	Group	36 / 60	72 - 120 / 120 - 200	168 - 204 / 280 - 340	240 - 288 / 400 - 840
		N = 10+ 10 controls	N = 20+ 10 controls	N = 20+ 10 controls	N = 20+ 10 controls
Body weight	Starch	+ 0.5	− 8.2*	− 14.6**	− 14.9**
Body weight	Water	− 3.9††	− 4.9††	− 7.0††	10.2††
Food (chow) intake	Starch	− 14.3**	− 21.4**	− 31.0**	− 27.5**
Food (chow) intake	Water	− 3.8†	− 6.0††	− 1.8†	− 5.1†
Water intake	Starch	− 8.2**	− 12.6*	− 2.8**	− 0.3**
Water intake	Water	− 4.2	− 17.0††	− 17.3††	− 17.3††
Body temperature	Starch	− 0.4	− 1.0	− 0.8	− 0.7
Body temperature	Water	− 1.1	− 1.0	− 0.8	− 0.9
Urinary volume	Starch	+ 23**	+ 11**	+103**	+112**
Urinary volume	Water	−111††	+362††	+712††	+963††
Urinary pH	Starch	+ 14.8*	+ 15.1**	+ 15.3**	+ 6.3
Urinary pH	Water	+ 22.2††	+ 10.2††	+ 10.8††	+ 4.0†
Urinary blood	Starch	absent	absent	absent	absent
Urinary blood	Water	absent	absent	present	present
Irritability on days 1 and 2	Starch	absent	absent	present	present
Drowsiness on days 1 and 2	Starch	absent	absent	present	present
Pallor on days 1 and 2	Starch	absent	absent	present	present
Hyporeflexia on days 1 and 2	Starch	absent	absent	present	present
Epistaxis on days 1 and 2	Starch	absent	absent	present	present
Abdominal bloating on days 1 and 2	Starch	absent	absent	present	present
Gastric rupture (days 1 and 2)	Starch	absent	absent	present	present
Death from pneumonia	Starch	absent	absent	present	absent
Death from gastrointestinal obstruction	Starch	absent	absent	present	present

†Numerical results are expressed as mean per cent change from controls given gastric tube insertion only, specifically as $((\overline{X}_d - \overline{X}_c)/\overline{X}_c) \times 100$ where $\overline{X}_d$ is the mean of 14 days of readings in rats given starch paste or distilled water and $\overline{X}_c$ the corresponding mean in rats given gastric tube insertions only. In animals given starch, * indicates that the mean per cent change was significantly different from that in rats given water at P = 0.05 to 0.02 and ** at P = 0.01 or less. In rats given distilled water, † indicates that $\overline{X}_d - \overline{X}_c$ differed from zero at P = 0.05 to 0.02 and †† at P = 0.01 or less. Clinical signs entered as present or absent were absent in all rats given distilled water except as noted.

tube insertion were probably due to mild psychic trauma or to interference with sleep patterns.

Increasing daily doses of starch produced increasing inhibition of growth and decreasing intake of laboratory chow, both changes being in part due to the effects of the large volume of water given with the starch. Water produced a marked diuresis, alkalinuria and decrease in water intake which occurred to the same or lesser degrees in animals fed starch paste. Both water and starch produced a slight decrease in body temperature which was significant when results for all doses were averaged but not for any one dose. Water also produced a hematuria at the higher doses.

In addition to inhibition of growth, starch variously caused irritability, drowsiness, pallor, hyporeflexia, epistaxis and abdominal bloating on the first two days of administration. These signs appeared to be associated with gastric distension, since most deaths from gastric rupture occurred at this time. Gastric rupture appeared in 50 to 75% of rats given starch in amounts of 168 g. per kg. and over. Of the survivors of gastric rupture, 5% died of pneumonia and 20% from bowel obstruction during the 14 days of starch administration.

Autopsy at 14 days revealed that rats adapted themselves to administration of large daily volumes of starch paste by enlargement of the gastrointestinal tissues, as shown in Table I. Enlargement of cardiac stomach also occurred in rats given the distilled water vehicle (that is, it was a response to liquid volume introduced into the stomach); the increase was mainly in dry weight, but large doses produced some hydration of gastric tissues. The pyloric or glandular part of the stomach was decreased in weight by starch administration owing to a decrease in dry weight of tissue.

When starch paste entered the small bowel, water was absorbed and the starch was pelleted into calculi and covered with mucus. The large pellets or calculi distended the bowel, and the extra work involved in propelling them along the intestine produced a marked hypertrophy of the muscularis mucosae and muscularis externa, as illustrated in Figs. 1a and 2a and c. As a result, there followed a marked increase in the weight of the small bowel, cecum and colon of starch-treated rats as indicated in Table I. Very large volumes of distilled water were not quickly absorbed from the small bowel, and the resulting distension produced some hypertrophy of in-

Fig. 1a

Fig. 1b

Fig. 1.—Photomicrographs illustrating (a) starch-induced hypertrophy of the muscularis mucosae and muscularis externa of cardiac stomach compared with that seen in rat a normal rat of the same body weight.

Fig. 2a

testinal smooth muscle but not to the same extent as that produced by the starch pellets. Distilled water had left the bowel before it reached the cecum and colon, which were not hypertrophied and actually lost weight at some doses as shown in Table I. Compared with distilled water controls, the greatest hypertrophy occurred in the cecum, then in the small bowel and least in the colon, as illustrated in Fig. 3. The water levels of the small bowel, cecum and colon tended to become somewhat increased in rats given starch but rarely in rats given only distilled water, in which they were sometimes decreased (Table I).

Shifts in weight of the remaining body organs are summarized in Table III. Large amounts of starch or distilled water produced a stress reaction as manifested by an increase in weight of the adrenal glands and a decrease in that of the thymus gland and spleen. The lungs, salivary glands and testicles and especially the brain were relatively resistant to loss of weight, the skin was particularly susceptible, and other organs lost weight at about the same rate as the residual carcass. Hypertrophy of the salivary glands, reported by Merkatz[2] in a woman who ate excessive amounts of starch, was not seen in these animals given starch directly into the stomach.

Data on water contents of remaining body organs are summarized in Table IV. While large

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Fig. 2b

Fig. 2d

Fig. 2.—Photomicrographs illustrating (a) starch-induced hypertrophy of smooth muscle of the small bowel and (c) cecum compared with that seen in a normal rat of the same body weight (b and d).

Fig. 2c

doses of starch produced some hydration of gastrointestinal tissues, the most common effect on other body organs was a slight dehydration due mainly to the effect of water in the administered starch paste.

In animals given progressively larger daily doses of starch for periods longer than 14 days, the causes of death were impaction of starch in the stomach or intestine or both, or pneumonia, if one excludes animals which died from stomach rupture or from regurgitation of starch paste with aspiration into the lungs and death from asphyxia. Deaths due to gastrointestinal impaction and obstruction from daily doses of starch of from 168 to 456 g. per kg. occurred between the 2nd and 49th days. Net body weight declined during the day before death; the weight of retained starch was subtracted from total body weight to obtain net body weight. Blood hemoglobin had not declined in any of these animals.

Histologically, the hypertrophy of smooth muscle in the gastrointestinal tract was found mostly in the inner circular muscle layer of the muscularis externa of the small bowel, cecum and colon (Figs. 2a and c). The stratified squamous epithelium and muscularis mucosae of

TABLE III.—WEIGHT OF BODY ORGANS IN ALBINO RATS AFTER 14 DAYS OF DAILY ORAL ADMINISTRATION OF STARCH AND OF DISTILLED WATER (DW)†

		Daily dose of starch (1st subheading line, g. per kg.) or distilled water (2nd subheading line, ml. per kg.)			
		36	*72 - 120*	*168 - 202*	*240 - 288*
Organ	*Group*	*80*	*120 - 200*	*280 - 340*	*300 - 380*
Adrenal glands	Starch	+2.9	+ 7.8	− 7.0	+ 5.2
Adrenal glands	D.W.	+3.1	+ 6.0	− 1.5	+19.2††
Brain	Starch	−0.5	− 1.9	− 5.5*	− 5.4
Brain	D.W.	+1.0	− 0.9	− 2.6	− 3.1*
Heart	Starch	−6.1	−11.3**	−21.0**	−22.2**
Heart	D.W.	—	− 3.4	− 5.5†	− 7.3††
Kidneys	Starch	+2.9*	−14.5*	−26.0**	−22.9**
Kidneys	D.W.	−5.8	− 7.0†	−13.3††	−15.1††
Liver	Starch	−3.3*	−12.1**	−21.2**	−21.1**
Liver	D.W.	−5.9	+ 1.9	− 6.2†	−12.1††
Lungs	Starch	−6.0*	− 8.7*	− 6.7	11.9**
Lungs	D.W.	+5.8	− 1.8	− 3.4	3.8
Muscle (ventral abdominal wall)	Starch	−9.5	−15.2	−22.6*	−27.1**
Muscle (ventral abdominal wall)	D.W.	—	−12.3††	−11.2††	17.1††
Skin	Starch	−0.8	−18.0**	−30.0**	−34.1**
Skin	D.W.	−2.2	− 2.7	−11.5††	−18.3††
Spleen	Starch	+1.1	−10.1	−35.3**	36.2**
Spleen	D.W.	1.7	− 6.1	−13.3††	−20.6††
Submaxillary salivary glands	Starch	+6.0	+ 9.1	−12.0**	−18.5**
Submaxillary salivary glands	D.W.	—	− 5.1	+ 2.5	− 1.5
Testicles	Starch	+1.9	− 3.3	− 8.2*	11.8*
Testicles	D.W.	+0.2	− 2.2	− 3.8	− 7.5††
Thymus gland	Starch	−6.5	−17.5**	−11.9**	16.3**
Thymus gland	D.W.	+0.8	+ 1.2	−16.9††	−16.9††
Residual carcass	Starch	−0.4	−11.0*	−20.8**	−21.8**
Residual carcass	D.W.	−3.3	− 1.5†	− 7.8††	−14.3††

†Weight of organs was fresh weight measured in g. The results are expressed as mean per cent change from controls given only gastric tube insertions, specifically as $(\bar{X}_d - \bar{X}_c)/\bar{X}_c \times 100$ where $\bar{X}_d$ is the mean in rats given starch or distilled water and $\bar{X}_c$ the mean in tube-insertion controls. In animals given starch, * indicates that the mean per cent change was significantly different from that in rats given distilled water at P = 0.05 to 0.02 and ** at P = 0.01 or less. In rats given distilled water, † indicates that $\bar{X}_d - \bar{X}_c$ was significant at P = 0.05 to 0.02 and †† at P = 0.01 or less.

cardiac stomach were also hypertrophied (Fig. 1a). The lamina propria and submucosa were hyperemic. A mild, diffuse fatty degeneration or early necrosis was occasionally present in the liver, and small areas of venous stasis and capillary hemorrhage were occasionally noted in the kidneys. Mild degrees of hyperemia or capillary-venous congestion were found in some adrenal glands, testicles, thymus glands, hearts, lungs and salivary glands. These minor changes were seen in controls given distilled water. In general, therefore, the microscopic appearance of body organs was normal or almost normal apart from gastrointestinal hypertrophy.

DISCUSSION AND CONCLUSIONS

The oral toxicity of starch was studied in this laboratory as part of a program designed to measure the toxicity of common foodstuffs in albino rats. This program in turn followed earlier attempts to demonstrate idiosyncratic skin reactions to drugs in albino rats. We had investigated the hypothesis that skin reactions to drugs could be due to marginal vitamin deficiencies, which may occur in man but are rarely seen in albino rats fed a standard high vitamin laboratory chow.[12] Adult rats were fed a group of diets used by nutritionists to demonstrate vitamin deficiencies in weanlings and were given benzylpenicillin in relatively large doses. A fulminating lethal reaction followed in rats fed certain of those diets. This lethal reaction could not be prevented by vitamin supplementation, and it appeared to be due to large amounts of certain foodstuffs in the diet. For example, in a diet deficient in pantothenic acid for rats, carbohydrate is provided as sucrose; it was found that rats fed this diet ate an amount of sucrose per day which would kill them if given at one time on an empty stomach.[13] Of the various foodstuffs investigated to date, starch is by far the least toxic.

The rat responds to oral administration of large amounts of starch as it does to large amounts of unabsorbable substances such as kaolin[14] and barium sulfate.[15] The excess matter is pelleted and excreted, and death occurs from bowel obstruction if the animal is unable to move these large calculi along the gastrointestinal tract. Bowel obstruction could be produced in over 50% of albino rats by adminis-

TABLE IV.—WATER CONTENT OF BODY ORGANS IN ALBINO RATS AFTER 14 DAYS OF DAILY ORAL ADMINISTRATION OF STARCH AND OF DISTILLED WATER (DW)†

Organ	Group	Daily dose of starch (1st subheading line, g. per kg.) or distilled water (2nd subheading line, ml. per kg.)			
		38	72 - 120	188 - 201	250 - 288
		60	120 - 200	280 - 320	400 - 480
Adrenal glands	Starch	+4.3	−4.7	−5.6	−2.7
Adrenal glands	D.W.	−0.9	−7.9	−5.5	+0.4
Brain	Starch	+1.7	−0.9	−1.9	−1.3
Brain	D.W.	−0.8	−0.8	−2.2†	−1.0
Heart	Starch	−1.2	−2.8	−1.8	−2.1
Heart	D.W.	−0.1	−2.5†	−3.2†	−2.9†
Kidneys	Starch	+1.5	−3.8	−3.6	+0.8*
Kidneys	D.W.	−0.4	−1.0††	−5.0††	−1.6†
Liver	Starch	−3.0	+0.2	+5.7*	+4.4*
Liver	D.W.	−4.5	−2.3	+0.8	−1.1
Lungs	Starch	−0.5	−0.5	−3.0	−2.7
Lungs	D.W.	−0.8	0.0	−2.4	1.9
Muscle (ventral abdominal wall)	Starch	−1.5*	−2.5	−0.8	−0.1*
Muscle (ventral abdominal wall)	D.W.	−1.9	−5.2††	−1.8†	−1.2
Skin	Starch	−1.1	+0.1	+1.4*	+3.6
Skin	D.W.	−1.1	−2.2	−4.3†	+1.5
Spleen	Starch	−1.1	−0.4	−2.1	0.0
Spleen	D.W.	−0.9	+0.2	2.2†	−0.9
Submaxillary salivary glands	Starch	−1.0	−3.0	−3.9	−2.6
Submaxillary salivary glands	D.W.	−4.7	−7.0††	−8.5††	−7.3††
Testicles	Starch	+0.5	−1.9	−1.3	2.3
Testicles	D.W.	−0.8	−2.8††	−1.3	−1.2
Thymus gland	Starch	−2.7	−1.3	−2.2*	−3.2
Thymus gland	D.W.	+1.3	−2.0	1.9†	−3.2
Residual carcass	Starch	+2.5	−0.8	+0.1*	0.0
Residual carcass	D.W.	+1.3	−2.3	−3.0	+0.1

†Water content was measured as g. per 100 g. dry weight. The results are expressed as mean per cent change from controls given only gastric tube insertions, specifically as $(\bar{X}_d - \bar{X}_c)/\bar{X}_c \times 100$ where $\bar{X}_d$ is the mean in rats given starch or distilled water and $\bar{X}_c$ the mean in tube-insertion controls. In animals given starch, * indicates that the mean per cent change was significantly different from that in rats given distilled water at $P = 0.05$ to 0.02 and ** at $P = 0.01$ or less. In rats given distilled water, † indicates that $\bar{X}_d - \bar{X}_c$ was significant at $P = 0.05$ to 0.02 and †† at $P = 0.01$ or less.

tration during one day of sufficient kaolin or barium sulfate, but starch had to be given daily for some seven weeks before it produced gastrointestinal obstruction in a similar percentage of animals. This occurred following absorption of most of the water from the starch paste, leaving usually a single large calculus or gastrolith in the stomach and many large starch calculi in the small bowel. Allan and Woodruff[5] reported a starch gastrolith in a young woman who had eaten three to four pounds of starch daily for one year. Hypertrophy of the gastrointestinal tissues, particularly of the circular fibres of the muscularis externa, permitted most rats to evacuate starch calculi in the stool.

Other signs of toxicity were inhibition of growth, anorexia, oligodipsia, mild hypothermia, diuresis, alkalinuria and a stress reaction. Mild toxic changes were occasionally noted microscopically in the liver and kidney, and these were associated with capillary-venous congestion of several body organs. These signs occurred in controls given equivalent volumes of distilled water and could have been due largely to the water, although convulsions, a characteristic of water intoxication,[16] did not occur.

Anemia, which has been reported frequently in women addicted to eating starch,[1-4] did not occur in our rats. This was due probably to the rats eating some 75% of their normal daily amounts of laboratory chow, a diet which contains vitamins and minerals in considerable excess of the animals' minimal needs. The results suggest that anemia in starch-addicted women is due to deficient intake of iron-containing foods rather than to starch. Edwards *et al.*[2] found the diet of pregnant cornstarch-eaters deficient in calories, protein, calcium, iron and most vitamins.

The response of the rat to absolute and partial starvation has been studied in this laboratory and the results suggest that starvation was not responsible for loss of body weight in rats given starch.[17,18] For example, starvation produces gastric ulcers which were not present in starch-treated rats. While part of the loss in body weight (actually inhibition of growth) was due to the water vehicle, loss was significantly greater in starch-treated rats and must be listed as a sign of subacute starch intoxication. The starch-treated animals were also more susceptible to pneumonia.

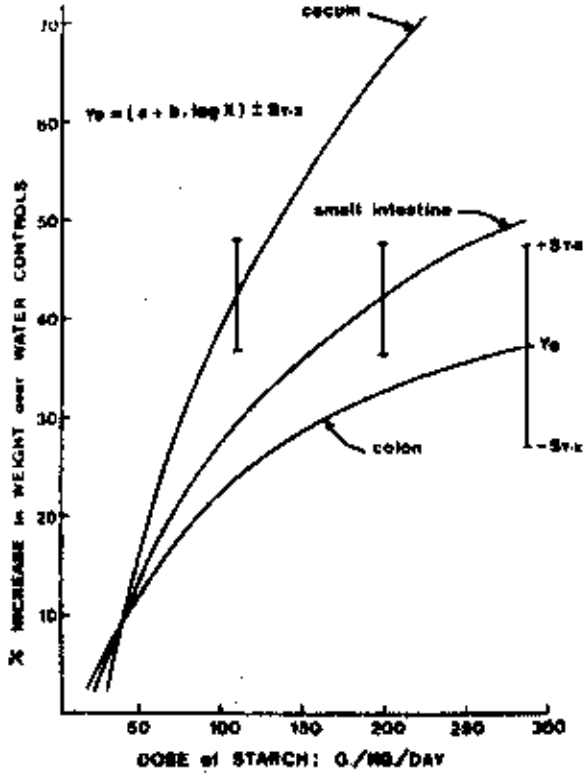

Fig. 3.—The regression, on daily dose of starch, of mean per cent increase in the weight of cecum, small bowel and colon over the respective weights in controls given distilled water. The values of "a" were −122, −49 and −44, of "b" 39.3, 18.3 and 18.8, and of Sy.x or the standard error (S.E.) 5.7, 4.0 and 9.3 in the respective estimated value of Y in the equation Y = (a + b log X) ± S.E.

Diets fed to albino rats seldom contain more than 75% starch.[8] Rats fed such a diet will eat from 40 to 100 g. of starch per kg. body weight per day, the younger animal eating relatively more.[8] When it is recalled that substances eaten with food over the 24 hours of the day are less toxic than when administered intragastrically at one time,[18] it would appear that a diet of 75% starch would produce no signs or minimal signs of toxicity due to the starch component.

Summary Addiction to starch eating has been reported mainly in Negro women of the United States of America. To investigate its toxicity, starch was given intragastrically as a paste in large doses to albino rats. Water was absorbed from the paste in the stomach and upper bowel, and the starch was converted to a calculus. Apparently in response to the extra work required to evacuate these calculi, there followed a considerable hypertrophy of the smooth muscle of the gastrointestinal tract. This permitted the administration of increasing daily doses of starch over a period of two to seven weeks. During this period doses of one-tenth body weight and above produced some inhibition of growth; doses of one-fifth body weight and above increased the susceptibility to pneumonia and bowel obstruction owing to the inability of the animal to evacuate the starch calculi. It would not appear that doses of this order could readily be taken by man, and smaller doses had insignificant toxicity. Anemia reported in human starch addicts would appear to be caused by dietary deficiencies rather than by the starch intake.

Résumé L'habitude de manger de l'amidon a été surtout constatée chez les négresses des États-Unis d'Amérique. En vue d'étudier la toxicité de cet aliment, nous l'avons donné à fortes doses sous forme de pâte, par voie intragastrique à des rats albinos. L'eau étant absorbée à partir de la pâte dans l'intestin et la première portion de l'intestin, l'amidon s'est transformé en calcul. On a alors constaté une hypertrophie considérable du muscle lisse du tractus gastro-intestinal, cette réaction étant probablement la conséquence du travail supplémentaire exigée de l'organisme pour évacuer ces calculs. Cette hypertrophie a permis d'administrer des doses quotidiennes croissantes d'amidon sur une période variant de deux à sept semaines. Pendant cette période, des doses quotidiennes allant jusqu'au 1/10ème du poids corporel ou au delà de ces doses ont entraîné un certain retard de la croissance; des doses d'au moins 1/5ème du poids ont augmenté la prédisposition à la pneumonie et à l'occlusion intestinale, causée par l'impossibilité pour l'animal d'évacuer les calculs d'amidon. Il ne semble guère possible de faire prendre à l'homme des doses aussi considérables et, d'autre part, les doses faibles n'ont guère de toxicité. Quant à l'anémie signalée chez l'homme qui est accoutumé à absorber de l'amidon, elle est probablement l'effet d'une insuffisance alimentaire et ne paraît pas pouvoir être reliée à l'ingestion d'amidon.

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NEOPLASM STUDIES

II. The Effect of Injecting Starch Grains into Transplanted Tumors [1]

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In recent studies on the positive chemotactic reaction of polymorphonuclear leukocytes to starch grains in tissue culture it was found that a progressive and continued accumulation of leukocytes occurred as long as the starch grains were too large to be engulfed by a single leukocyte. These observations suggested using starch grains of a relatively large size as a means of determining the effect of artificially induced leukocytosis on tumor tissues *in vivo*.

Arrowroot and corn starch were selected, the grains averaging 50-500 micra in diameter. The starch grains were sterilized by dry heat at a temperature of about 150° C. for one hour. They were then suspended in mammalian Ringer's solution just prior to use. This method of sterilization had no effect on attractability of the leukocytes. The tumors used were the transplantable mouse sarcomas 48 and 180, the Harding and Passey mouse melanoma, mouse carcinomas 5 and 63, and rat sarcoma 39.

Table 1: *Result of Inoculating Mice with Chopped Tumor Tissue Mixed with Suspensions of Foreign Material of Various Kinds*

Tumor	Material mixed with tumor	Number of mice	Mice after three weeks	
			With growth	Without growth
Sarcoma 180	Melanin granules	20	20	
Carcinoma 63	Bone charcoal	20	20	
Carcinoma 5	Bone charcoal	25	25	
Melanoma	Bone charcoal	20	20	
Carcinoma 63	Starch grains	36	9	27
Melanoma	Starch grains	13	1	12
Sarcoma 180	Starch grains	20	7	13
Carcinoma 5	Starch grains	26	20	6

Experiment I: Tissue cultures of finely chopped tumors mixed with starch showed that the presence of the starch grains in the explant had no effect on the proliferation and outgrowth of the tumor cells. The starch grains slowly disintegrated in the tissue culture medium (1), so that after several transplants over a period of ten days to two weeks all trace of starch was lost.

Experiment II: In the second group of experiments (Table I) mice were inoculated with mixtures of starch and chopped tumor tissue, while control

[1] With the aid of The International Cancer Research Foundation, Philadelphia, Pa.

TABLE II: *Mouse Sarcoma 180: Dimensions of Tumors after Injections of Arrowroot Starch*

	Gross weight of mouse	Measurements in millimeters							Remarks
		1st day	3rd day	7th day	10th day	12th day	21st day	37th day	
1.	14 gm.	R. 7×8* L. 3×3*	7×11 4×5	11×11* 4×4*	11×11* 5×5*	11×11* 5×5*	9×11 5×5		Animal died on 21st day with congested lungs. Tumor showed large areas of necrosis and marked leukocytosis
2.	22.5 gm.	10×12.5*	9×10	8×10*	15×17 (flat)		14×15		Animal killed on 22nd day. Tumor with areas of necrosis and leukocytosis
3.	23 gm.	8.5×11*	3×10 (soft)	Slight	None				Animal killed on 32nd day. Connective-tissue adhesions in site of tumor which had disappeared
4.	23 gm.	R. 12×14* L. 10×11*	12×17 11×10	11×16* 10×11*	11×15* 8×9*	12×15* Small	7×10 None	None	Animal killed on 37th day. Weight, 30 gm. Scar tissue with adhesions in place of tumor
5.	27.5 gm.	7.5×11*	9×10	8×13*	8×11*	8×11*	Open wound with pus	Small, dry scar	Animal killed on 81st day. Connective-tissue adhesions in place of tumor

* Starch injections made.

series received inoculations of chopped tumor mixed with a suspension of Ringer's solution of bone charcoal, manganese dioxide, and melanin granules. The melanin granules were obtained from a suspension of crushed Harding and Passey mouse melanoma, one portion of the non-cellular débris being used unboiled, the other boiled.

In none of the controls was any effect on tumor growth observed. A very definite effect, however, was obtained when the chopped tumor tissue was mixed with starch. Three of the tumors used—carcinoma 63, the melanoma, and sarcoma 180—were markedly inhibited. With carcinoma 5 the percentage of mice which developed tumors was relatively high. It is significant, however, that the mice injected with the starch mixtures, whether or not they developed growths, survived at least a month longer than those which had been inoculated with the other mixtures or with unmixed tumor tissue.

EXPERIMENT III: In the third set of experiments arrowroot starch was injected directly into growing tumors *in vivo*. The dry sterilized starch was mixed with Ringer's solution, about 15 per cent or just sufficient to make a dense suspension which could be readily drawn into a Luer syringe through a 22-gauge hypodermic needle, 1½ inches long. Before the suspension settled in the syringe, the needle was inserted through the skin to one side of the tumor, and the injection made as nearly as possible into the central region of the tumor. The amount injected was from 0.2 to 0.3 c.c. In the large tumors, injections were sometimes made into several areas by partially withdrawing the needle after one injection and re-inserting it for another. Control tumors which were injected with Ringer's solution alone showed the usual type of growth characteristic of untreated tumors.

In one series of experiments on mice with sarcoma 180 in one or both axillary regions, the dimensions of the tumors were recorded before and after the starch injections. The surface dimensions at the beginning of the experiments varied from about 5 to 20 mm. Two, three, and four injections were made at three-day intervals, the number of injections bearing no relation to the size of the tumor at the beginning of the experiment.

The surface dimensions of tumors injected with starch in 50 mice were measured at intervals on successive days. From these records five typical cases are given in Table II. The tumor in some instances decreased in size after the injections, in others not. Autopsy and microscopic examination showed a marked leukocytosis and necrosis in all 50 mice regardless of whether or not the tumors had decreased in size. When the regression had gone so far as to leave no external sign, it was found that the tumor had been replaced by scar tissue. In some of the fifty mice there was no external sign of regression. However, the significant feature is that an appreciable number of the injected mice survived long after the uninjected controls, all of which died with large tumors within three weeks of inoculation. Of the mice with starch-injected tumors, those which survived were vigorous and active fifty days after inoculation.

The results of another series of experiments, in which 33 mice, inoculated with sarcoma 180, were injected twice with starch at intervals of five days are shown in Table III. Several of the mice were apparently not affected by the injections, sections of the persisting tumors giving the usual histologic picture.

TABLE III: *Mouse Sarcoma 180: Results of Two Injections at Five-day Intervals of 0.2 c.c. Dense Suspension of Raw Corn Starch into Tumors Transplanted Ten Days Before First Injection*
(Tumor at time of injection varied from 5 × 4 to 8 × 15 mm. in the two surface dimensions)

	Tumor regressed or necrosed	Tumor persisted
Alive and healthy 73 days after injection with noticeable decrease in size of tumor between 17 and 27 days and disappearance noted between 27 and 47 days	16	
Killed at intervals from 12 to 47 days, showing intensive necrosis of tumor	4	
Killed at intervals from 15 to 30 days, showing tumor entirely replaced by connective tissue adhesions	3	
Died on 26th day, showing connective tissue adhesions	1	
Died on 12th and 29th day, the former with slight and the latter with extensive necrosis	2	
Killed on 26th day, showing a typical tumor		1
Died within 11 and 27 days with enlarged tumor showing no effect of injections		6
TOTAL	26	7

of sarcomatous growth. Of these animals, 6 died within twenty-seven days while a seventh was killed on the twenty-sixth day. The remaining 26 gave evidence either of extensive leukocytosis and necrosis of the tumor tissue or of its replacement by connective tissue.

In all histologic preparations of tumors injected with starch *in vivo*, a marked accumulation of polymorphonuclear leukocytes was observed, massed about the starch grains and dispersed throughout the tumor tissue. In the regions occupied by leukocytes there were pronounced necrosis of the tumor and more or less extended deposition of fibrous tissue. In sections of the tissues of mice in which no tumor could be palpated, the site of the original tumor showed only fibrous tissue with adhesions.

EXPERIMENT IV: In a fourth set of experiments, tissue cultures were prepared of tumors from mice killed in Experiment III. The mice were killed at twelve, seventeen, twenty-seven, and forty-seven days after the first starch injections. Tissue cultures were also made of fragments of carcinoma 63 and 5 into which starch had been injected previously *in vivo*. In all these cases polymorphonuclear leukocytes were observed migrating in large numbers from the explants within a few hours of culturing. In marked contrast to this were cultures of similar tumors removed from uninjected animals and then planted either with or without starch grains. The outgrowths from these cultures showed few if any leukocytes, indicating definitely that the pronounced leukocytosis observed in the cultures of tumors which had been injected with starch *in vivo* must have been due to an extensive migration into the tumor of leukocytes from the surrounding tissues and not from the neoplasm itself.

Discussion

The presence of leukocytes in tumors has been explained by Battaglia (2) as due to a positive chemotactic substance produced by the tumor cells. We have tested the chemotactic effect of aqueous extracts of necrotic tissue of various mouse tumors by the micro-capillary tube method recently described by us (1). The leukocytes of mouse blood and mouse spleen showed no sign of being attracted to these extracts, though they are markedly attracted to sugar solutions and to disintegrating starch. More recently Pavlovsky and Widakowich (3) have stated that eosinophilic leukocytes have a destructive effect on tumor cells. This is borne out, as far as leukocytes in general are concerned, by the results presented in this preliminary report. Our observations indicate that the injection into mouse tumors of starch grains, which are positively chemotactic to leukocytes, occasions a marked accumulation of polymorphonuclear leukocytes in and around the tumor. The accumulation definitely inhibits or slows down further growth of the tumor and in many cases results in the complete regression of the tumor through a process of aseptic necrosis and subsequent resorption of the necrotic material.

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NEOPLASM STUDIES

V. The Effect of Carbohydrate on the Accumulation of Granulocytes in Various Mouse Tumors [1]

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In a previous communication (1), it was shown that *in vivo* injections of starch and cellulose into various mouse tumors brought about regression of the tumor in a high percentage of cases. The experiments were based on the fact that carbohydrates exert a positive chemotactic effect specifically on blood granulocytes [2] and the injections were made to determine the consequence of inducing the accumulation of granulocytes in a tumor.

The chemotactic effect of substances on granulocytes has been demonstrated (2) by preparing tissue cultures with the material to be tested in microcapillary tubes alongside a growing fragment of a tissue. When the material is positively chemotactic, the granulocytes migrate into the open end of the capillary tube and accumulate there. When the attracting agent is a solution (*e.g.*, a soluble carbohydrate) the effect tends to be temporary, the granulocytes leaving the tube after several hours. When, however, the attracting agent is relatively insoluble, *e.g.*, starch or cellulose, the granulocytes continue to accumulate and remain around the particles for a long time.

The granulocytes form a massive accumulation about the starch grains and disperse only when the grains have disintegrated. It also has been shown that blood plasma, free of granulocytes, exerts a disintegrating action on starch. This action is much slower in the absence of granulocytes, indicating that the latter are capable of performing extracellular digestion.

The present report deals with injection experiments on about 3,000 mice, with histologic examination of tumors removed at intervals after the injections. Its purpose is mainly to present histologic data on the effect of the injections. For this reason large numbers of mice were killed which otherwise would have been kept to determine the ultimate effect of the injections on tumor regression.

Material and Methods

Four types of neoplasm were used: two transplantable tumors, viz., sarcoma 180 and carcinoma 63; a spontaneous carcinoma, and a chemically (methylcholanthrene) induced tumor. Most of the work was done with mouse sarcoma 180. This tumor has a very low incidence of spontaneous

[1] Aided by the International Cancer Research Foundation, Philadelphia, Pa., and the Anna Fuller Fund, New Haven, Conn.

We thank Dr William Kaufmann of the Department of Pathology, New York University Medical College, for his assistance in studying the histology of the tumors.

[2] In this report the term granulocyte signifies the polymorphonuclear blood leukocyte of which the neutrophil type is the most common in the mouse.

complete regression, is relatively non-vascular and, under normal circumstances, shows practically no infiltration of granulocytes. It grows rapidly and inoculated mice usually die within two or three weeks with large, localized growths at the site of the inoculation. Necrosis occurs spontaneously in various regions of the tumor but rarely results in complete regression.[5]

Carcinoma 63 is also classed among the rapidly growing tumors, and is relatively free of granulocytes. About 20 per cent of the transplants, before they have reached an appreciable size, regress spontaneously. When, however, the transplants become well developed, to about 9 mm. in diameter, the incidence of regression is low.

The methylcholanthrene-induced tumors were obtained from the Institute of Cancer Research, Columbia University, and the spontaneous tumors were of the C.F.W. strain obtained from the Carworth Farms Inc., New City, N. Y.[6] Both of these tumors are of relatively slow growth and, because of this, proved to be more satisfactory than the rapidly growing transplantable tumors for studying the effect of the injections. There was practically no infiltration of granulocytes in the untreated tumors and the incidence of spontaneous regression was extremely low. Metastases were common in mice with the spontaneous tumors.

The age of the sarcoma 180 selected for the experiments was seven to fifteen days, the average diameter of the tumor being 9 mm.; the age of the carcinoma 63 was ten to twenty-five days, the average diameter being 10 mm. The spontaneous tumors varied from about 7 mm. in diameter to about 23 by 33 mm., and the methylcholanthrene tumors from 10 by 15 mm. to about 25 by 37 mm.

Of the various starches the best proved to be the natural, uncrushed, relatively large-grained arrowroot and cassava. The grains were about 50 to 500 micra in diameter. Whole-grained starch persists longer after injection than the crushed material. The size is also of importance because it was found that a progressive and continued accumulation of leukocytes occurred as long as the grains were too large to be engulfed by a single leukocyte. Rice starch has very small grains which tend to be completely engulfed and, thereby, rendered incapable of further attraction.

The cellulose was obtained in a pure state from the Boyce Thompson Institute through the courtesy of Dr. Wanda K. Farr.

For controls various insoluble materials were used which are known to exhibit no chemotactic properties (2). They were bone charcoal, manganese dioxide, carmine and melanin granules suspended in Ringer's solution. The melanin was obtained by crushing fragments of Harding and Passey mouse melanoma in Ringer's solutions and centrifuging off the heavier débris. No difference was found between boiled and unboiled suspensions of the melanoma granules.

[5] The Crocker mouse sarcoma 180 was obtained from the Institute of Cancer Research of Columbia University, through the courtesy of Dr. Francis Carter Wood. According to unpublished data from that laboratory, the tumor has been designated as an undifferentiated fibrosarcoma. Its histologic morphology has remained constant through many years of propagation and only in rare instances (much less than 2 per cent) does spontaneous regression occur.

[6] The C.F.W. strain is reported by the Carworth Farms as giving 80 per cent spontaneous tumors among females which have borne many litters.

All the material was carefuly washed in several changes of pure 95 per cent alcohol and finally rinsed in distilled water. It was then dried at 60° C. for twenty-four hours and sealed in dry glass vials, which were autoclaved at 16 lb. pressure for half an hour. The sterilized dry material was mixed with Ringer's solution in a 25 c.c. vaccine bottle to about 15 per cent, a concentration which could readily be drawn into a 2 c.c. Luer syringe through a No. 22 gauge hypodermic needle, 1½ inches long. The suspension was used for injection only when found to be sterile by keeping it at body temperature for twenty-four hours, after which a sample was removed and planted on bacteriological agar.

For injections the hypodermic needle was inserted through the skin (sterilized with iodine) 2 cm. from the tumor and thrust along the subcutaneous tissue until it pierced the tumor. The injections were made either: (*a*) by depositing a given amount, about 0.2 to 0.5 c.c., at a single insertion of the needle, or (*b*) by partially withdrawing the needle after the first insertion, reinserting it and depositing similar amounts in two or three regions of the tumor. The tumors were injected either once or several times at intervals of three days. Measurements were always made just before the injections and at intervals thereafter.

The injection of particulate material into the tumor sometimes results in sudden death of the animal. It is possible that in these cases the material enters a blood vessel and causes death by embolism.

A certain number of the injected animals were killed at periods ranging from five hours to twenty-five days after the last injection. The tumors were then removed and fixed for histologic study in Zenker's fluid, Bouin's fluid, or a formalin-alcohol-acetic mixture. Paraffin sections were cut, 7 micra thick, and representative sections were selected throughout the block for staining. The stains used were Delafield's hematoxylin and eosin and Maximow's azur II-eosin with hematoxylin.

Tissue cultures were made of various regions of the tumors. The cultures were fixed *in toto* about forty-eight hours after incubation at body temperature with 2 per cent formol in Ringer's solution. Some were stained with dilute Delafield's hematoxylin and eosin and others with Giemsa's blood stain. The tissue culture technic gave another check for the aseptic conditions of the tumor.

In this report the results from the injection of starch and of cellulose have been described together because they are essentially the same. The cellulose injections were easier to perform, probably because of the greater uniformity of the cellulose particles in the suspensions. In the tumor, as in tissue culture media, the cellulose lasts much longer than the starch.

Observations

(1) *Spontaneous necrosis is unaccompanied by granulocytic accumulation:* Necrotic foci frequently appear spontaneously in the tumors during any stage of their development. These necrotic regions may increase in size and number during the continued growth of the tumor without destroying it. Of all the mice with sarcoma 180 used in this laboratory, the number in which spon-

FIG. 1. SPONTANEOUS NECROSIS IN SARCOMA 180 WITH PYKNOSIS AT MARGIN OF NECROTIC REGION
A. Early pyknotic stage. B. Later stage. C. Necrotic stage.

taneous complete regression occurred was less than 2 per cent, which is in accord with the records on sarcoma 180 of the Institute of Cancer Research.

A very early stage of a spontaneously appearing necrotic region in sarcoma 180, Fig. 1A, shows a localized area of pyknotic cells surrounded by normal appearing tumor cells with no evidence of granulocytes. Fig. 1B shows a later stage, in which the zone of pyknosis forms an advancing border around a central region of amorphous material. A still more advanced stage of necrosis is shown in Fig. 1C, in which the relatively small number of pyknotic cells probably indicates approaching cessation of necrosis. Fragments of this necrotic tissue, which was generally cheesy in consistency, were planted in a tissue culture medium containing blood granulocytes. In no case was there evidence of a positive chemotactic response of the granulocytes.

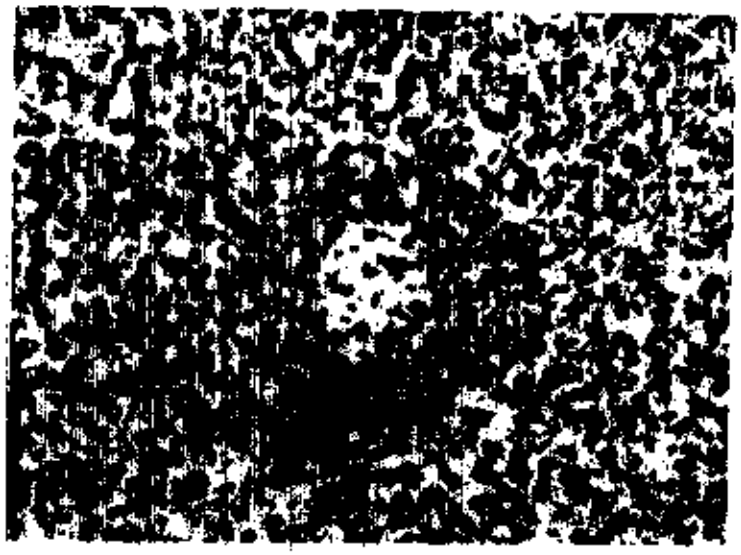

FIG. 2. STARCH GRAIN WITH GRANULOCYTES ACCUMULATED ABOUT IT IN SARCOMA 180, FORTY-EIGHT HOURS AFTER INJECTION

The absence of granulocytes in the regions of spontaneous necrosis is characteristic for all the tumors mentioned in this report.

(2) *Injection of inert material does not affect the normal histology of the tumors:* The inert materials injected were carbon, melanin, carmine, and manganese dioxide suspended in Ringer's solution. Tumors removed within twenty-four hours after the injections showed the material lying in clumps within the tumor, usually with spaces of various size between them and the tumor cells. Occasionally necrotic areas could be distinguished in the vicinity of the injections but they did not differ from those of normal, untreated tumors. When left *in situ*, the tumors continued growing. Histologic sections of tumors removed forty-eight hours subsequent to the injections, or later, showed tumor cells invading the injected masses which tended to become dispersed among the cells without changing the morphology of the tumor.

Mouse tumors of various sizes were also punctured several times *in vivo*,

in different regions, with a blank hypodermic needle to cause mechanical trauma. In other cases varying amounts of Ringer's solution (0.2 to 2.0 c.c.) were repeatedly injected. These tumors were removed and fixed at intervals from two to forty-eight hours after the operation. Except for occasional hemorrhagic areas, the preparations showed no pathological effects.

(3) *Injection of starch or cellulose produces* (*a*) *widespread granulocytic accumulation and necrosis with regression of tumor, or* (*b*) *restricted accumulation of granulocytes, or* (*c*) *no accumulation of granulocytes.*

(*a*) *Widespread granulocytic accumulation and necrosis:* (1) Sarcoma 180: When sugar solutions are injected into tumors, there is no accumulation of granulocytes, presumably because the sugar rapidly diffuses away. On the other hand, starch and cellulose tend to remain intact, the starch disappearing

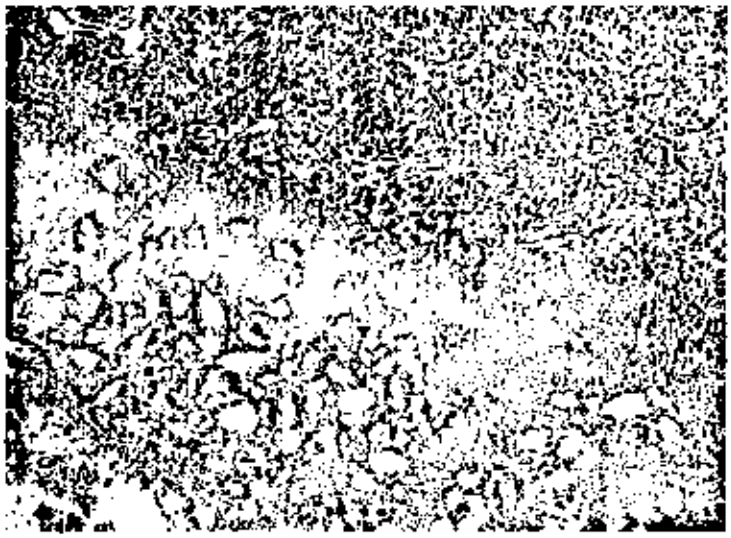

FIG. 3. LOW-POWER VIEW OF SARCOMA 180, SHOWING A CLUMP OF CELLULOSE (AT LOWER LEFT) WITH A MASSIVE ACCUMULATION OF GRANULOCYTES ALONG THE MARGIN SEVENTY-TWO HOURS AFTER INJECTION

in from several to eight or ten days after the injection while the cellulose lasts much longer. Fig. 2 shows a single starch grain densely surrounded by granulocytes in a section of mouse sarcoma 180 taken forty-eight hours after the starch had been injected. There is considerable necrosis about the region of the accumulation.

Induced necrosis of the tumor occurs only when the injection is followed by an accumulation of granulocytes in the tissue. A low-power view of the reaction in a zone adjoining the cellulose is shown in Fig. 3, from a section of sarcoma 180, seventy-two hours after the injection. The tumor tissue in the vicinity of the injected mass had undergone necrosis, with a marked accumulation of granulocytes. Beyond the zone of granulocytic invasion the tumor cells still appear normal.

Fig. 4 shows the zone between the necrosing region and the normal tumor

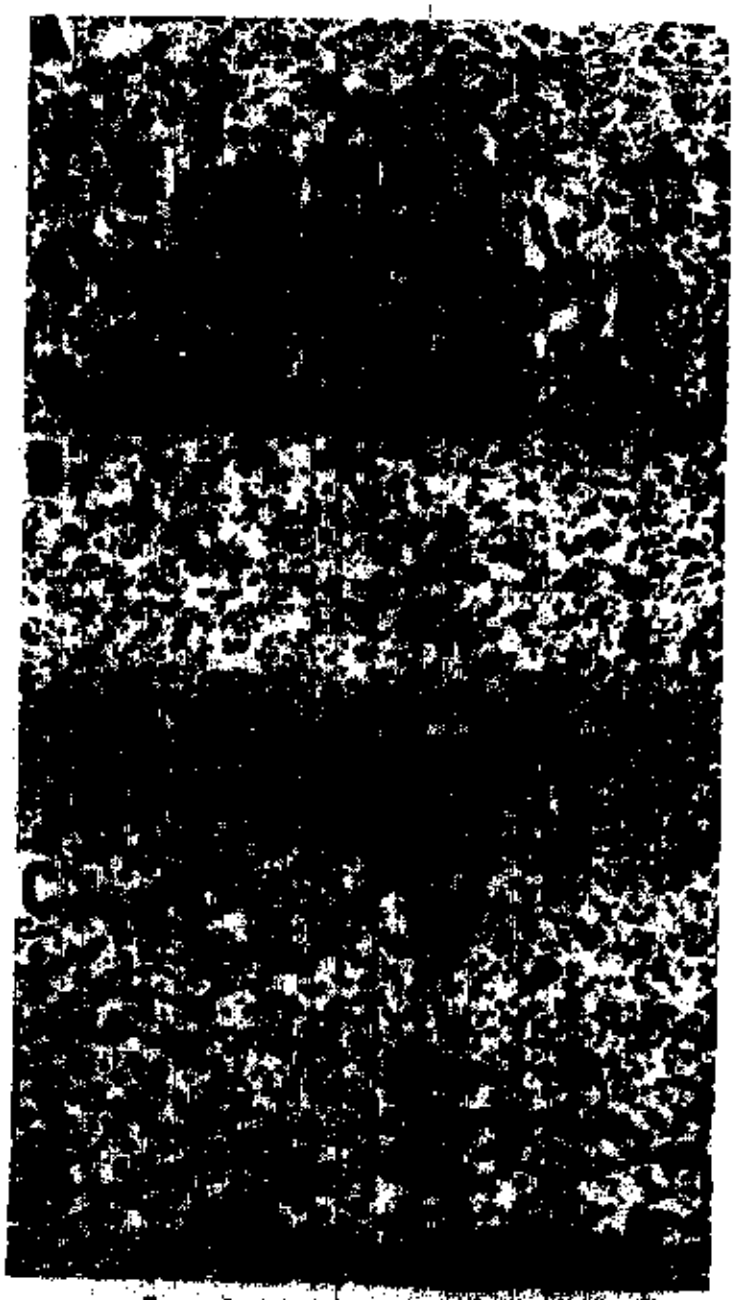

FIG. 4. SARCOMA 180 [illegible]

A. Tumor cells undergoing pyknosis in region of accumulated granulocytes. B. More advanced stage: necrosing tissue with scattered and accumulated granulocytes. C. Late stage of necrosis with extensive karyolysis.

FIG. 5. SPONTANEOUS MOUSE CARCINOMA, SHOWING MASSIVE ACCUMULATION OF GRANULOCYTES AND BREAKING DOWN OF SOME OF THE TUMOR ACINI

tissue under higher magnification. In 4A, pyknosis and necrosis of the tumor cells are seen to be restricted to the zone of greatest granulocytic accumulation, with a fairly sharp demarcation between healthy and necrosed regions. Granulocytes can be detected among the healthy tumor cells but they are few. A more advanced stage is shown in Fig. 4B, in which distributed granulocytes can be seen among the pyknotic tumor cells beyond the zone of the greatest accumulation. The fact that necrotic changes appear beyond the region where the granulocytes are massed indicates that the necrosing process is due to something more than the crowding of the granulocytes. The necrosing zone around the massed granulocytes keeps advancing.

A further advanced stage is shown in Fig. 4C. Granulocytes are distributed through the region and all the tumor cells are in varying stages of disintegration. The necrosis continues until the mass becomes cheesy, consisting of the disintegrated remains of tumor cells and granulocytes. Chromatic débris may be either present or absent. This disintegration spreads until the entire tumor is affected.

When the tumor is in an advanced stage of disintegration lymphocytes and macrophages in large numbers make their appearance in the connective-tissue capsule. Gradual absorption then takes place until the entire mass disappears and a fibrous scar forms at the site. Prior to absorption, the dimensions of the disintegrated tumor may remain constant for as long as twenty-five days after the injection.

(2) Other mouse tumors: A number of starch and cellulose injections were also made into carcinoma 63, into spontaneous and into chemically (methylcholanthrene) induced tumors. In carcinoma 63 the granulocytes, attracted by the injections, spread among the acini of the glandular tissue and necrosis

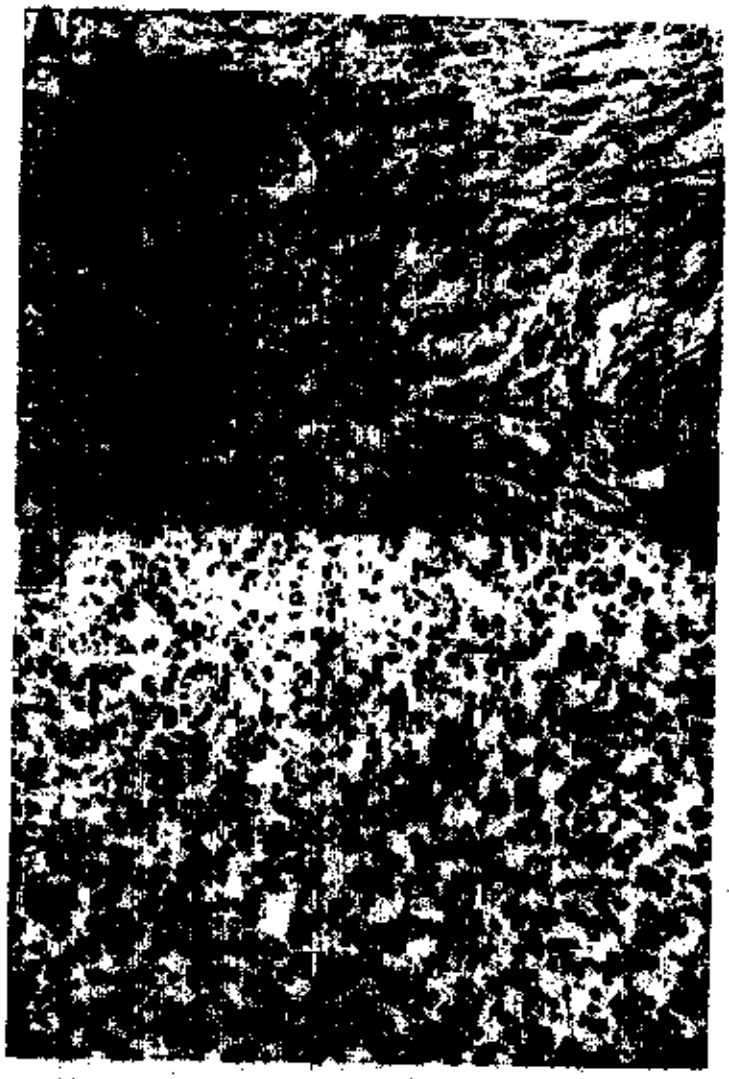

FIG. 6. METHYLCHOLANTHRENE TUMOR SIX DAYS AFTER INJECTION OF CELLULOSE

A. Granulocytes advancing beyond the region of necrosis and lying among normal appearing tumor cells. B. Generalized karyolysis of tumor cells in presence of granulocytes.

occurred wherever the granulocytes had collected. The phenomena accompanying the necrosis were identical with those described for sarcoma 180. The spontaneous and chemically induced tumors were all relatively slow growing and gave a definitely higher percentage of regression consequent to the injections than did the more rapidly growing carcinoma 63 and sarcoma 180.

All the spontaneous tumors which were removed for histologic examination were mammary carcinomata. A large number of these tumors were cystic, in which case the brownish bloody fluid of the cysts was aspirated to relieve pressure before the injection. The aspiration by itself was found to

have no effect on the growth of the tumor. Histologically the tumors removed at various intervals after injection showed an extensive granulocytic infiltration (Fig. 5).

The methylcholanthrene tumors reacted strikingly to the injections. The infiltrating granulocytes formed looser aggregations in this tumor than in sarcoma 180. This is shown in Fig. 6A, in which a considerable number of granulocytes can be seen among the healthy appearing tumor cells. Fig. 6B is from a region in which the tumor cells have undergone complete necrosis, with granulocytes predominating. This figure closely resembles Fig. 4C showing sarcoma 180.

(*b*) *Restricted accumulation of granulocytes:* In many cases the injected mass of starch or cellulose caused a massive accumulation of granulocytes

FIG. 7. SARCOMA 180 SHOWING TWO STARCH GRAINS WHICH DID NOT ATTRACT GRANULOCYTES AND ABOUT WHICH THE GROWTH OF THE TUMOR CELLS CONTINUED UNINTERRUPTED

The tumor cells on the margin of the starch grains appear to be crowded together. This is not actually the case, but is due to the pliable blade of the microtome knife glancing over the grain instead of cutting through it and hence rendering the immediate vicinity thicker than elsewhere.

which, however, became surrounded by a fibrous capsule. This condition occurred anywhere in the tumor either in the peripheral regions or deep within the tumor mass. There was no sign of necrosis outside the encapsulated material. Evidently there had been, at first, an attraction of granulocytes to the injected material which was later nullified by the formation of the fibrous capsule. This tumor, if left in the body of the animal, would have continued growing unaffected by the injection.

(*c*) *No accumulation of granulocytes:* In some instances the injected material gave no sign of its chemotactic property. An example of this is shown in Fig. 7, from a section of sarcoma 180 in which granulocytes are absent and two grains of starch can be seen closely invested with normal appearing sarcoma cells. This tumor had been injected ten days before its removal. The

traumatic space caused by the injection had become occupied by actively growing tumor tissue completely surrounding the starch. This indicates that the starch of itself has no deleterious effect on the tumor cells. The fact that the starch in this case, although not attracting granulocytes in the tumor, nevertheless possessed its usual chemotactic property, was demonstrated by removing the grains from the tumor tissue and planting them in tissue culture, where they were quickly surrounded by granulocytes present in the medium (Fig. 8). Very occasionally there were localized, necrotized zones without granulocytes about the masses of starch. The sharp demarcation and the lack of pyknosis along the margin of the necrotic masses indicated that the necrosis was not advancing. This may be spontaneous necrosis without relation to the starch.

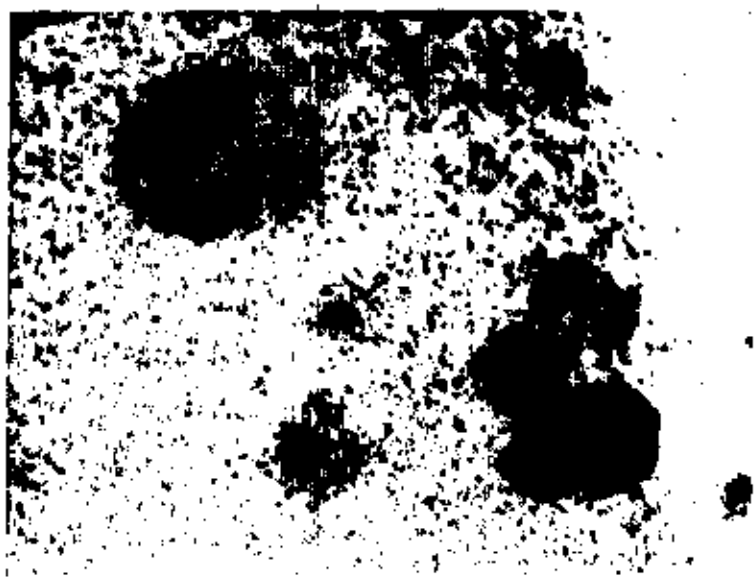

FIG. 8. SEVERAL STARCH GRAINS THICKLY SURROUNDED BY GRANULOCYTES IN A TWELVE-HOUR TISSUE CULTURE

(4) *Comparison of the frequency of widespread necrosis resulting from the injection of starch or cellulose in the various tumors:* By far the majority of the tumors used were sarcoma 180. Three hundred and ten mice were not killed for histologic examination, and in 130 of these the tumors began to decrease in size within eight to twenty days after the injections or remained stationary for twenty days and then decreased until all external appearance of tumor had disappeared in about thirty to forty days. These mice were living and healthy for six months after the injections and were eventually destroyed for final examination. The remaining 180 mice died with large tumors in about fifteen to twenty-five days.

The injection experiments with carcinoma 63 resulted in about the same percentage of regression, *viz.*, about 40 per cent.

The spontaneous and chemically induced tumors grow much more slowly and the animals remain alive for as long as six to seven months. Almost all

with injected tumors were killed at varying intervals. On histologic comparison the impression was gained that granulocyte accumulation and widespread necrosis occurred in a higher percentage of these slower growing chemically induced and spontaneous tumors than of the transplantable neoplasms, sarcoma 180 and carcinoma 63.

Discussion

Among all the mouse tumors examined there was found a high incidence of localized spontaneous necrosis. In none of them could granulocytes be found in appreciable numbers except for a relatively few obviously infected cases. The tissue culture method shows clearly the numbers of wandering cells. The blood granulocytes, if present, migrate out of the explant within a few hours of culturing. Numerous cultures were made of various tumors with and without spontaneous necrotic regions and in no case could granulocytes be obtained in appreciable numbers either from the healthy or necrotic tissue. The lack of chemotactic response to spontaneous necrotic tissue is also shown by the fact that there was no evidence of attraction when fragments of such tissue were planted in tissue culture with normal granulocytes.

The investigations described in this report are of interest in relation to the rôle of the polymorphonuclear leukocytes in induced necrosis. The results indicate that the injection of substances like starch and cellulose, which of themselves are non-toxic, will, in the event that a sufficient number of granulocytes are attracted, cause a widespread necrosis followed by regression of the tumor.

The destructive effect indirectly induced by carbohydrate material through its chemotactic property would explain some of the findings of Lustig and Wechstel (3). In contrast to a large number of substances which they mixed with minced carcinomatous tissue for inoculation, they found that glycogen inhibited the growth. Small tumors formed at the site of the inoculation but soon regressed with necrosis of the inoculated region. It is known that glycogen has the same chemotactic action on blood granulocytes as have other carbohydrates (2).

In all the work described in this report the chances of infection have been carefully ruled out. Moreover, the absence of a granulocytic reaction to the presence of foreign material *per se* has been borne out by the negative results from injecting substances like carbon, carmine, etc. Even the injection of starch may sometimes call forth no granulocytic response.

In the other cases of negative response to carbohydrate injection localized accumulations of granulocytes do occur, but the accumulations are walled off by fibrous encapsulation and there is no spreading of the granulocytes or of necrosis beyond the confines of the capsule, with the result that the tumor continues to grow.

In the literature there are increasing numbers of articles which stress the significance of the part played by induced, aseptic inflammatory processes in the destruction of tumors. Tyzzer (4) had already attributed the failure of development of implanted tumors in non-susceptible mice to the accumulation of polynuclear leukocytes about the implant until it becomes necrotic. This

has more recently been confirmed by Arima (5), who describes granulocytic infiltration into implanted, heterologous tumor fragments. Arima stated that such an infiltration was more pronounced in implants that did not take. We are obtaining similar findings in the heterologous inoculation of spontaneous mouse tumors (unpublished data).

Berenblum (6) used local irritants in experiments on the induction of mouse tumors with tar products, especially 1:2:5:6-dibenzanthracene. The irritant was mixed with the carcinogenic agent, which was applied locally. It was found that the presence of the strongest irritants, *viz.*, mustard gas and other related chemical compounds, prevented the induction of the tumor. Adair and Bagg (7) had already reported tumor destruction by the application of mustard gas locally to animal and human tumors. They ascribe the regressive effect to an induced inflammatory process. The substances used by Berenblum, Adair and Bagg are strong irritants. We have reported (8) that the mechanical irritation of normal tissue results in the liberation of substances which attract granulocytes.

The evidence is strong that, of the various non-irritating substances which have been injected, only the positively chemotactic agents may call forth a granulocytic response. Irritants such as mustard gas or transplanted heterologous tissue, according to our studies (8), would act by inducing the release of substances which serve as chemotactic agents. On the other hand, the reaction to the injection of starch or cellulose is a direct response to the presence of positively chemotactic foreign material.

The cause of necrosis resulting from granulocytic accumulation is still open to investigation. Fischer (9) has indicated in his observations on tissue cultures that the presence of a small number of leukocytes enhances growth in tissue culture while large numbers have a deteriorating effect. Evidence from our studies of the action of leukocytes on starch grains too large to be engulfed suggests the ability of the granulocytes to perform extracellular digestion. It is also possible that destructive substances are liberated by the granulocytes on their death. The progressive deterioration of blood granulocytes following diapedesis has been described by Clark (10) and Kredel (11). Opie (12) found that proteolytic enzymes can be extracted from autolyzed granulocytes, and Weiss and Czarnetzky (13) have extended these observations. These various reactions of the granulocytes are to be considered in the question regarding the initiation and spread of necrosis induced by experimental means.

Summary

1. The injection into a tumor of substances, *e.g.*, starch and cellulose, which are positively chemotactic to blood granulocytes may cause an extensive accumulation of granulocytes and a consequent widespread necrosis involving the entire tumor. The percentage of complete regression for the more rapidly growing transplantable tumors (sarcoma 180 and carcinoma 63) was about 40 per cent. For the more slowly growing spontaneous and methylcholanthrene-induced tumors it was somewhat higher.

2. The starch and cellulose injected tumors which did not regress showed either no accumulation of granulocytes or a restricted accumulation walled off from the healthy part of the tumor by a fibrous capsule.

3. No granulocytic response was observed in tumors to trauma or to hemorrhage; to the injection of suspensions in Ringer's solution of carbon, carmine, manganese dioxide, or melanin granules; or to sugars in aqueous solution.

4. Spontaneously appearing necrotic regions in mouse sarcoma 180, carcinoma 63, methylcholanthrene-induced and spontaneous tumors were all found to be relatively free of granulocytes. Spontaneous necrotic tissue gave no evidence of attraction to granulocytes in tissue culture.

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Effect of Starches on Protein Utilization and Liver Lipid Composition in Rats

SUMMARY—The effect of starches on utilization of protein and lipid composition in the liver of rats was investigated. The carbohydrates tested were rice starch, corn starch, wheat starch, and arrow root starch. Criteria for evaluation of protein utilization were growth response, digestibility of protein, and availability of lysine. Arrow root starch was inferior to corn starch, rice starch, and wheat starch in effect upon protein utilization. Corn starch and wheat starch were similar in effects on protein utilization. Studies of growth and protein efficiency ratios indicated that rice starch is inferior to corn starch and wheat starch. The distribution of fatty acids in the liver lipid was somewhat different in rats fed arrow root starch from that in rats fed the other three starches. Lysine supplementation did not alter the fatty acid pattern in the liver lipid of rats from that in the unsupplemented group.

INTRODUCTION

Although potato starch, both raw and cooked is inferior to sugars, dextrin, and complex carbohydrates such as corn starch in effects on protein utilization (Harper and Katayama, [illegible]; Booher *et al.*, 1951; Jelinek *et al.*, 1952; Chang, [illegible]), a few questions have arisen following certain studies. First, are other starches derived from tubers also inferior to the other carbohydrates on protein utilization, as is potato starch? Secondly, do the starches derived from the cereal grains differ from each other in effects on protein utilization?

Scattered reports have demonstrated that carbohydrates may have a possible role in the development of heart disease. [illegible] (1967) reported that the incidence of heart disease [illegible] showed a better relationship to [illegible] than any other major foodstuff. Evidence has also shown that the type of carbohydrate in the diet affects the level of lipid as well as the fatty acid composition in the blood lipid of man and in the liver lipid of animals (MacDonald, 1962, 1964).

Since cereal grains such as rice, wheat, and corn are widely used as food, it would seem of importance to investigate how the starches derived from these foods influence protein metabolism and fat metabolism as compared with the tuber starch, such as arrow root starch.

EXPERIMENTAL METHODS

Ninety-six weanling rats were divided into two series of 4 groups, each containing 12 rats. The 4 diets in series 1 included the following carbohydrates: rice starch, corn starch, wheat starch, and arrow root starch. For convenience the rats on the different starch sources are referred to herein as "rice rats," "corn rats," etc. The 4 diets in series 2 contained the same carbohydrates as in series 1 plus 0.7% of L-lysine added to each diet. The diets had the following ingredients (in g per 100 g diet): wheat gluten, 24; hydrogenated fat, 8; carbohydrate, 61; salt mixture (USP 14), 4; cod liver oil, 2; and vitamin mix in sucrose, 1. The vitamin mix supplied (in mg/kg of diet): thiamine, 5; pyridoxine, 5; menadione, 5; cobalamin concentrate (3 mg/g), 5; riboflavin, 10; nicotinic acid, 20; Ca pantothenate, 20; d-alpha-tocopheryl succinate, 75; folic acid, 5.5; biotin, 0.3; inositol, 400; and choline chloride, 1,000. The addition of L-lysine to the diets in series 2 was compensated for by subtracting the same percentage of carbohydrate.

The experimental diets were fed to the rats when they

Table 1. Total weight gain and protein efficiency for rats fed various carbohydrate diets (9 weeks on experiment).

CHO* starches	Initial weight (g)	Final weight (g)	Total weight gain (g)	Total food consumed (g)	Total protein intake (g)	Protein* efficiency ratio
Series 1 (without lysine)						
Rice starch	81.9	196.5	74.6[c] ± 4.9	643.9	115.9	0.64[c] ± 0.03
Corn starch	82.7	175.7	93.0[a] ± 4.5	647.3	116.5	0.80[a] ± 0.04
Wheat starch	83.4	176.7	93.3[a] ± 4.8	699.9	126.0	0.74[a] ± 0.02
Arrow root starch	84.2	146.9	62.2[b] ± 4.2	669.5	120.5	0.52[b] ± 0.03
Series 2 (with 0.7% L-lysine)						
Rice starch	88.9	353.7	270.8[c] ± 4.8	956.8	172.2	1.57[c] ± 0.03
Corn starch	88.8	374.5	285.7[a] ± 7.7	967.9	174.2	1.64[a] ± 0.05
Wheat starch	88.5	374.1	285.6[a] ± 4.8	1014.1	182.5	1.56[a] ± 0.02
Arrow root starch	88.4	346.3	257.9[b] ± 5.8	1147.9	206.6	1.25[b] ± 0.03

* Carbohydrate.
* Protein efficiency ratio = weight gain per g of protein eaten.
Means having the same superscript are not significantly different ($P < 0.01$).

were four weeks old and weighed 80–85 g. Food and water were provided *ad libitum*. Body weight and food consumption for each rat were recorded weekly. At the end of 9 weeks, the animals were fed a protein-free diet. Feces excreted during the first three days were discarded. Thereafter, the feces from each rat were collected daily for 5 days for the determination of metabolic fecal nitrogen and lysine. After the protein depletion period, each group was fed its previous tested ration. Feces were again collected for a 5-day period for the determination of total fecal nitrogen and lysine. In changing from one diet to another, three days elapsed before the feces were again collected. The procedures for treatment of the fecal samples and determination of fecal nitrogen and fecal lysine were essentially as described previously (Chang, 1962). At the end of 12 weeks, the animals were anesthetized by intraperitoneal injection of sodium amytal. The rat livers were removed for the analysis of liver lipid, and fatty acid composition in the liver lipid of the rats was determined by gas chromatography (Miller and Ellis, 1965). Differences in means were tested using Duncan's multiple range test (Steel and Torrie, 1960).

RESULTS

Growth and protein efficiency ratios

The average increase in weight of the rats is shown in Table 1. With or without lysine supplementation, the growth rate of arrow root rats was lower than that of corn and wheat rats. The weight gains of corn and wheat rats were similar. The weight gain of rice rats was lower than that of corn and wheat rats. Table 1 also shows the protein efficiency ratio as determined by dividing the total weight gain by the grams of consumed protein during 9 weeks. Without lysine supplementation, the protein efficiency ratios of arrow root rats was lower than that of rats fed the other three diets, and that of rice rats was lower than that of corn and wheat rats. With lysine supplementation, the protein efficiency ratios were similar for rice, corn, and wheat rats and lowest for arrow root rats. Lysine supplementation increased protein efficiency ratios greatly, regardless of type of carbohydrate.

Digestibility of protein and availability of lysine

The digestibility of protein was lowest in arrow root rats and practically identical in the others (Table 2). In both

Table 2. Digestibility of protein by rats fed various carbohydrates (5-day period).

CHO* starches	Total N intake (g)	Total N in feces (g)	Metabolic fecal N (g)	Fecal* food N (g)	Total N* absorbed (g)	Digestibility* (%)
Series 1 (without lysine)						
Rice starch	2.270	0.086	0.019	0.067	2.203	97.0[a] ± 0.2
Corn starch	1.919	0.095	0.021	0.074	1.845	96.1[c] ± 0.3
Wheat starch	2.123	0.097	0.017	0.080	2.043	96.2[a] ± 0.2
Arrow root starch	2.105	0.287	0.093	0.194	1.911	90.7[b] ± 1.2
Series 2 (with 0.7% L-lysine)						
Rice starch	2.572	0.119	0.024	0.095	2.477	96.3[a] ± 0.4
Corn starch	2.596	0.157	0.030	0.127	2.469	95.1[b] ± 0.4
Wheat starch	2.739	0.148	0.028	0.120	2.619	95.6[b] ± 0.3
Arrow root* starch	.....	.....	.....	.....	.....	.....

* Carbohydrate.
* Severe diarrhea, collecting of feces was impossible.
* Fecal food N = total N in feces minus metabolic N.
* Total N absorbed = total N intake minus fecal food N.

* True digestibility of protein = $\frac{\text{total N absorbed}}{\text{total N intake}} \times 100$.

$f > g$ ($P < 0.01$).

Table 3. Availability of lysine for rats fed various carbohydrates (5-day period).

CHO* diets	Total lysine intake (mg)	Total lysine in feces (mg)	Metabolic lysine (mg)	Fecal[b] true lysine (mg)	Total[c] lysine absorbed (mg)	True lysine[d] availability[e] (%)
Series 1 (without lysine)						
Rice starch	230.5	25.1	4.6	20.5	210.0	91.1[a] ± 0.4
Corn starch	209.8	29.0	7.0	22.0	187.8	89.5[b] ± 2.7
Wheat starch	209.9	27.2	5.2	22.0	187.9	89.5[b] ± 0.3
Arrow root starch	185.1	76.7	29.4	47.3	137.8	74.4[c] ± 2.8
Series 2 (with 0.7% L-lysine)						
Rice starch	850.3	25.6	6.4	19.2	831.1	97.7[a] ± 0.7
Corn starch	857.2	31.9	7.3	24.6	832.6	97.1[b] ± 1.0
Wheat starch	943.1	41.7	7.3	34.4	909.3	96.4[b] ± 0.2
Arrow root starch	.....	.....	.....	.....	.....	.....

[a] Carbohydrate.
[b] Fecal total lysine or total lysine in feces minus metabolic lysine.
[c] Total lysine absorbed = total lysine intake minus fecal total lysine.
[d] True lysine availability = $\frac{\text{total lysine absorbed}}{\text{total lysine intake}} \times 100$.
[e] $f > g$ ($P \leq 0.01$).

series, arrow root rats showed distention of the cecum and diarrhea, especially in the lysine- supplemented group; collection of feces of the arrow root rats in series 2 was therefore impossible. The availability of lysine is shown in Table 3. Without lysine supplementation, lysine availability was lowest in arrow root rats. In both series, lysine availability was similar for rats fed the other three sources of carbohydrates.

Liver fat and fatty acid composition

The liver lipid and fatty acid composition are shown in Table 4. Without lysine supplementation, arrow root rats showed the lowest liver lipid concentration. With lysine supplementation, there were no significant differences in liver lipid of the four dietary groups. Studies of the fatty acid composition in the liver lipid indicated that arrow root rats showed the highest stearic and lowest palmitic and myristic acid concentration in both series except that in series 2 the difference in myristic acid concentration between arrow root and rice rats were not statistically significant. There were no significant differences in fatty acid concentration in the liver lipid of rats among the other groups. Lysine supplementation did not alter the fatty acid composition in the liver lipid of any of the groups.

DISCUSSION

The effect of arrow root starch on protein utilization appears to resemble that of potato starch in a general way. Previous studies showed enlargement of the cecum and diarrhea in rats fed raw potato starch (Chang, 1962). Symptoms were similar in our arrow root rats. The low digestibility of protein and availability of lysine are additional evidences indicating the resemblance between these two starches in physical and chemical nature. Since no significant differences between corn and wheat rats were observed in growth rate, protein efficiency ratio, digestibility of protein, or availability of lysine, these two types of starch are presumably nutritionally equivalent in the sense that they may be converted into the same product upon digestion. The slower growth and lower protein efficiency of rice rats indicates that rice starch is inferior to corn starch and wheat starch as a source of carbohydrate. Evidently, data reported in this paper and a previous paper (Chang, 1962) demonstrate that starches derived from tubers such as arrow root starch or potato starch are inferior to the starches derived from cereal grain in protein utilization.

The role of dietary carbohydrate in lipid metabolism has

Table 4. Total lipid and fatty acids in the liver of rats fed various carbohydrate diets.

CHO* diets	Total[a] lipid (%)	Myristic acid (%)	Palmitic acid (%)	Palmitoleic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Arachidonic acid (%)
Series 1 (without lysine)								
Rice starch	5.5[a] ± 0.2	1.0[a] ± 0.1	26.4[a] ± 0.6	6.3 ± 0.6	13.0[a] ± 0.7	27.1 ± 0.8	16.3 ± 0.6	9.9 ± 0.9
Corn starch	5.8[b] ± 0.3	1.6[b] ± 0.1	27.8[b] ± 0.6	6.4 ± 0.4	14.1[b] ± 0.7	26.4 ± 1.0	16.7 ± 0.3	7.7 ± 0.7
Wheat starch	5.6[b] ± 0.3	1.6[b] ± 0.1	28.7[b] ± 0.5	6.3 ± 0.7	13.0[a] ± 0.1	28.6 ± 0.9	16.1 ± 0.5	10.5 ± 0.2
Arrow root starch	4.5[c] ± 0.1	0.8[c] ± 0.1	23.0[c] ± 0.8	5.9 ± 0.7	20.0[b] ± 0.7	24.4 ± 0.8	16.3 ± 0.5	10.0 ± 0.6
Series 2 (with 0.7% L-lysine)								
Rice starch	5.1[a] ± 0.2	0.6[a] ± 0.1	26.0[a] ± 0.7	7.4 ± 0.5	13.8[a] ± 0.5	25.6 ± 0.6	18.6 ± 0.3	8.0 ± 0.3
Corn starch	5.2[a] ± 0.2	0.9[a] ± 0.1	26.5[a] ± 0.3	7.1 ± 0.6	13.2[a] ± 0.5	26.2 ± 0.5	19.0 ± 0.4	7.4 ± 0.5
Wheat starch	5.3[a] ± 0.2	0.8[a] ± 0.1	27.0[a] ± 0.6	6.5 ± 0.7	13.6[a] ± 0.6	25.4 ± 0.7	17.6 ± 0.7	7.1 ± 0.4
Arrow root starch	5.2[a] ± 0.1	0.5[a] ± 0.1	23.2[a] ± 0.8	6.8 ± 0.9	17.7[b] ± 0.4	24.3 ± 0.7	18.4 ± 0.4	9.0 ± 0.4

[a] Carbohydrate.
[b] Percent of lipid in fresh liver.
Means having the same superscript are not significantly different ($P \leq 0.01$).

come to the attention of many researchers (Grant and Fahrenbach, 1959; MacDonald and Braithwaite, 1964; Yudkin, [illegible]). MacDonald (1962) reported that the feeding of sucrose to rabbits was associated with more liver lipid than was feeding with liquid glucose, and the latter with more liver lipid than was feeding with starch. The same author (1964) also reported that starch diet caused a fall in the level of serum lipid whereas the sucrose diet caused a rise in serum lipid of man. Data in this paper furnish evidence that different starches may differ in their influence on lipid metabolism. There is no adequate explanation for the effect of dietary carbohydrate on the fatty acid metabolism noted here. The mechanism of such influence [illegible] further investigation.

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EFFECT OF GLUCOSE, FRUCTOSE, SUCROSE AND STARCH ON NITROGEN UTILIZATION IN RATS FED HIGH AND LOW PROTEIN DIETS

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ABSTRACT

Starch-fed rats did not retain more nitrogen than rats fed sucrose, glucose or fructose on equal calorie and nitrogen intake and at two levels of dietary casein intake, either 18 or 11%. On long-term feeding, starch-fed animals gained more weight than those fed glucose, fructose or sucrose.

Nitrogen balance studies with both low and high protein diets in ad lib. fed animals indicate that the stimulating effect of corn starch or dextrin on growth, as compared with sucrose, is due to increased food intake and not to better utilization of protein (1-3). However, in pair-fed animals on diets containing low levels of amino acids, N utilization was reported to be more efficient with dextrin than with sucrose (7, 8).

Pepper found that sucrose-fed animals gained weight more rapidly than those pair-fed with glucose (4). On the other hand, in experiments of Mitchell et al. (9) the gain in body weight was identical on both rations. With the same amount of food, glucose-fed rats gained more weight than those fed fructose (9).

There is thus a consensus as to the effect of different carbohydrates on N utilization in ad lib. fed animals but lack of agreement as to the effect in pair-fed animals. It is to be noted that in the above-mentioned pair-fed experiments only two different carbohydrates were compared at a time. The effect of any carbohydrate on N retention in relation to other carbohydrates, however, cannot be evaluated unless the carbohydrates concerned are studied at the same time.

Three parallel experiments were therefore carried out to compare the effects of glucose, fructose, sucrose and starch on N utilization in pair-fed animals, on both high and low protein diets.

MATERIAL AND METHODS

In each of the three experiments, six groups of local strain male albino rats weighing 60 to 70 g were studied. Each group contained four animals, each of which was fed a different diet, containing either glucose, fructose, sucrose or starch. The composition of the diets is summarized in Table 1. The first experiment was performed with diets containing 18% casein, and the second and third experiments with diets containing 11% casein.

TABLE 1. *Percent composition of the synthetic diets by weight*

Diet	*Glucose*	*Fructose*	*Sucrose*	*Starch*
		18% protein		
Casein	18	18	18	18
Corn starch	0	0	0	72
Sucrose	0	0	72	0
Glucose	72	0	0	0
Fructose	0	72	0	0
Butter	5	5	5	5
Salt[a] and vitamins[b]	5	5	5	5
		11% protein		
Casein	11	11	11	11
Corn starch	0	0	0	79
Sucrose	0	0	79	0
Glucose	79	0	0	0
Fructose	0	79	0	0
Butter	5	5	5	5
Salt[a] and vitamins[b]	5	5	5	5

[a] USP salt mixture No. II.
[b] Containing 0.2 mg thiamine, 0.3 mg riboflavin, 0.1 mg pyridoxine, 1.5 mg calcium pantothenate, 5 mg niacin and 100 mg choline chloride per 100 g.

The rats were transferred to individual metabolic cages, fed ad lib. for seven days, and during the following five days the food ration was adjusted to pair-feeding; i.e. each of the four animals was given its specific diet but the amount for each member of the same group was restricted to that consumed by the animal with the smallest intake.

In the first and second experiments the animals were studied for three periods of five days each (total 15 days) and in the third for eight five-day periods (total 40 days). Animals were weighed at the beginning and end of each period. Pair-feeding was maintained and thus at the end of each five-day period the food consumption of each of the four animals in any group was almost equal. During each period the feces were collected, dried and pooled; the urine was continuously collected in a receptacle containing 10 ml of 4% H_2SO_4. At the end of each period the cages, funnels and funnels were washed with distilled water. The washings were added to the collected urine and diluted up to a final volume of 100 ml. Aliquots were examined for N content by Kjeldahl's method (10). A preliminary control comparing the Nessler and Kjeldahl methods gave equal values for the N content in the urine. The pooled feces of each five-day period were separated from adherent hair and homogenized in 40% ethyl alcohol and then diluted to 100 ml. Aliquots of 1.0 ml with 0.5 g K_2SO_4 and 10 ml of 4% $CuSO_4$ and 1.0 ml H_2SO_4 were burned in Kjeldahl flasks on an electric plate for 1 to 2 hr. A few drops of H_2O_2 were added from time to time, and the N content was determined by the Kjeldahl method (11). The N content of the diets was determined similarly.

RESULTS

The N retention and resulting weight gain during Experiments 1 and 2 are shown in Table 2, and those of Experiment 3 in Table 3. The data of body weight, N excreted in stools and N retained (N intake minus N excreted in urine and stools) were submitted to covariance analysis. The factors involved in this analysis were the N concentration in the food and the type of carbohydrate diet. The adjusting variables were: a) the total N intake, used for the analyses of body weight and N excreted in stools; and b) the amount of N absorbed (N intake less N excreted in stools) for the analysis of the N retained.

The amount of N excreted in the stools was significantly affected by the type of carbohydrate diet ($F = 11.34$, d.f.: 3.39, $P<0.001$). Multiple comparisons between the various carbohydrate diets by an extension of Scheffé's method (12) yielded the following significant differences: a) The N excretion in stools on starch diet was significantly higher than on both fructose and glucose diets at significance level 0.01. b) The N excretion on a starch diet was also significantly higher than on a sucrose diet at significance level 0.05.

The mean excretion in stools after adjustment for N intake was as follows: starch, 176 mg; sucrose, 144.4 mg; glucose, 129.8 mg; fructose, 124.1 mg.

The amount of N retained, after adjustment for the amount absorbed, was significantly affected only by the concentration of N in the diet ($F = 14.92$, d.f.: 1,39, $P<0.001$). For carbohydrate diets, $F = 0.56$, d.f.: 3.39; for interaction, $F = 0.02$.

TABLE 2. *Mean values for N intake and excretion and body weight during three five-day periods, by type of carbohydrate diet and N concentration in food*

	Starch	Sucrose	Glucose	Fructose
Experiment 1: 18% casein (6 rats in each group)				
N intake, mg	5,401.0	5,381.0	5,346.0	5,381.0
N in stool, mg	188.3	187.4	185.5	133.2
N in urine, mg	2,054.0	1,864.0	1,965.0	1,952.0
N retained, mg	3,158.0	3,370.0	3,208.0	3,295.0
Body weight gain, g	55.3	52.2	45.0	53.0
Experiment 2: 11% casein (6 rats in each group)				
N intake, mg	4,552.0	4,489.0	4,529.0	4,534.0
N in stool, mg	166.7	120.7	165.3	115.4
N in urine, mg	1,042.0	890.0	1,088.0	982.0
N retained, mg	3,343.0	3,478.0	3,377.0	3,437.0
Body weight gain, g	35.0	31.5	32.0	32.3

See text for covariance analysis by Scheffé's method (12) for body weight, N in stools and N retained.
Effect of types of carbohydrate on body weight gain: $F = 2.18$, d.f.: 3,39. $P > 0.10$. (n.s.)
" " " " N excretion: $F = 11.34$, d.f.: 3,39. $P < 0.001$.
" " " " N retained: $F = 0.56$, d.f.: 3,39. (n.s.)
Effect of N content in diet on N retained: $F = 14.92$, d.f.: 1,39. $P < 0.001$.

TABLE 3. *Mean weight gain and mean N intake and excretion by type of carbohydrate in diets containing 11% casein, during 40 days of pair-feeding. Experiment 3: (6 rats in each group)*

	Starch	Sucrose	Glucose	Fructose
N intake, g	16.22	16.21	16.21	16.21
N in stool, g	0.572	0.490	0.478	0.422
N in urine, g	6.159	5.600	5.990	5.893
N retained, g	9.493	10.138	9.742	9.895
Body weight gain, g	140.8	126.5	104.2	118.5

See text for covariance analysis by Scheffé's method (12) for body weight, N in stools and N retained.
Effect of type of carbohydrate on body weight gain: $F = 7.12$, d.f.: 3,19, $P < 0.01$.

In the 15-day study period (Experiments 1 and 2), no significant difference was found between the carbohydrate types in relation to body weight in either the high or low protein diets ($F = 2.18$, d.f.: 3,39, $P > 0.10$), while in the 40-day study period (Experiment 3, 11% casein) the effect of the type of carbohydrate in the diet on weight increase was significant ($F = 7.12$, d.f.: 3,19, $P < 0.01$) mainly because of the difference between the starch-fed group and the other groups. No significant difference was found by Scheffé's method between animals fed glucose, fructose or sucrose; although the mean difference between the animals fed sucrose and those fed glucose is large, this difference was not found to be significant due to the large spread in the weight increases.

DISCUSSION

The N retention studies in pair-fed animals during the three experiments showed that there was no significant difference between glucose, fructose, sucrose or starch in either the high or low protein diets. These results coincide with those reported for ad lib. fed animals (2, 4, 6, 7). The fraction of total N excreted in stools of the starch-fed animals was significantly higher than in the other groups. This difference was, however, very small in relation to the urine N so that it did not affect the overall N excretion. The weight gain of the animals on the different carbohydrate diets was not significantly different

during the short-term periods (15 days). On long-term (60 days) pair-feeding, there was a definitely better growth rate in the starch-fed animals, although there was no significant difference in the growth rate between animals fed glucose and fructose.

The stimulating effect of starch and dextrin on the growth of rats and chicks has been observed by many investigators in ad lib. fed animals. Monson et al. (1) reported that chicks fed a dextrin diet grew faster than chicks fed a sucrose diet, and attributed the superiority of dextrin to better utilization of dietary protein or amino acids due to prolonged residual time of dextrin in the gastrointestinal tract. Studies by Spivey et al. (2) and by Chang (4) do not support this hypothesis. These authors found that the food intake by rats fed dextrin and potato starch was usually higher than that of rats fed sucrose or glucose.

Our studies show that on equal caloric and N intake there was no difference in N utilization between glucose, fructose, sucrose and starch diets. Nevertheless, on long-term feeding the starch-fed animals gained more weight. This gain could be due to either water retention or added tissue. It is pertinent that carcasses of both ad lib. and pair-fed animals fed starch and dextrin have been found to contain more fat than those fed sucrose and glucose (6, 8, 9).

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CARCINOGENS FORMED IN THE HEATING OF FOODSTUFFS. FORMATION OF 3,4-BENZPYRENE FROM STARCH AT 370-390° C.

[illegible] and J. R. WELLINGHURST

From the [illegible], University of [illegible], Australia

Received for publication [illegible]

THE present investigation is to determine whether well known detectable carcinogens are formed in normal domestic situations, for example by the heating of foods under [illegible] approximating to those applying in household cookery. Though 3,4-benzpyrene (BP) [illegible] in the [illegible] the "smoking" of foods and [illegible] the smoked food [illegible] the wood which produces the smoke [illegible] Miller, 1957; [illegible] Kipp and Sick, 1954; Bailey and Dugan [illegible] these [illegible] fuel oil, when burned, give rise to carcinogens. Thus nine polycyclic aromatic hydrocarbons including BP have been detected in coffee soots, though they were not present in the coffee beans before heating (Kurokawa and Hueper, 1956). Moreover in the "char" formed in making "coffee" (U.S.A.) BP is detected by a spectrophotometric method which as used by Kurokawa (1956) is considered completely reliable (Fieser, 1957). Another group of polycyclic hydrocarbons containing BP is present in tobacco smoke though not in the original tobacco (Hieserway and Lindsey, 1956). With regard to the temperature attained, it is estimated (Kurokawa and Hueper, 1956, p. 39) that the coffee beans on roasting may be exposed to a temperature of 540° C., and it seems likely that the wood and tobacco are exposed to even higher temperatures. These materials, and many foodstuffs which are heated, contain polysaccharides; hence it was decided to examine the products obtained by heating starch, which is present in floury mixtures which are sometimes strongly heated.

A temperature of 370–390° C. was chosen as this is likely to be reached in some cooking operations, e.g. we have found that the surface temperature of cooking bread may reach 390–400° C., for this is the temperature rapidly attained when a [illegible] thermocouple is placed in the same position as bread in an electric toaster. It may be that some temperatures in cooking are even higher, [illegible] considers that fats in contact with hot metal may reach 400–500° C. This is probably the temperature range used by Barnett (1918) who destructively distilled starch (apparently with a flame), and who claimed toluene, phenols, furans, furfuran, fatty acids, and a compound $C_{10}H_{12}O$; however, most of his products seem to have been obtained when a mixture of starch with zinc dust was distilled, i.e. in a reducing atmosphere.

In the present work the starch was pyrolysed in an iron pot (equipped with a distillation head) in a metal bath which was electrically heated at 370–390° C. The apparatus, [illegible] of ordinary pressure, contained a small amount of [illegible]; the [illegible] after some preliminary

[illegible] gave a [illegible] with [illegible] (Bailey and Case, [illegible] type. The aqueous [illegible] by [illegible].

The [illegible] the pot contained much oxygen (Found: 0; [illegible] per cent, 11·0 [illegible]) and the methylene chloride [illegible] was chromatographed on silica [illegible] compounds from which on further [illegible] of BP was [illegible] the use [illegible] by the fluorescence [illegible].

EXPERIMENTAL

3:4-Benzopyrene detection (BP).—A fluorescence method was used. The [illegible] Chance 9X1 filter being used [illegible] quartz condenser lens was used [illegible] of a Hilger medium spectro- [illegible] as to make use of the greater [illegible] some three times greater [illegible] considerably increased. [illegible] 7 mm. and the other 0·5 [illegible].

[illegible] investigating the fluore- [illegible] to [illegible], a slow [illegible] of BP could be [illegible] materials 0·001 [illegible].

[illegible].—All organic solvents, [illegible] adsorbents and the aqueous [illegible] BP. The [illegible] was a [illegible] mentioned [illegible] has sometimes been [illegible] used contained small [illegible].

[illegible], volume about 4 litres [illegible] by an iron [illegible] (300 g.) [illegible]

[illegible] 65 to 100 [illegible] removed [illegible] chloride [illegible] four

[illegible] was carefully purified, [illegible] extracted (Soxhlet) with about [illegible] ml. of [illegible] and [illegible] ml. of [illegible] ether mixed. These four [illegible] combined, extracted [illegible] with water and then dried [illegible] a short column [illegible] brown oil whose [illegible] of the lactone, was [illegible] treatment, [illegible] amounting [illegible] of even [illegible] by [illegible] (A) un- [illegible] with the other [illegible] fraction from [illegible] (A) only [illegible] (A) and (B) [illegible] of using [illegible] It was [illegible] (80 ml.). [illegible] and [illegible] represented [illegible] from the [illegible] removed by [illegible]

[illegible] the methylene chloride extract [illegible] with the [illegible]

A preliminary study of the chemical types present in the methylene chloride [illegible] was undertaken. It is a complex solution. The alkali soluble material was [illegible] [illegible] C, [illegible] per cent; H, [illegible] per cent; [illegible] C, [illegible] per cent; H, [illegible] per cent) [illegible] to a mixture of palmitic and stearic acids, [illegible] from the fatty material in the stomach. Phenols were not [illegible] of the [illegible] but in [illegible] the neutral [illegible] yielded [illegible] C, [illegible] per cent; H, [illegible] per cent; [illegible] C, [illegible] per cent; H, [illegible] per cent) [illegible] [illegible] to [illegible]

[illegible] not yet been examined.

[illegible]—A series of BP solutions [illegible] the same [illegible] of [illegible] material as the unknown was used as a standard. An [illegible] of the [illegible] of [illegible] material was [illegible] by [illegible] absorption [illegible], by weight, and by fluorescence [illegible]. The unknown [illegible] solutions were [illegible] on the same plate; [illegible] was by means of a [illegible]. [illegible] BP [illegible] used. When [illegible] 404, [illegible], 427, [illegible] and [illegible].

As a result of [illegible] (D), [illegible] to be [illegible] BP, while [illegible] /100 g. [illegible] to [illegible] /100 g. of [illegible].

That the method [illegible] by the addition of [illegible] of [illegible] 100 g. [illegible] and [illegible] of [illegible].

The amount of BP found [illegible] is [illegible] the [illegible] (D) is [illegible].

DISCUSSION

The yield of BP was very minute, being 0·07 μg./100 g. of starch, much lower than that of Gilbert and Lindsey ([illegible]) who obtained 17 μg. of BP per 100 g. of starch which had been heated to [illegible] C. This yield is probably [illegible] than that of [illegible] ([illegible]) who reported 1 to 7 μg./100 g. of "char" [illegible] a [illegible] asbestos surface.

The fluorescence method of detecting BP is so sensitive that there is danger of contamination from [illegible]. Hence a blank experiment with the same amount of starch and [illegible] with the same amount of [illegible] as when the starch was heated, was [illegible]. No [illegible] of BP [illegible], though less than 0·01 μg. of BP per ml. of [illegible] can be [illegible] by the [illegible]. Moreover it is significant that the [illegible], when [illegible] to the [illegible] treatment as the [illegible] BP.

[illegible] that [illegible] was [illegible] and [illegible] of BP [illegible] 1000 [illegible] of the [illegible]

[illegible] to [illegible] from the BP. Nevertheless the [illegible] unrecorded low temperature of [illegible] of larger amounts at the same [illegible] starch is exposed to oxidation [illegible]. Such experiments are being [illegible]

SUMMARY

Commercial [illegible] been "destructively distilled" at atmospheric pressure and at a [illegible] C. [illegible] was essentially excluded. The charred [illegible] per 100 g. "char". The [illegible]

Thanks are due to [illegible] A. N. [illegible] for advice, and to the Anti-Cancer Council of [illegible]. The [illegible] were carried out by Dr. [illegible]

[illegible]

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Effect of Clay and Cornstarch Intake on Women and Their Infants[1]

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THE OCCURRENCE of the intriguing practices of dirt- and starch-eating (pica) has been recorded for centuries (1). In recent years, Dickins and Ford (2), Hertz (3), Whiting (4), and Edwards *et al.* (5,6) have reported the continued existence of these unusual dietary habits in southern communities. Though much concern is shown in prenatal and public health clinics over the possible consequences of ingestion of these unusual substances, the metabolic effects of these practices have not been delineated.

The powerful influences of superstition, custom, and tradition oppose the classification of these practices as food fads. In contrast, the motivation to consume clay and cornstarch appears to be deep seated and unyielding. These practices are not related to weight control, nor are the products peddled by food "quacks." They are self-chosen and, apparently, sex-linked (6).

The ready availability of a large number of women who came as patients to prenatal clinics at the John A. Andrew Memorial Hospital and the close attachment over many years of this group to Tuskegee Institute prompted a research team, including an obstetrician, nutritionist, dietitian, social worker, clinical technician, and public health nurses, to explore further this unusual departure from normal dietary patterns.

In previous reports by the authors (5,6), information concerning these practices, as reported by 297 women and by 47 health agencies and 91 persons engaged in health work, either professionally or as midwives, was presented. In this paper, analyses of blood and excreta and observations on the newborn infants of selected members of the original group of women are reported.

Experimental Procedures

The sixty-two Negro subjects were selected from patients who attended the prenatal clinic at the John A. Andrew Memorial Hospital, Tuskegee Institute, Alabama. From this group, forty-six gave

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[1]The data are taken in part, from the theses of Alta M. Kemp and Doris Laing, submitted in partial fulfillment of the requirements for the degree of Master of Science, Tuskegee Institute. Appreciation is expressed to the Alpha Kappa Alpha sorority for the grant which made this investigation possible. Received for publication April 8, 1963.

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fasting blood samples. With the exception of controls, these subjects collected food and excreta, both during a period when clay or cornstarch was eaten and after a period, usually seven to ten days, when the substance had been omitted from the diet. Forty-two of the forty-six subjects who gave blood samples and twenty additional women from similar socioeconomic backgrounds delivered infants at the hospital.

The women lived in rural or suburban communities adjacent to Tuskegee Institute. The socioeconomic backgrounds of the subjects and their usual food patterns have been reported (6).

COLLECTION OF FOOD AND EXCRETA

Because the practices of clay and cornstarch ingestion appear to be deeply entwined in superstition and evoke strong emotions, it was impossible to maintain balance periods of the usual duration. In previous work with women of this group, we have observed that the extent of the patient's cooperation is inversely proportional to the demands made on her. Great faith appeared to be required on the part of the women to follow through with collections of excreta and food. For this reason, collection of diets and excreta were limited to a 24-hr. period. In the opinion of the authors, these factors also contribute to the paucity of information available in the literature on the subject of unusual dietary practices.

Procedures for collection of food and excreta were discussed with each subject, and the importance of adhering strictly to these procedures was stressed. Mimeographed instruction sheets, a urine bottle containing 10 ml. glacial acetic acid, containers for the collection of food and feces, and a capsule containing carmine to be used as fecal markers were provided for each woman.

On the morning following the 24-hr. collection of food and excreta, the subjects returned these containers to the clinical laboratory and gave fasting blood samples. They were examined by an obstetrician and then served a hot breakfast in the hospital dietary department. During the interview which followed, the nutritionist recorded the woman's statement of her previous 24-hr. food intake. A quantity of clay or cornstarch equal to that ingested by the woman was given to the interviewer at this time to indicate the amount consumed.

The dietary record was used to check the contents of the collection cans and as a basis for calculating the caloric and vitamin values of the previous day's food intake. The women were given funds to compensate for the expense of transportation to and from the hospital and the cost of food samples collected. This procedure was followed to encourage continued cooperation during the study.

After the first collection period, the obstetrician requested each clay- or cornstarch-eater to refrain from the practice until the next interview. The obstetrician was well known and revered by these women, many of whom had had previous babies delivered by him. Collections of food and excreta and fasting blood samples were obtained again. Control subjects collected samples for only one 24-hr. period.

CHEMICAL ANALYSES

Food, urine, and feces were refrigerated as soon as they were brought to the research or clinical laboratory. Feces were weighed and mixed prior to analysis; the 24-hr. food collection was homogenized in a Waring Blendor. These and urine samples were frozen and maintained at —20°C. until analyzed.

Feces, aliquots of food homogenates, and samples of clay were dried and ashed by standard laboratory procedures. These ashed samples were used for subsequent assays of calcium and iron.

Routine clinical procedures were used for analyses of hemoglobin, serum protein, calcium, phosphorus, and glucose, which were conducted in the clinical laboratory. Concentrations of hemoglobin, serum protein, calcium, and glucose were determined in blood. Urine was analyzed for total nitrogen, calcium, phosphorus, and total solids. Total nitrogen, calcium, and iron were determined in feces.

Nitrogen assays of excreta and diets were conducted by the Kjeldahl-Gunning method. Determinations of iron were made on feces and food by the method of Ventura and White (7). Calcium assays of feces and food were conducted, using the procedure of Patton and Reeder (8).

"AVAILABILITY" OF MINERALS IN CLAY

To estimate whether any of the calcium and iron in clay might be available to the women, *in vitro* studies were conducted. Clay was suspended in 0.1 N hydrochloric acid, comparable in pH to gastric juice. Suspensions were mechanically shaken for 1 hr. at room temperature. The liquid was decanted and the residue washed several times with distilled water to remove adhering acid-soluble substances. Washings were combined with the decanted acid solution and the composite dried and ashed for iron and calcium determinations.

RATINGS OF DIETS AND INFANTS

The procedure used to rate the diets as "excellent," "good," "fair," or "poor" has been described (6). The nutritive value of the diets usually consumed was calculated from 24-hr. recall records obtained from each woman by the staff nutritionist or dietitian during three interviews over a period of five to six months. Information from these three sources was averaged for each woman.

TABLE 1 **Total and available iron, calcium, and ash in clay and cornstarch***

SUBSTANCE	IRON		CALCIUM		ASH	
	Total	Available†	Total	Available	Total	Available
	mg./100 gm.	*mg./100 gm.*	*mg./100 gm.*	*mg./100 gm.*	*%*	*%*
Clay	20.8	0.03	21.4	0.20	92.0	0.28
Cornstarch	5.6	0	4.0	‡	0.06	0.00

*Dry weight basis; averages of duplicate analyses of two samples from different sources.

†Per cent available = $\frac{\text{amount of iron, calcium, or ash in extract}}{\text{amount of clay or cornstarch extracted}} \times 100$.

‡Negligible.

Infants were examined by a pediatrician according to routine hospital procedure. He did not know whether the mother had consumed clay or cornstarch.

Results and Discussion

ANALYSES OF CLAY AND CORNSTARCH

Moisture and ash contents of four clay samples from different sources were determined. Average moisture content was 23.2 per cent, ranging from 20.3 to 24.6 per cent. Total ash in the samples averaged 76.8 per cent, with a range of 75.6 to 79.7 per cent. Total iron content was 20.8 mg. per 100 gm., though only 0.03 mg. per 100 gm. of this appeared to be "available" by chemical tests (Table 1). Average total calcium content of clay samples was 21.4 mg. per 100 gm., but of this amount, only 0.2 mg. per 100 gm. was "available" in the *in vitro* tests.

The physical features of the edible clay and the reactions of the women to this substance are of interest. The quantity of clay consumed ranged from about 6 to 130 gm. per day. Most of the women obtained the clay from the bank of a stream or river near their homes. Some baked the clay in the oven before eating it.

Usually the samples were light grey in color and free of rocks and foreign material; this clay was usually chosen in preference to the red because it was smoother. Sometimes a pink coloration in the clay suggested the presence of iron. According to the women, the material had a sour taste.

The most important mineral substances in clay are iron, aluminum, magnesium, and potash. Other workers have analyzed (9) clay obtained directly from clay banks and samples prepared commercially for eating purposes. The essential substances found in both are subangular quartz (silicon dioxide), ilmenite (ferrous oxide and titanium oxide), and coal fragments.

Clay is rarely free from foreign ingredients, some of which are visible to the eye. The commonest of these are: organic matter, such as humus and fossils; carbonate of lime; sulphide of iron, such as pyrite or marcasite; oxides of iron, which stain the clay from a dull to a bright red; and sand and silt, which frequently are coarse particles of detrital silica.

Though only small amounts of calcium and iron appeared to be available in clay, the *in vitro* studies suggest that 0.28 per cent of the total ash might be available. It seems possible, therefore, that small amounts of other minerals may have been provided by the practice of clay-eating.

The quantity of cornstarch ingested ranged from 15 gm. to 2 lb. a day. The women preferred the Argo brand, which they purchased for about 8 cents a box.

The total ash in Argo starch was 0.06 per cent. The starch contained 4 mg. per 100 gm. calcium, but a negligible fraction of this was "available" in the *in vitro* tests. Starch contained 5.6 mg. per 100 gm. iron; however, none of this was "available" by the procedure used.

CALORIES AND PROTEIN

Young *et al.* have reported (10) that the 24-hr. recall and seven-day food record may be used interchangeably for pregnant women for all nutrients, there being no detectable differences between means of estimates obtained by the two methods. When subject cooperation and time are of prime concern, the recall by the subject of her food intake during the previous 24 hr. has often been used and was employed here as the method of expediency.

The caloric intakes of the twenty-seven pregnant clay- and cornstarch-eaters in this study were less during the 24-hr. period when clay or cornstarch was omitted from the diets (Table 2) than during a comparable period when these substances were eaten. These data suggest that the substances may have stimulated the appetite of these women or that an emotional reaction, brought about by abstinence from them, may have had a negative effect on appetite.

Payton *et al.* have reported (11) similar observations. In a survey of the dietary habits of 571 pregnant Negro women, they observed that 17 per cent ate cornstarch. Average caloric intakes of these cornstarch eaters were substantially higher than those of the total group—2218 and 1665 calories, respectively. Consumption of other nutrients was similar to that of the total group.

Protein intakes (dietary N × 6.25) in the present investigation were low in all groups except the non-

TABLE 2 Calories, protein, calcium, and iron in diets and excreta of clay- and cornstarch-eaters and controls

SOURCE	CLAY-EATERS				PREGNANT CORNSTARCH-EATERS*		CONTROLS	
	Pregnant		Non-pregnant					
	Additive†	No additive	Additive	No additive	Additive‡	No additive	Pregnant	Non-pregnant
Number of subjects	20	20	3	3	7	7	7	8
Calorie intake	2013	1418	1343	1203	2525	1692	1390	1825
Protein								
In diet (gm./day)	41	34	30	27	47	19	38	54
Dietary nitrogen (gm./day)	6.5	5.4	4.8	4.2	7.5	3.0	6.1	8.8
Urinary nitrogen (gm./day)	3.0	3.6	6.3	5.9	1.6	2.2	4.2	6.1
Fecal nitrogen (gm./day)	1.1	1.6	1.5	1.2	1.5	0.6	0.9	1.3
Calcium								
In diet (gm./day)	0.81	0.43	0.44	0.92	0.85	—	1.00	0.61
Consumed as additive	§	—	§	—	—	—	—	—
Urinary (gm./day)	0.06	0.06	0.12	0.06	0.05	—	0.06	0.06
Fecal (gm./day)	0.29	0.21	0.26	0.42	0.13	—	0.51	0.31
Iron								
In diet (mg./day)	6.6	6.6	13.7	4.6	2.4	1.4	4.0	9.7
Consumed as additive (mg./day)	8.3	—	10	—	¶	—	—	—
Fecal (mg./day)	5	7	14	15	4	—	5	3

*Because there was only one non-pregnant cornstarch-eater, data are omitted.
†Clay.
‡Cornstarch.
§9–12 mg. per day.
¶Negligible.

pregnant controls. The low dietary nitrogen values of non-pregnant clay-eaters were accompanied by high urinary nitrogen excretions.

Intakes of vitamin A, thiamine, riboflavin, niacin, and ascorbic acid compared favorably with recommended allowances, vitamin A intakes being two to five times higher.

SERUM PROTEIN AND GLUCOSE

Average concentration of serum protein was higher in non-pregnant controls than in other groups. However, all values were within the normal range. No significant differences in blood glucose were observed.

HEMOGLOBIN

Average hemoglobin concentrations in experimental groups ranged from 8.7 to 10.1 gm. per 100 ml. (Table 3), indicating mild to moderate anemia. Individual values ranged from 3.9 to 12.8 gm. per 100 ml. No attempt was made to determine whether hydremia was present or if the anemia was due to nutritional or other causes.

Ferguson reported (12) the hemoglobin levels of pregnant women in rural southern Mississippi. Of his 1000 patients, 52.2 per cent had hemoglobin levels below 11 gm. per 100 ml. blood, which was considered indicative of anemia. Adair, Dieckmann, and Grant observed (13) a mean hemoglobin concentration of 11.6 gm. per 100 ml. in their pregnant subjects. They suggest a minimum level of 10 gm. per 100 ml. during pregnancy. Davidson, Fullerton, and Campbell found (14) an average hemoglobin level of 10.8 gm. per 100 ml. in 818 pregnant women, ranging in age from fifteen to forty-four years. In non-pregnant women, fifteen to forty-four years old, the average hemoglobin was 11.2 gm. per 100 ml.

IRON INTAKE AND EXCRETION

Assays of diets during the period of clay ingestion showed approximately 7 and 14 mg. iron in the food of the pregnant and non-pregnant clay

TABLE 3 Blood constituents of clay- and cornstarch-eaters and control subjects of Table 2

SUBJECTS	HEMOGLOBIN	SERUM PROTEIN	CALCIUM	GLUCOSE
	gm./100 ml.	*gm./100 ml.*	*mg./100 ml.*	*mg./100 ml.*
Clay-eaters				
Pregnant	8.7	6.1	12.4	97
Non-pregnant	8.8	5.0	14.4	88
Pregnant cornstarch-eaters	10.1	6.0	11.3	85
Controls				
Pregnant	10.1	6.7	10.1	85
Non-pregnant	9.8	7.2	12.7	81

groups, respectively (Table 2). Values ranged from about 2 to 10 mg. in the cornstarch and control groups.

Though ingestion of cornstarch did not appear to augment mineral intake, the quantities of clay consumed may have provided 8 to 10 mg. iron daily to the pregnant and non-pregnant clay-eaters, respectively (Table 2). The iron intake of non-pregnant clay-eaters was less during the period of omission of clay.

The data of Moore *et al.* (15) point to the efficacy of reducing substances in the diet in stimulating iron absorption. Since the clay-eaters described the taste of clay as being "sour and pucker-ish," it would be of interest to explore the possible presence of reducing substances in clay which may facilitate the absorption of iron from this substance.

CALCIUM INTAKE AND EXCRETION

Blood calcium concentrations of control, non-pregnant subjects were higher than those of pregnant women, with values ranging from 7.6 to 11.0 mg. per 100 ml. in the pregnant group, and from 8.0 to 16.3 mg. in the non-pregnant group. Means were 10.1 and 12.7 mg. per 100 ml. for the pregnant and non-pregnant groups, respectively. The lower serum calcium level is attributed to the large demands made on the mother by the growing fetus.

Usual urinary excretion of calcium ranges from 0.2 to 0.5 gm. per day; in this study, values were low by comparison. Individual values ranged from 0.002 to 0.2 gm. per day.

PHOSPHORUS AND TOTAL SOLIDS IN URINE

Excretion of phosphorus in the urine was usual for all subjects during both periods. Considerable variation in the excretion of total solids was observed (Table 4). The significance of these observations is not apparent.

CONDITION OF INFANTS AT BIRTH

Sixty-two infants were delivered at the hospital by subjects in the study. The ingestion of clay and cornstarch did not appear to affect the birth weight, length, head and chest size, or the period of gestation of these infants (Table 5).

Only 27 per cent of the infants of clay eaters and 33 per cent of those of cornstarch eaters were rated as "good," whereas 79 per cent of the infants of controls were so rated.[4] Twenty-three per cent of infants of women in the clay group and 33 per cent of infants delivered to the cornstarch group were rated "poor" or "very poor," whereas only 11 per cent of the infants of controls were rated in this manner.

A higher percentage of infants in the cornstarch group was stillborn. In addition, 18 and 25 per cent of infants of mothers in the clay and cornstarch groups, respectively, died in four days, compared with 11 per cent of the control infants.

Sixty-eight per cent of the diets of mothers

TABLE 4 Total solids in urine of control and selected experimental subjects

SUBJECTS	NUMBER OF SUBJECTS	TOTAL SOLIDS	
		Additive used	No additive
		gm./day	*gm./day*
Clay-eaters			
Pregnant	8	23.9	26.9
Non-pregnant	3	32.1	36.9
Cornstarch-eaters			
Pregnant	2	30.9	14.3
Non-Pregnant	1	16.4	21.9
Controls			
Pregnant	4	—	35.9
Non-pregnant	7	—	28.5

[4] A baby rated as "excellent" is one who cries immediately, has good sucking reflexes and no abnormalities; a "good" baby is one who has delay in respiration for a few seconds, borderline weight, and average physical abilities; a "fair" baby is one who needed oxygen and stimulation and whose respiration was delayed for a minute or longer. Babies rated as "poor" or "very poor" usually died within two to four days.

TABLE 5 Condition at birth of infants of clay- and cornstarch-eaters and controls

CRITERIA	INFANTS OF CLAY-EATERS		INFANTS OF CORNSTARCH-EATERS		INFANTS OF CONTROLS	
	Average	Range	Average	Range	Average	Range
Number of full-term infants	22		12		28	
Birth weight (lb.)	7.5	5.0– 9.8	7.0	5.2– 8.4	7.1	5.4– 9.8
Birth length (in.)	19.8	17.5–22.0	18.8	17.0–20.0	19.2	17.0–21.5
Head size (in.)	13.1	12.0–14.5	13.5	11.5–14.5	13.1	11.0–14.5
Chest size (in.)	12.8	11.5–14.5	12.7	12.0–14.5	12.7	11.5–14.0
Length of gestation (months)	8.9	8.0– 9.0	9.0	9.0	8.7	8.0– 9.0
Classification of infant						
Good	6		4		22	
Fair	11		5		3	
Poor	2		2		1	
Very poor	3		1		2	
Premature births	2		0		4	
Stillbirths	1		2		1	
Infant deaths within 4 days	4		3		3	

TABLE 6 Complications experienced during pregnancy by clay- and cornstarch-eaters and controls

SUBJECTS	NUMBER OF SUBJECTS*	PRE-ECLAMPSIA		HYPERTENSION		EDEMA		NUTRITIONAL ANEMIA†	
		Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent	Number of cases	Per cent
Clay-eaters	22	1	5	3	14	3	14	6	27
Cornstarch-eaters	12	1	8	1	8	1	8	2	17
Controls	28	2	7	2	7	2	7	2	7

*Only women who delivered infants at the hospital are included.
†Moderate or severe.

whose infants were rated "fair" to "good," were rated "good" or "fair." Seventy-five per cent of the diets of mothers who had infants rated "poor" to "very poor" had diets rated "poor."

Only six premature births occurred in the sixty-two infants born. This small number does not justify more than a statement that no influence of clay or cornstarch-eating on prematurity was suggested by the data. Coodin reported (16) 15.9 per cent prematurity in Negro infants and 6.4 per cent in white infants in a group of 608 prematures. Brockway and co-workers observed (17) somewhat higher prematurity rates, 20.0 per cent and 18.5 per cent in Negro and white infants, respectively.

COMPLICATIONS DURING PREGNANCY

The incidence of toxemia was approximately the same in the clay, cornstarch, and control groups, 5, 8, and 7 per cent, respectively (Table 6). Approximately twice as many cases of hypertension and edema occurred in the clay-eating mothers as compared with the cornstarch and control groups. Lundgren and McDaniel, in a report (18) of toxemia among patients at Margaret Hague Hospital, observed in a group of 1024 pregnant women, 11.9 per cent toxemia, 7.9 per cent pre-eclampsia, and 1.6 per cent hypertension.

Twenty-seven per cent of the mothers who ate clay and 17 per cent of those who ingested cornstarch in this study were anemic. In the control group, the percentage was only 7. It is possible that hydremia rather than anemia may have been present.

MOTIVATION FOR CLAY AND CORNSTARCH EATING

A review of the case histories and interview records of these subjects would suggest that the desire for clay and cornstarch cannot be explained on the basis of one motive alone. (a) In some women, it appears to represent a traditional practice handed down through generations and looked on with favor within the immediate community. Thus, to some women, to become pregnant would imply that she should take up the "custom" of clay-eating. Superstition is interwoven with tradition in this instance, as many women feel that the baby would not be normal if clay were not eaten. (b) In other women, the practices of clay- and cornstarch-eating appear to be similar in etiology to smoking. In many, the initial experiences with the unusual dietary substances are accompanied by a sensation of relaxation, appetite stimulation, or the pleasure of chewing (6). Into this same category one is inclined to place the effect derived from filling the stomach, because the ingestion of clay and starch would ward off hunger pangs. This latter effect, no doubt, represents the chief objection to the practice. Clay and cornstarch are substituted for foods which would provide a variety of nutrients.

Other needs appear to be satisfied by the practice. The comments of a cornstarch-eater who had completed her college work illustrate this point:

> I had been bothered by a queer sensation several weeks after I learned that I was pregnant. I nibbled on many foods, chewed gum, smoked, but found no relief. One day as I was ironing, I tasted the starch which I had just prepared. It satisfied the inner feeling completely, as no other substance had done. I found myself eating 1 to 2 boxes a day. After my baby was delivered, I wanted no part of it.

There are, no doubt, other explanations for the initiation and perpetuation of the habit. The possibility that the desire for clay and cornstarch expresses a physiologic need must receive additional attention in carefully controlled investigations. Cornstarch provides calories, often inadequately supplied in the diets of low-income groups. It seems apparent that, in many instances, it serves as a partial replacement for other staple carbohydrates, such as rice and potatoes, though it is not commonly ingested with meals. The possibility that iron and calcium from clay may be available to those who ingest it is, indeed, worth further study.

Summary

Analyses of diets, blood, and excreta from a group of pregnant clay- and cornstarch-eaters and the condition of their infants at birth are reported. Total nitrogen, calcium, and iron were determined in food, feces, and urine; assays of phosphorus and total solids were also made on the latter samples. Determinations of hemoglobin, glucose, serum protein, and calcium were made on blood. Samples of clay were analyzed for total ash, iron, and calcium.

The omission of clay or cornstarch from the diets of pregnant women appeared to influence appetite for food as reflected by lower caloric intakes when these substances were not eaten. Protein intakes were low in all groups except the non-pregnant controls. Average daily intakes of calcium and iron were considerably below the recommended allowances, except in a few instances. Hemoglobin concentrations indicated mild anemia in clay-eaters. Serum protein and glucose levels were within normal ranges.

Though no differences were observed in birth weight, length, head and chest size, or length of gestation, the average rating of infants born to the control group was higher than that for the infants of mothers in the clay and cornstarch groups.

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Air-Conditioning May Lift Pork Production

Air-conditioned quarters and "pellets," segregated feeding of controlled diets can raise pork production by at least one piglet per litter, researchers at the Ohio Agricultural Experiment Station, Wooster, believe.

Most brood sows now give birth to only 70 per cent of the offspring of which they are capable, researchers have found, but they feel that litters can be increased to more than 95 per cent of potential by raising the "standards of living" of the brood sows. In a $60,000 project, partially supported by eight electrical utilities in Ohio, test sows are being slaughtered twenty-five days after fertilization, and their reproductive tracts examined to determine how many piglets would actually have been born and how many more might have been if ideal temperature and humidity during gestation had prevailed.

W. L. Roller, agricultural engineer at the station, is reported by a staff reporter of the *Wall Street Journal* to feel that even a modest increase in hog production would allow farmers to use fewer brood sows and less equipment for the same pig production. In twelve Corn Belt states, farmers' capital investment costs might be reduced by about $100 million annually.

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Dietary Starch: Effect on Cholesterol Levels

By Daniel B. Farnell and Moore J. Burns

Weanling rats were fed for 21 days on one of four cholesterol-free diets identical in fat content and containing either soybean protein or casein as the source of protein. The carbohydrate source was either dextrose or a mixture of equal parts of cornstarch and dextrose. All four diets contained the same percentage of protein, fat, and total carbohydrate. The inclusion of starch in both the soybean protein and casein diets resulted in significantly lower liver cholesterol levels. In the soybean protein diet, starch resulted in increased serum total cholesterol. Starch in the casein diet did not change serum total cholesterol. The possible mode of action and epidemiologic implications of these effects of starch are discussed.

IT HAS been shown that people who subsist on diets mainly or exclusively of vegetable origin generally have lower serum cholesterol levels than those whose diets contain more animal-derived foods.

Although the possible relation of the cholesterolemic effects of these diets to their relative content of various fats, sterols, and proteins has undergone extensive investigation, less attention has been given to the role of carbohydrate, particularly, of starch.

Kempner[1] reported that a diet composed mainly of rice, fruits, and dextrose or sucrose had a hypocholesterolemic effect. "Pure" vegetarians have been found to have lower serum cholesterol than either lacto-ovo-vegetarians or nonvegetarians.[2] Portman et al.[3] found that starch instead of sucrose in a cholesterol-supplemented diet lowered serum cholesterol in rats.

Starch substituted for either glucose or sucrose in a cholesterol-supplemented diet resulted in lower serum cholesterol levels in germ-free and normal chickens.[4] Sucrose was replaced by cornstarch in a special diet used to induce liver and kidney damage and elevate blood cholesterol in rats.[5] Cornstarch in this case resulted in lower liver cholesterol but did not significantly alter serum cholesterol levels.

Differing results were reported by Guggenheim et al.[6] who found that starch gave higher liver cholesterol levels than either sucrose or dextrose in cholesterol-supplemented diets. Starch in diets without added cholesterol resulted in only slightly higher liver cholesterol levels than resulted from sucrose; however, there was no difference in the effects of starch and dextrose. Serum cholesterol levels were not affected by varying the carbohydrate.

Pillice et al.[7] noticed a hypocholesterolemic effect of starch in cholesterol- and cholic acid-supplemented diets; this effect disappeared after 3 weeks. Tissue cholesterol was not determined. Dextrin[8] had no effect on serum

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cholesterol levels when used instead of sucrose or glucose in cholesterol-supplemented diets. Again, tissue cholesterol was not measured.

The purpose of the present experiment was to demonstrate the effect of starch on serum and liver cholesterol levels when used in cholesterol-free diets containing either high quality animal protein (casein) or low quality vegetable protein (soybean protein).

Methods

Twenty-four weanling male Sprague-Dawley rats (starting weight 43 to 54 Gm.) were distributed randomly into individual suspended wire bottom cages and given access *ad lib.* to clean water and purified diets as described in table 1. The diets were fed for 21 days, after which the animals were fasted overnight, anesthetized with ether, and killed by exsanguination.

The method of Searcy and Bergquist[9] was used for determining serum total cholesterol. The fresh livers were first weighed, then each was ground thoroughly with purified sand and absolute ethyl alcohol. The mixture was centrifuged and the supernatant decanted into a volumetric flask. Three additional extractions were performed, the first with hot absolute alcohol, and the last two with a mixture of two parts absolute alcohol to one part ether. All extracts were added to the volumetric flask, and the volume adjusted with the alcohol-ether mixture. The liver extract was then filtered, and an aliquot evaporated to dryness. The acetone-ether solvent used by Searcy and Bergquist[9] was then added to the residue, and their method used to determine the cholesterol content.

Results

The effects of the various diets on total weight gain during the 21 day period and on serum and liver cholesterol levels are shown in table 2.

Table 1.—*Content of Diets*

	No. 1 Soybean protein (%)	No. 2 Casein (%)	No. 3 Soybean protein + starch (%)	No. 4 Casein + starch (%)
Soybean protein[1]	21.07	—	21.07	—
Casein[2]	—	21.07	—	21.07
Cerelose[3]	40.0	40.0	20.0	20.0
Starch[4]	—	—	20.0	20.0
Crisco[5]	25.0	25.0	25.0	25.0
Alphacel[6]	8.93	8.93	8.93	8.93
Vitamin mix[7]	1.0	1.0	1.0	1.0
Mineral mix[8]	4.0	4.0	4.0	4.0

[1]Soybean protein—purified.
[2]Casein—hot-alcohol extracted.
[3]Cerelose—dextrose.
[4]Starch—cornstarch.
[5]Crisco—hydrogenated vegetable oil.
[6]Alphacel—ground cellulose.
[7]Vitamin mix—This mix supplied the following per kilogram of diet: thiamine hydrochloride 2.3 mg.; riboflavin 2.3 mg.; pyridoxine hydrochloride 1.5 mg.; calcium pantothenate 7.6 mg.; niacin 15.2 mg.; alpha-tocopherol acetate 30.0 mg.; menadione 3.8 mg.; choline chloride 1.[illegible] Gm.; vitamin B_{12} 11.5 μg., folic acid 1.5 mg.; vitamin A 15,[illegible] I.U., and vitamin D [illegible] I.U.
[8]Mineral mix—composition (%): $CaCO_3$, [illegible]; KH_2PO_4, [illegible]; KCl, 14.[illegible]; NaCl, 7.70; $MgCO_3$, [illegible]; $MgSO_4 \cdot 7H_2O$, 7.[illegible]; $FeSO_4 \cdot 7H_2O$, [illegible]; $CuSO_4 \cdot 5H_2O$, 0.1[illegible]; $MnSO_4 \cdot H_2O$, [illegible]; $KAl(SO_4)_2 \cdot 12H_2O$, [illegible]; KI, [illegible]; and alphacel, [illegible].

Table 2.—*Effect of Diets on Weight Gain and Cholesterol Levels*

Diet	No. of animals	Total weight gain (Gm.)	Serum cholesterol (mg./100 ml.)	Liver cholesterol (mg./100 Gm.)
1. Soybean protein	6	23.0 ± 11.2	129 ± 22	597 ± 129
2. Casein	6	65.7 ± 23.4	95 ± 16	662 ± 38
3. Soybean protein + starch	6	30.2 ± 6.0	158 ± 17	702 ± 47
4. Casein + starch	6	94.6 ± 15.9	88 ± 9	580 ± 70

All values are mean ± standard deviation.

The data in table 2 were subjected to "t"-tests at a 5 per cent probability level to determine the significance of the difference between mean values obtained with and without starch in the diet.

No significant difference in weight gains resulted from adding starch to the diets.

The addition of starch to both the soybean protein and casein diets resulted in significantly lower levels of liver cholesterol than occurred in rats fed the corresponding starch-free diet. On the other hand, serum cholesterol levels were increased by the addition of starch to the soybean protein diet. Starch added to the casein diet had no effect on serum total cholesterol.

Both of the casein diets resulted in lower liver and serum cholesterol levels than either of the soybean protein diets, which substantiates previous reports of the cholesterol-lowering effect of casein versus soybean protein.

Discussion

Although the difference in the effect of starch on liver and serum cholesterol levels may at first suggest inconclusive results, it is more likely that this difference is not only characteristic of the effects of starch under conditions similar to those in this experiment but may suggest a reason for the effects.

These results are similar to those reported by Adams et al.,[8] who employed special hypercholesterolemic diets, presumably containing added cholesterol. They observed that starch lowered liver cholesterol; however, starch gave lower serum cholesterol than dextrose, while there was no difference between the effects of starch and sucrose on serum cholesterol. Their diets apparently were fed for a considerably longer period of time than those used in the present experiment.

The major pathway of cholesterol excretion in both rats[10] and man[11] is by conversion to bile acids. It has been shown that dietary starch increases turnover and excretion of bile acids.[12,13] Normal rats excrete about twice the amount of bile acids as germ-free rats under similar conditions.[14] Both starch and dextrin reduce the dietary requirement for riboflavin, presumably by providing a more favorable environment for the synthesis of riboflavin by intestinal microorganisms.[15] Harrill et al.,[16] using starch-free diets containing either 5 per cent or 20 per cent fat, found that serum cholesterol levels increased with increasing ingestion of riboflavin and that liver cholesterol levels were either lowered or not affected, depending on the relative amount of sucrose and fat used. They also showed that the flavin adenine dinucleotide

(FAD) content of the liver was increased by higher levels of dietary riboflavin.

In view of this evidence, it seems reasonable to suppose that riboflavin may be a necessary precursor to some coenzyme, such as FAD, which may be required for the mobilization and subsequent degradation of cholesterol to bile acids, and that in cholesterol-free diets the effect of starch on cholesterol levels is a result of an increase in available riboflavin. Possibly riboflavin is one of the cofactors contained in the yellow-colored supernatant preparation of liver or heart tissue reported to promote degradation of cholesterol by rat or mouse mitochondria.[17]

It is emphasized that the diets used in the present experiment were cholesterol-free. Consequently, any observed changes in serum and tissue cholesterol necessarily were due either to a change in the degree of cholesterol biosynthesis, the distribution of cholesterol between serum and tissues, or in the degradation and excretion of cholesterol. A simple redistribution of cholesterol would not result in increased bile acid excretion. If the effect of the starch were to increase cholesterol biosynthesis, then the liver as well as serum levels should be increased. If the effect were simply a decrease in synthesis, the serum levels would not be expected to increase; also, a decrease in cholesterol synthesis would be expected to give lower, not greater, bile acid excretion. It is therefore concluded that dietary starch lowers liver cholesterol but may raise serum levels, probably by increasing the mobilization and excretion of cholesterol.

Related questions presently being investigated are whether arterial wall cholesterol is affected by starch in the same way as liver cholesterol, and whether prolonged administration of similar cholesterol-free starch diets will result in lower serum as well as liver cholesterol.

Because the diets in this experiment were fed ad lib., the question arises as to whether the effect of the starch was related to an increase in the total food consumption, since starch reportedly does increase food intake.[18] The lack of difference in weight gain as a result of starch in the diets suggests that no significant difference in food consumption occurred. Even if the animals on starch did consume more food, it does not seem likely that this would decrease liver cholesterol, especially in view of the finding[18] that various degrees of dietary restriction in rats had no effect on liver cholesterol levels.

The results of this experiment do not in some respects support the findings previously referred to of Guggenheim et al.[5]

The possible relation between these effects of starch on cholesterol levels and the epidemiology of atherosclerosis is worth mentioning. For one thing, it is evident that measurements of serum cholesterol alone do not necessarily give an accurate indication of the tissue cholesterol levels. Also, the effects of starch could be responsible in certain cases for the apparent lack of correlation between dietary habits and incidence of atherosclerosis, as in this example: Although the Japanese people reportedly have a diet considered to be "antiatherogenic," the people of Thailand have an "atherogenic" diet.[19]

The incidence of atherosclerosis in Japan is similar to that in the United States, but atherosclerosis is rarely reported in Thailand. Examination of the data concerning reported average daily food intake reveals that except for one set of data, the validity of which the author cast doubt upon, the Thai consume a diet consisting of a higher percentage of carbohydrate, derived almost entirely from rice, than do the Japanese.

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Starch hydrolysis in man: an intraluminal process not requiring membrane digestion

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FOGEL, MICHAEL R., AND GARY M. GRAY. *Starch hydrolysis in man: an intraluminal process not requiring membrane digestion.* J. Appl. Physiol. 35(2): 263–267. 1973.—Although pancreatic amylase is secreted into the intestinal lumen, it may act primarily after binding to the mucosal surface. To study the relative contribution to starch hydrolysis of intraluminal amylase versus amylase adsorbed to the microvillous membrane, jejunal perfusion and test meal experiments were performed in normal human subjects as well as in patients with exocrine pancreatic insufficiency (PI). Normal controls were able to hydrolyze 7.3 g of a 9.0-g infused starch load/hr to an average molecular weight of 610 g, whereas PI patients could only hydrolyze 4.3 g/hr to an average molecular weight of 1,836 g. The hydrolytic capacity of the intraluminal contents in both groups was more than adequate to explain the observed starch hydrolytic rate despite the fact that PI patients had only 10% of the hydrolytic capacity of the control subjects. Starch given in a starch-protein-fat test meal was hydrolyzed rapidly enough to suggest that PI patients should utilize starch-containing foods as a source of calories. Since the available intraluminal hydrolytic capacity is greatly in excess of that needed to account for the observed rates of starch hydrolysis in man, membrane digestion of starch is probably of secondary importance.

intestinal amylase; starch digestion; pancreatic insufficiency; membrane digestion; intestinal perfusion; maltose hydrolysis

STARCH IS A MAJOR CONSTITUENT of human diets and perhaps the leading source of calories in underdeveloped countries (5). Until recently, it was generally believed that this polysaccharide was completely degraded within the intestinal lumen to maltose, maltotriose, and α-limit dextrins by the action of secreted pancreatic amylase (4, 17). However, Ugolev, in a series of experiments in rats, has suggested that starch is hydrolyzed by pancreatic amylase that has adsorbed to the surface of the jejunum and that this "membrane digestion" is the most important pathway for the digestion of starch (13, 20). This theory, that the surface of the microvillus is the primary locus for starch digestion, complements the generally accepted membrane digestion thesis of disaccharide hydrolysis (16, 21) by constitutive disaccharidases.

In studies of digestion in normal man, Dahlqvist and Börsgtrom (7) found that sufficient amounts of intraluminal amylase were present after a test meal to rapidly hydrolyze the ingested starch. Subsequently, Auricchio et al. (2) have shown that infants hydrolyze starch rapidly in the duodenum by an intraluminal mechanism that reaches maximal efficiency at 6 months of age. These experiments in man cast some doubt on the requirement for a human membrane digestion system for starch.

To ascertain the importance of intestinal mucosal membrane digestion of starch in man, we have performed starch perfusion and test meal studies in normal volunteers. Since patients with pancreatic insufficiency (PI) presumably have defective starch hydrolysis, we have studied five PI patients and compared their results with those of the normal group.

METHODS

Subjects

Nine normal volunteers (age 21–50 years) and five patients with PI (age 35–54 years) secondary to chronic pancreatitis were intubated with a double-lumen polyvinyl tube, constructed so that 30 cm separated the distal and proximal orifices. After the subjects fasted overnight, the tube was allowed to pass into the upper jejunum and then positioned with radiological monitoring so that the proximal orifice was located just distal to the ligament of Trietz. Recently, the triple-lumen tube has been utilized in perfusion experiments to assure adequate mixing with endogenous secretions and to minimize the problem of reflux (19). Since none of the substances to be analyzed; glucose, maltose, or starch, are normally present in the intestinal lumen after a 12-hr fast, the double-lumen technique was felt to be adequate for the materials studied.

The PI patients all had abnormally low pancreatic enzyme secretion in response to a Lundh test meal (15). Their fecal fat excretion ranged from 12 to 50 g/day. The patients had been maintained on oral pancreatic extract (Viokase, Viobin Co.) which was discontinued 36–48 hr prior to the study.

Intestinal Perfusion

A 1% solution of amylopectin (soluble starch, Sigma) was delivered through the proximal orifice in the jejunum by a constant infusion pump (model 600-1200 Harvard Apparatus Co., Dover, Mass.) at 15 ml/min for 90 min. After a 30-min period without perfusion, the starch perfusion was repeated for 1 hr. After another 30 min rest period, a 1% maltose solution was perfused for an additional 1 hr at 15 ml/min. Both solutions contained polyethylene glycol (PEG) (5 g/liter) as a nondigestable marker. The effluent

from the distal orifice was collected by siphonage in a polyethylene container surrounded by acetone and Dry Ice. The frozen specimens were stored at −20 C until analyzed.

Test Meal Technique

The subjects ingested homogenized test meals consisting of: 50 g of soluble starch (amylopectin, Sigma), 20 g of egg albumen (Fisher Scientific Co.), 20 g of peanut oil (Planter Co.), 5 g of PEG, 5 ml of Sucaryl (Abbott), 5 ml of lemon extract (McCormack, 6% ethanol), and water to 500 ml total volume. After ingestion of this test meal, the effluent from the proximal orifice just distal to the ligament of Treitz was continuously collected by siphonage for 3 hr in 30-min aliquots.

Analytic Methods

The collected specimens were analyzed in the following manner.

Total carbohydrate was determined by a modification of the anthrone method of Scott and Melvin (18), whereby the volume of samples and reagents was reduced by 2.5 times without loss of sensitivity.

Glucose was determined by the hexokinase-glucose-6-phosphate dehydrogenase NADP coupled system, as previously described (9).

Maltose and maltotriose were measured together by the 3,5-dinitrosalicylate (DNS) method of Bernfeld (6). Since some of the oligosaccharide products of starch hydrolysis are further hydrolyzed to glucose by the action of brush border maltase, glucose would usually be present in the collected samples. In the DNS assay, the glucose present produces twice the color reaction of maltose per unit weight. Therefore, the true maltose equivalent was determined by subtracting one half the glucose found by the specific assay for glucose described above.

The maltose result, as determined by comparison with a standard curve of known amounts of maltose, includes maltotriose, which on Sephadex G-15 column chromatography of the test meal samples from normal volunteers accounted for approximately 15% of the hydrolytic products present. Since the color produced by 2 mg of maltose is equivalent to that produced by 3 mg of maltotriose in the DNS method, the maltose equivalents underestimate the amount of maltose-maltotriose by 5% in the normal subject; we have disregarded this small error because it is within the reproducibility of the perfusion technique and the assay methods used. For patients with PI, however, the amount of maltotriose present as a hydrolytic product was 45% of the total hydrolytic products, making the maltose equivalent 15% less than the actual amount. We have adjusted the results of the PI patients to include this variable by multiplying the maltose equivalent by 1.15.

The amylase activity of the collected samples was determined by the DNS method. The reaction mixture of 0.5 ml of the test solution and 0.5 ml of 1% amylopectin in a 0.9% NaCl-phosphate buffer at pH 6.9 was incubated for 3 min. The amount of maltose equivalents released was determined after subtracting the maltose present in the test solution prior to the amylase assay. One unit of amylase activity equaled 1 mg of maltose produced at 37 C in 1 hr.

The degree of polymerization (DP) of glucose in the saccharide molecule was determined by dividing the total glucose units (anthrone method) by the number of free reducing groups (maltose-maltotriose DNS method) in the sample (7).

The average molecular weight of the carbohydrates in each sample was calculated by multiplying the number of glucose units per molecule (DP) by the molecular weight of glucose.

PEG was determined by the method of Hyden (12) which in this laboratory shows a stable linear relationship of concentration and absorbance at 60 min.

Polyacrylamide disc electrophoresis was performed on salivary and duodenal secretions by the method of Davis (8) in order to separate the salivary and pancreatic amylases.

Gel filtration chromatography was carried out on selected intestinal contents by use of a 45 x 2.5 cm column of Sephadex G-15. Specimens were dialyzed at 4 C for 24 hr against a phosphate buffer at pH 7 prior to placing them them on the column.

Calculations

After the initial 30-min equilibration period, starch hydrolysis, carbohydrate absorption, and amylase activity were estimated from samples collected over the ensuing 30 min.

$$\text{starch in effluent} = \text{total carbohydrate} - (G_1 + G_2) \quad (1)$$

$$\text{starch hydrolysis} = (St_i \cdot V - St_e \cdot V \cdot P_i/P_e) \quad (2)$$

carbohydrate absorption

$$= (St_i \cdot V - St_e \cdot V \cdot P_i/P_e) - ((G_1 + G_2) \cdot V \cdot P_i/P_e) \quad (3)$$

where V = volume perfused and the following symbols indicate concentrations: St_i = starch perfused, St_e = starch remaining in the effluent, P_i = PEG perfused, P_e = PEG in effluent, and $(G_1 + G_2)$ = maltose + maltotriose as determined by DNS reaction (see text for maltose determination).

Hydrolytic capacity. To determine intraluminal hydrolytic activity in test subjects, we have derived a figure for the potential maximal starch digestion per hour for each perfused subject based on the amylase activity of collected specimens and labeled it as hydrolytic capacity (HC).

$$HC = (\text{amylase units/ml}) \cdot V \cdot P_i/P_e \quad (4)$$

Statistical analysis was performed using the Student t test for small groups (22).

RESULTS

Starch Hydrolysis, Absorption, and Intraluminal Hydrolytic Capacity

Normal man. During the first starch perfusion in normal volunteers, 9.0 g of starch were perfused every hour. The

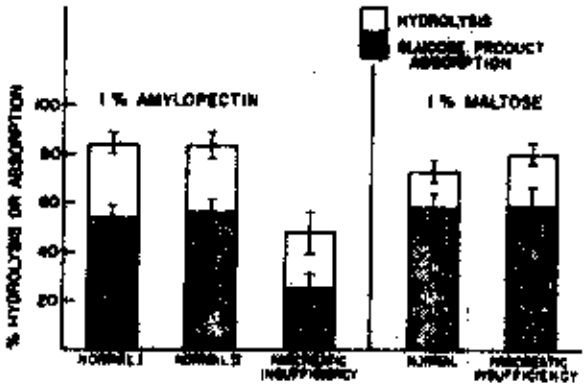

FIG. 1. Comparison of the hydrolysis and glucose product absorption of 1% starch and 1% maltose perfusion solutions in nine normal and five PI patients. Brackets correspond to one standard error of the mean. Normal I is the initial starch perfusion and normal II is the second starch perfusion following a 30-min rest period.

hydrolytic rates shown in Fig. 1 demonstrate a rapid starch hydrolysis (84%) which led to a 51% absorption of the infused starch. The effluent used to calculate the starch hydrolytic rates was very high in amylase activity; when the result was interpreted in terms of the number of grams of starch that could be theoretically hydrolyzed, intraluminally, in 1 hr by that amount of amylase (hydrolytic capacity), there was 40 times as much intraluminal hydrolytic capacity than was necessary to explain the observed starch hydrolysis (Table 1).

Starch Hydrolysis Following a Rest Period

If pancreatic amylase is adsorbed to the microvillus membrane, it might be expected to wash out during a perfusion but return after a rest period. Ugolev describes such a "washout" phenomenon in animal perfusion experiments, finding that starch hydrolysis decreases with time and partially returns to its original value after a rest period. By contrast, we found no change in the first 20–30 min of perfusion. Furthermore, as shown in Fig. 1, the hydrolysis and absorption of starch in perfusion II was no different than in perfusion I.

Starch Hydrolysis in Pancreatic Insufficiency

The patients with pancreatic insufficiency were not able to hydrolyze starch as well as the normal subjects. As can be seen in Table 1, the rate was approximately one-half that of the normal subjects, and the intraluminal hydrolytic capacity was even further reduced to only 10% of that found in normals. Nevertheless, there was still enough intraluminal amylase activity (hydrolytic capacity) to account for the observed starch hydrolysis; the intraluminal hydrolytic capacity measured on collected samples always exceeded the observed rates of starch hydrolysis by a factor of 2–19. However, the statistical difference in hydrolytic capacity between the two groups was just above the $P < 0.05$ level.

Size of Hydrolytic Products

The average molecular weight of the hydrolytic products of the perfusion experiments was determined for both the normal and the PI patients. As Table 1 indicates, the hydrolysis of starch in the PI patients was much less complete than that found in the normal subjects.

Maltose Perfusion

As a control, maltose, a carbohydrate not requiring amylase action for hydrolysis, was perfused for 1 hr after the completion of the starch perfusion. As shown in Fig. 1, there were no differences between the patients with PI and the normal volunteers in their digestion of maltose. These results for maltose hydrolysis are similar to those previously reported for normal subjects (10).

Test Meal

By the time an ingested meal containing 50 g of starch had reached the ligament of Trietz, the mean degree of polymerization (glucose units) in aspirated meal samples of the controls was only 3.0. In PI patients, this value ranged from 3.4 to 7.4 with a mean of 4.5, but the difference between the groups is not statistically significant. There was a marked difference in the amount of amylase present in response to a test meal, normal subjects having 4,200 units/ml (range 1,400–7,000) and PI patients producing only 187 units/ml (range 18–300). However, the smaller amount of amylase activity present in the lumen of PI patients under these conditions was sufficient to account for the observed starch hydrolysis.

Molecular Size of Starch Breakdown Products

Separation of hydrolytic products into individual carbohydrates is the most precise way to estimate the extent of digestion. Gel filtration chromatography on Sephadex G-15 was performed on the samples collected following the test meals. Typical graphs for both PI patients and normal subjects are presented in Fig. 2. The position of glucose and maltose was determined by specific reactions for these sugars (10); the position of maltotriose and maltotetrose was determined by comparing the sample results to the column chromatography of known sugars of 3 (raffinose-cotton seed) and 4 (stachyose-artichoke) glucose units. The results demonstrate a relative increase in

TABLE 1. *Jejunal perfusion of 1% amylopectin and comparison with in vitro luminal hydrolytic capacity*

	No. of Subj	In Vivo Hydrolysis, g/hr	Hydrolytic Capacity, g/hr	Avg Mol Wt
Normal subj	9	7.6 ± 0.3*†	306 ± 78*‡	610 ± 30*‡
PI subj				
TC		4.6	82	756
RH		4.8	19	1,224
BC		6.4	56	576
EW		1.6	8	3,018
DE		4.1	10	3,600
Totals	5	4.3 ± 0.8*†	35 ± 22*‡	1,836 ± 576*‡

* Values are means ± SE. † Difference significant at the $P < 0.05$ level. ‡ Difference not significant at the $P < 0.05$ level.

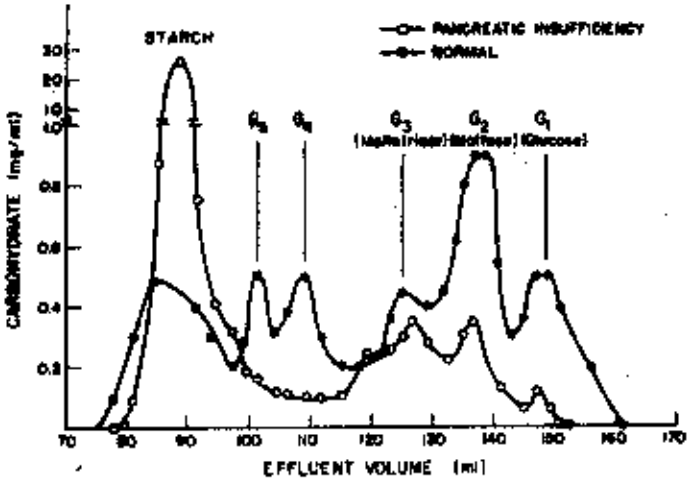

FIG. 2. Comparison of gel chromatography (Sephadex G-15) on test meal aspirates from one representative normal subject and one PI patient. The starch peak was in the void volume. G_1, G_2, G_3, . . . are saccharides of 1, 2, 3, . . . glucose units.

the amount of maltotriose and larger hydrolytic products in PI patients as compared to normals, indicating that hydrolysis of the carbohydrate meal is less complete.

Source of Amylase in PI Patients

Although amylase was reduced in PI patients, the amount in the intestinal lumen was adequate enough to hydrolyze starch. In response to this unexpected finding of markedly reduced but adequate amounts of amylase in PI patients, we attempted to determine whether the amylase present was of pancreatic or salivary origin. The technique of polyacrylamide disc electrophoresis was used to separate pancreatic from salivary amylase (3). As shown in Fig. 3, pancreatic amylase was found to be present in the intestinal fluid, but no salivary amylase was identified in the PI patients. A small amount of salivary amylase was found in the intestinal fluid of normal controls.

DISCUSSION

The results of our test meal and perfusion studies confirm the work of previous authors who found that starch was

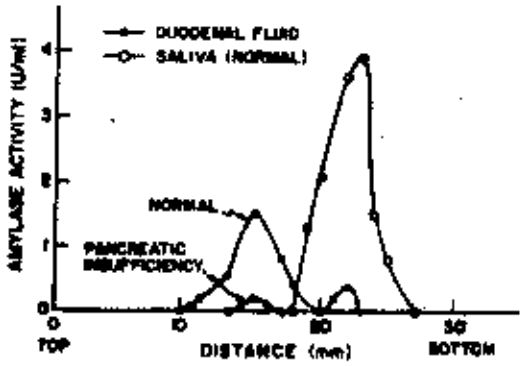

FIG. 3. Separation of amylase activity on polyacrylamide disc electrophoresis from normal and PI duodenal aspirates and from normal saliva.

rapidly hydrolyzed by the excessive amount of secreted pancreatic amylase. Furthermore, the intraluminal hydrolytic capacity under intestinal perfusion conditions is many times the amount required for the observed hydrolysis in normal subjects and is more than adequate in the PI patients. The measurement of amylase activity was done under ideal in vitro conditions and may tend to overestimate the true hydrolytic capacity of the intraluminal fluid although the substrate concentration for the in vitro assay and the starch perfusate concentration were similar. Therefore, the values of hydrolytic capacity should not be considered as absolute quantitative findings but they do represent a strong indication of the more than adequate amounts of intraluminal amylase (especially following a meal) present in the bowel lumen.

The relative amounts of the hydrolytic products formed, as shown by Sephadex G-15 column chromatography, differed in the two groups. Since starch and its partial degradation products appear in the void volume, we have no exact measure of those products above 5 glucose units. Thus, the relationship between Fig. 2 and the DP for each subject is only qualitative. Auricchio et al., however, showed that infants below 6 months of age, who have poor pancreatic amylase production, hydrolyzed starch less completely than did other older children. Similarly, in PI patients, relatively more maltotriose than maltose was produced. Consequently, more glucose was formed in the normal controls, presumably by the hydrolysis of the higher concentrations of maltose by intestinal maltase. Since maltose hydrolysis and absorption were similar in both the normal and PI subjects, the lower levels of glucose production and absorption in the PI group are directly related to incomplete starch hydrolysis which leads to an insufficient amount of substrate at the brush border for maximal maltose hydrolysis.

In his animal studies, Ugolev demonstrated a very high rate of starch hydrolysis that could not be explained by the hydrolytic capacity of the intraluminal contents. Our studies are at variance with this work since the hydrolytic capacity of the intestinal contents in our normal and patient groups, in response to both perfusion and test meal studies, was always greater than the observed hydrolysis in vivo. The most important difference between the studies may be in the perfusion rates utilized: 6-12 ml/min in small laboratory animals as opposed to 15 ml/min in man. Some authorities consider that 0.5-2 ml/min is an optimal perfusion rate for rats (14), while in man 10-15 ml/min is commonly used. It seems very likely that a rapid rate would wash most of the pancreatic amylase out of the lumen, thereby producing low levels of intraluminal amylase after the initial few minutes. Furthermore, in these experiments there was no way for pancreatic amylase to flow from the pancreatic ducts to the perfused segment. Since pancreatic amylase is very difficult to wash off the intestinal mucosa (1), an artificial preparation was created in which little intraluminal amylase was present in the small intestine, but in which pancreatic amylase was adherent to the mucosa. Thus, a membrane digestion of starch was uncovered by greatly reducing the intraluminal amylase activity. In fact, he found that as the perfusion rate increased, the mem-

brane digestion rate of starch increased. Hence, the technique employed probably made it impossible to assess the importance of intraluminal amylase because of the inordinately high perfusion rate utilized.

Recently, Hubel and Parsons (11), using a perfusion technique, demonstrated a statistically significant but very small enhancement of starch hydrolysis by washed rat jejunal mucosa in vivo. Importantly, this enhancement occurred only at low concentrations of intraluminal amylase. In normal subjects and even in PI patients, we continually demonstrated an excess of intraluminal amylase as measured by the hydrolytic capacity of the intraluminal fluid.

The disc electrophoresis studies clearly demonstrate that pancreatic amylase is present, albeit in small amounts, in the intestinal lumen of PI patients in response to a test meal. Furthermore, since these patients were able to adequately hydrolyze starch at the ligament of Trietz, it seems reasonable to assume that more complete hydrolysis would occur as the test meal passed on down the jejunum. This adequate hydrolysis of starch in PI explains why the starch tolerance test for chronic pancreatitis has been virtually abandoned as a clinical test. In fact, we should encourage PI patients to eat food containing starch which, as we have shown, will be hydrolyzed and absorbed without the addition of exogenous enzyme preparations.

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CARIES EXPERIENCE OF RATS FED VARIOUS STARCHES FOR STUDY OF EXPERIMENTAL CALCULUS FORMATION

by
GÖRAN FROSTELL
PAUL N. BAER

INTRODUCTION

Diets containing large quantities of starch have been found to enhance the formation of dental calculus when fed to weanling Sprague-Dawley rats (*Baer & White*, 1967). In some of the experimental groups rampant dental caries was observed. The purpose of the present investigation was fourfold:

1) to determine the ability of various starches to induce dental caries,

2) to determine whether pregelatinization of the starches influences their cariogenicity,

3) to determine whether amylopectin, the insoluble fraction of starch, or amylose, the soluble fraction, is the most effective in inducing dental caries,

4) to determine whether there is a correlation between calculus formation and dental caries experience.

I. EFFECT OF DIFFERENT STARCHES

MATERIALS AND METHODS

The experiments on calculus formation have been described in detail by *Baer & White* (1967). Eleven groups of weanling Sprague-Dawley rats of

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Table I
*Diets**)

Unmodified starch (66 %)	Pregelatinized starch (66 %)
Wheat (a)	Wheat (a)
Rice (b)	Potato (c)
Arrowroot (b)	Tapioca (d)
Tapioca (c)	Amioca (d)
Amioca (d)	
Potato (e)	
Cornstarch (e)	

*) In addition, all diets contained 32 % skim milk and 2 % liver powder

Sources: (a) Hercules Powder Co., New York; (b) Morningstar Paisley, Inc., Clifton, N. J.; (c) Stein Hall Co., New York; (d) National Starch & Chemical Co., New York; (e) Clifton Corn Processing Co., New York.

both sexes, containing 60 animals per group, were fed a powdered diet containing 32 per cent skim milk, 2 per cent liver powder and 66 per cent of one of the commercially available starches listed in Table I. Distilled drinking water was administered *ad libitum*.

Usually 36 animals in each group were sacrificed after a 30-day experimental period and an additional 24 per group after 60 days. The remainder were kept for study of calculus formation only and were not available for caries scoring. Upon termination of the experimental period the animals were decapitated, defleshed by the use of dermestid beetles and the molar teeth scored for calculus (*Baer, Stephan & White*, 1961) and for caries (*Keyes*, 1958). The total number of carious enamel areas was determined for each animal.

RESULTS

After a 30 day experimental period there were no statistically significant differences in the total number of carious enamel areas between animals on the regular corn starch and those on unmodified rice. Animals on unmodified amioca and unmodified potato starch had a tendency to have higher caries rates ($P < 0.05$). (Table II, Fig. 1.)

After 60 days (Table IIIa) there were no statistically significant differences between the animals fed the regular corn starch and the unmodified wheat, unmodified rice, unmodified tapioca and unmodified arrowroot. For the

Table II

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

Diet No	Diet Regimen (Starch)	N	Calculus scores X	S.E.	N	Caries scores X	S.E.	Vs Control t	F	Level of significance
S—1	Unmodified wheat	24	3.13	0.519	—	—	—			
Control	Cornstarch, regular	24	3.67	0.428	10	0.72	0.351			
S—2	Pregelatinized arrowroot	—	—	—	12	17.17	2.764			
S—3	Pregelatinized potato	24	1.63	0.300	12	34.67	5.855			
S—4	Unmodified rice	23	2.65	0.473	11	3.72	1.830	2.0211	0.10>P>0.05	—
S—5	Unmodified arrowroot	23	1.57	0.402	—	—	—			
S—6	Unmodified tapioca	23	2.70	0.442	—	—	—			
S—7	Pregelatinized sagoo	22	0.77	0.130	10	26.00	3.952			
S—8	Pregelatinized tapioca	23	1.61	0.265	12	18.25	3.499			
S—9	Unmodified sagoo	24	3.38	0.496	12	7.00	2.871	2.6564	0.05>P>0.01	*
S—10	Pregelatinized wheat	23	1.78	0.398	11	24.18	5.784			
S—11	Unmodified potato	15	0.47	0.369	9	3.67	1.607	2.4157	0.05>P>0.01	*

Table III a

Effect of various starches on calculus formation and dental caries. 69 day experimental period

Diet No	Diet Regimen (Starch)	N	Calculus scores $\bar{X}$	S.E.	N	Caries scores $\bar{X}$	S.E.	Vs Control t	P	Level of significance
S—1	Unmodified wheat	24	4.79	0.478	24	2.50	0.812	0.5343	0.6>P>0.5	—
Control	Cornstarch, regular	23	4.57	0.555	12	1.83	0.694			
S—2	Pregelatinized arrowroot	—	—	—	12	24.50	3.258			
S—3	Pregelatinized potato	23	2.48	0.338	23	33.52	3.491			
S—4	Unmodified rice	24	4.67	0.476	24	0.50	0.456	1.6378	0.20>P>0.10	—
S—5	Unmodified arrowroot.	23	2.17	0.528	23	2.87	0.737	0.9119	0.40>P>0.30	—
S—6	Unmodified tapioca	21	3.71	0.594	21	4.14	0.874	1.8126	0.10>P>0.05	—
S—7	Pregelatinized amioca	22	1.77	0.886	22	18.05	3.708			
S—8	Pregelatinized tapioca	24	2.67	0.238	24	32.25	2.653			
S—9	Unmodified amioca	21	4.60	0.339	24	4.79	0.866	2.2339	0.05>P>0.01	*
S—10	Pregelatinized wheat	24	2.83	0.280	24	6.42	1.268			
S—11	Unmodified potato	19	0.37	0.114	18	16.67	5.334	2.2452	0.05>P>0.01	*

*) 2

Table III b

Effect of pregelatinization of various starches on their caries-inducing properties. Values from Table II and IIIa.

Substance	Day	N	Unmodified $\bar{X}$	S.E.	N	Pregelatinized $\bar{X}$	S.E.	Vs Control t	P	Level of significance**)
Wheat	60	24	2.50	0.812	24	6.42	1.268	2.6028	0.05>P<0.01	*
Arrowroot	60	23	2.87	0.737	12	24.50	3.253	8.5624	P<0.001	***
Potato starch	30	9	3.67	1.607	12	34.67	3.855	4.4578	P<0.001	***
	60	18	16.67	5.334	23	33.52	3.491	2.7364	0.01>P>0.001	**
Tapioca	60	21	4.14	0.874	24	32.25	2.653	9.5896	P<0.001	***
Amioca	30	12	7.80	2.871	10	25.00	3.952	3.7620	0.01>P>0.001	**
	60	24	4.79	0.866	22	18.05	3.708	3.6213	P<0.001	***

**) If calculated on the number of lesions all the differences were significant on the 0.1 per cent level (***).

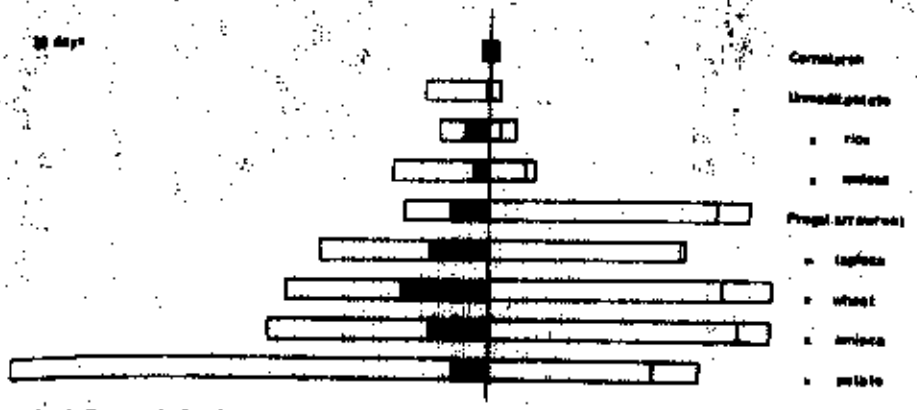
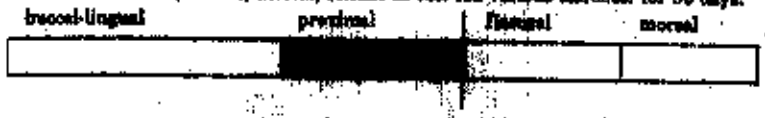


Fig. 1. Bar graph showing variations in the pattern of smooth-surface (buccal, lingual, proximal) and sulcal (fissural, morsal) lesions in rats fed various starches, for 90 days.



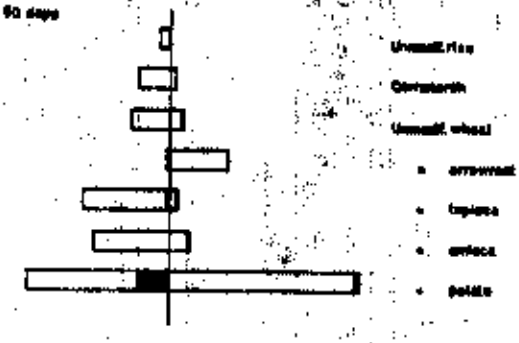


Fig. 2 a. Bar graph showing variations in the pattern of smooth-surface and sulcal caries in rats fed various unmodified starches for 60 days.

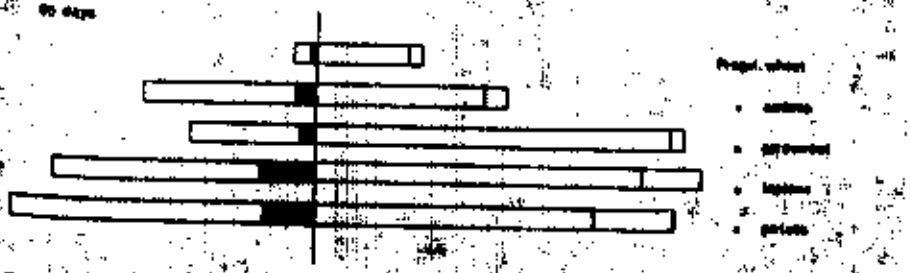


Fig. 2 b. Bar graph showing variations in the pattern of smooth-surface and sulcal caries in rats fed various pregelatinized starches for 60 days.

unmodified amioca and the unmodified potato starch diets there was a tendency towards a higher number of carious areas ($P<0.05$).

Animals on all the pregelatinized starch diets (arrowroot, potato, amioca, tapioca and wheat) had several times more carious enamel areas than those fed the regular corn starch diet and those fed the unmodified starches. The differences were significant or highly significant, (Table IIIb), except for wheat for which there was a strong tendency (Figs. 2a and b.)

II. EFFECTS OF AMYLOSE AND AMYLOPECTIN

MATERIALS AND METHODS (A.)

Four groups of weanling Sprague-Dawley female rats, containing 20—25 animals per group, were fed for an experimental period of 30 days, one of the four diets shown in Table IV. Other experimental conditions were the same as in part I.

RESULTS

Animals on the corn amylose and the corn amylopectin diets (Table V) had more carious enamel areas than those animals on the high amylose corn starch. For amylopectin the difference was significant on the 1 per cent level.

Animals on the corn amylopectin diet had more carious enamel areas than animals on the corn amylose diet, but the difference was not statistically significant ($P>0.10$). The calculus scores are given in Table VI. For additional information about the weight gain of the animals see *Baer & White* (1967).

Table IV
Diets)*

Diet*) (55 %)	Percent amylose or amylopectin contained in each starch fraction: Amylose	Amylopectin
a. Regular cornstarch (1)	28	72
b. High amylose cornstarch (2)	70	30
c. Corn amylose (3)	100	—
d. Corn amylopectin (3)	—	100

*) In addition, each diet contained 33 % skim milk and 2 % liver powder.

Sources: (1) Clinton Corn Processing Co., New York; (2) National Starch & Chemical Co., New York; (3) A. E. Staley Mfg. Co., Decatur, Ill.

Table V

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

Substance	Day	N	Unmodified caries-scores $\bar{X}$	S.E.	N	Amylose caries-scores $\bar{X}$	S.E.	N	Amylopectin caries-scores $\bar{X}$	S.E.
Cornstarch, regular	30		—	—	20	7.60	1.297	20	10.85	2.071
	60	20	3.10	0.767		—	—		—	—
	90	40	5.50	0.914		—	—		—	—
Cornstarch, high amylose	30	17	2.71	1.325		—	—		—	—
	60	16	3.38	1.221		—	—		—	—
Potato starch	30	19	5.78	1.420	20	12.45	1.991	19	[illegible]	4.447
	60	26	15.42	1.992		—	—			
	60	28	16.43	2.954	19	34.00	4.438	17	47.29	[illegible]

	Day	t	F	Level of significance
High starch				
Amylose — corn starch, high amylose	60	[illegible]	P>0.1	—
Amylose — corn starch, high amylose*)	30	[illegible]	0.05>P>0.01	*
Amylopectin — corn starch, high amylose*)	30	[illegible]	0.01>P>0.001	**
Amylopectin — amylose	30	[illegible]	P>0.1	—
Potato starch				
Amylose — unmodified potato starch	30	2.7013	0.05>P>0.01	*
		[illegible]	P>0.1	—
	60	3.4001	0.01>P>0.001	**
Amylopectin — unmodified potato starch	30	[illegible]	P<0.001	***
		[illegible]	P<0.001	***
	60	[illegible]	P<0.001	***
Amylopectin — amylose	30	[illegible]	P<0.001	***
	60	1.8005	0.10>P>0.05	—

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

Table VI

Effect of amylose vs. amylopectin on calculus scores

Diet	N	Calculus Scores $\bar{X}$	S.E.
Corn starch, regular	40	4.48	.41
High amylose cornstarch	40	1.45	.50
Corn amylose	40	0.65	.18
Corn amylopectin	37	2.89	.37

The difference between groups on the regular corn starch diet and those on the high amylose corn starch diet was not statistically significant ($P > 0.10$).

MATERIALS AND METHODS (B.)

Groups of weanling Sprague-Dawley female rats containing approximately 20 animals per group, were fed for an experimental period of 30 and 60 days, unmodified potato starch, potato amylose and potato amylopectin diets, respectively. Other experimental conditions were the same as in previous experiment.

RESULTS

Animals on the potato amylopectin diet for 30 days had significantly more carious enamel areas than those on the potato amylose diet ($P < 0.001$). (Table V). After 60 days there was still a difference, but this difference was not statistically significant ($0.10 > P > 0.05$).

Animals fed the potato amylopectin diets had significantly more carious areas than animals fed the unmodified potato starch diets ($P < 0.001$).

There was no statistically significant difference in the number of carious enamel areas between animals on the potato amylose diet for 30 days and those on the unmodified potato starch diet, although there was a tendency for animals on the amylose diet to have more caries. After 60 days the difference was statistically significant ($0.05 > P > 0.01$).

III. CORRELATION BETWEEN CALCULUS FORMATION AND CARIES EXPERIENCE

MATERIAL AND METHODS

Since group means for both calculus formation and caries experience were available for some of the different diets, a study was made of the

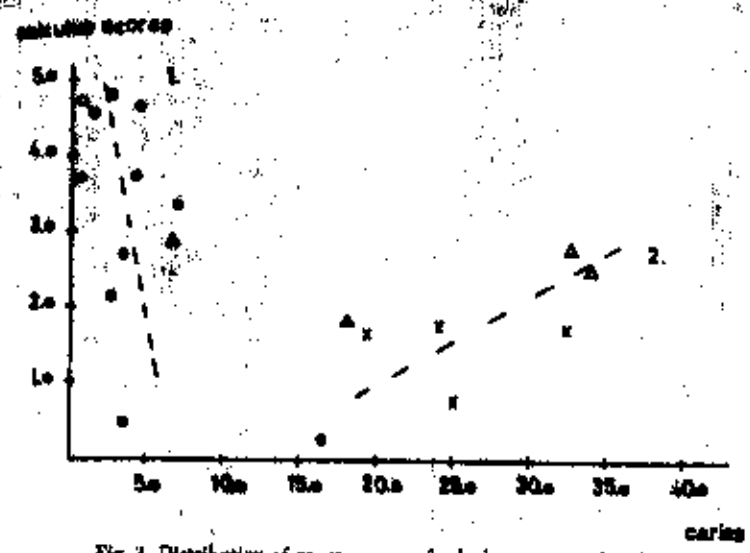


Fig. 3. Distribution of group means of calculus scores and caries scores.

1. Unmodified starches. 2. Pregelatinised starches.

○ unmodified starches 30 days
● unmodified starches 60 days.
△ pregelatinised starches 30 days.
+ pregelatinised starches 60 days.

correlation between calculus formation and caries experience. At the time the scoring of dental caries was finished the scores for the individual values of calculus formation in the different animals were not available. Thus, it was not possible to carry through a detailed statistical study of the correlation between calculus formation and caries experience in the individual animal.

For those groups in which group means for calculus formation and caries experience were available, a correlation diagram was set up.

RESULTS

No clear correlation was found between calculus formation and caries experience (Fig. 3). In general, those animals fed the unmodified starches had very high calculus scores and very low caries scores. However, in experiments in which the animals were fed pregelatinised starches, there was a tendency for those groups with high caries scores also to have high calculus scores. Thus, those groups fed the pregelatinised starches usually had high caries scores and high calculus scores.

DISCUSSION

Since all the diets contained 32 per cent skim milk, which contains lactose, the lactose content of the diet may to some extent have contributed to the caries experience of the animals. Lactose, however, is considered to be only moderately cariogenic, a belief which is confirmed by the experiments with the unmodified starches.

Pregelatinization, however, caused a significant increase in cariogenicity of all the starches tested in all the experimental series. This finding is in accordance with the results of *Neff* (1967), who showed that cooked starch caused a considerable decrease in pH of dental plaques whereas raw starch did not. His results have been confirmed (*Frostell*, in press).

Amylopectin, the insoluble, branched constituent of starch, was generally more cariogenic than amylose, the soluble, linear constituent of starch. The cariogenicity of amylose was of the same order of magnitude as that of pregelatinized starch.

Thus, the results tend to show that the processing of starch increases its cariogenicity considerably and that this property is mainly ascribable to its amylopectin content and to a lesser extent to its amylose content, since regular starch contains much more amylopectin than amylose and since amylopectin was somewhat more cariogenic than amylose. Since in these experiments no polysaccharide production from sucrose was possible and still considerable plaque accumulation on smooth surfaces occurred, the question is raised whether amylopectin — which is not completely hydrolyzed by alfa-amylase and which chemical composition resembles that of the bacterial dextrans — may replace bacterial dextrans and favour plaque formation.

Acknowledgement. The authors wish to express their gratitude to Dr. B. Larsen for valuable help and advice.

SUMMARY

Groups of weanling Sprague-Dawley rats were fed a diet containing 66 per cent starch, amylose or amylopectin. Twelve different types of unmodified or pregelatinized starches were studied. The diets were administered for 30 or 60 days in nine experiments and caries and calculus were scored.

There was no statistically significant difference in the number of carious enamel areas between animals fed the regular corn starch control diet and those fed unmodified wheat, unmodified rice, unmodified arrowroot and unmodified tapioca, whereas animals fed the unmodified potato starch and

[illegible] had a tendency to have more carious areas. Animals on the [illegible] (arrowroot, potato, amioca, tapioca and wheat) had several times more carious areas than those on the regular corn starch diet.

Animals fed corn amylose or corn amylopectin had significantly more carious areas than those on unmodified corn starch diets. Animals on amylopectin diets had more caries than animals on amylose. For potato amylose and amylopectin the difference was highly significant after 30 days on the diets, but not after 60 days.

No clear correlation was found between calculus formation and caries experience. In general, animals fed the unmodified starches had very high calculus scores and very low caries scores. However, in experiments in which the animals were fed pregelatinized starches there was a tendency for groups with high caries scores to have high calculus scores also.

RÉSUMÉ

CARIE DENTAIRE CHEZ DES RATS NOURRIS AVEC DIFFÉRENTES FÉCULES AU COURS D'UNE ÉTUDE SUR LA FORMATION EXPÉRIMENTALE DU TARTRE

Un régime contenant 66 % de fécule, d'amylose ou d'amylopectine a été administré à des groupes de rats Sprague-Dawley venant d'être sevrés. Douze types différents de fécule non modifiée ou prégélatinisée ont été étudiés. Les régimes ont été administrés pendant 30 ou 60 jours; la carie et le tartre ont été enregistrés.

Il n'y avait pas de différence statistiquement significative dans le taux de la carie entre les animaux ayant reçu le régime témoin à la fécule de maïs ordinaire et ceux qui étaient nourris à l'amidon de blé non modifié, la fécule de riz non modifiée, la fécule d'arrowroot non modifiée et la fécule de tapioca non modifiée, tandis que les animaux nourris à la fécule de pomme de terre non modifiée avaient un nombre significativement plus élevé de zones carieuses. Les animaux recevant les fécules prégélatinisées (arrowroot, pomme de terre, amioca, tapioca et blé) avaient plusieurs fois plus de zones carieuses que ceux qui recevaient le régime à la fécule de maïs ordinaire.

Les animaux nourris à l'amylose de maïs ou à l'amylopectine de maïs avaient un nombre significativement plus élevé de zones carieuses que ceux qui recevaient le régime à la fécule de maïs ordinaire. Les animaux recevant les régimes à l'amylopectine avaient plus de cavités que les animaux recevant l'amylose. En ce qui concernait l'amylose et l'amylopectine de pomme de

terre, la différence était hautement significative après 30 jours au régime mais pas après 60 jours.

ZUSAMMENFASSUNG

KARIESBEFALL BEI RATTEN DENEN VERSCHIEDEN STÄRKESORTEN GEGEBEN WURDEN UM EXPERIMENTELLE ZAHNSTEINBILDUNG ZU STUDIEREN

Gruppen von Sprague-Dawley-Ratten, die eben abgewohnt waren, wurde eine Diät gegeben, die 65 Prozent Stärke, Amylose oder Amylopektin enthielt. Zwölf verschiedene Sorten von nichtmodifizierten oder pregelatinisierten Stärkesorten wurden untersucht. Die Diäten wurden den Tieren während 30 oder 60 Tagen gegeben und Karies und Zahnsteinbildung wurden registriert.

Die Verschiedenheit im Kariesbefall zwischen Tieren, denen die nichtmodifizierte Kartoffelstärke (Maisstärke) gegeben wurde und derjenigen die nichtmodifizierte Weizenstärke, nichtmodifizierte Reisstärke, nichtmodifizierte Arrowrootstärke und nichtmodifizierte Tapiokastärke gegeben wurde war nicht statistisch signifikant, aber Tiere die nichtmodifizierte Kartoffelstärke und nichtmodifizierte Amylopektin bekommen hatten einen Tendenz nach mehr Karies als die Kontrolltiere. Tiere, denen pregelatinisierten Stärkesorten gegeben wurde, hatten vielmals mehr kariierte Zahnflächen als Tiere der Kontrolltiergruppe.

Tiere, denen Maisamylose oder Maisamylopektin gegeben wurde, hatten signifikant mehr Karies als Tiere der Kontrastdiätgruppe (High amylose corn starch). Tiere der Amylopektindiätgruppen hatten mehr Karies als Tiere der Amylosediätgruppen. Zwischen Kartoffelamylose und Kartoffelamylopektin war der Unterschied stark signifikant nach dreissig Tagen aber nicht nach sechzig.

Manchmals keine klare Korrelation zwischen Zahnsteinbildung und Kariesbefall finden. Tiere, denen nichtmodifizierte Stärkesorten gegeben wurde, wiesen starke Zahnsteinbildung und niedrigen Kariesbefall auf. Doch wenn Tieren pregelatinisierte Stärkesorten gegeben wurde, fand man bei Gruppen mit starkem Kariesbefall auch eine Tendenz starker Zahnsteinbildung.

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Starch and Iron Absorption (32431)

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The habitual ingestion of large amounts of laundry starch is a common practice among Negro females in certain geographic areas of the United States(1). The amount ingested may exceed two pounds of starch daily and is frequently associated with marked iron deficiency(2). The severity of "starch eaters anemia" seems greater than expected from an iron deficient diet alone(3) and led us to postulate that starch interfered with iron absorption. The present investigation was performed to study this hypothesis and reports the acute and chronic effects of starch ingestion upon iron absorption in rats.

Materials and methods. Male albino rats, Walter Reed Carworth Farms strain, weighing 140 to 200 g were obtained and measurements of iron absorption and kinetics were performed when animals on a normal high protein diet weighed 170 to 200 g. The principles of laboratory animal care as promulgated by the National Society for Medical Research, were observed. The standard laboratory diet contained 25% protein and 9 mg of iron per 100 g dry weight. Special diets were made with starch (Argo), sucrose (USP), casein (USP) and corn oil (Mazola) and were supplemented with an iron-free commercial salt mixture and vitamins. Sufficient ferrous sulfate [illegible] to [illegible] the prepared diets

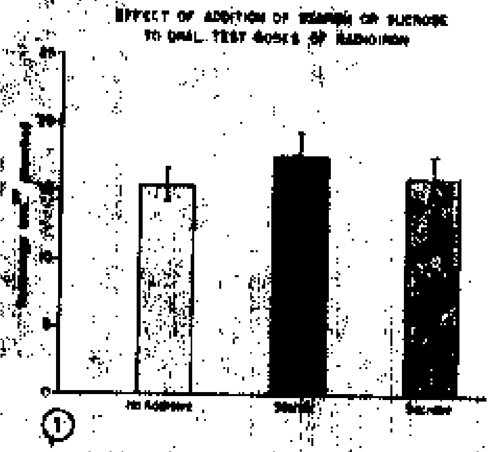
to bring the iron content to 9 mg per 100 g. Diets were assayed for iron content by a modification of the method of [illegible]. Animals were fed special diets for [illegible] weeks before study.

Iron absorption studies were performed in rats fasted for 16 hours. Oral test doses containing 0.5 μc of ferrous[59] [illegible] (Abbott 0.4 μc/[illegible]) and 0.50 mg of [illegible] commercial ferrous sulfate (Vitamin [illegible]) were introduced into the stomach through a 17 gauge [illegible] esophageal [illegible]. Whole body retention of the radioiron 7 days later was considered equivalent to absorption. Whole body radioactivity was measured in [illegible] [illegible] ARMAC[illegible], as previously described(8).

[illegible] microhematocrit determinations and measurements of the serum iron and iron binding capacity was obtained from the [illegible] [illegible] plasma with heparinized [illegible]. The serum iron concentration [illegible] binding capacity was determined by the "[illegible]" method (9).

Plasma clearance studies were performed following [illegible] injection of iron[59] labeled [illegible] serum for 15 minutes at 37°C before injection of 0.25 ml into the dorsal vein of the [illegible]. The iron binding capacity of the [illegible] which was not exceeded. At 10, 20, [illegible] and [illegible] minutes following injection, 0.02 ml of whole blood was obtained from the tail vein of the rat with a calibrated hemoglobin [illegible]. The blood was diluted into 2 ml of water and counted in a well-type crystal scintillation detector (PACKARD Auto Gamma Spectrometer, Model 410 A). The plasma iron clearance (T/2) was determined from the best fitted plot of these points on semilogarithmic graph paper. The whole blood volume was estimated to be [illegible]% of the [illegible] weight. The plasma volume was calculated from the hematocrit and estimated blood volume. Daily iron turnover was calculated from the serum iron concentration, plasma volume and plasma iron clearance studies(7,8). The incorporation of iron[59] into red [illegible] was [illegible] [illegible] [illegible] specimens obtained 20 hours after intravenous injection of labeled plasma(8,9). The nonheme iron concentration was measured in homogenates of weighed aliquots of liver(10).

Results. Fasted rats, raised on a normal iron-replete diet, were given 5 ml oral doses of labeled iron (10.5 mg) containing either 1 g of starch, 1 g of sucrose or no additive to ascertain if starch had a direct intraluminal effect upon iron absorption. Normal control animals absorbed 15.6% (SE 1.5%) of the test dose of iron. Rats dosed with the starch



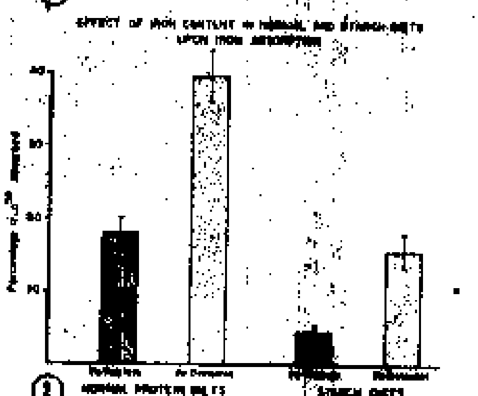


FIG. 1. Addition of either 1 g of starch or sucrose to oral test doses of iron[59] had no significant effect upon iron absorption.

FIG. 2. Rats were fed either a normal high protein or starch diet that contained either 5 or 90 μg of iron per gram of food. Animals ate these diets for 3 weeks before receiving an oral test dose of radioiron. Rats fed an iron replete high protein diet absorbed 18% of the test dose of iron. Iron absorption by animals maintained on other diets was 39% (iron deficient normal diet), 4% (iron replete starch diet) and 15% (iron deficient starch diet).

[illegible] and the sucrose solution absorbed [illegible] (SE, 3.0%) and 16.4% (SE, 1.8%) of the oral test dose of radioiron, respectively (Fig. 1).

Rats fed a protein depleted starch diet for [illegible] days lost body weight (1 g/day); whereas, [illegible] that consumed a normal high protein diet gained 2 to 3 g daily. Comparative studies of iron absorption showed no significant difference between rats fed the iron-replete high protein diet and animals that ate an iron deficient starch diet. However, studies of rats maintained on iron deficient protein replete diet and animals fed a protein deficient diet with iron added showed that protein depletion caused a relative inability to absorb appropriate quantities of iron from oral test doses (Fig. 2).

Rats weighing 150 g were fed diets containing various quantities of protein and either starch or sucrose for 2 weeks before study. Animals fed a high protein diet consumed 15 g of food and gained more than 2.5 g of body weight each day. In contrast, rats fed protein depleted starch or sucrose diets ate only 8 to 9 g of food and lost approximately 1 g of body weight daily. Protein depletion and weight loss were accompanied by significant decreases in iron absorption, red cell incorporation of radioiron, iron turnover and total iron-binding capacity with prolongation of the plasma Fe^{59} clearance and an increased serum iron concentration. The addition of small amounts of casein (5 g/100 ml) to the starch diet made it more acceptable for consumption; the rats gained weight (1 g/day), absorbed 7.5% of the oral test dose of iron and had improvement of iron kinetic studies. To ascertain if this partial correction of iron metabolism was caused by weight gain or dietary protein, rats were fed limited quantities of a normal high protein diet (7.5 g/day). These animals sustained a weight loss comparable to rats on a protein depleted starch or sucrose diet. However, iron absorption and kinetic studies were similar to those observed in rats gaining weight on a 5% casein diet. (Table I).

Discussion. The normal rat grows continuously throughout life. Fed a normal high protein diet, these animals gain 2 to 3 g of body weight and must absorb at least 120 μg of iron daily to maintain a normal body iron concentration of 60 parts per million (11,12). Erythropoiesis keeps pace with growth to maintain a normal hemoglobin concentration in the circulating blood. Conversely, iron absorption and erythropoiesis must be diminished with a retarded growth rate or marked anorexia and polycythemia should occur; normally, iron excretion and hemolysis are limited (9,13,14).

Previous investigations showed that both the rate of erythropoiesis and the quantity of iron in body tissues [illegible] influence iron absorption (5). Starvation and protein depletion [illegible] the concentration of iron in [illegible] organs and cause marked decrease in the rate of erythropoiesis and iron absorption (5,15,16).

In the rat, starch and sucrose diets seem to produce a combination of the effects of starvation and protein depletion. These animals [illegible] and lose weight to produce relative [illegible] increase in iron requirements and decrease iron absorption. Experiments with rats fed limited quantities of a high protein diet show increased erythropoiesis and absorption of more iron than rats maintained on protein-free food.

In starch-fating humans iron overloading can not be a major factor because starch diets are less deficient and weight loss is not a prominent clinical feature in adult females with this syndrome. However, protein deficiency may contribute to the severity of iron deficiency by decreasing erythropoiesis because of a relative unavailability of essential substrates from dietary protein for hemoglobin synthesis (16).

Summary. Decreased iron absorption was observed in rats fed protein deficient starch and sucrose diets. This was not caused by a direct intraluminal effect of starch or sucrose upon iron absorption. The observation was attributed to both a retarded rate of growth and a relative deficiency of protein for hemoglobin synthesis. We postulate that "starch induced anemia" is caused by a lack of dietary [illegible] for a decreased [illegible] of protein

TABLE 1. Effect of Dietary Composition upon Iron Absorption and Kinetics

		Normal diet, unrestricted intake	Normal diet, unrestricted intake	Normal diet, restricted intake	Starch diet, unrestricted intake	Sucrose diet, unrestricted intake	Starch casein, unrestricted intake
Additions to diet (g/100 g)	Casein	27	27	27			5
	Starch	56		60	86	86	81
	Sucrose		60				
	Corn oil	10	10	10	10	10	10
Dietary composition (g/100 g)	Protein	25	25	25	1	0	5
	Chos.	53	55	53	85	86	71
	Fat	10	10	10	10	10	10
	Iron	.009	.009	.009	.009	.009	[illegible]
Food consumption (g/day/rat)		15.2	15.4	7.5	8.3	8.7	11.8
Mean wt of rats (g)		186	184	134	136	131	167
Iron absorption (%)		17.5 (2.1)	18.3 (1.8)	6.6 (1.2)	2.7 (.3)	2.2 (.4)	7.6 (1.3)
RBC incorporation of radioiron (%)		41 (1.8)	40 (2.4)	24 (2.9)	5 (.4)	6 (.5)	26 (1.7)
Serum iron (μg/100 ml)		128 (8.2)	119 (5.6)	149 (12.7)	168 (11.4)	175 (14.3)	142 (9.7)
Total iron binding capacity (μg/100 ml)		472 (18.9)	465 (20.4)	418 (13.4)	382 (14.3)	316 (17.3)	411 (18.0)
Plasma iron[a] clearance (T/2)		67 (5.1)		89 (8.2)	122 (8.9)		94 (7.2)
Daily iron turnover (μg)		103 (6.3)		65 (5.1)	54 (4.9)		73 (7.8)
Liver iron concentration (μg/g)		105 (8.7)		173 (18.7)	270 (12.6)		156 (14.2)

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The effect of uncooked and roll-dried wheat starch, alone and mixed in equal quantity with sucrose, on dental caries in the albino rat

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1. Eighty weanling albino rats, five from each of sixteen litters were distributed between five dietary groups in sixteen randomized blocks. Each block was formed from within a litter and each animal in the block received a different diet.

2. The main features of the diets were: group 1, 72 % sucrose; group 2, 72 % uncooked wheat starch; group 3, 72 % roll-dried wheat starch; group 4, 36 % sucrose and 36 % uncooked starch; group 5, 36 % sucrose and 36 % roll-dried starch.

3. The rats were killed after 20 days on the diets and assessed for dental caries. The rats consuming diets containing sucrose (groups 1, 4 and 5) had significantly more caries than animals receiving diets in which starch was the sole carbohydrate. The diet containing roll-dried wheat starch produced significantly more caries than uncooked starch. The mixture of uncooked starch and sucrose was more cariogenic than the mixture of roll-dried starch and sucrose.

It has been shown (Green & Hartles, 1967) that rats fed on a diet containing sucrose as the sole source of carbohydrate had a greater caries incidence than those fed on diets containing a similar quantity of either uncooked or roll-dried maize starch. Sucrose diets were also more cariogenic than diets containing equal amounts of sucrose and either of the two forms of maize starch. The roll-dried maize starch produced significantly more caries than the uncooked starch. Somewhat surprisingly it was found that a mixture of sucrose and uncooked maize starch produced significantly more caries than a mixture of sucrose and roll-dried maize starch.

Grenby (1965) reported that he could not demonstrate any significant difference in caries incidence in Wistar rats receiving diets containing either uncooked or cooked wheat starch. The total consumption of maize starch in Britain is very small compared with that of wheat starch. In view of this and the findings of Grenby (1965) it was decided to repeat our earlier studies in which maize starch had been used but in this case to substitute wheat starch for maize.

EXPERIMENTAL

Eighty weanling albino rats of both sexes, five from each of sixteen litters, were assigned, one from each litter, to the five dietary groups. The animals were housed and managed as described by Green & Hartles (1966*a*, *b*). The rats were weighed at the beginning, and at the end of the experiment. They were maintained on the diets for 20 days and then killed by diethyl ether inhalation.

Diets

Diet RC 14 (group 1) contained 72% sucrose in the form of icing sugar (Green & Hartles, 1966*b*).

Diet RC 27 (group 2) was as RC 14 except that sucrose was replaced by uncooked wheat starch (Energen Limited).

Diet RC 28 (group 3) was as RC 14 except that sucrose was replaced by roll-dried wheat starch (Energen Limited).

Diet RC 29 (group 4) was as RC 14 except that half the sucrose was replaced by uncooked wheat starch.

Diet RC 30 (group 5) was as RC 14 except that half the sucrose was replaced by roll-dried wheat starch.

The diets and deionized water were provided in unrestricted amounts.

Dental caries was assessed as described by Green & Hartles (1966*a*).

RESULTS

Growth of animals. The animals remained healthy throughout the experiment and grew normally.

Caries incidence. The results are given in Table 1; dental caries is expressed as the number of lesions per rat in the first and second mandibular molars. Cumulative T

Table 1. *Caries experience of five groups each of sixteen rats maintained on different high-carbohydrate diets for 20 days*

Group	Diet	Cumulative T (all lesions). Mean	Cumulative B (more severe lesions). Mean
1	RC 14, sucrose	7·2	2·1
2	RC 27, uncooked wheat starch	0·9	0
3	RC 28, roll-dried wheat starch	3·2	0·1
4	RC 29, sucrose and uncooked starch	8·1	2·2
5	RC 30, sucrose and roll-dried starch	6·5	1·1
	Pooled standard error of group means	±0·40	±0·31

includes all lesions (maximum value is 12), and cumulative B includes the more severe lesions (see Green & Hartles (1966*a*) for description of classification).

The total number of lesions present was subjected to an analysis of variance in which the total variation of the experiment was divided into that due to treatments, that due to blocks, and the residual or error variation. The total treatment variation was then further divided into a series of orthogonal comparisons so that each comparison could be given a valid level of significance, using Snedecor's *F*-test. The

within-litter comparisons which have been made together with their levels of significance are shown in Table 2. The comparisons permit the following conclusions.

(1) Diets containing sucrose were significantly more cariogenic than those containing starch alone.

(2) Roll-dried wheat starch produced significantly more caries than uncooked starch.

(3) The mixture of uncooked wheat starch and sucrose was significantly more cariogenic than the mixture of roll-dried starch and sucrose.

Table 2. *Within-litter comparisons of the total number of carious lesions arising as a result of different dietary carbohydrates, with the significance level for each comparison*

(The number of rats compared is shown in parentheses)

Dietary treatments compared			Level of significance
Pure starches. Groups 2 and 3 (32)	against	diets containing sucrose. Groups 1, 4 and 5 (48)	$P < 0{\cdot}001$
Uncooked starch. Group 2 (16)	against	roll-dried starch. Group 3 (16)	$P < 0{\cdot}001$
Sucrose. Group 1 (16)	against	sucrose/starch mixtures. Groups 4 and 5 (32)	NS
Sucrose and uncooked starch. Group 4 (16)	against	sucrose and roll-dried starch. Group 5 (16)	$P < 0{\cdot}025$

NS, not significant.

DISCUSSION

In the production of dental caries wheat starch behaves in a similar manner to maize starch when incorporated in the diet of the caries-susceptible rat.

Diets containing uncooked wheat starch resulted in a low incidence of dental caries, and the roll-drying of the starch significantly increased its cariogenicity. However, the really intriguing finding was that a diet containing equal quantities of sucrose and uncooked starch was more cariogenic than one containing the same amounts of sucrose and roll-dried starch. This result is similar to that obtained with maize starch (Green & Harding, 1967) and poses the interesting question as to why the consumption of a mixture of highly cariogenic sucrose with a virtually non-cariogenic starch should lead to more dental caries than when sucrose is mixed with moderately cariogenic roll-dried starch. We suggested earlier (Green & Harding, 1967) that in the case of roll-dried maize starch there may be present other metabolizable substrates which might lessen the utilization of sucrose by the flora of the dental plaque, but so far we have not obtained any evidence to support this view. We are now establishing techniques to determine the frequency of eating of rats on different diets, for this may prove to be a factor in the relative cariogenicity of diets.

Our finding that roll-drying of wheat starch increases its cariogenicity is apparently in conflict with results obtained by Grenby (1965). He prepared samples of precooked starch in his own laboratory and the resulting material was probably different

from that produced by commercial roll-drying. These results focus attention on the possible effects of methods of processing on the caries-producing potential of starches.

We wish to thank Miss B. M. Morrison for her skilled technical assistance, the Medical Research Council for their financial support and Messrs Tate and Lyle Limited for the gift of icing sugar.

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THE INFLUENCE OF COOKED AND RAW WHEAT STARCH ON DENTAL CARIES IN THE RAT

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Summary—The effects of cooking wheat starch on its cariogenicity in rats and in decalcification tests *in vitro* were investigated. When rats of a caries-susceptible strain were fed for 8 weeks from weaning on diets containing 66% wheat starch, 32% skim milk powder and 2% dried liver powder, the incidence of caries was extremely low, no matter whether the starch was cooked or raw. In decalcification tests *in vitro*, mixtures of cooked wheat starch and saliva brought about significantly more dissolution of ground molar enamel than mixtures containing raw wheat starch. The behaviour of starch in caries tests is discussed.

INTRODUCTION

IN A PREVIOUS paper the initiation of experimental caries in a colony of rats was described, and evidence was given that the nature of the carbohydrate fraction of the diet determines the incidence of caries. The teeth of rats fed on a diet containing 66% raw wheat starch contained only a few small carious lesions, whereas the incidence was much higher in rats which received sucrose or glucose instead of starch (GRENBY, 1963).

One of the ultimate objects of this work is to investigate the cariogenicity of different types of bread and other processed cereal foods along the lines of tests which have already been carried out on flour and other fractions of the wheat grain (KÖNIG and GRENBY, 1964). White flour contains approximately 65–70% starch, the structure of which is radically altered by cooking with water, as in the bread-making process. Wheat starch consists of a mixture of granules of different sizes from 0·002 to 0·05 mm dia. When it is heated with water the granules swell irreversibly and may eventually rupture, depending on the amount of heating and the volume of water present. In baking bread the starch granules become distended but generally retain their structure.

It is possible that these changes may increase the cariogenicity of the starch by facilitating attack by enzymes and altering its physical characteristics so that it adheres to the teeth more tenaciously than raw starch. Accordingly in the experiments reported here, cooked and raw wheat starch were compared in the diet of caries-susceptible rats. The action of bacterial and salivary enzymes on the cooked and raw starch was also investigated in experiments *in vitro*.

MATERIALS AND METHODS

The animal experiments were carried out as previously described (GRENBY, 1963), using a strain of Wistar rats originally obtained from the Nederlands Instituut voor

Volksvoeding. In each experiment, groups of animals matched for sex, and when possible for litter, were put on the different diets. The diets were fed as dry powders *ad libitum* for a period of 8 weeks from weaning. They were based on the diet No. 580 of STEPHAN and HARRIS (1955) which contains sucrose 66%, skim milk powder 32% and dried liver powder 2%. This diet was fed to control groups of animals to confirm that the animals had retained their susceptibility to caries. To compare cooked and raw starch, all the sucrose in the diet No. 580 was replaced by starch.

Caries was assessed by the method of Shaw *et al.* (1944). Both left and right mandibular teeth were ground down in planes parallel to the occlusal surface and examined for lesions under a magnification of × 25. Almost all the lesions were in the fissures of the first and second molars. Each lesion was given a number from one to five, depending on how far it had progressed. These numbers were added to give a caries score for each animal.

The methods used in the experiments *in vitro* were similar to those of JENKINS *et al.* (1959). 0·600 g starch, 3 ml water and 8 ml pooled saliva were mixed with 0·050 g ground rat dental enamel and incubated at 37·4°C for 24 hr. The calcium and phosphorus in solution, the pH and the quantity of titratable acid present were determined before and after incubation. Thus the acid produced by the action of salivary micro-organisms on the food could be measured and the effects of different foods in the demineralizing mixtures could be compared. The method of separation of rat dental enamel was based on that of GILDA (1951) and gave a product which was 98 per cent pure.

Raw wheat starch was obtained from Energen Ltd. as a fine, white powder (protein content less than 0·5 per cent). To prepare cooked starch, five parts were made into a slurry with four parts water. This was poured into flat pans and placed in a steam oven at about 100°C for 90 min. The thin cakes were allowed to dry slightly in air cut into small pieces and dried in a current of air at 60°C for about 6 hr. The hard product was pulverised in a hammer-mill. The moisture contents of the cooked and raw starch were 11·9 per cent and 13·2 per cent respectively. Microscopic examination showed that, whereas the raw starch had the characteristic granular structure, the cooked starch was almost amorphous.

The proportion of the cooked starch subject to attack by beta-amylase under standard conditions was 80 per cent, compared with 2 per cent for the raw starch. The reducing power, expressed as mg glucose per 100 g dry starch, rose from 16 for the raw starch to 856 for the product after cooking (Samogyi reducing value determination on the fraction of the starch soluble in boiling water).

RESULTS

A. Animal experiments

Two experiments were carried out in which cooked and raw starch were compared at a level of 66 per cent in the diet of rats. In Experiment I (Table 1) forty-seven rats were randomized with respect to litter, but approximately the same number of males and females were fed on each diet. In Experiment II (Table 2) forty-two rats were divided into two groups matched for both sex and litter.

TABLE 1. CARIES SCORES OF GROUPS OF RATS FED FOR 8 WEEKS FROM WEANING ON DIETS CONTAINING

i 66% raw wheat starch; 32% skim milk powder; 2% liver powder (twenty-four rats)

ii 66% cooked wheat starch; 32% skim milk powder; 2% liver powder (twenty-three rats)

Litter	Raw starch diet				Cooked starch diet			
	Males		Females		Males		Females	
A	1		3	2	0	0	4	5
			0	3				
B	0	0	1	0			3	0
	0	0						
	2							
C	2	1	1	0	2	4	8	
					5	2		
					3	7		
D	1	5	1		4	0	0	
	3				1	0		
E	1	0	1	2	4		0	6
							5	4
Mean scores	1·2		1·3		2·5		3·5	

The difference between the mean caries scores of males and females fed on the same diet was not significant in either experiment. When the mean score of all the animals on the raw starch diet was compared with that for cooked starch, the difference was very small in both experiments. In Experiment I the mean caries score of 2·9 on the cooked starch diet was significantly higher than the figure of 1·25 for raw starch ($p<5\%$). In Experiment II, however, there was no significant difference between the mean caries scores of 2·95 on the raw starch diet and 2·13 for cooked starch ($p>5\%$). In a further trial with a smaller number of animals (twenty-four in all) there was again no significant difference between the mean scores on the raw and cooked starch

TABLE 2. CARIES SCORES OF GROUPS OF RATS FED FOR 8 WEEKS FROM WEANING ON DIETS CONTAINING

i 66% raw wheat starch; 32% skim milk powder; 2% liver powder (twenty-one rats)

ii 66% cooked wheat starch; 32% skim milk powder; 2% liver powder (twenty-one rats)

Litter	Raw starch diet					Cooked starch diet				
	Males		Females			Males		Females		
F	D	1	3	2	3	2	2	0	3	0
G	2	3	3			2	1	1		
H	5	1	0			D	0	5		
I	8	3	3			4	4	2		
J	3		0	3		D		D	1	
K	1	2	6	7		0	D	7	D	
Mean scores of surviving rats	2·9		3·0			1·9		2·4		

(D = rat died before end of the experiment).

diets. When a control group of six rats was fed on a diet containing 66 per cent sucrose instead of starch for 8 weeks from weaning the mean caries score was 23·5.

It is concluded that the differences in caries scores on raw and cooked starch diets are marginal and of no significance because of variation between experiments.

The pattern of caries attack was similar on both the diets. All the lesions were small (score 1 or 2 by Shaw's system); lesions were most frequently found in the central fissure of the first molar and the anterior fissure of the second molar.

Weight gains during the experiments were recorded. Over the 8-week experimental period rats on the cooked starch diet gained about 1·7 g/rat/day, and those on raw starch about 1·3 g/rat/day.

B. Experiments in vitro

The effects of mixtures of cooked and raw starch and saliva on the demineralization of ground rat dental enamel were compared in a series of four separate tests (Table 3).

TABLE 3. CHANGES AFTER THE INCUBATION OF STARCH-SALIVA MIXTURES WITH GROUND RAT DENTAL ENAMEL AT 37·4°C FOR 24 hr
(Mean of 4 experiments)

Constituents of mixture	Increase in concentration μg/ml	
	Calcium	Phosphorus
Raw starch, saliva, enamel	183	132
Cooked starch, saliva, enamel	288	172
Raw starch, saliva } controls	27	53
Cooked starch, saliva } controls	24	15
After subtraction of control figures:		
Raw starch, saliva, enamel	156	79
Cooked starch, saliva, enamel	264	157
Standard error	±27	±16 (6 Degrees of freedom)
"t" for difference between results for cooked and raw starch	2·85	3·41
Probability	$2\% < p < 5\%$	$1\% < p < 5\%$

The quantity of acid produced and the quantities of calcium and phosphorus dissolving were variable, depending on the sample of saliva used, but the final pH of the mixture was always between 4·0 and 4·6, and the acid produced in 24 hr was equivalent to an average of 0·72 ml of 0·05 N. Control experiments omitting enamel were carried out, and the mean results were subtracted from the means in the presence of the enamel. Both the calcium and phosphorus figures were significantly higher with cooked starch than with raw starch ($p < 5\%$) when the appropriate error variances of all of the results were taken into account. The changes in Ca and P concentrations summarized in Table 3 were smaller than those in other experiments in which sucrose was used instead of starch.

DISCUSSION

Wheat starch is a polysaccharide which under certain conditions can be degraded to simple sugars, mainly maltose. There is much evidence that mono- and disac

charides such as glucose and sucrose are cariogenic, but both in the present experiments and in earlier work (GREENBY, 1963) very little caries occurred in rats fed on starch diets in the absence of sucrose and glucose.

The extremely low incidence of caries on starch diets suggests that the quantities of simple sugars formed by degradation of the starch in the mouth are insufficient to promote caries, or that these particular sugars are not suitable substrates for caries-producing oral micro-organisms. In this connexion, unpublished evidence has been obtained in these laboratories that *in vitro* oral lactobacilli grew faster on starch than on sucrose, while numbers of oral streptococci were higher after a 24-hr incubation with sucrose than with starch.

In the present work, however, it has been shown that oral micro-organisms incubated with starch can produce considerable quantities of acid in 24-hr tests *in vitro*, and that this acid can dissolve calcium and phosphorus from rat dental enamel. It is possible that these processes do not contribute to caries *in vivo* either because the starch does not remain in the mouth long enough for appreciable acid production, or because starch is prevented by plaque or some other barrier from reaching the tooth surface. On the other hand sucrose and other cariogenic sugars appear to be able to penetrate this barrier and form acids at the tooth surface (see KÖNIG and GREENBY, 1966).

The present experiments were designed to test the effect of cooking starch on its cariogenicity. The intact granules of raw starch are only slowly attacked by amylases. When wheat starch is cooked as in the present work, the granules swell irreversibly and are ruptured. The starch can then be attacked by α- and β-amylases, and its digestibility is increased. Tests *in vitro* showed that incubated mixtures of cooked starch and saliva dissolved significantly more rat dental enamel than raw starch mixtures (Table 3).

It was thought possible that by cooking the starch, and thereby increasing the extent of the enzymic degradation to fermentable sugars, its cariogenicity would also be increased. The results of the present experiments do not support this view. The cariogenicity of a particular food depends not only on its chemical composition and effect on oral micro-organisms, but also on its physical characteristics, especially the extent to which it adheres to the teeth. In the present work, however, the destruction of the starch granules by cooking did not appear to increase its cariogenicity by virtue of any alteration in its physical characteristics. It cannot be assumed from this that starch is of no importance in caries in man, because in a mixed diet which also contains sugars, the starch may help to bring the sugars into close contact with the tooth surface and thus promote caries, even though it appears to be non-cariogenic in the absence of sugars.

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Résumé—Les effets de l'amidon de blé cuit sur la carie chez le rat et sur des tests de décalcification *in vitro* sont déterminés. Lorsque des rats d'une espèce susceptible à la carie sont nourris pendant 8 semaines, suivant leur sevrage, à l'aide de régimes contenant 66% d'amidon de blé, 32% de poudre de lait écrémé et 2% de poudre de foie déséché, la fréquence de la carie est très peu élevée, que l'amidon soit utilisé cru ou cuit. Au cours des tests de décalcification *in vitro*, des mélanges d'amidon de blé cuit et de salive ont provoqué une dissolution significativement plus élevée de poudre d'émail dentaire de rat que des mélanges contenant de l'amidon de blé cru. Le comportement de l'amidon aux pours des dents expérimentales est discuté.

Zusammenfassung—Die Wirkungen gekochter Weizenstärke auf deren kariogenen Effekt bei Ratten und bei Entkalkungsversuchen *in vitro* wurden untersucht. Wenn Ratten eines kariesanfälligen Stammes nach der Entwöhnung 8 Wochen lang mit Diäten gefüttert wurden, die 66% Weizenstärke, 32% Magermilchpulver und 2% getrocknetes Leberpulver enthielten, war das Auftreten von Karies extrem niedrig, unabhängig davon, ob die Stärke gekocht oder roh war. Bei Entkalkungsversuchen *in vitro* brachten Mischungen von gekochter Weizenstärke und Speichel signifikant mehr Auflösung von gemahlenem Rattenzahnschmelz hervor als Mischungen, die rohe Weizenstärke enthielten. Die Wirkungsweise von Stärke bei Kariesuntersuchungen wird diskutiert.

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THE INFLUENCE OF DIETARY CARBOHYDRATE ON LEVELS OF AMINO ACIDS IN THE FECES OF THE WHITE RAT[1]

By Alfred E. Harper, Mitsuru C. Katayama, and Bohdan Jelinek

Abstract

The effect of various carbohydrates on the levels of amino acids excreted in the feces of rats has been studied. Potato starch, autoclaved potato starch, or dextrin each induced much greater levels of amino acids in the feces than either sucrose or glucose. The amino acids appear to be derived mainly from endogenous nitrogen. However, when rats were fed rations containing unmodified potato starch, undigested dietary protein apparently contributed a significant amount of [illegible] acids found in the feces. The inclusion of 10% lactose in a ration containing sucrose as the source of carbohydrate caused a statistically significant increase in the levels of lysine and histidine in the feces. The influence of [illegible] to changes in the intestinal flora. The incorporation of [illegible] 5% casein diet had no significant effect on the amounts of amino acids in the feces.

Introduction

The nature of the diet has been shown to influence the quantity of amino acids excreted in the feces. Pearson and Panzer (11) found less valine, histidine, and methionine in the feces of rats which were fed corn oil in their diet than in the feces of rats which were fed diets free of fat. Ross (12) observed that breast-fed infants excreted more free amino acids in the feces than did infants receiving formulas. The present study demonstrated that the carbohydrate component of the diet may influence the quantity of certain [illegible] amino acids excreted in the feces of rats.

Experimental

Male weanling rats of the Wistar strain, weighing between 40 and 50 gm., were housed in individual cages having elevated screen bottoms. Groups, consisting of five to six animals each, were fed the experimental rations for a period of either four or six weeks. The animals were weighed weekly and individual food consumption records were kept.

The 9% casein rations were fed ad libitum but the food consumption of rats fed the rations containing 18% casein was controlled by restricting the amount of ration offered. Equal quantities of food were offered during the first few days, but when the average food intake reached 10 gm. per day, the ration thereafter offered was restricted to this amount. This procedure was followed in order to ensure that the nitrogen intake of the various groups receiving 18% casein would be similar.

During preliminary experiments it had been observed that the food intake of rats receiving, ad libitum, rations containing 18% casein with either sucrose or [illegible] starch as the source of carbohydrate, gradually increased. The male

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animals grew at a rate of 40 to 44 gm. per week, which is considered to be excellent growth. When, on the other hand, the ration contained potato starch, food intake did not increase much above 10 gm. per day and normal growth could not be obtained.

The percentage composition of the basal ration was as follows: casein 9, carbohydrate 41.6, salt mixture* 4, corn oil 4, inositol 0.1, choline chloride 0.2, whole liver powder (Wilson's) 0.1. Vitamins were added so as to provide, per kilogram of ration: thiamine 5 mgm., riboflavin 10 mgm., pyridoxine 5 mgm., nicotinic acid 5 mgm., calcium pantothenate 25 mgm., *p*-aminobenzoic acid 300 mgm., α-tocopherol 25 mgm., menadione 2 mgm., folic acid 2 mgm., biotin 200 μgm., liver oil to provide 60,000 I. U. vitamin A and 12,000 I. U. vitamin D. The various rations fed are indicated in Table I. All supplements were included in the basal diet at the expense of the carbohydrate.

Feces excreted during the first three days of the experiment were discarded. Thereafter, the feces from each rat were collected at least twice weekly from absorbent paper that had been placed beneath the cage. The feces were screened from food particles and hair, and were then air-dried at room temperature, weighed, and ground for the analyses.

The proteins in the feces were hydrolyzed by autoclaving 1 gm. of feces in 20 ml. of 5 *N* hydrochloric acid for 10 hr. at 120° C. The amino acids were determined by the microbiological procedure of Henderson and Snell (3). *Leuconostoc mesenteroides* P-60 was used as the test organism for lysine determinations, *Lactobacillus arabinosus* for valine, and *Streptococcus faecalis* for methionine and histidine. A Cannon titrator (2) with a quinhydrone electrode was used throughout for titrations.

The influence of various carbohydrates on the digestibility of casein was studied. The animals were housed in cages with screen bottoms of ¼ in. mesh. The cages were supported by metal funnels. To collect the feces, a fine mesh screen was placed a few inches above the inverted apex of each funnel. The feces were removed every second day and were air-dried for 24 hr. The nitrogen in each sample was determined by the micro-Kjeldahl method (1). The techniques followed were similar to those used by Mitchell (7). The endogenous nitrogen loss for each group, which consisted of three young male rats, was determined by placing the rats on a ration containing 4% egg albumen as the sole source of protein. Each group was then fed a test ration containing 9% casein. In changing from one ration to another, at least three days were allowed to elapse before the feces were again collected and analyzed. The low nitrogen diet had the same general composition as the basal ration except that 4% egg albumen was substituted for 9% casein and the difference was made up by increasing the amount of carbohydrate.

According to Mitchell (7) the nitrogen excreted during the feeding of a low protein or nitrogen-free ration is a measure of the loss of endogenous nitrogen

* Salt mixture 351 (4) with the following changes: NaF was omitted; $MnSO_4 \cdot 4H_2O$ was increased from [illegible] gm. to 14.2 gm.; 60 gm. $FeSO_4$ was substituted for 20.5 gm. of $FePO_4 \cdot 4H_2O$; 800 gm. of Na_2HPO_4 and 0.[illegible] gm. of $MnCl_2$ were added.

which is derived from the intestinal tract and its secretions. The value, calculated as [illegible] excreted per gram of food consumed, is used as a correction factor in the calculation of the "true" digestibility of the protein in an experimental ration. The true digestibility is then calculated according to the formula:

$$\text{True digestibility} = \frac{\text{N intake} - (\text{fecal N} - \text{endogenous fecal N}) \times 100}{\text{N intake}}.$$

Results and Discussion

The growth rates of the rats fed the various rations are reported in Table I. There were no marked growth differences among groups which were fed sucrose, glucose, dextrin, or autoclaved potato starch as the source of carbohydrate. However, when unmodified potato starch was used as the only source of carbohydrate in the diet, the growth rate of the rats was low. The rats on this ration [illegible] white, bulky feces which were found by microscopic examination to consist largely of undigested starch granules which gave a blue color with iodine. Also, the digestion of dietary protein was shown to be impaired when unmodified potato starch was used as the sole source of carbohydrate.

TABLE I

GROWTH RESPONSE OF RATS FED DIETS CONTAINING VARIOUS CARBOHYDRATES

Expt. No.	Ration No.	% Casein	Ration	Mean weekly gain in weight, gm.	Mean daily food intake, gm.
I	1	18	Potato starch	13.5	9.8
	2		Sucrose	21.9	9.7
	3		Autoclaved potato starch*	22.2	9.9
	4		Glucose	22.2	9.7
	5		Dextrin**	20.3	9.8
II	6	18	Potato starch	8.8	8.9
	7		Potato starch and 30% lactose	17.5	9.6
	8		Sucrose	23.5	9.6
	9		Sucrose and 10% lactose	22.1	9.6
III	10	9	Potato starch and sucrose	13.6	9.5
	11		Potato starch and sucrose + 0.619% N***	12.4	9.0
	12		Autoclaved corn starch*	13.8	8.4
	13		Autoclaved corn starch* + 0.619 N***	8.8	6.8
	14		Corn starch	10.8	8.2
	15		Corn starch + 0.619% N***	9.1	7.7

* Autoclaved starches were prepared by [illegible] the starch with water and autoclaving at 120° C. for two hours. The protein was dried in the air at room temperature and then ground in a Wiley mill.

** Dextrin was prepared by [illegible] starch in an oven at 145° C. for eight hours with [illegible] on addition of iodine.

*** [illegible] bicarbonate.

TABLE II

EFFECT OF CARBOHYDRATES ON THE DIGESTIBILITY OF CASEIN

Ration	Rat No.	Initial weight, gm.	N intake, mgm.	Fecal N, mgm.	Endogenous fecal N, mgm.	True digestibility, %
Potato starch	4	[illegible]	100	40	25	85
	5	[illegible]	126	49	29	84
	6	[illegible]	94	33	12	78
Autoclaved potato starch	7	[illegible]	78	[illegible]	14	92
	8	[illegible]	101	34	19	95
	9	[illegible]	75	17	13	95
Sucrose	[illegible]	[illegible]	56	6	5	98
	[illegible]	[illegible]	99	8	11	100
	[illegible]	66	81	4	6	100

Apparently the lower rate of growth of rats fed the unmodified potato starch ration was due to the poor utilization of unmodified potato starch and to the coincident decrease in the extent of digestion of the dietary protein.

The inclusion of lactose at a level of 20% in the unmodified potato starch ration improved the growth rate slightly, whereas the incorporation of 10% lactose into the [illegible] ration did not cause any observable change in the growth of rats. [illegible] (9), using diets containing various levels of lactose [illegible] with corn starch, found that the growth of rats was practically [illegible] on diets containing 30% or less of lactose. Similarly Outhouse, [illegible], Mowitz and White (10) observed that there was no alteration in growth when [illegible] sucrose or lactose replaced an equivalent amount of corn starch in the [illegible]. The beneficial effect obtained when unmodified potato starch was partially replaced by lactose may have been due to the utilization of lactose per se by the rat.

The rate of [illegible] of rats was not improved by supplementing 9% casein rations with [illegible] salts. Similar results were obtained by Lardy and [illegible] (6) when [illegible] citrate into rations containing [illegible] or [illegible] meal.

The [illegible] in Table III [illegible] the effects of various carbohydrates [illegible] weekly [illegible] of lysine, valine, histidine, and [illegible]. Potato starch, autoclaved potato starch, and [illegible] of amino acids in the feces than [illegible], and Beadles (8) found that [illegible] losses of organic nutrients in the [illegible]. They [illegible] that carbohydrates may have [illegible] of the organic nutrients contained in [illegible]. In the [illegible] here, a significant loss of dietary protein [illegible] protein was almost com[illegible] potato starch or sucrose

TABLE III

[illegible] VARIOUS DIETS ON THE WEEKLY LEVELS OF AMINO ACIDS IN THE FECES

(Amino acid levels are expressed as mgm. per week [illegible] values ± standard error of the mean)

No.	Diets	Week of exp.	Lysine	Valine	Histidine	Methionine
1	Potato starch	1	46 ± 6.0	50 ± 3.3	18 ± 2.1	17 ± 1.7
		2	79 ± 9.1	71 ± 7.0	24 ± 2.9	22 ± 2.4
		3	84 ± 6.3	100 ± 9.8	37 ± 3.0	33 ± 2.0
		4	100 ± 11.5	109 ± 8.2	33 ± 2.7	31 ± 2.6
		5	127 ± 20.5	108 ± 9.6	35 ± 2.5	39 ± 2.2
2	Sucrose	1	14 ± 1.2	13 ± 1.3	5.0 ± 0.43	5.3 ± 0.45
		2	14 ± 1.0	17 ± 1.0	5.1 ± 0.71	5.3 ± 0.39
		3	21 ± 1.3	18 ± 1.7	5.9 ± 0.77	6.5 ± 0.43
		4	30 ± 1.8	37 ± 1.6	6.8 ± 0.74	9.1 ± 0.77
		5	26 ± 1.6	30 ± 1.4	6.8 ± 0.55	8.0 ± 0.55
3	Autoclaved potato starch	1	48 ± 3.3	32 ± 3.0	13 ± 0.9	14 ± 0.7
		2	67 ± 4.0	78 ± 6.5	19 ± 1.3	19 ± 1.9
		3	71 ± 6.0	80 ± 6.1	21 ± 1.2	24 ± 1.8
		4	66 ± 6.1	74 ± 6.7	21 ± 1.4	21 ± 2.3
		5	80 ± 9.8	61 ± 9.1	30 ± 2.8	24 ± 2.1
4	Glucose	1	17 ± 2.2	30 ± 4.3	12 ± 1.9	7.3 ± 1.52
		2	14 ± 2.4	30 ± 4.2	10 ± 1.4	6.3 ± 1.10
		3	24 ± 2.0	40 ± 2.8	15 ± 1.8	10.8 ± 1.36
		4	20 ± 2.6	37 ± 3.0	9 ± 1.1	6.6 ± 0.92
		5	27 ± 2.4	32 ± 2.2	11 ± 1.0	9.1 ± 0.90
5	Dextrin	1	75 ± 8.4	45 ± 3.4	31 ± 2.8	19 ± 1.8
		2	72 ± 15.9	80 ± 11.6	30 ± 6.4	18 ± 3.6
		3	97 ± 13.0	85 ± 10.8	38 ± 4.6	31 ± 4.0
		4	78 ± 10.2	70 ± 9.9	31 ± 4.0	26 ± 3.4
		5	103 ± 15.3	88 ± 9.2	48 ± 4.3	35 ± 3.5

was used as the source of carbohydrate (Table II). The extent to which dietary protein contributed amino acids in the feces was apparently modified by the type of carbohydrate fed.

The levels of amino acids excreted in the feces were generally lower during the first week of the experiment than during the following weeks. During the first week the quantity of food consumed by the rats was least. Since Mitchell (7) has shown that the endogenous nitrogen excreted in the feces is proportional to the food intake, the smaller quantity of amino acids from endogenous sources could account, in part at least, for the lower levels of amino acids found in the feces during the first week.

That the intestinal secretions and the intestinal microorganisms probably contribute the major portion of the amino acids found in the feces is supported by the data in Table II, which show that endogenous nitrogen makes up a large portion of the nitrogen excreted in the feces. Also, unmodified potato starch and autoclaved potato starch contributed relatively large excretions of endogenous nitrogen, and these diets produced the highest levels of amino acids

in the feces. It thus appears reasonable to conclude that the type of carbohydrate ingested by the rat may influence the amounts of amino acids from endogenous [illegible] which are found in the feces.

The effect of lactose on the fecal excretion of lysine, valine, and histidine is shown in Table IV. The inclusion of 20% of lactose in the potato starch ration apparently did not influence the amino acid content of the feces; however, the inclusion of 10% of lactose in the sucrose ration caused an increase in the amino acid content of the feces. The t test (5) was applied and the increases in the lysine and histidine were found to be statistically significant ($t = 3.73$, $P < 0.01$, and $t = 2.80$, $P \triangleq 0.02$ respectively). The increase in the valine value was not statistically significant ($t = 1.64$, $P > 0.1$). Ross (12, 13) has shown that breast-fed infants excrete greater quantities of free amino acids in the feces than do infants receiving prepared diets. The high lactose content of human milk was mentioned as possibly influencing the amount of free amino acids excreted in the feces. Ross also

TABLE IV

EFFECT OF LACTOSE ON THE LEVELS OF AMINO ACIDS IN THE FECES

The amino acid levels are expressed as mgm. per week
(mean values ± standard error of the mean)

No.	Ration	Lysine	Valine	Histidine
6	Potato starch	67 ± 6.2	88 ± 7.0	38 ± 2.4
7	Potato starch and lactose	73 ± 4.9	88 ± 3.9	41 ± 2.9
8	Sucrose	21 ± 0.9	26 ± 1.3	7.7 ± 0.22
9	Sucrose and lactose	29 ± 2.1	31 ± 2.4	9.0 ± 0.45

found that in the feces of the breast-fed infants the predominant organism was *Lactobacillus bifidus* which comprised almost the whole flora; whereas with the infants on artificial diets the fecal organisms were mixed, and *L. bifidus* was either scanty or absent. Mitchell (9) showed that there was an increase of *Bacillus acidophilus* in the intestinal flora of the rat when 15% of lactose was incorporated into the diet. Thus it seems possible that an alteration of the intestinal flora may be associated with the increased levels of amino acids found in the feces of rats receiving supplementary lactose in the sucrose ration. The slow rate of removal of lactose from the intestinal tract may permit the growth of a greater and more varied flora. However, the laxative effect of lactose, which may cause greater losses of ingested protein, cannot be entirely disregarded.

Since the food intake influences the amount of endogenous nitrogen excreted, the amounts of [illegible] of protein ingested are reported

in Table V, [illegible] the groups receiving supplementary nonprotein nitrogen. On this basis, there was a tendency for an excretion of slightly higher levels of amino acids in the feces when ammonium salts were included in the rations containing either unmodified potato starch or autoclaved corn starch. However, this increase was not found to be statistically significant.

TABLE V

EFFECT OF AMMONIUM SALTS ON THE LEVELS OF AMINO ACIDS IN THE FECES

The results are expressed as mgm. of fecal amino acid per gram of protein ingested (mean values ± standard error of the mean)

No.	Ration	Lysine	Valine	Histidine	Methionine
10	Potato starch	13.7 ± 0.[illegible]	[illegible] ± 0.[illegible]	6.6 ± 0.33	3.0 ± 0.84
11	Potato starch and N	16.1 ± 1.[illegible]	[illegible] ± 1.41	7.4 ± [illegible]	3.0 ± 0.[illegible]
12	Autoclaved corn starch	11.8 ± 0.07	[illegible] ± 0.07	4.1 ± 0.22	3.8 ± 0.32
13	Autoclaved corn starch and N	12.5 ± 1.[illegible]	[illegible] ± 0.[illegible]	4.8 ± 0.[illegible]	3.8 ± 0.28
14	Corn starch	4.8 ± 0.[illegible]	[illegible] ± 0.40	2.0 ± 0.[illegible]	1.3 ± 0.04
15	Corn starch and N	3.7 ± 0.[illegible]	[illegible] ± 0.[illegible]	2.0 ± 0.16	1.1 ± 0.07

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THE INADEQUACY OF UNMODIFIED POTATO STARCH AS DIETARY CARBOHYDRATE FOR THE ALBINO RAT[1]

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Abstract

Unmodified (raw) potato starch was poorly utilized, whereas unmodified corn starch and wheat starch were almost completely digested by the rat. The ingestion of unmodified potato starch caused a distention of the caecum and intestines. This enlargement resulted from the accumulation of food residue in the caecum and the consequent formation of gas from fermentation. The utilization of unmodified potato starch was not appreciably improved by the inclusion of either lactose or sucrose in the ration. However, the utilization of potato starch by the rat was improved when the starch was heated in an autoclave at 120° C., in a dry oven at 145° C., or was finely ground. During digestion studies performed *in vitro* with pancreatin pronounced hydrolysis of both corn starch and wheat starch occurred, but hydrolysis of unmodified potato starch was very slight. Ground potato starch was hydrolysed very rapidly. The low digestibility of unmodified potato starch was attributed to the resistance of the outermost layer or layers of the granules to attack by alpha-amylase.

Introduction

It is known that certain starches, mainly those derived from tubers, are better protected against the action of amylases than the starches of cereals. A review of the literature revealed many reports which gave clear evidence that unmodified potato starch was poorly utilized by rats (9), dogs (4), guinea pigs (13), hens (7), mice (3), and sheep (6). Several investigators have reported (1, 5, 10, 11) that man digests cereal starches much more rapidly than potato starch.

Various modifications (physical and chemical) render the starch granules more susceptible to enzymatic attack. Such modifications include: autoclaving in the presence or absence of water at temperatures around 100° C., soaking in dilute acids, and grinding. The digestibility of potato starch was shown to be greatly improved by cooking or mechanically rupturing the granules (12).

The present study was designed to determine whether the utilization of raw potato starch could be improved by dietary supplements and whether other native starches might be poorly utilized. The potato starch was fed with other carbohydrates in an attempt to obtain better utilization of this starch by the rat; and raw potato, wheat, and corn starches, which were carefully prepared in the laboratory to conserve as nearly as possible the native state of the starch granules, were used for feeding trials and enzyme studies.

Experimental

Animal-feeding Techniques

Groups of weanling male rats (Wister strain) of 40 to 50 gm. body weight (21 to 25 days old) were used in this experiment. The five rats which comprised each group were housed in individual cages with raised screen floors and

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were given water ad libitum. The food intake was controlled by restricting the amount of ration offered each rat to 10 gm. per day. Each animal was weighed weekly and individual food consumption records were kept. Corrections were made for spillage.

The percentage composition of the basal ration was as follows: total carbohydrate 73.6, casein 18.0, salt mixture* 4.0, corn oil 4.0, inositol 0.1, choline chloride 0.2, whole liver powder (Wilson's) 0.1. Vitamins were included so as to provide, in milligrams per kilogram of ration, thiamine hydrochloride 5, riboflavin 10, pyridoxine hydrochloride 5, niacin 5, calcium pantothenate 25, p-amino benzoic acid 300, alpha-tocopherol 25, menadione 2, folic acid 2, biotin 0.2. Vitamins A and D were added as halibut liver oil to provide 60,000 I.U. and 12,000 I.U. respectively per kilogram of ration.

The various rations are listed with the growth data in Table I. In each ration total carbohydrate made up 73.6% by weight, the starch being reduced in amount when a ration was supplemented by other carbohydrate. To maintain equivalent caloric intakes, rats fed the ration which contained 7.5% celluflour were each offered 10.7 to 10.8 gm. of ration daily. The utilization of the carbohydrate component was assessed by (*a*) its capacity to promote growth and (*b*) its effect on the quantity of faeces excreted by the rat.

In Experiment II, laboratory preparations of corn, wheat, and potato starches were compared as the source of carbohydrate in 20% casein rations. The composition of these rations was basically the same as shown above but 20% casein was substituted for the 18% casein and the carbohydrate was reduced accordingly. After the experimental period of three weeks, one rat from each group was sacrificed and the gastrointestinal tracts were compared.

Preparation of Starches

Wheat starch was prepared by working wheat flour into a dough, allowing the dough to stand for one hour, then washing it repeatedly with water in order to separate the starch from gluten. The starch granules were allowed to settle from the washings, the supernatant liquid was decanted, and the granules were repeatedly washed with water, separated on a Buchner funnel, and dried at about 55° C.

Potato starch was prepared from potatoes which were peeled, washed, and ground in a meat grinder. The minced product was mixed thoroughly with water and then the pulp was separated from the starch granules by filtering the ground mass through a cotton cloth. After allowing the starch granules to settle, the supernatant liquid was decanted. After repeated washings with water, the starch was separated on a Buchner funnel and dried at about 55° C.

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* *Salt mixture was of the following composition:*

$CaCO_3$	543.0 gm.	$FeSO_4$	60.0	gm.
$MgCO_3$	25.0 "	KI	0.08	"
$MgSO_4$	16.0 "	$MnSO_4 . 4H_2O$	16.2	"
$NaCl$	69.0 "	$Al_2(SO_4)_3 . K_2SO_4 . 24H_2O$	0.17	"
KCl	112.0 "	$CuSO_4$	0.90	"
KH_2PO_4	212.0 "	$ZnCl_2$	0.52	"
Na_2HPO_4	300.0 "			

This is salt mixture No. 351 (8) with certain changes.

Corn starch was prepared by shaking a quantity of corn meal with about five volumes of water in a large glass jar for one hour on the mechanical shaker. The starch granules, suspended in ice-cold water, were transferred to another container and allowed to settle. The starch was repeatedly washed with ice-cold water. Finally the supernatant liquid was discarded and the corn starch thus obtained was separated on a Buchner funnel and dried at approximately 55° C.

Ground potato starch was prepared by subjecting commercial potato starch for four hours to the action of a ball-mill containing porcelain balls.

Autoclaved potato starch was prepared by moistening the starch with water and heating it in an autoclave at 120° C. for two hours. The product was dried at approximately 55° C. and then ground in a Wiley mill.

The dextrin was prepared by heating thin layers of potato starch (1 in.) in an oven at 145° C. for eight hours with frequent stirring. The product gave a purple coloration on addition of iodine.

Enzymatic Degradation of Starches

The rate of hydrolysis by pancreatin USP of the various starches prepared in the laboratory was determined *in vitro*. The enzyme solution was prepared by shaking 250 mgm. of pancreatin with 50 ml. of distilled water. The suspension was centrifuged and the supernatant liquid was used as the source of alpha-amylase.

The rate of hydrolysis of each of the prepared starches was determined as follows: 250 mgm. of the starch was placed in a 50 ml. rubber-stoppered cylinder. To this was added distilled water, *ca.* 40 ml.; phosphate buffer (Sørensen's) *M*/15, pH 7, 2 ml.; enzyme solution 5 ml.; and water to make 50 ml. The starch granules were suspended by shaking and the cylinder was placed in a horizontal position in an incubator at 30° C. After 0, 1, 3, and 20 hr. of incubation 5-ml. aliquots were taken and reducing sugar was determined by the Somogyi method (15).

Results and Discussion

The average rates of growth of rats fed various rations containing raw potato starch and the respective average weights of their faeces are shown in Table I.

The incorporation of 7.5% of celluflour into the basal ration did not appear to influence the utilization of potato starch by the rat. The increase in the weight of the faeces excreted by the rats fed this ration was probably due to the presence of undigested celluflour. The inclusion of 5% of either lactose or sucrose in the basal ration resulted in a slight increase in the rate of growth with a coincident decrease in the amount of faeces excreted. The incorporation of 20% of either lactose or sucrose into the basal ration accentuated the increase in the rate of growth and the decrease in the amount of faeces. It seems probable that the disaccharides were completely assimilated, while the potato starch in the ration remained poorly utilized.

TABLE I

THE EFFECT OF VARIOUS STARCHES ON THE GROWTH RESPONSE OF RATS

Experiment number	Casein in the diet, %	Ration	Gain in weight per week, gm. (mean ± S.E.)	Weekly weight of air-dried feces, gm. (mean ± S.E.)
IA	18	Potato starch, raw	13.5 ± 1.1	28.7 ± 1.1
		" " " + 7.5% cellufloor	14.7 ± 0.9	33.4 ± 1.3
		" " " + 5 % lactose	16.8 ± 1.4	25.5 ± 1.3
		" " " + 20 % lactose	19.1 ± 0.9	22.6 ± 0.8
		" " " + 5 % sucrose	16.8 ± 1.1	25.4 ± 1.2
		" " " + 20 % sucrose	19.4 ± 0.1	21.8 ± 1.0
		Sucrose (control)	22.6 ± 0.1	2.1 ± 0.2
IB	18	Autoclaved potato starch	22.2 ± 0.1	4.8 ± 0.2
		Dextrin from potato starch	22.2 ± 0.1	9.3 ± 1.0
		Sucrose (control)	21.9 ± 0.2	1.7 ± 0.1
II	20	Potato starch, raw (laboratory preparation)	16.5 ± 1.6	
		Potato starch, ground	23.0 —	
		Wheat starch (laboratory preparation)	28.6 ± 0.6	
		Corn starch (laboratory preparation)	29.8 ± 0.6	
		Sucrose (control)*	32.3 ± 0.5	

* *Much higher weight gain was due to higher percentage of casein in the diet.*

The data in Experiment I B* are presented for comparison with the present results. The average rate of growth of rats fed rations containing autoclaved potato starch or dextrin as the source of carbohydrate was approximately the same as that of rats fed rations with sucrose as the carbohydrate. Apparently, potato starch which was modified by autoclaving or dry heating was rendered susceptible to enzymatic attack in the gastrointestinal tract of the rat.

In Experiment II, the laboratory preparations of corn starch and wheat starch each supported a rate of growth which was only slightly lower than that obtained with sucrose. Two rats, when fed the 20% casein ration with ground potato starch as the source of carbohydrate, grew at an average rate of 23.0 gm. per week. It would appear that raw wheat and corn starches are well utilized, and that the grinding of the potato starch improved its utilization by the rat. Langworthy and Deuel (10) reported that raw corn and wheat starch were completely utilized by human subjects. Normally wheat and corn starches are subjected to some chemical treatment during their manufacture. Apparently there is no difference in the utilization of the raw starches as compared with the commercially prepared ones.

Meteorism (an extension of the abdomen by gas), which occurs with the ingestion of unmodified potato starch, was exhibited by rats fed the laboratory preparation of potato starch. A comparison of the gastrointestinal tracts of the rats fed the different starches is shown in Fig. 1.

* *Harper, Kalayana, and Jelinek. Unpublished data, 1951.*

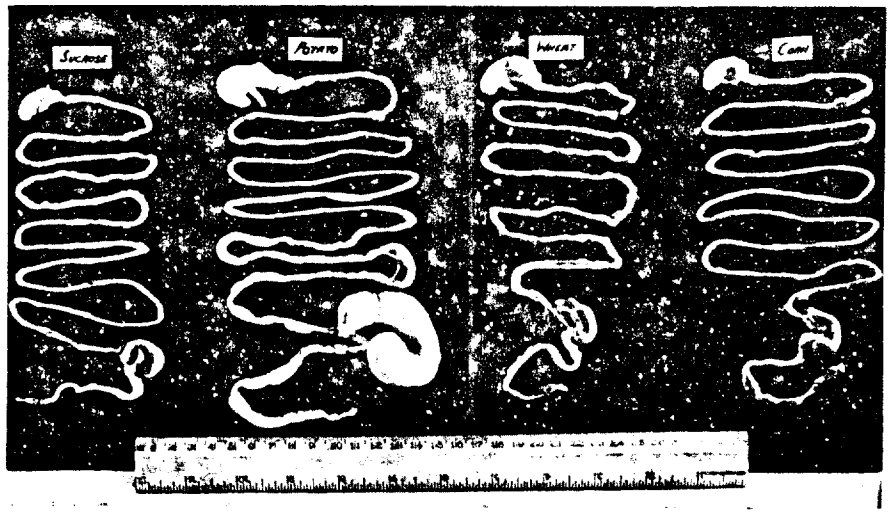

FIG. 1. Comparison of the gastrointestinal tracts of rats fed starches prepared in the laboratory with that of a sucrose-fed control.

The ingestion of unmodified potato starch caused a distention of the caecum and intestines. The main causative factors for this enlargement were thought to be the accumulation of food residue in the caecum and the accumulation of gases from fermentation of the food residue. (The food residue present in the caecum gave a blue coloration with iodine, when raw potato starch was used as the source of carbohydrate in the diet, thus indicating the presence of amylose.) It was found that the average dry weight of the complete gastrointestinal tract of five rats fed on 18% casein ration with raw potato starch as the source of carbohydrate was 9.9 gm., whereas the corresponding average weight when rats were fed a ration with sucrose as the source of carbohydrate was 3.5 gm. This difference in the weight of the gastrointestinal tracts was probably caused by a greater accumulation of food residue in the caeca, when raw potato starch was ingested. Langworthy and Deuel (10), working with human subjects, found that the ingestion of potato starch caused excessive formation of gas and frequent intestinal cramps. Beazell, Schmidt, and Ivy (1) later confirmed these findings and further suggested that bacterial fermentation may account for an important part of the breakdown of unmodified potato starch which occurs during passage through the alimentary tract.

The extent of hydrolysis by pancreatin, of unmodified starches prepared from potato, wheat, and corn, as well as of potato starch modified by mechanical grinding is illustrated in Table II.

Ground potato starch was hydrolyzed very rapidly whereas the hydrolysis of unmodified potato starch was very slight. Pronounced hydrolysis occurred with both corn starch and wheat starch. Considerable growth of microorganisms was noticed in the corn starch suspensions after 20 hr. of incubation and, since reducing sugars had been consumed, the extent of hydrolysis of the corn starch could not be determined at this point. Sherman, Walker, and Caldwell (14) reported that boiled potato starch showed a rate of enzymatic hydrolysis equal to, or slightly greater than, that of the cereal starches. Beazell *et al.* (1) found that the quantity of reducing sugar obtained from raw potato starch by pancreatin never exceeded 7.6% of that obtained from the digestion of boiled starch. Since lengthening the incubation period to 20 hr. did not increase the

TABLE II

THE RATE OF HYDROLYSIS OF STARCHES BY ALPHA-AMYLASE (PANCREATIN)

The reducing sugar was calculated as maltose

Starch	%, Hydrolysis after:			
	0 hr.	1 hr.	3 hr.	20 hr.
Potato, raw	0	0.3	1.0	1.9
Potato, ground	0	45.1	53.5	58.3
Wheat	1.5	5.9	16.9	32.5
Corn	2.0	5.2	12.6	Fermentation

quantity of raw potato starch digested, they suggested that as a result of mechanical rupture of the envelope of the starch granule during milling, a constant fraction of the raw starch may have been more easily digested than the remainder. In view of the results obtained by these investigators and the observations made during this study, it is likely that unmodified potato starch is appreciably digested by alpha-amylase only when the outermost layer or layers of the starch granule are ruptured.

Since this work has been completed a publication by Booher, Behan, and McMeans (2) on biological utilization of unmodified and modified food starches has appeared. These authors presented information regarding the utilization of a wide variety of starches by the rat. They suggested that the relatively low digestibilities of such unmodified starches as those from the potato, arrowroot, and sago palm are entirely attributable to the properties of the outer layers of the granules. They suggest that a firmer degree of hydrogen-bonding of the molecules in the outer layers may occur in these starches than occurs in starches from cereal grains and cassava root.

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DIGESTION OF STARCH *IN VIVO* AND *IN VITRO* IN A RAT INTESTINE

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SUMMARY

We have confirmed UGOLEV's hypothesis that starch hydrolysis in the gut is not merely an intraluminal process but the most important hydrolytic activity occurs on the mucosa itself.

Pancreatic amylase (α-1,4 glucan 4-glucanohydrolase, EC 3.2.1.1) was selectively adsorbed on the intestinal mucosa. The so-called "membrane digestion" is an important contribution for a new concept of the digestion mechanism.

Using perfusion *in vivo* of starch solutions in a rat gut, the experiments suggest some typical characteristics of this type of digestion.

1. The adsorption of amylase took place in the proximal parts of the gut, and seemed to decrease in areas where the villi were shorter.

2. This surface phenomenon was reversible, as a prolonged perfusion liberated the bound amylase from the intestinal mucosa, whereas after a short rest-time a partial regeneration of membrane digestion occurred.

INTRODUCTION

The mechanism of starch hydrolysis in the gut is still not clear. Using incubation *in vivo* techniques on human beings and on animals, BORGSTRÖM AND DAHLQVIST[1,2] suggested that the amylase (α-1,4 glucan 4-glucanohydrolase, EC 3.2.1.1) activity in the intestinal contents was high enough to hydrolyse the starch in a few minutes. They are convinced that the hydrolysis of starch to disaccharides and oligosaccharides, in contrast with the hydrolysis of disaccharides, occurs entirely in the intestinal lumen.

Recently another type of digestion, which occurs on the intestinal mucosa, was demonstrated, and the importance of the "membrane digestion" was shown[3]. The so-called membrane- or contact-digestion takes place in the pores of the striated border under the influence of enzymes fixed on the external surfaces of the cellular membranes. The final stages of hydrolysis occur mainly in the pores of the striated border. UGOLEV[4] also showed that the membrane-digestion markedly stimulates the

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initial stages of hydrolysis in the lumen of the small intestine[4]. This mechanism seems to be a general one: it has been found in several species of animals[5].

We have investigated the relative significance of luminal and membrane digestion in starch hydrolysis experimentally.

The rate of starch hydrolysis was studied in different parts of the small intestine of white rats (directly in the intestine and under the effect of the amylase liberated into the perfusate). This enabled us to determine: a, the intensity of the processes taking place in the lumen of the small intestine (the so-called technique *in vitro*) and b, a total hydrolysis in the intestine caused by the enzymes acting in the gut lumen and on its surface (the so-called technique *in vivo*).

METHODS

In vivo

A buffered starch solution in Ringer pH 7.0 containing 0.075, 0.375 or 0.75 % of soluble starch was perfused through the lumen of an isolated part of the gut of a nembutal-anaesthetized white rat. The perfusion rate was kept constant at 1 ml in 5 to 10 sec. At various times, 1 or 2 ml of the perfusate was quantitatively collected in a cooled (0°) graduated test-tube. After stopping the reaction with 1 ml 1 N HCl, the rate of hydrolysis was determined in 1 ml perfusate from the difference in absorbance of a starch–iodine colouration between inlet and outlet of the perfusion system (modified SMITH–ROY reaction).

The digestion in % per ml and per min was estimated by the following equation:

$$\text{Digestion } \%/\text{ml/min} = 100\left(1 - \frac{C_{\text{starch out}}}{C_{\text{starch in}}}\right) \cdot \frac{\text{perfusion rate/min}}{\text{volume perfused gut}}$$

In vitro

At the same time 1 or 2 ml of the perfusate were taken for the determination of the hydrolysis rate *in vitro*, which is directly correlated with the enzyme concentration in the perfusate. After incubation at 38° until hydrolysis of the starch content was complete (negative iodine staining), 0.5 ml of the perfusate was incubated during 5 or 10 min at the same temperature as in the rat gut with 0.5 ml of a starch solution (in the same final concentration as *in vivo*). The reaction was stopped by addition of 1 ml 1 N HCl and the same colour reaction was carried out as mentioned above. We have shown elsewhere[6] that repeated incubation of the perfusate did not affect the level of its amylolitic activity.

RESULTS AND DISCUSSION

Our results, summarized in Table I, led us beyond the original purpose of the research.

It has been demonstrated that membrane digestion *in situ* is more important than luminal digestion, some characteristics of the former being as follows.

The distribution of membrane digestion capacity seems to be unequal in different part of the gut; the membrane digestion deteriorates rapidly whereas the luminal digestion seems to be more constant; after a short rest-time a regeneration of the membrane digestion occurs.

UGOLEV's suggestion[4] about the important role of the membrane digestion of starch in the intestinal tract has been confirmed by these experiments. As seen from

TABLE I

Expt. No.	Rat No.	Piece of gut	Starch concentration (%)	Vol. perfused intestine	Perfusion rate / Vol. intestine	Digestion: In vivo (mg starch/ml/min)	Digestion: In vitro (mg starch/ml/min)	In vivo / In vitro
1	6	Duoden.	0.75	2.5	4.16	22.8	5.8	3.9
		Duoden.	0.75	2.5	4.40	22.5	5.8	3.8
					3.76	10.5	3.7	3.5
2	13	Duoden.	0.375	3.3	2.1	6.2	4.4	1.4
		Duoden.	0.375	3.3	2.3	5.6	4.35	1.3
3	1	25 cm d–j	0.375	3.3	3.02	12.5	1.46	8.6
			0.375	3.3	3.10	7.25	1.47	4.9
4	2	25 cm d–j	0.375	3.3	1.4	3.20	0.26	12.4
		25 cm d–j	0.375	3.3	1.5	4.42	0.81	5.5
5	3	25 cm d–j	0.375	2.4	3.34	12.2	5.65	2.18
6	7	25 cm d–j	0.75	5.2	1.54	2.82	1.2	2.32
7	14	25 cm d–j	0.375	2.7	1.84	3.25	0.97	3.35
					1.56	2.40	0.97	2.48
8	15	25 cm d–j	0.375	5.3	1.2	0.71	0.15	4.85
					0.83	1.38	0.66	2.30
9	18	25 cm d–j	0.375	3.7	1.05	2.06	1.00	2.06
					1.60	2.92	1.20	2.45
10	5	Jej–Il	0.075	9.1	2.02	0.79	0.33	2.36
11	10	Proximal jej	0.075	2.5	1.4	0.58	0.14	4.15
					1.4	0.52	0.12	4.32
		Distal jej	0.075	2.5	0.9	0.23	0.068	3.4
					0.7	0.10	0.045	2.22
12	11	Proximal jej	0.075	1.4	2.0	1.20	0.24	5.0

Abbreviations: Duoden, duodenum; d–j, duodenum–jejunum; Jej–Il, jejunum–ileum.

Table I, the hydrolysis of starch in every part of the gut was significantly more important than the hydrolysis effected by the free amylase in the intestinal cavity.

In his investigation on digestion and absorption of carbohydrates in the lumen of the gut, DARLQVIST[2] suggested that the amylase activity in the intestinal content was sufficiently high to hydrolyse the starch completely. Using his investigation techniques, he could hardly draw a conclusion about the real nature of the hydrolysing mechanism to which starch is submitted. His methods, using only a partial collection of the intestinal juices by intubation and collecting these for rather long periods (30 and 60 min), will increase the possibility of a higher digestion of the perfusate (*in vitro*) than in the intestine (*in vivo*).

The technique used by UGOLEV[4] makes it possible to collect the complete perfusate at the same rate as the starch inlet. Even at higher amylase concentrations, the hydrolysis *in vitro* was completely stopped by cooling the rather small fractions. This inhibition technique has been confirmed by running parallel tests using HCl as inhibitor of the amylase activity. Both series of experiments have given identical results.

A linear relationship has been found between the ratio *in vivo*/*in vitro* in function of the perfusion rate in ml/min for a given volume of a gut piece. Using a proportional pump (Technicon), with calibrated tubings, giving a rate of 1.2 to 8 ml/min in the same piece of the intestine, we have seen that the ratio *in vivo*/*in vitro* is a linear function of the perfusion rate for a given gut volume (Fig. 1).

The results proved that the role of the membrane digestion increases with increase

in the rate of perfusion. This phenomenon is accounted for by hydrodynamic characteristics of both fast and slow flows of liquid. As is well known, the turbulence of a liquid increases with flow rate, thus contributing to a better contact of the dissolved substrate with the intestinal epithelium surface. In natural conditions the contact of the chyme with the striated border is ensured by the motility of the small intestine.

These experiments have enabled us to show the difference between DAHLQVIST's[3] and UGOLEV's[4] observations. Experimental data of BORGSTRÖM AND DAHLQVIST[1] correspond to the left part of our curve (Fig. 1); it is evident that under their experimental conditions the role of membrane digestion could not be ascertained.

Other characteristics of the membrane digestion were observed in our experiments.

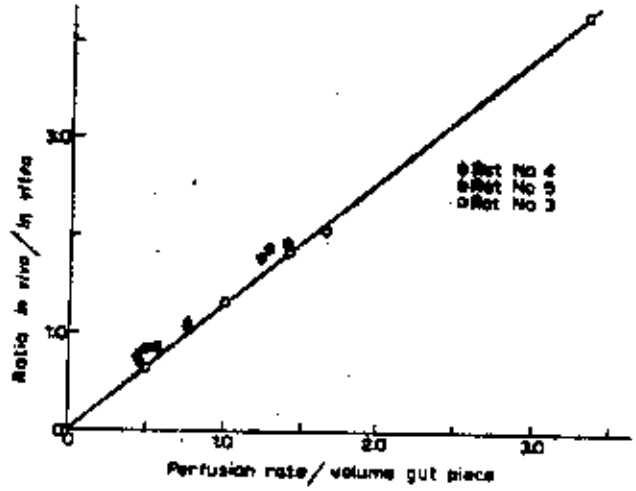

Fig. 1. Linear relation digestion *in vivo/in vitro* in function of the perfusion rate

1. *The distribution of membrane digestion capacity in the gut*

In these experiments the duodenum was perfused, the pancreatic juice continuing to pour into the small intestine. As may be seen from Fig. 2 and Table I, the amylase activity of the intestinal wall seems to be unequally distributed in different parts of the gut. Membrane digestion is more important in the proximal parts of the gut (jejunum) than in the distal parts (distal jejunum and ileum). The distribution gradient corresponds to the distribution gradient observed for saccharase[4] and seems to decrease in areas where the villi are shorter. This difference in the amylase adsorption capacity of the gut ought to be investigated to determine the nature of this surface phenomenon.

2. *The adsorption lability of amylase*

The adsorption lability is presented in Fig. 3. In the same rat a decrease of the membrane digestion as a function of the perfusion time was noticed both in the duodenum and in the perfused jejunum-ileum part of the gut, whereas no significant diminution of the activity *in vitro* appeared. It seems as if prolonged perfusion liberates the bound amylase from the intestinal mucosa; during that time the amylase

concentration in the intraluminal cavity seems to be constant. It is interesting to notice that invertase is structurally bound in the intestinal mucosa, as it is not liberated during the perfusion.

3. Regeneration of membrane activity

It was observed that after a short rest-time, varying between 10 and 30 min, a partial regeneration of the membrane digestion occurred (Fig. 4) without changing the amylase content in the collected juices. This phenomenon could be followed in prolonged experiments even after 2.5 h rest time. An experiment with 5 periods of

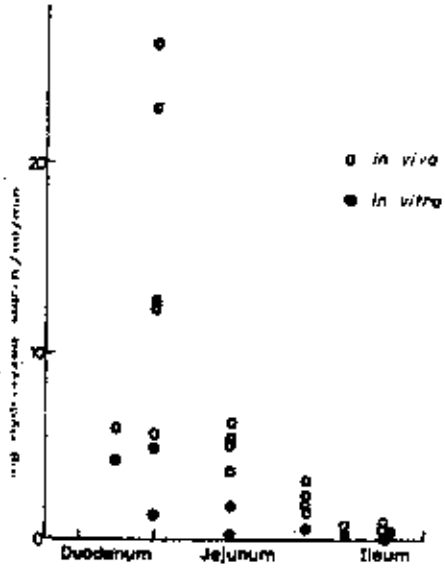

Fig. 2. Probable distribution of the membrane digestion in the intestine of a rat.

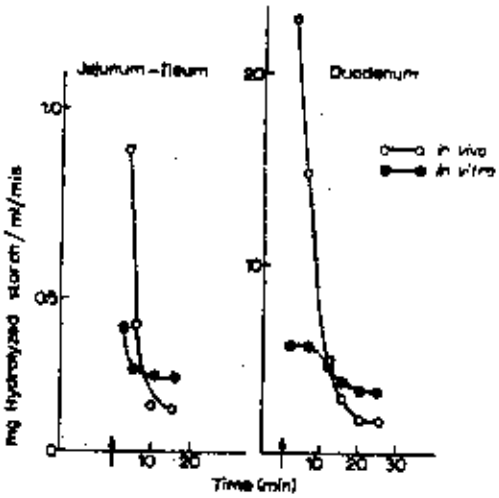

Fig. 3. The adsorption lability of amylase in the striated border as a function of the perfusion rate.

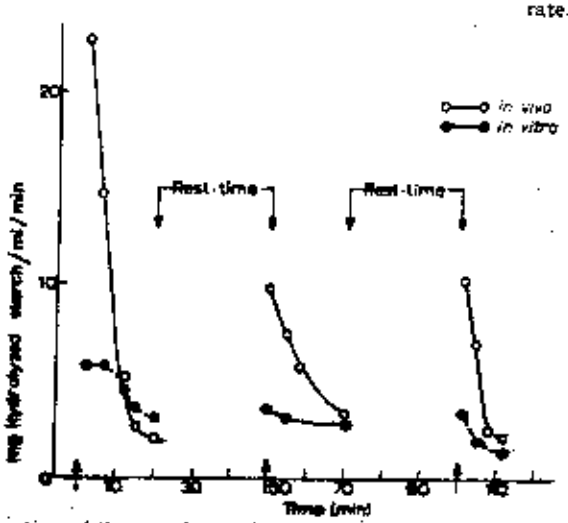

Fig. 4. Regeneration of the membrane digestion after an interruption of the perfusion.

perfusion followed by rest-times showed the same picture. This means that the adsorption of amylase was completely reversible. We did not succeed in binding any bacterial glucamylase (Ned. Gist en Spiritus Fabriek, Delft, Holland) to the mucosa of a rat.

These experiments have shown that there are no contradictions between the results obtained by DAHLQVIST AND UGOLEV[2], and that the discovery of membrane digestion is an important contribution towards a new concept of the digestion mechanisms.

ACKNOWLEDGEMENTS

This work was done in Koltouchi-Pavlovo and in Gent, with a grant from the Foundation for Belgian-Soviet cultural agreement. Part of the experiments were conducted with the collaboration of D.L. during his sojourn in Koltouchi. The rest of the experiments were carried out independently in Gent and Koltouchi, in accordance with a planned programme. The results of the last group of experiments differ only in details which might be due to insignificant differences in experimental conditions, characteristics of the animals or their maintenance.

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F.A.O. Nutrition Meetings Report Series 46A:7, 60-92.

ACCEPTABLE DAILY INTAKES (ADI) FOR MAN OF MODIFIED STARCHES

Substance[a]	ADI (mg/kg body weight)
Amylose, amylopectin	No limit[2]
Enzyme-treated starches	No limit[2]
White and yellow dextrins	No limit[2]
Acid-treated starches	No limit[2]
Bleached starches	No limit[2]
Oxidized starches	No limit[1,2]
Alkali-treated starches	No limit[2]
Mono starch phosphate	No limit[2,4]
Starch acetate	0 - 12.5[1]
Starch sodium succinate	No ADI
Hydroxypropyl starch	0 - 25[1]
Distarch phosphate (phosphorous oxychloride)	No ADI
Distarch phosphate (sodium-trimetaphosphate)	0 - 35[3,4]
Phosphated distarch phosphate	0 - 35[3,4]
Acetylated distarch phosphate	No ADI
Hydroxypropyl distarch phosphate	No ADI
Distarch glycerol	No ADI
Acetylated distarch glycerol	No ADI
Hydroxypropyl distarch glycerol	0 - 12.5[1]
Acetylated distarch adipate	0 - 12.5[1]

[a] Specifications will be published for these compounds (see Annex 1, Ref. 18) except for the first three items. The specifications are tentative for those items which have not been given an ADI.

[1] Temporary acceptable daily intake - details on further information required are given in the respective monographs.

[2] Not limited except by good manufacturing practice.

[3] As distarch phosphate (sodium-trimetaphosphate) or phosphated distarch phosphate or the sum of both.

[4] Subject to limits of phosphorous load (see Annex 1, Ref. 7, p. 88).

Note: No monographs were prepared for certain other modified starches (see Annex 1, Ref. 18, section 3.2.1).

MODIFIED STARCHES

AMYLOSE AND AMYLOPECTIN

Biological Data

Normal native starches consist of a mixture of 15-30 per cent. amylose and 70-85 per cent. amylopectin. Amylose structurally is a linear polymer of anhydroglucose units, of molecular weight approximately between 40 000 and 340 000, the chains containing 250 to 2000 anhydroglucose units. Amylopectin is considered to be composed of anhydroglucose chains with many branch points; the molecular weight may reach as high as 80 000 000.

One hundred per cent. amylopectin or "waxy" starches are commercially available having been extracted from special mutants of the parent plant e.g. waxy corn or waxy rice. Starches with a very high amylose content have also been produced, but on a limited scale for special application. The granules of native starches are extracted from the plant source by purely physical means, washed and dried. Even in this way a certain small amount of modification takes place so that the raw starch is strictly speaking not the same as it existed in the plant. The changes which have taken place are, however, only of academic interest. From the nutritional point of view it is important to realize that the human gastro-intestinal tract digests very efficiently raw cereal and root starches such as wheat, maize, rice and cassava but is not able to digest easily raw potato, arrowroot and canna starches (Becher et al., 1951).

Digestibility of starches may be assessed by studying the action of pancreatin or other sources of amylase (Leach & Schoch, 1961) (Sandstedt et al., 1962) or of taka-diastase (Kihara & Kawase, 1949) in vitro on the raw granular starch, or by feeding the raw starch to animals such as rats and chicks. But from the standpoint of use in the human diet it is the gelatinized (cooked) form that requires evaluation. From the work of Sandstedt et al. (1962) and Borchers (1963) it is clear that both in vitro studies and animal feeding establish the resistant character of some high amylose corn starches to amylolytic attack.

Nevertheless it does not appear likely that in practice high amylose corn starches in gelatinized form will present any problem of digestibility to man, who has no difficulty with gelatinized potato starch. The digestibility of raw granular potato starch in vitro was 18.98 per cent. under conditions where corn starch yielded a value of 45.64 per cent. (Kihara & Kawase, 1949). In the studies by Sandstedt et al. (1962) the susceptibility of potato starch in vitro to pancreatic digestion was far lower than that of the most resistant high-amylose corn starch. Although in vivo digestibility invariably exceeds the in vitro value, it is unlikely that potato starch would prove more digestible than high-amylose corn starch, either raw or gelatinized.

Comments

These native starches should be regarded as food rather than food additives.

EVALUATION

Not limited except for good manufacturing practice.

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ENZYME-TREATED STARCHES

Enzyme modification results in the production of dextrose and glucose syrups and also of intermediate products such as maltodextrins and gums. Toxicological testing is not required.

Comments

This treatment results in the production of normal food constituents. Specification of the final product should make it possible to ensure freedom from any undesirable microbiological contaminants or products and the enzymes must be derived from acceptable sources.

EVALUATION

Not limited except for good manufacturing practice.

WHITE AND YELLOW DEXTRINS

Biological Data

White dextrins are prepared by heating dry starch in the presence of an acid at a temperature generally below 130°C. White dextrins may also be obtained by further continuing the acid process for making thin boiling starches. Because of the nature of the application as well as their flavour their use in food is restricted. Dextrins are a stage in the normal digestion of starch occurring in the human gastro-intestinal tract. They represent a broad range of products with considerably smaller molecular size than native starch.

Yellow dextrins are prepared in a similar manner but at a higher temperature and using less acid. Apart from depolymerization, a good deal of internal re-arrangement occurs with formation of highly branched molecules. These materials are used in foods in limited quantities as adjuvants in flavour encapsulation and similar minor uses.

Comments

These substances are regarded as intermediates of normal digestion of starch and are normal constituents of food.

EVALUATION

Not limited except for good manufacturing practice.

ACID-TREATED STARCHES

Biological Data

These modified starches represent an intermediate stage in the normal enzymatic digestion of food starch in the human body. This process also occurs in the human stomach and results in hydrolysis of some of the linkages between adjacent anhydroglucose units with a reduction of the mean molecular weight of these starch molecules. In the commercial process hydrochloric, sulfuric or phosphoric acid is used and the excess is neutralized with sodium hydroxide or sodium carbonate.

Subsequent treatment of the modified starch ensures that only traces of either sodium chloride sodium sulfate or sodium hydrogen phosphate remain behind.

Comments

These starches should be regarded as normal constituents of food.

EVALUATION

Not limited except for good manufacturing practice.

BLEACHED STARCHES

Biological Data

Bleaching by very light oxidation is carried out using sodium hypochlorite, sodium chlorite, hydrogen peroxide, potassium permanganate, peracetic acid, or ammonium persulfate with sulfur dioxide. Interaction with the starch molecules must be very small since no change occurs in the physical properties of the starch or its solution except in its colour. Theoretically, there will be production of a few aldehyde or carbonyl groups. Only trace amounts of sodium chloride, sodium sulfate or sodium acetate remain in the final product. If potassium permanganate is used, there may be residues of manganese sulfate up to 50 ppm calculated as Mn.

Comments

This treatment involves no more, or very little more, oxidation than might occur in food or in the gut as evidenced by the absence of viscosity changes. Since starches of this kind are unlikely to constitute more than 10 per cent. of a finished consumer product, the resulting residue of manganese dioxide will be insignificant.

EVALUATION

Not limited except for good manufacturing practice.

OXIDIZED STARCHES

Biological Data

Whatever oxidizing agent is used only minor modifications of the starch molecule are achieved in normal manufacturing practice. These are equivalent to the introduction of 1 per cent. w/w of carboxyl (-COOH) or 0.5 per cent. w/w of beta (-CO) groups or, 3.6 carboxyl and 3.9 carbonyl groups per 100 glucopyranose units. Some depolymerization takes place by rupture of the bonds between glucose units. No chlorine is introduced into the molecule and the final products usually contain only residues of sodium chloride, sodium sulfate and sulfur dioxide.

Oxidation with hypochlorite

Biochemical aspects

Early comparative experiments pointed to inhibition of amylolysis (Tremolières et al., 1958). *In vitro* digestibility by pancreatin or saliva was used to compare lightly and highly oxidized corn starch with unmodified corn starch and a reference starch. Maltose production after a fixed interval of enzyme action was taken as a measure of digestibility. The oxidized starch was 10-15 per cent. less digestible by pancreatin than unmodified starch but there was no obvious difference as regards salivary digestion (Shuman, A. C. & Morta, E. T., 1959). The digestibility of oxidized wheat starch (conditions not stated) was examined in rats by matched-feeding technique using the modified starch as the sole source of carbohydrate at a level of [illegible] per cent. (dry basis) of the diet. The degree of assimilation by and the general effects on groups of 6 rats over a feeding period of [illegible] days were [illegible] from consideration of body weight changes, faecal residues, digestibility coefficients for starch and post-mortem appearance of the animals and their gastro-intestinal tracts. The digestibility coefficients were calculated from the starch content of ingested food and residues found in faeces and post-mortem gastrointestinal contents. Body weight gain and digestibility coefficients were practically indistinguishable from those obtained for wheat starch or corn starch. Nothing abnormal was noted on post-mortem examination (Bowker et al., 1951).

Other studies, in 3 groups of 2 rats each, used corn starch oxidized with 3.9 per cent., 4.5 per cent., or 5.5 per cent. hypochlorite calculated as chlorine. This corresponds to the introduction of 0.57 per cent., (2.04 COOH groups per 100 glucopyranose units), 0.8 per cent. (2.86 COOH groups per 100 glucopyranose units) and 0.9 per cent. (3.57 COOH groups per 100 glucopyranose units) carboxyl groups. To 5 g basal diet were added 1, 2 or 4 g modified or control starch and this diet was fed to rats for 10 days. Comparison of digestibilities showed an apparent decrease with increasing oxidation but no effect on caloric values. No tissue damage was associated with the diarrhoea and caecal enlargements observed in groups receiving 2 g or 4 g starch in their feed. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates (White, 1962).

The digestibility of oxidized starches at levels of 2.5 per cent., 6 per cent. and 42.2 per cent. calculated as chlorine, equivalent to a carboxyl content of 0.32 per cent. (1.15 COOH per 100 glucopyranose units), 0.8 per cent. (2.61 COOH per 100 glucopyranose units) or 5.4 (?) per cent. (5.23 COOH per 100 glucopyranose units), was studied in groups of 6 male and 6 female rats. The animals were kept for 7 days on 5 g basal diet and then given either

1 g or 2 g starch supplements for 31 days. Poor weight gain with diarrhoea were noted only with the highly oxidized material at all dietary levels. One rat from each of the high dietary level groups showed marked caecal dilation only in animals fed the heavily oxidized starch (Whistler & Belfort, 1961).

Acute toxicity

None supplied.

Short-term studies

Rat. Starch treated at a level of 0.375 per cent. chlorine was fed to weanling albino rats at 70 per cent. of their diet for 10 weeks with corn starch as control. Feeding was either unrestricted or by paired-feeding technique. No toxic effects were noted. No details of this work, carried out in 1944-5, were available (Garton Bone & Co. Ltd., 1967).

Long-term studies

None supplied.

Comments

Hypochlorite-oxidized starch has been investigated by digestibility studies *in vitro* and *in vivo*. One short-term study over 10 weeks was reported only in outline and referred to one particular commercial sample. Assimilation difficulties and delayed *in vitro* enzymic degradation occur only at high levels of oxidation together with diarrhoea and caecal dilatation. Hypochlorite oxidation affects the physical properties of the starch; provided the chemical change is limited to the introduction of no more than 1 carboxyl group per 20 anhydroglucose units and a carbonyl content which is negligible, the biological effects of the modified starch do not appear to be deleterious. Despite the paucity of animal data and lack of any information in man it would be unwarranted to exclude the use of oxidized starches (containing no more than 1 carboxyl group per 20 anhydroglucose units) as food additive pending the provision of metabolic studies preferably in man.

EVALUATION

Temporarily not limited except for good manufacturing practice.

Further work required by June 1972

Metabolic studies in several species.

REFERENCES

Becker, L. B., Rohan, I., & McNamara, E. (1951) J. Nutr., 46, 75

Garton Bone & Co. Ltd., (1967) Submission to U.K. authorities

Shuman, A. C. & Harris, E. T. (1959) Unpublished report No. 4 of Shuman Chemical Lab. Inc. to Corn Industries Research Foundation

Tremolières, J., Barnier, J. J. and Lowy, R. (1959) Nutritio et Dieta, 1, 100

Whistler, R.L., and Belfort, A. M. (1961) Science, 133, 1569

White, T. A. (1963) Cereal Science Today, 8, 48

ALKALI-TREATED STARCHES

Modification is carried out by treatment with a maximum of 1 per cent. alkali hydroxide. No chemical transformation except depolymerization seems to occur. If any amylose appears in crystalline form it may not be susceptible to enzymic degradation.

Comments

This treatment results in normal food constituents.

EVALUATION

Not limited except for good manufacturing practice.

MONO STARCH PHOSPHATE

Biochemical aspects

These starches contain probably simple phosphoric ester group, attached to some of the hydroxyl radicals of the glucopyranose units. Residual phosphate amounts to approximately 2 per cent, phosphate calculated as P. They are made by the use of alkali orthophosphates.

In vitro enzymatic hydrolysis of mono starch phosphate by wheat α-amylase was compared with unmodified wheat starch. No difference was noted in the rate of production of reducing substances. The metabolic behaviour of the phosphate radical in starch phosphate was studied *in vivo* by comparing the distribution of P^{32}, after oral administration of labelled starch phosphate to rats, with that from either labelled orthophosphate or pyrophosphate. The percentage activity excreted in urine and faeces over 48 hours as well as the percentage activity retained in liver, kidney, blood plasma and bone showed no significant difference for the 3 types of phosphate examined. The phosphate moiety of starch phosphate probably behaves metabolically like any other ionic phosphate (Laboratories of International Minerals & Chemical Co., 1958).

Acute toxicity

None available.

Short-term studies

None available.

Long-term studies

None available.

Comments

An adequate metabolic study has shown that this modification does not affect the digestibility of the starch. Whatever the manner of attachment of the phosphate groups may be, their metabolic disposition after digestion follows the same pathways as free orthophosphate or pyrophosphate. This material is not to be regarded as toxicologically different from native starch of the same botanical origin provided the phosphate content is limited.

EVALUATION

Not limited[1] except for good manufacturing practice.

REFERENCES

Laboratories of International Minerals and Chemical Corporation (1958)
Unpublished report to American Maize Products Co.

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[1] Subject to limits of phosphorous load given in 7th Report of the Joint FAO/WHO Expert Committee on Food Additives, Wld Hlth Org. techn. Rep. Ser., 1964, 281, 21

STARCH ACETATE

Biological Data

Esterification is achieved by using either acetic anhydride (up to 4 per cent.) or vinyl acetate (up to 7.5 per cent.). A maximum of 2.5 per cent. acetyle groups is introduced corresponding to a maximum degree of substitution of 0.1.

Biochemical aspects

The digestibility of acetylated starches was measured by the biochemical oxygen demand of incubated samples. As the acetyl contents increased so the BOD values decreased and, in parallel, the digestibility. Starch acetates containing 2.5 per cent. acetyl groups are only 83.7 per cent. as digestible as native starch (Turner, 1961). Digestibility by fungal amyloglucosidase was shown to be 80.5 per cent. of that of native starch (Turner, 1961). Calorie values were determined in groups of 10 male rats fed 4 weeks on a diet supplemented with graded doses of 0, 1.5 g, 3.0 g, 4.5 g and 6.0 g dextrose (equivalent to 0, 6, 12, 18 and 24 calories). The dose response curve was used to estimate the caloric value of supplements of 3 g and 4.5 g of acetylated (1.8 per cent. acetyl) or native starch. No significant difference was found between the starch samples with regard to calorie value (Geer, 1961).

Acute toxicity

None available.

Short-term studies

Rat. Groups of 10 male rats were fed for 28 days diets containing 60 per cent. of various starch acetates (the degree of acetylation varied from 0, 1.34 per cent., 2 per cent., 2.56 per cent. to 2.86 per cent.). Weight gain was reduced in groups receiving starch acetates with more than 2 per cent. acetylation but feed efficiency remained unaffected. Diarrhoea occurred at 2 per cent. and higher degrees of acetylation and there was noticeable caecal enlargement at the same levels. No tissue damage or inflammation were noted in association with the diarrhoea (Turner, 1961). In another experiment potato starch acetates (acetylated to 1.86 per cent.) were fed for 13 weeks to groups of 10 male and 10 female rats at levels of 2 per cent., 15 per cent. and 45 per cent. of the diet. The 2 per cent. level was fed for only 4 weeks. No animal died. Growth rates and haematological findings were not significantly affected. The relative weights of liver, kidney, adrenal, pituitary and thyroid showed some significant differences compared with controls, being generally lower except for male thyroids. Male caecal weights were higher than controls and distended caeca were seen at the 15 per cent. and 45 per cent. dietary levels. No other histopathological changes due to starch acetate were seen (Furen et al., 1967).

Long-term studies

None available.

Comments

Commercial acetylation processes introduce considerable modifications in the starch molecule. An increasing degree of acetylation reduces *in vitro* enzymatic digestibility and leads to diarrhoea and caecal enlargement when the

product is fed at high dietary levels. The short-term study in rats was inadequately designed. However, a no-effect level for the study can be derived. In view of the comparatively large modification in the starch metabolic it would be desirable to carry out life-span studies in one species and metabolic studies in man.

EVALUATION

Level causing no toxicological effects in the rat

Five per cent. (= 50 000 ppm) in the diet equivalent to 2500 mg/kg body weight/day

Estimate of acceptable daily intake for man

	mg/kg body weight
Temporary acceptance	0-12.5

Further work required by June 1972

Adequate 90-day studies in 2 species (one a non-rodent mammal).

REFERENCES

Feron, V. J., Til, H. P. and de Groot, A. P., (1967) Report No. [illegible] by Centraal Instituut voor voedingsonderzoek (TNO)

Oser, M., (1961), Unpublished report No. [illegible] b & c by Food and Drug Research Laboratories Inc., 20 April 1961. Submitted by National Starch and Chem. Corp.

Turner, A. W., (1961) Unpublished report to Avebe, 17 October 1961. Submitted by Avebe, Aardappelmeel de Mate.

STARCH SODIUM SUCCINATE

Modification is performed by 4 per cent. succinic anhydride to form the succinic acid ester. The extent of esterification is not known. The material used in the tests described below was prepared in the laboratory, where a high reaction efficiency (80 per cent., or 3.2 per cent. of succinic anhydride reacted, a degree of substitution of about 0.03) may have been attained.

Biological Data

Biochemical aspects

No enzymatic hydrolysis data about *in vitro* behaviour are available. Caloric value was determined in groups of 10 male rats fed for 4 weeks a basal diet supplemented with either starch, starch succinate or sucrose, the last in graded amounts. All rats were normally active and remained healthy. The caloric equivalent of the starch ester, as determined from comparison with the dose response curve for sucrose at 0, 0.75 g, 1.5 g, 3.0 g and 4.5 g (equivalent to 0, 3, 6, 12 and 18 calories per day), was identical with that of native starch (Carson, 1960).

Acute toxicity

None available.

Short-term studies

Rat. Groups of 3 male and 3 female rats were fed for 10 weeks on diets containing 70 per cent. of starch or starch sodium succinate as their sole source of carbohydrate. Groups were fed *ad lib* and also *pair-fed*. No significant differences were observed in growth rates, food efficiency or haemoglobin levels between the various groups. No histopathological examinations were reported (Chow, 1965).

Long-term studies

None available.

Comments

The biological data for this starch ester are inadequate. Adequate metabolic studies preferably in man are desirable and 90-day studies in at least two species (one a non-rodent species) on material of known degree of substitution are required.

EVALUATION

Not possible on the data available.

REFERENCES

Carson, S., (1960) Unpublished report by Food and Drug Research Laboratories Inc., No. [illegible], 1 December 1960.

Chow, B. L., (1965) Unpublished report by Food Research Laboratories Inc., No. [illegible], 18 February 1965.

HYDROXYPROPYL STARCH

Modification is carried out by propylene oxide at levels up to 25 per cent. and the resultant starch is usually lightly oxidised, bleached or acid modified after etherification. Substitution may amount to a maximum of 4 ether linkages per 10 glucopyranose units if 25 per cent. propylene oxide is used and 4-8 ether linkages per 100 glucopyranose units if 5 per cent. propylene oxide is used.

Biological Data

Biochemical aspects

In vitro digestibility by pancreatin was estimated by comparing the amount of reducing material liberated with that formed from native wheat starch. No significant difference could be detected between low (1 in 10) and high (4 in 10) substituted starches compared with unmodified starch (Kay & Calandra 1962). Calorie value could not be estimated because of diarrhoea.

Acute toxicity

Animal	Route	LD_{50} mg/kg body weight	Reference
Rat	oral	≥ 10 000	Hercules Powder Co., 1958a

Application of powder or solutions produced mild irritation in rabbits' eyes (Pailotta, 1959). The Schwartz Prophetic Patch Test on 210 human subjects using powdered high and low modified starch, as well as native starch as control, showed no difference after 72 hours initial exposure and no evidence of sensitization on 72 hour challenge after 2 weeks (Majors & Ruben Koenig, 1958). The Repeat Insult Patch Test in 23 human subjects showed no irritation after 8 twenty-four hour exposures and no evidence of sensitization on 24 hour challenge after 2 weeks (Ruben Koenig, 1959).

Short-term studies

Rat. Groups of 10 male and 10 female rats were fed for 90 days diets containing 0, 2 per cent., 5 per cent., 10 per cent. and 25 per cent. of highly modified starch (25% propylene oxide) and 25 per cent. unmodified wheat starch. No systemic toxicity was noted. There were no adverse effects regarding mortality, urinalysis or haematology at any level. There was slight reduction in growth rate at the highest dietary level with lower food utilisation and without an equivalent increase in food consumption. Mild diarrhoea occurred at 25 per cent. dietary level. No adverse effects occurred at any other level. At autopsy there were no significant differences in the organ weights of liver, kidney, spleen, gonad, heart or brain. The observed increased ratios at 25 per cent. dietary level for liver/body weight and kidney/body weight were due to the relatively lower body weight. Gross and histological examination of all major tissues revealed no abnormalities due to the feeding of highly modified starch (Kay & Calandra, 1961). In another experiment groups of 10 male and 10 female rats were fed for 90 days on diets containing 0, 5 per cent., 15 per cent. and 45 per cent. of low modified starch (5 per cent. propylene oxide). Haematological findings at 12 weeks were comparable for all groups. Body weights did not differ significantly from controls but were consistently lower in male rats only. Food efficiency was similar in all groups. Caecal

enlargement was seen at the 15 per cent. and 45 per cent. dietary levels. Diarrhoea occurred at the 45 per cent. level and very slightly at the 15 per cent. level. No histological abnormalities were detected in any major organs, which were due to the test substance. The enlarged caecum showed no evidence of inflammation or changes in the muscular coat (Feron et al., 1967).

Long-term studies

None available.

SUPPLEMENTARY INFORMATION

Biological data on Propylene Chlorohydrin

Biochemical aspects

Wesley et al. (1965) identified propylene chlorohydrin as the residue formed in foods fumigated with propylene oxide. The chlorohydrin is formed by the reaction of the epoxide with the chloride of food. Previously, it had been believed that propylene glycol, formed by reaction with water in the food, was the residue. Propylene chlorohydrin is also formed in starches modified by hydroxypropylation. Both propylene chlorohydrin isomers have been identified in fumigated foods (Ragelis et al. 1966).

When volatilization was precluded, a combination of high temperature and prolonged time in cooking did not appreciably alter the propylene chlorohydrin content of food, but when volatilization was possible, the chlorohydrin content was reduced 80 per cent. by cooking (Wesley et al. 1965). When propylene chlorohydrin was added to a standard ground laboratory rat diet, 30 minutes of mixing in an open mixer at room temperature resulted in a 45 per cent. decrease in the propylene chlorohydrin content (USFDA, 1966).

Acute toxicity

Animal	Route	LD_{50} (mg/kg body weight)	Reference
Rat	oral	216	USFDA, 1969
Dog	oral	150 mg/kg - no deaths 300 mg/kg - 1/7 deaths 250, 300 mg/kg - 4/8 deaths	USFDA, 1969

Short-term toxicity

Rat. Groups of 10 male and 10 female five-week-old rats were fed, for 28 weeks, diets to which propylene chlorohydrin had been added.

The planned dietary levels were 0, 1000, 2500, 5000, and 10 000 ppm, but analysis of the 10 000 ppm diet after mixing in the test compound (open mixer, 30 minute mixing time, room temperature) showed an actual concentration of 3500 ppm or 35 per cent. of the planned level. The 2-chloro isomer constituted 37 per cent. of the total found. The actual level in this diet, after seven days exposure to laboratory conditions, was reduced to 838 ppm, with 38 per cent. of the 2-chloro isomer, or less than 10 per cent. of the planned concentration. Weight gain in both sexes on the 5000 and 10 000 ppm levels was depressed. The depression was slight in the males on the 5000 ppm level and both groups of females and moderate in the males on the 10 000 ppm level. Food consumption was slightly decreased in these groups but food efficiency was normal. The average liver and kidney weights of the males and the liver weight of the females on the 10 000 ppm level were decreased but the organ weight/body weight ratios were normal. The decreased spleen weights and spleen/body weight ratios in the males and other minor organ weight variations appeared to be unrelated to the treatment. No effects on haematological values, mortality, or gross or microscopic lesions in the tissues were observed (USFDA, 1969).

Propylene chlorohydrin was administered to groups of 10 male and 10 female eight-week old rats by stomach tube in doses of 0, 25, 50, 75, and 100 mg/kg/day for 22 weeks. The dose for the high level was increased from 100 mg/kg to 150 mg/kg in the eleventh week, to 200 mg/kg in the fourteenth week, and to 250 mg/kg in the sixteenth week. Doses of 200 mg/kg and less did not increase mortality. All the rats on the high level were dead by the nineteenth week with all but one of the deaths occurring between the sixteenth and nineteenth weeks after the dose had been increased to 250 mg/kg. On the high level, weight gain was moderately depressed in the males and slightly depressed in the females while the dose was 100 or 150 mg/kg. Both sexes lost weight when the dose was increased to 200 mg/kg. Weight gain was slightly, but not significantly, decreased in both sexes on the 75 mg/kg level. Food consumption was slightly decreased in the males of the high level while the dose was 200 mg/kg and decreased to a greater extent when the dose was raised. The females on the high level also showed a slight decrease in food consumption when the dose was increased. With the rats losing weight when the dose was increased to 200 mg/kg, the food efficiency values have no meaning. The liver weight/body weight ratios of both males and females on the 75 mg/kg dose and the liver weight and liver weight/body weight ratio of the males on the 25 mg/kg dose were increased, but this increase was not accompanied by gross or microscopic alterations in the liver. Other organ weight and organ weight/body weight ratio changes did not appear to be related to the treatment. No haematological effects or gross or microscopic effects on the tissues of the treated rats, at a dose of 75 mg/kg or less, were seen. The tissues of the high level rats were not examined microscopically (USFDA, 1959).

Comments

Although in vitro enzymatic digestion tests revealed no abnormalities there was some evidence that a high degree of substitution rendered the product less digestible at the 20 per cent. dietary level. The 90-day tests are adequate and point to 10 per cent. in the diet as the no-effect level. Information is desirable on the metabolic behaviour preferably in man - of the moiety containing the propylene glycol ether.

EVALUATION

Level causing no toxicological effect in the rat

Ten per cent. (@ 100 000 ppm) in the diet equivalent to 5000 mg/kg body weight/day.

Estimate of acceptable daily intake for man

Temporary acceptance	mg/kg body weight 0 - 25

Further work required by June 1973

Ninety-day tests in a non-rodent mammalian species using material modified to the highest degree employed as food additive.

REFERENCES

Feron, V. J., Til, H. P. & de Groot, A. P. (1967) Unpublished report by Centraal Instituut voor Voedings onderzoek TNO, No. [illegible]

Kay, J. H. & Calandra, J. C. (1961) Unpublished report by Industrial Bio-Test Laboratories, Inc. 17th November 1961

Kay, J. H. & Calandra, J. C. (1962) Unpublished report by Industrial Bio-Test Laboratories, Inc. 22nd January 1962

Majors, P. A. & Rubenkoenig, H. L. (1959) Unpublished report by Hill Top Research Institute, Inc. 18th May 1959

Pelletts, A. J. (1959) Unpublished report by Hazleton Laboratories, Inc. 22nd May 1959

Ragelis, E. P., Fisher, B. B. & Klimeck, S. A. (1968) J.O.A.C., 48, 862

Rubenkoenig, H. L. (1959) Unpublished report by Hill Top Research Institute, Inc. 13th May 1959

United States Food and Drug Administration (1969) Unpublished data

Wesley, F., Rourke, B. & Darbishire, O. (1965) J. Fd. Sci., 30, 1067

DISTARCH PHOSPHATE

(prepared using Phosphorus Oxychloride)

Biological Data

Cross-linking of starch chains may be produced also by the use of phosphorus oxychloride. Although theoretically likely, there is no relevant chemical evidence available to show that modification by phosphorus oxychloride in fact produces distarch phosphate. The maximum number of phosphate bridges could reach one per 100 glucopyranose units.

Biochemical aspects

In vitro digestibility by pancreatin of corn or potato starch modified with 0.05 per cent. or 0.1 per cent. phosphorus oxychloride was found to be similar to the unmodified starch. (Janssen, 1968). Calorie value was determined for starch treated with 0.05 per cent. $POCl_3$ in groups of six male and six female rats receiving 85 per cent. of distarch phosphate for six weeks as sole carbohydrate source in their diet. No differences were noted between modified and unmodified starches (Oser, 1964).

Acute toxicity

None available.

Short-term studies

None available.

Long-term studies

None available.

Comments

Phosphorus oxychloride appears, at least theoretically, to produce distarch phosphates similar to those formed from trimetaphosphate. However there is no evidence that the chemically more reactive phosphorus oxychloride might not cause reactions leading to undetermined by-products. No short or long-term studies are available. The metabolic behaviour of the moiety containing the phosphate groups has not been studied. Adequate 90-day studies in at least two species (one a non-rodent mammal) and metabolic studies preferably in man are required.

EVALUATION

Not possible on the data available.

REFERENCES

Janssen, G. J. (1968) Unpublished report submitted by Association des Amidonneries de Zaire

Oser, B. L. (1964) Unpublished report No. 66180 a-1 by Food and Drug Laboratories submitted by American Maize Products Co.

DISTARCH PHOSPHATE

(prepared using sodium trimetaphosphate)

Biological Data

Native starches are known to contain phosphoric acid esters equivalent to 0.004 per cent. P and some potato starches up to 0.1 per cent. Distarch phosphate is made by the use of sodium trimetaphosphate which cross-links starch chains at an approximate rate of one phosphate link per 650 glucopyranose units. The amounts of phosphate introduced are at the most 0.04 per cent. P (Graefe, 1964).

Biochemical aspects

In vitro digestion of a distarch phosphate using trimetaphosphate by salivary, pancreatic and intestinal amylase was measured by the production rate of reducing sugar. No deleterious effect was shown on enzymic depolymerization (Beaver, 1958).

Caloric value and digestibility of a distarch phosphate using trimetaphosphate were tested in groups of 10 rats fed for seven days on 4g basal diet with either 0.0 g or 3.0 g starch supplement by observing the gain in body weight and the organ weights of liver, kidney, heart and spleen after the feeding period. No significant differences were noted between the modified and the unmodified starches (Hinson, 1960). Distarch phosphate using trimetaphosphate of undisclosed treatment was fed to groups of male and female rats on 8 g diets as 1 g or 2 g supplements over 21 days. Weight gains were comparable for modified and unmodified starches tested. Animals appeared normal at autopsy (Whistler & Belfort, 1961).

Acute toxicity

Animal	Route	LD_{50} mg/kg body weight	Reference
Mouse, female	oral	>24 000	Hodge, 1954
		>18 000	Hodge, 1958
Rat, female	oral	>20 000	Hodge, 1954
		>25 000	Hodge, 1958
Guinea-pig	oral	>8000	Hodge, 1954
		>18 000	Hodge, 1958
Rabbit	oral	> 7000	Hodge, 1954
		>10 000	Hodge, 1958
Cat	oral	> 6000	Hodge, 1954
		> 8000	Hodge, 1954

Only small numbers of animals were used but no deaths occurred from the quantities administered. Livers and kidneys of guinea-pigs, rabbits and cats showed no histological abnormalities related to the administration of the modified starch (Hodge, 1954; Hodge, 1958).

Short-term studies

None available.

Long-term studies

None available.

Comments

The toxicological information shows that cross-linking by means of trimetaphosphate does not affect the digestibility or caloric value to any significant extent. Moreover the actual extent of phosphate cross-linkage is very small. No short or long-term studies are available. The metabolic behaviour of the moieties containing the phosphate groups has not been studied. Despite the scanty available toxicological information this modified starch may be included in the evaluation of the highly modified phosphated distarch phosphates, being a preliminary stage in the manufacture of the latter. Adequate metabolic studies preferably in man, and 90-day studies in two species, one a non-rodent mammal, are required.

EVALUATION

See phosphated distarch phosphate.

REFERENCES

Graefe, G. (1964) "Die Stärke", 16, 158

Hixson, O. F. (1960) Unpublished report H-1004 by Roemer-Hixson Laboratories 3rd February 1960

Hodge, H. C. (1964) Unpublished report from University of Rochester, Division of Pharmacology & Toxicology, 5th October 1964

Hodge, H. C. (1958) Unpublished report from University of Rochester, Division of Pharmacology & Toxicology, 26th May 1958

Roemer, L. (1960) Unpublished report H-1004-1 by Roemer-Hixson Laboratories, 4th March 1960

Whistler, R. L. & Belfont, A. M. (1961) Science, 133, 1509

PHOSPHATED DISTARCH PHOSPHATE

Biological Data

These modified starches are prepared by the combined use of sodium tripolyphosphate and sodium trimetaphosphate which results in cross-linking and esterification of starch chains. The overall extent of modification is still small, the residual phosphate being of the order of 0.4 per cent. P.

Biochemical aspects

The *in vitro* digestibility of this modified starch by pancreatic amylase was somewhat reduced compared with normal unmodified starch (Kohn & Kay 1963a). *In vivo* digestibility was examined in groups of 10 male rats fed for 10 days 5 g basal diet supplemented by 1 g, 2 g, or 4 g unmodified or modified starch. Weight gains were identifical for both types tested at all three levels of supplementation. No unusual behavioural reactions were observed (Kohn & Kay 1963a).

Acute toxicity

None available.

Short-term studies

Rat. Groups of 10 male and 10 female rats were fed on a diet containing 10 per cent. rising to 35 per cent. of phosphated distarch phosphate for a total of 90 days. Female rats showed a consistent reduced weight gain throughout the test. Although four test and two control animals died during the test these incidents were regarded as unrelated to the test substance. All animals behaved normally. Haematological examination and urinalysis were normal and comparable in the various groups. The absolute liver weights of male rats were lower for the test group than for controls and the absolute kidney weight were lower for both sexes but these findings were not associated with any gross or histopathological changes. (Kohn et al., 1964).

Groups of 25 male and 25 female rats were fed diets containing 0.2 per cent., 1.0 per cent. and 5.0 per cent. modified or unmodified starch for 90 days. Eleven controls and three test animals died from intercurrent disease. There were no obvious gross or histopathological changes attributable to the test substance. Organ weights and haematological examination (days 45 and 90) were normal in both groups. Pooled urinalysis was comparable for all three groups. (Kohn et al., 1964).

Dog. Groups of three male and three female beagles were given daily for 90 days gelatine capsules containing 50, 250 and 1250 mg modified starch/kg body weight. No adverse effects were observed as judged by behaviour, body weight changes, mortality, haematological studies, blood chemistry, urinalysis, liver function tests, organ weights, gross and histopathological findings (Cervenka & Kay, 1963b).

Long-term studies

None available.

Comments

Although the extent of the modification is small there is some evidence of reduced *in vitro* digestibility by enzymes. The levels tested in rats and dogs represent 3.5 per cent. - 5 per cent. of the diet and did not attain an effect level. Further metabolic studies preferably in man are desirable. Evaluation is based on the available short-term studies but further 90-day studies in two species at levels higher than those studied hitherto are desirable.

EVALUATION

Level causing no toxicological effect in the rat

Five per cent. (5 50 000 ppm) in the diet equivalent to 2500 mg/kg body weight/day.

Estimate of acceptable daily intake for man

Unconditional acceptance	mg/kg body weight 0 - 25[a]

REFERENCES

Cervenka, H. & Kay, J. H. (1963) Unpublished report[b] submitted by Corn Products Co.

Kohn, F. E. & Kay, J. H. (1963a) Unpublished report[b] submitted by Corn Products Co.

Kohn, F. E. & Kay, J. H. (1963b) Unpublished report[b] submitted by Corn Products Co.

Kohn, F. E., Kay, J. H. & Calandra, J. C. (1964a) Unpublished report[b] submitted by Corn Products Co.

Kohn, F. E. & Kay, J. H. (1964b) Unpublished report[b] submitted by Corn Products Co.

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[a] Includes distarch phosphate prepared using trimetaphosphate or phosphated distarch phosphate or the sum of both. Subject to limits of phosphorous load given in the seventh report of the Joint FAO/WHO Expert Committee on Food Additives, Wld. Hlth Org. techn. Rep. Ser., [illegible], p.[illegible].

[b] By Industrial Bio-test Laboratories.

ACETYLATED DISTARCH PHOSPHATE

Modification is usually performed by the use of up to 0.1 per cent. of phosphorus oxychloride and five per cent. acetic anhydride. Vinyl acetate may be used as an alternative acetylating agent. Maximum acetylation amounts usually to 2.5 per cent. acetyl groups.

Comments

No biological data are available for evaluation. Adequate 90-day studies in two species (one a non-rodent mammal) and metabolic studies preferably in man are required to determine the combined effects of acetylation and phosphate cross-linkage.

EVALUATION

Not possible on the data available.

HYDROXYPROPYL DISTARCH PHOSPHATE

Modification is carried out with 0.1 per cent. phosphorus oxychloride and 5-10 per cent. of propylene oxide. Cross-linkage would be no greater than is experienced on modification with phosphorus oxychloride alone and other linkages would probably not exceed 20 per 100 anhydroglucose units.

Biological Data

Biochemical aspects

Digestibility was tested in groups of five rats fed for seven days a basal diet supplemented by 0, 1 g and 3 g modified or control starch. No difference in weight gain was observed (Prior, 1961).

Acute toxicity

None available.

Short-term studies

None available.

Long-term studies

None available.

Comments

There are virtually no suitable data available for evaluation. Adequate metabolic studies preferably in man are required to elucidate the fate of this compound and the moieties containing the various modifying radials. Ninety-day studies in two species (one a non-rodent mammal) are also required.

EVALUATION

Not possible on the data available.

REFERENCES

Prior, E. T. (1961) Unpublished report by Wisconsin Alumni Research Foundation No. 1021247/6, submitted by Stein, Hall & Co. Inc.

DISTARCH GLYCEROL

Treatment with epichlorhydrin under alkaline conditions up to 0.3 per cent. tends to produce cross-linking at a rate of one glycerol diether linkage per 200 - anhydroglucose units.

Biological Data

Biochemical aspects

Much evidence in the literature points to etherified starches being somewhat resistant to enzymic degradation. Probably small fragments containing the ether linkage will remain and will not be absorbed readily. Any material absorbed is likely to be excreted by the kidney. No direct experimental evidence exists for this particular hypothesis. (French, 1960). The metabolic fate of methylglucose is cited as collateral evidence. Although methylglucose is absorbed by rats (Bols, 1956) no demethylation is believed to occur (Csáky & Wilson, 1956) and excretion is essentially quantitative (Csáky & Glenn, 1957). The caloric value of two starches treated at levels of 0.07 per cent. and 0.30 per cent. epichlorhydrin were estimated by comparing the weight gain obtained after feeding a basal diet supplemented with 3 g of each starch against a dose-response curve obtained by feeding daily sucrose supplements of 0, 1.5 g, 3 g, 4.5 g and 6 g equivalent to approximately 0, 5, 12, 18 and 24 calories. Distarch glycerols were only slightly lower in caloric value than unmodified starch, the differences not being significant. No details were available on the number of rats used or the length of feeding of a given supplementation (Oser, 1960).

Acute toxicity

None available.

Short-term studies

Rat. Groups of 10 male and 10 female rats were fed for 90 days unmodified starch or starch treated with 0.07 per cent. and 0.30 per cent. epichlorhydrin at 71 per cent. of their diet. The efficiency of food utilization was similar as between all groups. Haematological investigations showed no abnormalities related to the feeding of modified starches. Non-protein nitrogen and sugar levels in the blood remained normal in all groups. The weights of kidney, liver and adrenals were normal and no histopathological changes were seen in the liver or kidney. Only a summary report was available (Oser, 1960).

Long-term studies

None available.

Comments

Although only minor structural modifications are stated to be present in this material the biological information provided lacks full particulars and was not adequate for evaluation. No experimental evidence was provided on the metabolic fate of this compound. Adequate metabolic studies preferably in man as well as 90-day studies in two species (one a non-rodent mammal) are required.

EVALUATION

Not possible on the data available.

REFERENCES

Caffey, T. E. & Wilson, J. B. (1956a) Biochem. Biophys. Acta., 22, 185

Caffey, T. E. & Glenn, J. E. (1956b) Amer. J. Physiol., 185, 159

French, D. (1960) Communication to T. A. White, 20th January 1960

Oser, B. L. (1960) Unpublished report by Food and Drug Research Laboratories Inc., 4th January 1960, submitted by National Starch Products Inc.

Sala, A. (1956) Rev. esp. Fisiol., 12, 17

ACETYLATED DISTARCH GLYCEROL

Modification is carried out with epichlorhydrin to a maximum of 0.3 per cent. and acetic anhydride to a maximum of five per cent. The amount of acetyl groups introduced does not exceed 3.5 per cent. Further treatment, such as bleaching, is often carried out.

Biological Data

Biochemical aspects

No separate tests on enzymic hydrolysis are available but it is very likely that this modified starch behaves like starch acetate and distarch glycerol, both of which are somewhat less accessible to enzymic degradation. The caloric value was determined by reference to a dose-response curve established in groups of 10 male rats given a basic diet with graded supplements of 0, 0.075 g, 1.5 g, 3.0 g and 4.5 g increase equivalent to 0, 3, 6, 12 and 18 calories per day. Starches modified by the use of 0.1 per cent. epichlorhydrin + 5.5 per cent. acetic anhydride and 0.3 per cent. epichlorhydrin + 5.5 per cent. acetic anhydride were tested against native starch at levels of supplementation of 1.5 g and 3.0 g per day for 30 days. Weight gain on modified starch supplement was slightly reduced compared with that on unmodified starch. All rats remained normally active and healthy. The caloric estimates were similar for both levels of epichlorhydrin treatment (Oser, 1961).

Acute toxicity

None available.

Short-term studies

Rat. Groups of 15 male and 15 female rats were fed for 90 days on diets containing 50 per cent. either normal or modified starch (0.3 per cent. epichlorhydrin + 10.5 per cent. acetic anhydride). Two modified starches of this sort were tested, each containing 3.1 per cent acetyl. There were no deaths. The growth rate of male rats was significantly lower compared with controls on unmodified starch. The full and empty caecal weights of both male and female rats in the test groups were significantly greater than in controls. Haematology, blood chemistry, urinalysis, organ weights and gross as well as histological examination were normal (Oser, 1964).

Comments

The available data do not permit the establishment of a no-effect level. The interpretation of increased caecal weights is discussed in the thirteenth report (see Annex 1, Ref. 15). Adequate metabolic studies preferably in man and 90-day studies in two species (one a non-rodent mammal) are required.

EVALUATION

Not possible on the data available.

REFERENCES

Ouer, H. (1961) Unpublished report by Food & Drug Research Laboratories Inc. No. 61774, 1st June 1961, submitted by the National Starch and Chemical Corporation

Ouer, H. L. (1964) Unpublished report by Food & Drug Research Laboratories Inc. No. 66664, 16th October 1964, submitted by the National Starch and Chemical Corporation

HYDROXYPROPYL DISTARCH GLYCEROL

Modification is performed by the use of 0.1 per cent. epichlorhydrin and 10 per cent. propylene oxide. Cross-linkage would be no greater than expected on modification with 0.1 per cent. epichlorhydrin. Substitution by treatment with 10 per cent. propylene oxide would probably add no more than 15-20 ether linkages per 100 glucopyranose units.

Biological Data

Biochemical aspects

None available.

Acute toxicity

None available.

Short-term studies

Rat. Groups of 25 male and 25 female rats were fed for 13 weeks on a diet containing one per cent. and five per cent. of modified starch or five per cent. of native starch. The general appearance and behaviour of test rats was comparable with the controls. One male and one female rat died at the lowest test level. Growth, food consumption, body weight haematology at one and three months, urinalysis at one and three months, gross and histopathological findings were comparable in all groups (Knapp, 1967a).

Dog. Three groups of four male and four female beagle dogs were given in their diet one per cent, and five per cent of modified starch or five per cent. of native starch for 13 weeks. Haematological studies, blood sugar, blood urea, serum GPT and alkaline phosphatase as well as urinalysis were comparable for all groups. Appearance and behaviour were normal. Body weight changes were unrelated to the administration of the test substance. Gross and histopathology showed no consistent abnormalities. Organ weights of thyroid, heart, liver, spleen, kidneys, adrenals and testes showed no test-related changes (Knapp, 1967b).

Long-term studies

None available.

Comments

No biochemical data are available on enzymatic hydrolysis or the fate of the moiety containing propylene glycol radical. Metabolic studies, in man, are desirable. The 90-day tests reveal no significant abnormalities and are used as a basis for evaluation.

EVALUATION

Level causing no toxicological effect in the rat

Five per cent. (= 50 000 ppm) in the diet equivalent to 2500 mg/kg body weight/day.

Estimate of acceptable daily intake for man

	mg/kg body weight
Temporary acceptance	0 - 12.5

Further work required by June 1973

Metabolic studies in several species.

REFERENCES

Knapp, W. A. jr (1967a) Report by Hazleton Laboratories Inc., 14th April 1967 submitted by the International Latex and Chemical Corporation

Knapp, W. A. jr (1967b) Report by Hazleton Laboratories Inc., 28th February 1967 submitted by the International Latex and Chemical Corporation

ACETYLATED DISTARCH ADIPATE

Modification is carried out by the use of five per cent. acetic anhydride and a maximum of 0.12 per cent. adipic acid, the latter acting as cross-linking agent. The maximum number of acetyl groups introduced is 2.5 per cent. The number of adipic cross-links does not exceed more than one in about 1000 anhydroglucose units, or not more than 0.08 per cent. adipyl groups introduced on the starch.

Biological Data

Biochemical aspects

In vitro studies with pancreatin have shown that only the acetate ester bond splits easily while the adipic acid ester linkages are not attacked. No free adipic acid could be demonstrated in the hydrolysate (Morgareidge, 1966a). The metabolic fate of adipate-modified starches was therefore investigated in male rats *in vivo* using adipic acid labelled at C1 and C6 with C^{14}. The rate of appearance of $^{14}CO_2$ was compared between starch modified with 1,6 - C^{14} adipic acid and a physical mixture of untreated starch and equivalent amounts of 1,6 - C^{14} adipic acid. Starch adipate is absorbed and/or metabolised more slowly as shown by the delayed appearance of $^{14}CO_2$ in the respired air. Almost all the C^{14} activity of the free adipic acid was recovered in the respired air; only a small amount appeared in the urine while none was detected in the faeces, in the gastro-intestinal tract or in the carcass. More than half of the C^{14} activity of the esterified adipic acid appeared in the respired air and a proportionate amount in the urine, the rest was found in the faeces. No activity appeared in the carcass (Morgareidge, 1966a). The calorie equivalent of the modified starch was determined in groups of 10 male rats fed for 28 days on a basal diet containing either 1.8 or 3.0 g of starch supplements. The modified starch had been treated with 0.2 per cent. adipic anhydride and 5.5 per cent. acetic anhydride. Native starch was used as control. Caloric values were determined from a dose/response curve obtained by the use of 0, 0.75 g, 1.5 g, 2.0 g and 4.5 g of sucrose supplements equivalent to 0, 3, 6, 12 and 18 calories per day. There was no difference in caloric value as between the modified and unmodified starches (Oser, 1961).

Acute toxicity

None available.

Short-term studies

Rat. A 90-day feeding study was carried out in groups of 15 male and 15 female rats at a dietary level of 50 per cent. modified or unmodified starch. The growth rate of males was significantly lower in the test group and the full and empty stomach weights of both sexes were significantly greater in rats fed the treated starch. All rats survived the test period and no differences were observed between the groups in respect of liver and kidney weights, haematology, blood chemistry, urinalysis, gross and histopathology. The modified starch used had been treated with 0.12 per cent. adipic acid and 10.5 per cent. acetic anhydride resulting in 2.1 per cent. of acetyl groups being present (Oser, 1964).

Long-term studies

None available.

Comments

The metabolic studies show that adipate cross-linking interferes with enzymatic breakdown and possibly delays absorption. A study with the C^{14} labelled material showed that the moiety containing the adipic acid was metabolised normally more slowly. No retention of label was found in the carcass. The available 90-day studies were only done at one dose level and do not establish a no-effect level although the pathological findings appear to be related only to the acetylation. Estimation of the acceptable daily intake may be based on the no-effect level for rats for acetylated starch.

EVALUATION

Levels showing no toxicological effect in the rat (for starch acetate)

Five per cent. (= 50 000 ppm) in the diet equivalent to 2500 mg/kg body weight/day.

Estimate of acceptable daily intake for man

	mg/kg body weight
Temporary acceptance	0 - 12.5

Further work required by June 1974

Adequate 90-day studies in two species (one a non-rodent mammal).

REFERENCES

Morgareidge, K. (1969a) Unpublished report No. 78523 by Food and Drugs Research Laboratories Inc., 20th November 1969 submitted by National Starch and Chemical Corporation

Morgareidge, K. (1969b) Unpublished report No. 79408 by Food and Drugs Research Laboratories Inc., 28th October 1969 submitted by National Starch and Chemical Corporation

Ower, N. (1961) Report No. 81774 by Food and Drugs Research Laboratories Inc., 1st June 1961 submitted by National Starch and Chemical Corporation

Ower, N. L. (1964) Report No. 40555 by Food and Drugs Research Laboratories Inc., 14th October 1964 submitted by National Starch and Chemical Corporation

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Effects of Different Ratios of Mixture of Starch and Casein, or Starch and Fat, in Diets on Lipogenesis in Rats

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Studies were made on the incorporation of ^{14}C of acetate-1-^{14}C into the lipids of the liver and carcass, and changes in the concentrations of nucleotides, citric acid, and free fatty acids in the liver, using rats fed diets consisting of starch and casein, or starch and corn oil, at different ratios. Lipogenesis was stimulated with an increase in the content of starch both in the starch-casein and starch-corn oil diets. There was a rapid drop in lipogenesis in rats fed the diet with a little increment in the content of corn oil. Lipogenesis decreased gradually with an increment in the content of casein in the starch-casein diet. A positive correlation was found between lipogenesis and the level of ATP in the liver. The concentration of citric acid decreased with an increase in lipogenesis in the liver. Changes in dietary composition did not produce any significant alteration in the concentration of free fatty acids in the liver.

It has been reported that the incorporation, both *in vivo* and *in vitro*, of ^{14}C-acetate or ^{14}C-glucose into the lipids of rats varies with dietary composition, resulting in a marked reduction when carbohydrate has been replaced by fat or the level of fat increased in the diet.[1-3] It has also been known that the lipogenesis is influenced rapidly, in a matter of a few hours, after one diet is changed to another.[4]

Adenylnucleotides, citrate, and free fatty acids have been known to be regulatory metabolites of the rate-limiting enzymes of glycolysis, gluconeogenesis or lipogenesis. Phosphofructokinase is inhibited by ATP and citrate,[5,6] and fructose-diphosphatase by AMP, which is partially reversed by ATP.[7] Acetyl CoA carboxylase is stimulated by citrate,[8] but glucose-6-phosphate dehydrogenase is inhibited by free fatty acids.[9]

In the present study, different ratios of carbohydrate to fat and carbohydrate to protein in diets were examined for the effect on lipogenesis in the liver and carcass. Investigation was also made to establish any relationship between lipogenesis in the liver and the concentrations of nucleotides, citrate, and free fatty acids in this organ.

EXPERIMENTAL

Male rats of the Wistar-Donryu strain were trained to such extent for 2 weeks as to consume a 25%

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2) E. G. Brice and R. Obey, *ibid.*, 218, 107 (1956).
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casein diet[10] for only 2 hr once a day.

After the training period, the rats were fed 5 g of starch and casein, or 6 g of starch and fat mixture (Table I), for 2 hr. Immediately after the feeding

TABLE I. COMPOSITION OF STARCH-CASEIN AND STARCH-CORN OIL DIETS

	Starch (g)	Casein (g)	Corn oil (g)	Cellulose powder (g)
Casein:Starch		Starch-casein diet		
5 : 95	4.75	0.25	—	—
25 : 75	3.75	1.25	—	—
50 : 50	2.50	2.50	—	—
75 : 25	1.25	3.75	—	—
Corn oil:Starch		Starch-corn oil diet		
5 : 95	5.30	—	0.28	0.42
25 : 75	3.60	—	1.20	1.20
50 : 50	2.00	—	2.00	2.00
75 : 25	0.90	—	2.70	2.40

period they were injected intraperitoneally with 10.0 μCi of acetate-1-^{14}C, having a specific activity of 10.0 mCi/mmole, and placed in a metabolic chamber. The respiratory carbon dioxide was collected by trapping into 2 N sodium hydroxide solution. One hour after injection, the rats were sacrificed by decapitation. In experiments without injection of labelled acetate, 1 hr after the feeding period for 2 hr the rats were sacrificed by decapitation or anesthetized with ether and the blood from the heart was collected with a syringe. In either case, the livers were removed quickly from them, placed in acetone containing solid carbon dioxide, and used for analysis. The carcass, from which the dietary residue in the stomach had been removed, were kept frozen until they were used for analysis. The frozen carcasses were ground by passing them through a meat grinder several times.

The lipids of the liver and carcass were isolated and purified by a modification of the method of Folch *et al.*[11]. Then they were estimated gravimetrically. The purified lipids were dissolved in a definite volume chloroform-methanol (2:1, v/v), and placed for counting.

The radioactivities of the carbon dioxide and lipids were determined by a windowless gas-flow counter. The results on the incorporation of ^{14}C were shown with ratios of the radioactivity in each of the three fractions *i.e.*, the respiratory carbon dioxide, the lipids of the liver, and the lipids of the carcass, to the total of radioactivity found in these three fractions.

Liver cholesterol was extracted and precipitated with digitonin by a modification of the method of Webster *et al.*[12]. The precipitate was dissolved with definite volume of glacial acetic acid, and the cholesterol content was measured by absorption of the solution with anthron reagent at 625 mμ. For the assay of radioactivity of the cholesterol, the digitonide obtained by the method mentioned above was washed twice with acetone-ether (1:2, v/v) and once with ether. The purified digitonide was suspended in methanol and plated for counting.

Liver glycogen was isolated by the method of Stetten and Boxer[13] and estimated by phenol-sulfuric acid method.[14]

The concentration of nucleotides in the liver were estimated in the following manner. A specimen weighing 10 g was collected from some rats, was homogenized with 20 ml of 0.6 N perchloric acid, and centrifuged at 10,000×*g* for 15 min. The resultant supernatant was neutralized with 30% potassium hydroxide solution and again centrifuged. The nucleotides in given volume of the supernatant were eluted with a formic acid-ammonium formate mixture using Dowex 1×8[15]. A resin bed 0.6 cm in diameter and 40 cm in height was used and each 5 ml of eluate was collected. The quantity of the nucleotides in a given fraction was determined by the absorption at 260 mμ. The concentration of the sum total of GTP and UTP was calculated on an assumption that these substances might be contained in equal amount in the fraction showing the maximum density.

For the assay of citric acid in the liver, the frozen liver was homogenized in 4 volumes of 10% trichloroacetic acid solution and centrifuged at 10,000×*g* for 15 min. The citric acid in the resulting supernatant was determined by the method of Natelson *et al.*[16] The free fatty acids in the liver and serum

10) T. Kimura and K. Ashida, *Agr. Biol. Chem.*, 32, 1440 (1968).
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were determined by the method of Novák.[17] The equivalent concentration of fatty acids was obtained by the standard of palmitic acid.

RESULTS

Lipogenesis and concentrations of liver nucleotides following administration of starch-casein diets.

The results in Table II show the relative recovery ratio of ^{14}C into the respiratory carbon dioxide, and the lipids of the liver and carcass. One hour after injection of acetate-1-^{14}C, the increase in the incorporation into the lipids of the liver was observed by increasing the content of starch. Similar tendency was observed in the lipids of the carcass.

In comparing the level of nucleotides, it seemed preferable to calculate the relative value of each nucleotide. This value was expressed as percentage to the level of ADP, which was relatively constant against changes in the dietary components (Table III). Table III shows these relative values of nucleotides. The level of ATP and sum total of GTP and UTP increased, but the level of UMP decreased, as the content of starch in a given diet increased. There was, however, no appreciable change in the concentration of the other nucleotides.

Lipogenesis and concentrations of liver nucleotides following administration of starch-corn oil diets.

As shown in Table IV an increase in the

Table II. Relative Recovery Ratio of ^{14}C from Respiratory Carbon Dioxide, Liver, and Carcass Lipids One Hour after Injection of Acetate-1-^{14}C in Rats Fed Diets for Two Hours

Diet Casein : Starch	No. of rats	Body wt. (g)	Relative recovery ratio* Respiratory CO_2 (%)	Liver lipids (%)	Carcass lipids (%)
5 : 95	4	135±3**	74.2±3.16	7.2±0.65	18.6±3.50
25 : 75	4	132±4	74.8±3.68	5.9±1.55	19.3±2.89
50 : 50	4	134±4	78.9±5.70	5.3±1.56	15.8±4.84
75 : 25	4	133±5	84.7±2.96	4.0±0.75	11.3±2.93

* The ratio of radioactivity in each of the three fractions, *i.e.*, the respiratory carbon dioxide and lipids of the liver, and those of the carcass, to the sum total of radioactivity found in these three fractions.

** Mean±S.E.

Table III. Relative Concentrations* of Nucleotides in Livers of Rats Three Hours after Feeding Diets

Diet Casein : Starch	CMP	AMP	GMP	IMP	UMP	ADP	UDPGlcNAc +UDPGlc**	GDP	UDP	ATP	GTP +UTP
5 : 95	11***	73	7	19	18	100 (1.35)****	44	6	48	120	27
25 : 75	12	63	6	15	31	100 (1.42)	40	17	35	101	24
50 : 50	8	97	8	22	43	100 (1.46)	49	16	60	76	25
75 : 25	11	80	9	26	55	100 (1.63)	42	15	51	49	18

* The concentration of ADP is taken as the standard for nucleotide concentrations.

** UDP-N-acetylglucosamine and UDP-glucose.

*** Each experiment was carried out with pooled liver of 4 rats.

**** Figures in parentheses are the concentration of ADP, μmole/g of wet tissue.

17) M. Novák, *J. Lipid Res.*, 6, 431 (1965).

TABLE IV. RELATIVE RECOVERY RATIO OF ^{14}C FROM RESPIRATORY CARBON DIOXIDE, LIVER, AND CARCASS LIPIDS ONE HOUR AFTER DILUTION OF ACETATE-1-^{14}C IN RATS FED DIETS FOR TWO HOURS

Diet Corn oil : Starch	No. of rats	Body wt.(g)	Relative recovery ratio* Respiratory CO_2(%)	Liver lipids (Cholesterol)%	Carcass lipids(%)
5 : 95	4	117±7**	67.4±4.50	4.7±1.80 (0.068)***	28.0±4.40
25 : 75	4	121±3	79.5±0.96	1.5±0.16 (0.065)	19.0±1.29
50 : 50	4	118±4	86.5±1.68	1.4±0.14 (0.046)	12.2±1.89
75 : 25	4	113±4	90.6±1.54	0.9±0.09 (0.042)	8.5±1.39

* The ratio of radioactivity in each of the three fractions, i.e., the respiratory carbon dioxide and lipids of the liver, and those of the carcass, to the sum total of radioactivity found in these three fractions.
** Mean±S.E.
*** The values show the ratio of radioactivity in the liver cholesterol to the sum total of radioactivity in the respiratory carbon dioxide and lipids of the liver, and those of the carcass.

TABLE V. RELATIVE CONCENTRATIONS* OF NUCLEOTIDES IN LIVERS OF RATS THREE HOURS AFTER FEEDING DIETS

Diet Corn oil : Starch	CMP	AMP	GMP	IMP	UMP	ADP	UDPGlcNAc +UDPGlc**	GDP	UDP	ATP	GTP +UTP
5 : 95	6***	76	11	10	43	100	44	23	28	104	25
25 : 75	19	132	17	30	29	100	36	9	27	54	11
50 : 50	8	92	12	12	49	100	31	6	84	16	12
75 : 25	13	100	13	20	39	100	39	6	43	30	11

* The concentration of ADP is taken as the standard for nucleotide concentrations.
** UDP-N-acetylglucosamine and UDP-glucose.
*** Each experiment was carried out with pooled liver of 4 rats.

starch content in the diet caused an increase in lipogenesis both in the liver and carcass. However, the incorporation of ^{14}C into the liver lipids was much more distinct in rats fed a diet in which the relative content of starch to corn oil was 95:5 than in rats fed one of the other 3 kinds of diet. Namely, the relative recovery ratio from the liver lipids over 3 times greater in the former rats than in those fed diet in which the relative content of starch to corn oil was 75:25, but the change in the liver cholesterol was relatively small.

The concentrations of the liver nucleotides as determined after feeding diets containing different contents of starch and corn oil are shown in Table V. The concentration of ATP was remarkably high in rats fed a diet containing the largest amount of starch of the diets used. The level of ATP was about 3 times higher in rats fed a 95:5 starch-corn oil diet than in those fed a 75:25 starch-corn oil diet. Accordingly, the change in the concentration of ATP coincided with that in the incorporation of ^{14}C into the liver lipids.

Concentrations of liver citric acid, free fatty acids and glycogen, and serum free fatty acids in rats fed a starch, a starch-casein or a starch-corn oil diet.

Table VI shows the concentrations of citric acid and free fatty acids in the liver of rats fed a diet consisting of starch alone, of starch and casein, or starch and corn oil. The concentration of citric acid increased in rats fed a starch-corn oil diet. As shown in Table

TABLE VI. CONCENTRATIONS OF CITRIC ACID AND FREE FATTY ACIDS IN LIVER OF RATS THREE HOURS AFTER FEEDING DIET

Diet			No. of rats	Body wt. (g)	Citric acid ($\times 10^{-4}$M)	Free fatty acids μeq/g wet wt.
Starch	Casein	Corn oil				
100	0	0	5	182±9*	1.56±0.010	12.1±1.76
70	30	0	5	182±8	1.88±0.016	14.1±1.61
70	0	30	5	182±7	2.22±0.015	12.8±2.29

* Mean±S.E.

TABLE VII. CONCENTRATIONS OF LIVER GLYCOGEN AND SERUM FREE FATTY ACIDS OF RATS THREE HOURS AFTER FEEDING DIETS

Diet			No. of rats	Body wt. (g)	Liver glycogen mg/g wet wt.	Serum free fatty acid μeq/ml
Starch	Casein	Corn oil				
100	0	0	5	219±7*	35.1±5.0	0.15
70	30	0	5	215±15	12.8±1.5	0.34
70	0	30	5	220±7	19.3±2.8	0.55
Before feeding			5	219±20	2.0±0.6	1.15

* Mean±S.E.

IV, lipogenesis of the liver decreased remarkably in rats fed a starch-corn oil diet in which the content of corn oil had been raised from 5% to 25%, while it decreased gradually in rats fed a starch-casein diet with an increment in the casein content of the diet. Therefore, it is assumed that the concentration of citric acid in the liver may be related to lipogenesis in this organ. The changes in dietary composition had little effect on the concentration of free fatty acids in the liver.

Table VII indicates that liver glycogen was higher in rats fed a starch-corn oil diet than in those fed a starch-casein diet, although the highest content of liver glycogen in the liver resulted from the feeding of starch alone. When a diet consisting of starch alone was given, serum free fatty acids became the lowest. A higher content of free fatty acids in the serum was observed in rats fed a starch-corn oil diet than in rats fed a starch-casein diet.

DISCUSSION

In the present experiments, lipogenesis was stimulated with an increase in the content of starch both in rats fed a starch-casein diet and in rats fed a starch-corn oil diet (Table II and IV). An increase in the content of corn oil from 5% to 25% caused a sudden drop in lipogenesis especially in the liver. Similar results were already shown by Hill *et al.*[9] In rats fed a starch-casein diet, however, lipogenesis decreased gradually with an increment in casein content.

In Fig. 1, the ratios of AMP, UMP, GDP, and ATP in the liver are plotted against the starch content of a given diet. Also in it, the ratio of the total radioactivity in the liver lipids to that in the respiratory carbon dioxide is plotted in a similar way. Of the curves of nucleotides drawn by plotting, only the curve of ATP was similar to those of lipogenesis both in rats fed a starch-casein diet and in rats fed a starch-corn oil diet. Accordingly, the correlation between the ATP-ADP ratio and lipogenesis in the liver was clearly demonstrated in the ratio of ATP to ADP which had been plotted against the ratio of the total radioactivity of lipids in the liver to that in the respiratory carbon dioxide in Fig. 2. Figure 2 seems to indicate that there may be a correlation between lipogenesis in the liver and

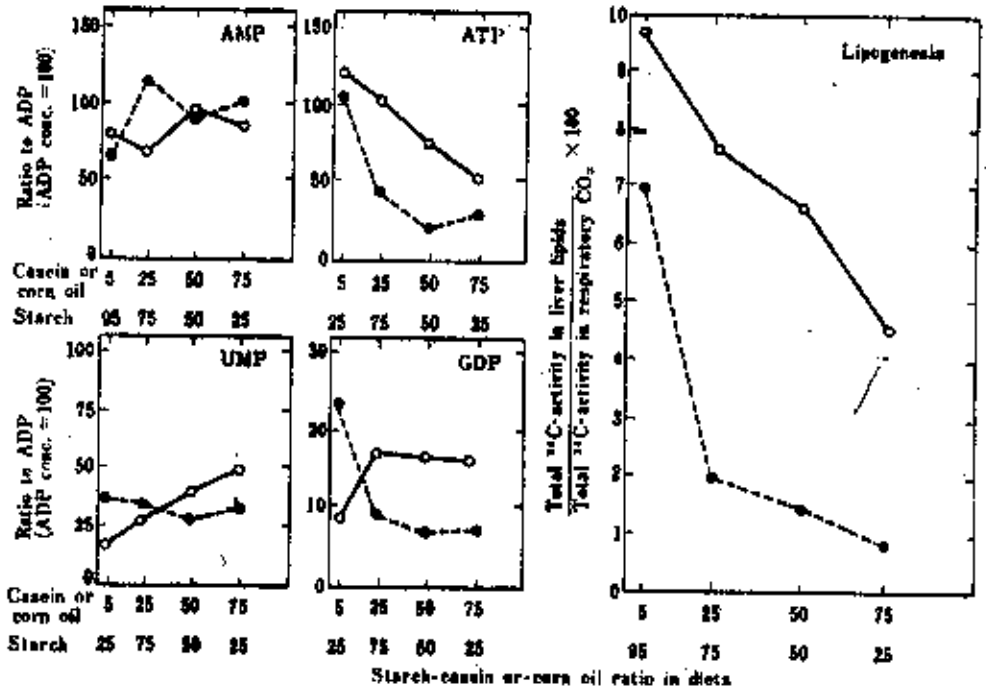

FIG. 1. Effect of Different Ratios of Starch-Casein or -Corn oil in Diets on Relative Ratio of Nucleotide Concentrations and Lipogenesis in the Liver.
Starch-casein ○——○, Starch-corn oil ●----●

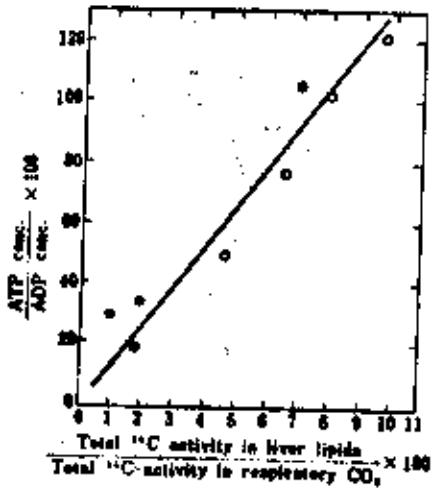

FIG. 2. Relationship between ATP-ADP Ratio and Lipogenesis in the Liver.
Starch-Casein ○, Starch-Corn oil ●

the changes in the concentration of ATP of this organ. Munro *et al.*[18,19] affirmed that the levels of ATP and glycogen in the liver were reduced after administration of protein. Knoch and Hartman[20] demonstrated that the concentration of ATP decreased after starvation to one-half of that in normal state, but Thorn and Jacob[21] could not find any significant change in the level of ATP during starvation. It was proved that the ratio of ATP to ADP in the total tissue-extract of the liver decreased upon addition of fatty acids to

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perfused rat liver.[22] Masoro *et al.*[23] stated that an increase in the activity of ATPase would be the cause for the inhibition of lipogenesis during fasting. These facts would also lend support to the possibility that lipogenesis may be correlated positively to the concentration of ATP.

One of the postulated extra-mitochondrial functions of citrate is a source of acetyl CoA in the cytoplasm. Kornacker and Lowenstein[24] demonstrated that the activity of liver citrate-cleavage enzyme was suppressed on starvation and restored on refeeding. Therefore, it is postulated that the level of citrate may decrease when lipogenesis is stimulated, or increased, in response to accelerated fatty acid oxidation. In fact, the level of citric acid in the total tissue increased in the heart,[25] muscle,[26] and liver[26] of alloxan-diabetic rats and in the liver of fasting rats.[27,28] The perfusion with fatty acids led to an increase in the content of citric acid in the heart[29] and muscle,[30] although Spencer and Lowenstein[31] reported that no dietary condition caused any alteration in the concentration of citric acid. The data presented in Table VI may also suggested that a decrease in the concentration of citrate occurs with the stimulation of lipogenesis.

On the other hand, the importance of citrate as a regulatory metabolite of the cellular enzyme system has been discussed recently by many workers. A regulator for the rate-limiting enzymes of lipogenesis and glycolysis is acetyl CoA carboxylase and phosphofructokinase,[32] respectively. Citrate is known to activate acetyl CoA carboxylase,[33,34] and inhibit phosphofructokinase.[33,35] The feeding of starch resulted in a reduction in the content of citric acid in the liver (Table VI), which is in favor of the activity of phosphofructokinase, but not in favor of that of acetyl CoA carboxylase. Accordingly, a possibility remains that acetyl CoA carboxylase may be regulated by the change in the intracellular location rather than by total content of citrate in the liver. It is not clear, however, whether citrate actually regulated the metabolic pathway *in vivo* in the present experiments or not. It was reported by Yugari and Matsuda[36] that the activity of glucose-6-phosphate dehydrogenase was inhibited by free fatty acids, especially long-chain fatty acids. This suggests that the level of liver free fatty acids may vary with the kind of diets; that is, a diet of starch alone, of starch and casein, or of starch and corn oil. As shown in Table VI, however, changes in dietary composition did not produce any significant alteration in the level of free fatty acids. The concentration of serum free fatty acids and the content of liver glycogen were higher in rats fed a starch-corn oil diet than in those fed a starch-casein diet. Randle *et al.*[30] demonstrated that the perfusion of fatty acids into the heart inhibited the utilization of glucose. Accordingly, after the suppression of the utilization of carbohydrate, the glycogen content may increased in response to the increase in concentration of serum free fatty acids.

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EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS FED CHOLESTEROL-FREE DIETS

PART 2. INFLUENCE OF VARIOUS CARBOHYDRATES

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SUMMARY

It has been shown that the addition of saturated fat to a semi-synthetic, cholesterol-free ration is atherogenic for rabbits. Experiments were carried out in which the carbohydrate component, which comprised 40 % of the diet, was either glucose, sucrose, starch or hydrolyzed starch. This diet was fed for 33 weeks. Starch was the most hypercholesteremic and atherogenic of the carbohydrates used and glucose was the least. Hydrolyzed starch resulted in higher serum cholesterol levels than did sucrose, but in slightly lower atheromata. Serum β-lipoprotein cholesterol levels were elevated in all four dietary groups, least in the glucose-fed rabbits. The glucose group exhibited normal serum triglyceride and phospholipid levels. Serum phospholipid levels were only slightly elevated in the other dietary groups. Serum triglycerides were twice normal in the sucrose-fed rabbits and were elevated 3- and 5-fold in the starch and hydrolyzed starch groups, respectively. Liver cholesterol levels were elevated (4–6-fold) in all four carbohydrate-fed groups. Aortic cholesterol levels were elevated in all the experimental groups, and the aortic free/ester cholesterol ratio was much lower than that observed in the aortas of normal levels. The biliary bile acids were determined and showed deoxycholic acid to be the predominate component (over 90 %) in all groups.

The data suggest that, in rabbits fed semi-synthetic diets, glucose is the least and starch the most atherogenic carbohydrate. In all rabbits fed the semi-synthetic diet, hypercholesteremia, elevations in serum β-lipoprotein cholesterol and atheromata were observed.

INTRODUCTION

Our studies on the factors which cause a semi-synthetic, cholesterol-free diet to be atherogenic for rabbits, whereas a chow diet of similar protein–fat–carbohydrate

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composition is not[1,2], suggest that the carbohydrate portion of the diet may be an important factor. Earlier studies on the semi-synthetic, atherogenic diet used glucose[3], sucrose[4] or glucose-starch[5] as the carbohydrate source, but there has been no direct comparison of these different carbohydrates. Reports on the effects of different carbohydrates upon cholesteremia and atherosclerosis in rabbits[6-8] have been carried out, using cholesterol-containing diets. This report describes the effects of glucose, sucrose, starch and hydrolyzed starch upon the induction of atherosclerosis in rabbits fed a semi-synthetic, cholesterol-free diet.

MATERIALS AND METHODS

Male rabbits of the Dutch-belted strain (average starting weight 1740 g) were fed one of four experimental diets for a period of 39 weeks. The basic diet consisted of casein (25), carbohydrate (40), salt mix, USP XIV (5), cellulose (15), vitamin mix (1) and hydrogenated coconut oil (14). The rabbits were divided into four groups whose dietary carbohydrate component was either glucose (G), sucrose (S), starch (T) or a partially hydrolyzed starch (H). The latter was a commercial product (Amidex) prepared by acid hydrolysis of corn starch. A fifth group of rabbits (CO) was maintained on the common atherogenic regimen of rabbit chow, augmented with 2 % cholesterol and 6 % corn oil. The semi-synthetic diets were prepared to our specifications by General Biochemicals, Chagrin Falls, Ohio.

At the end of the feeding period the rabbits were bled and killed and the livers and aortas removed. Aortic atheromata were graded visually on a 0–4 scale. The serum cholesterol was isolated as the digitonide using the SPERRY-WEBB[9] procedure, and color development was carried out, using the MAKS[10] reagent. Serum triglycerides[11] and phospholipids[12] were also determined. The serum α- and β-lipoproteins were separated by dextran sulfate precipitation[13] and analyzed for total cholesterol[9]. Aliquots of the liver were saponified in 15 % Alc-KOH and the cholesterol was extracted and assayed colorimetrically[10]. The aortas of each group were pooled, extracted with chloroform–methanol (2:1, v/v) and the extract analyzed for free and total cholesterol[9,10]. The gall bladder bile of each group was pooled, deproteinized, and, after hydrolysis of the bile acid conjugated with NaOH under pressure, the bile acids were purified by the $FeCl_3$ method of DOUBILET[14]. The bile acid content was assayed by gas–liquid chromatography using the triple component column described by OKISHIO AND NAIR[15].

RESULTS AND DISCUSSION

The autopsy results are presented in Table 1. We have included data obtained from 6 normal rabbits who were killed at the same time, but who had not been maintained in our colony for the duration of this experiment. The greatest mortality was observed in the cholesterol-fed group (CO), whereas the survival rate of all the carbohydrate-fed groups was similar. The rabbits in the four carbohydrate groups main-

TABLE 1

AUTOPSY DATA ON RABBITS FED SPECIAL DIETS (33 WEEKS)

Diet	Survival ratio	Weight change (g)	Liver wt. (g)	Liver cholesterol (g/100 g)	Atheromata arch	Atheromata thoracic
Glucose (G)	7/15	— 21	77.1	1.66 ± 0.03	1.1	0.9
Sucrose (S)	6/15	— 93	60.5	1.23 ± 0.18	1.9	1.0
Starch (T)	6/15	+ 37	63.3	1.90 ± 0.27	2.3	1.2
Hydr. starch (H)	6/15	— 30	73.8	1.31 ± 0.24	1.7	1.0
Cholesterol (CO)	3/15	+401	265.0	9.48 ± 0.30	4.0	4.0
Normal (N)	6	—	64.2	0.33 ± 0.03	0.0	0.0

tained their starting weight, within limits, but did not show the increases one might expect over an 8-month period.

There is no simple explanation for the mortality rates observed in the course of this experiment. In an earlier experiment involving semi-synthetic diets[1] the mortality in most groups was much lower. The weight losses observed in the surviving animals are not large enough to suggest malnutrition and the greatest mortality was observed in the group (CO) whose survivors showed greatest weight gain.

Average liver weights of the four carbohydrate-fed groups were slightly above those found in the normal rabbits. The average weight of the normal rabbits used was 2140 g and the liver weight represented 3.00 % of the body weight. The liver weights as percentage of body weight for the carbohydrate-fed groups were: G: 4.49 %; S: 4.15 %; T: 3.56 % and H: 4.32 %. The liver size of the cholesterol-fed group was much larger, 12.37 % of body weight. The liver cholesterol levels of all the carbohydrate-fed groups were 4–6 times normal, with the lowest level observed in the sucrose-fed group. The liver cholesterol levels of group S (sucrose) were lower than those of groups G (glucose) and T (starch), below the 5 % level of probability ($0.05 > P > 0.01$).

The atheromata were most severe in the group of rabbits fed cholesterol, as expected. Among the groups fed different carbohydrates, that fed starch exhibited the most severe atheromata, the glucose fed group exhibited the least. The arch atheromata in these groups were glucose, 1.07 ± 0.25; and starch, 2.25 ± 0.31 ($0.05 > P > 0.01$). The atheromata observed in the groups fed sucrose and hydrolyzed starch were intermediate between those of the glucose- and starch-fed rabbits. The level of arch atheromata in the sucrose group (1.92 ± 0.55) was quite high, but the difference between this group and the glucose group was not statistically significant. The level of arch atheromata in group H was 1.69 ± 0.25.

The finding that sucrose is more atherogenic than glucose is not surprising, since GRANT AND FAHRENBACH[8] had demonstrated a similar difference in atheromata in rabbits fed diets containing cholesterol and glucose or fructose. The even higher atherogenicity of starch has not been reported previously, but this material has been shown to be more hypercholesterolemic than either glucose or sucrose in cholesterol–

carbohydrate-fed rabbits[8], and we, too, found this diet to be the most cholesteremic. Whether this is generally true or whether the starch-induced hypercholesteremia is peculiar to the rabbit must await further investigation. PORTMAN *et al.*[16] found starch to be less cholesteremic than either glucose or sucrose in rats fed cholesterol and cholic acid. Similar results were obtained by STAUB AND THIESSEN[17], but they also found that potato starch was more cholesteremic than either wheat or corn starch. We observed[18] when rats were fed the identical diets as were the rabbits, the cholesterol levels of the starch, sucrose and glucose groups were not significantly different. STAUB AND THIESSEN[17] made a similar observation when these carbohydrates were fed to rats in cholesterol-free diets. The data reviewed in the foregoing paragraph emphasize how various animal species may react differently to similar dietary situations. The results which we and others have communicated cannot, at present, be directly related to human atherosclerosis. Continuing studies in various animal species and observation in man will show eventually where there are general similarities in response to high carbohydrate diets and where the various species diverge.

TABLE 2

AORTIC LIPIDS OF RABBITS ON SPECIAL DIETS (33 WEEKS)

Group	No.	Atheromata		Cholesterol (mg/100 g)			Triglyceride (mg/100 g)	Phospholipid (mg/100 g)
		arch	thoracic	total	free	F/E		
G	7	1.1	0.9	177	153	3.0	49.6	138.6
S	6	1.9	1.0	170	106	1.5	134.2	80.0
T	8	2.3	1.2	192	77	0.7	66.6	89.5
H	7	1.7	1.0	134	91	2.1	66.1	84.4
CO	3	4.0	4.0	287	187	1.9	53.2	184.0
N	6	0.0	0.0	0.80	lost	—	33.2	65.0

Table 2 summarizes the lipid spectrum of the pooled thoracic aortas of the various groups of rabbits. The free/ester cholesterol ratio is usually between 10 and 20 in normal rabbit aorta[2,19,20], whereas in cholesterol-fed rabbit aortas it is between 0.4 and 1.0. Similar differences have been observed between the aortas of young children and adults[21]. Our present findings bear out the earlier reports. The total cholesterol levels of the thoracic aortas were roughly parallel to the visual grading. The aortic triglycerides were higher than normal in all of the groups, with only the starch group exhibiting a gross elevation. The aortic triglyceride content in rabbits is generally constant during the course of cholesterol feeding and does not exhibit the gross elevation observed in the aortic cholesterol levels[22]. The aortic phospholipid was elevated only in the cholesterol- and glucose-fed groups. The rabbit aorta synthesizes phospholipid during the development of cholesterol-induced atherosclerosis[23]. The elevations in aortic phospholipid observed in the aortas of the carbohydrate-fed rabbits may also reflect a response to cholesterol deposition.

The serum lipids of the rabbits used in this experiment are presented in Table 3. The serum cholesterol levels of the starch-fed group were significantly higher than those of the glucose ($P < 0.001$) or sucrose ($0.05 > P > 0.01$) fed groups. The serum

TABLE 3

SERUM LIPIDS OF RABBITS FED SPECIAL DIETS (33 WEEKS)

Diet	No.	Cholesterol (mg/100 ml) total	free	ester	α-LP[a]	%α	α/β	Triglyceride (mg/100 ml)	Phospholipid (mg/100 ml)
G	7	209 ± 31[b]	53	156	31	14.8	0.17	44 ± 7[b]	38 ± 5
S	6	310 ± 66	101	209	15	4.9	0.05	79 ± 30	52 ± 6
T	6	640 ± 97	186	454	17	2.7	0.03	104 ± 36	50 ± 13
H	8	400 ± 77	146	254	18	4.5	0.05	145 ± 30	68 ± 19
CO	3	1469 ± 178	593	876	99	6.7	0.07	181 ± 41	522 ± 84
N	6	57 ± 10	22	35	16	28.1	0.39	30 ± 2	34 ± 2

[a] α-LP = serum α-lipoprotein.
[b] Standard error.

cholesterol level of the group fed hydrolyzed starch was also significantly higher than that of the glucose group ($0.05 > P > 0.01$). The serum cholesterol levels of the carbohydrate fed groups are roughly in the order of the severity of their atheromata. All of these groups also showed elevations in serum β-lipoprotein cholesterol levels and reductions in the ratio of serum α-/β-lipoprotein cholesterol compared to the normal rabbits. The α-/β-lipoprotein cholesterol ratios of groups G and N are identical with those observed in an earlier experiment[9]. These results confirm the observation of many investigators that elevations of β-lipoprotein cholesterol predispose to atherosclerosis. This is true even with the relatively modest cholesteremias found in this experiment. The serum triglycerides of the carbohydrate-fed rabbits were higher than normal levels, but with the exception of group H the elevations were 2–3 times normal, whereas cholesterol levels were 4–6-fold higher. The triglyceride level of group H was significantly higher than that of group G ($P < 0.01$). The serum phospholipid levels of group G were normal and those of the other groups were not significantly elevated.

Since dietary carbohydrates have been shown to affect biliary cholic acid levels[24] and bile flow[25] in rats, we determined the bile acids present in the pooled gall bladder bile of the rabbits in the various dietary groups. Table 4 shows that, as PORTMAN *et al.*[24] found in the rat, glucose and sucrose did reduce the level of cholic

TABLE 4

BILIARY BILE ACIDS (%) OF RABBITS FED SPECIAL DIETS (33 WEEKS)

Diet	Deoxycholic acid	Cholic acid
G	100	—
S	98	2
T	91	7
H	96	4
CO	93	7
N	97	3

acid when compared with starch. The level of cholic acid was highest in the two groups (T, CO) which had the most severe atheromata, but in view of the small amount of cholic acid found in all of the samples it is doubtful whether this plays a role in the development of atherosclerosis in the rabbit.

These experiments indicate that the type of carbohydrate present in a semi-synthetic diet which is atherogenic for rabbits, may significantly affect the cholesteremia and severity of atherosclerosis. These experiments are being extended to include other carbohydrates. The effect of fructose will be of special interest.

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Direct Starch Analysis Using DMSO Solubilization and Glucoamylase

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ABSTRACT

A new enzyme-based method for the direct analysis of corn, wheat, and potato starch and wheat flour is described which employs dimethylsulfoxide (DMSO) to dissolve the sample. Quantitative enzymatic hydrolysis is performed in the DMSO-citrate buffer solvent, and the glucose produced is measured either by an enzymatic colorimetric technique or by a glucose oxidase technique. This solubilization method avoids starch degradation, and glucoamylase allows quantitative conversion to glucose. The sample in mg. quantities is dissolved in warm DMSO. The conditions for optimum glucoamylolysis in this solvent are described. A technique for [illegible] the [illegible] or only glucose present before enzymatic hydrolysis is also described. Comparative data relating this technique to polarimetric and gravimetric methods of starch analysis are discussed.

Techniques for the quantitative analysis of starch can generally be classified as polarimetric or hydrolytic techniques. In either case, the starch polymer must be dissolved or dispersed in a manner that is compatible with subsequent reactions or measurements. Acidic gelatinization has been used extensively for polarimetric techniques, although calcium chloride solubilization is preferred. Acidic conditions are often used to degrade the starch polymer to reducing saccharides, but the [illegible] of [illegible] ingredients such as proteins often [illegible] of the reducing saccharides. [illegible] techniques for starch liquefaction and hydrolysis have also been [illegible]. [illegible] is [illegible] by the thermal instability of [illegible] (1). Enzymatic hydrolysis by [illegible] is [illegible], usually because terminal alpha

[illegible]

(1 → 4) bonds or alpha (1 → 6) bonds at branch points resist hydrolysis. Furthermore, the maltose produced by alpha-amylolysis is a competitive inhibitor of alpha-amylase. Donelson and Yamamoto have adapted their damaged-starch technique to an analytical method for starch in wheat by gelatinizing the sample with heat prior to diastase hydrolysis (2). The conversion to reducing sugars is about 65% complete and the utility of the method requires strict adherence to the prescribed treatment of the sample. Their enzymatic results were converted by a correction factor of 1.58 and were slightly higher than polarimetric results.

Glucoamylase[1] hydrolyzes both the alpha (1 → 4) and alpha (1 → 6) glucosidic linkages in starch to form glucose as the exclusive product. Glucoamylase has been used after heat-gelatinization of starch for starch analysis in dietetic foods (3), in resistant dextrin (4), in a wide variety of other products (5), and to estimate starch gelatinization (6). It is now clear that glucoamylase is applicable to starch hydrolysis in a wide variety of systems. The glucose produced after 3 hr. of incubation with glucoamylase at 50°C. was identified by thin-layer chromatography as the only monosaccharide produced, and was quantitatively measured by a glucose oxidase-peroxidase procedure (3). Glucoamylase was also shown to hydrolyze sugars of the maltose series present in flour, but did not decompose glucose (3).

Although the chemical specificity and the quantitative extent of glucoamylolysis has been demonstrated, the solubilization of starch has employed the time-consuming heat-gelatinization process. The extent and rate of dissolution of starch in dimethylsulfoxide (DMSO) at room temperature (7) and a description of the molecular interactions of this solvent with amylose and amylopectin (8) have been reported.

This paper describes a new method for quantitative starch analysis that employs rapid solubilization of starch in aqueous DMSO, quantitative hydrolysis with glucoamylase, and either automated colorimetric analysis of reducing sugars or enzymatic assays of the glucose produced. Optimum conditions for analysis and comparisons to polarimetric and gravimetric techniques are discussed.

MATERIALS AND METHODS

Corn starch, potato starch, and glucoamylase (*Rhizopus*) were obtained from Sigma Chemical Co., St. Louis, Mo. Glucose oxidase-peroxidase was obtained from Worthington Biochemical Corp., Freehold, N.J., as the Glucostat Special kit. In this purity state, glucose oxidase is essentially free of carbohydrate activity. Ayten wheat starch was obtained from General Mills. All other chemicals were reagent grade and were used without purification.

The sodium 2,2-bicinchoninate reagent for autocatalytic reducing sugar analysis was prepared by dissolving with heat 4.0 g. of the reagent in 20 ml. of 50% sodium hydroxide and 300 ml. of water. One hundred grams of potassium sodium tartrate was dissolved in this solution and a few drops of Ralgro (Hoechst Corp., Charlotte, N.C.) antifoam were added to improve the subsequent flow pattern in the [illegible] system. Dilution to 1 liter with distilled water. The stock buffer [illegible] was prepared by adding [illegible] sodium hydroxide and to adjust the pH to [illegible] and was kept in a [illegible] bottle.

Thin-layer chromatograms employed a Kieselguhr G adsorbent, a butanol-ethanol-water (50-50-20) solvent, and an anisaldehyde spray reagent. Gas-chromatographic analysis of the starch hydrolysates used conventional trimethylsilylation techniques and a stainless-steel column 1/8 in. o.d. X 6 ft., packed with 10% UC-[illegible] (methylvinylsilicone) on Chromosorb AW (80- to 100-mesh) run at 210°C. with flame ionization detection.

The polarimetric experiments followed either AOAC Method 13.035 or AACC Method 76-20; a Rudolph polarimeter calibrated with National Bureau of Standards sucrose was used. The glucostat special reagent kit was prepared by dissolving one vial of Chromogen and one vial of Glucostat Special in 30 ml. of distilled water.

Dissolution

The starch and flour samples were Soxhlet-extracted for 16 hr. with an equivolume mixture of methanol, butanol, and benzene. The extract was dried and extracted with chloroform, and the lipid content was calculated directly as the percent soluble in chloroform. The final sample concentration of 0.03 to 0.04% (w./v.) was established by mixing an accurately weighed sample of starch or flour with 20 ml. of 90% DMSO preheated to 55°C. in a 50-ml. flask. This solution was covered, kept at 55°C. for 20 min., and diluted, with maceration, to 50-ml. total volume with citrate buffer (pH 4.88), also preheated to 55°C. This solution was placed in a 45°C. bath and after it had cooled to that temperature, the volume was readjusted to 50 ml. with a solution of 10% DMSO in the citrate buffer, also heated to 45°C. Under these conditions, the starch was completely dissolved and the solution had an apparent pH of 4.4.

Enzymatic Hydrolysis

The glucoamylase reagent was stored under refrigeration and was prepared by dissolving 200 to 300 mg. in citrate buffer to a total volume of 100 ml., filtering through Whatman GF/A glass-fiber paper, and rediluting to 100 ml. with citrate buffer. This solution was heated to 45°C. and mixed with an equal volume of starch solution for a 20-min. enzyme reaction at 45°C. At this point in the method, the solution is 18%, by volume, DMSO. A 0.04% solution of dextrose was treated in an identical manner to serve as a calibration point for the colorimetric measurement of glucose by the 2,4-dinitrophenolate method.

Initial Reducing Value

The quantity of glucose produced by glucoamylolysis was corrected for the reducing substances naturally present in starch and flour by a procedure identical with that above, except that glucoamylase was omitted from the system. At the concentrations used for hydrolysis, the absorbance due to the natural reducing substances was too low for accurate measurement on the AutoAnalyzer colorimeter. A standard addition technique was adopted to improve the accuracy of measuring the initial reducing value. The sample size was increased from 30 to 40 mg. to 100 mg. and a 0.04% glucose solution replaced the glucoamylase in the citrate buffer, giving a final concentration after mixing of 1 mg. per ml. starch and a 0.02% glucose solution. In this way, the absorbance differences between the starch solutions and a 0.02% glucose solution were measured in a more accurate range of the absolute absorbance scale.

Automated Glucose Analysis

The conventional Technicon AutoAnalyzer modules were used as shown in Fig. 1 for the thermochromic colorimetric procedure for reducing sugars based on sodium 2,4-dinitrophenolate (6). Samples of the starch and flour solutions and solutions for initial reducing value and for calibration were placed directly on the sampler module.

Enzymatic Glucose Analysis

Standard solutions of glucose were prepared in the concentration range of 0 to 10 mg. glucose per 100 ml. by a procedure identical with that for starch samples to compensate for any absorption at 425 mμ due to DMSO, citrate, or glucoamylase. The concentration of glucose produced by glucoamylase was reduced by one-half by dilution with distilled water before the glucose oxidase-peroxidase method was used.

The concentration of glucose present in samples before glucoamylolysis is measured similarly by replacing glucoamylase with an equivalent amount of DMSO and citrate buffer.

RESULTS AND DISCUSSION

DMSO Dissolution

The temperature of dissolution was selected to approximate that of maximum glucoamylase activity. Although the results of starch dissolution in 90% DMSO show that the percent solubility is minimal at neutral apparent pH values (8) and that about 16 to 20 hr. is required for greater than 90% solubility (7,8), our results show that at 55°C., the DMSO solubilization technique is essentially complete within 5 min. at an apparent pH value of 4.4. Under these conditions, the maximum solubility of these starches is about 0.1% by weight.

Prolonged soaking in anhydrous DMSO gave reduced enzymatic conversion of starch, which agrees with Leach and Schoch's observation that anhydrous DMSO was capable of suspending starch granules but did not swell or disperse the granule (7). Starch digestion in 90% DMSO at apparent pH values of 2 and 10 also reduces the susceptibility of the starch to glucoamylase hydrolysis.

The percent glucose recovered after solubilization and enzymatic hydrolysis was

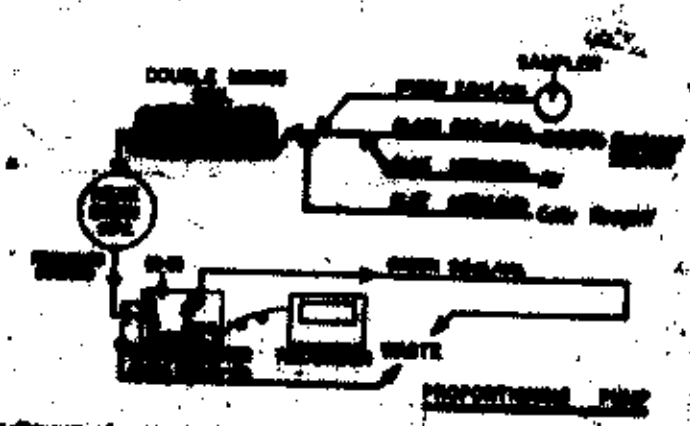

Fig. 1. Flow diagram of automated colorimetric analysis of reducing sugars.

measured after 5, 10, 15, 20, and 40 min. of dissolution in 90% DMSO at 55°C. These glucose data vary slightly with starch variety, but the conversion after 5 min. of dissolution is nearly complete. Twenty-minute dissolution time was selected as the most appropriate for the three starch varieties and two flour samples studied here.

Cave et al. (10) have shown that amylose maintains its helical configuration in DMSO solutions. There is substantial evidence that this is also the case for amylopectin (8). In addition, Leath and Schoch have reported, on the basis of specific viscosity data, that a corn-starch solution is virtually unchanged after standing 60 days in 90% DMSO at room temperature (7). They also report that potato starch is degraded by DMSO and discuss a DMSO solution of primary linkage in the branched portion of the polymer.

Differences between corn and potato starch were also observed in this study at 55°C. Phase-contrast microscopy of the starch solutions after 20 min. at 55°C. shows that the granules have lost their birefringence and that wheat and potato starch have lost their granular appearance entirely. Corn starch still displays some granular appearance after 20 min. at 55°C., but the subsequent enzymatic digestion serves to eliminate these [illegible] the dissolution.

Thin-layer chromatography (TLC) of the starch solutions shows no evidence of starch degradation, although the specific viscosity data (7) are probably more diagnostic of degradation than TLC.

Glucoamylase

Glucoamylase was filtered prior to the final dilution in citrate buffer to remove an insoluble material. The infrared spectrum and microscopic morphology of the insoluble material indicate a siliceous material. The specific activity of glucoamylase was not determined at these solvent conditions of 10% (by volume) DMSO, but the enzymatic hydrolysis is complete in 10 min. or less. The glucoamylase activity, measured as mg. glucose produced after 20 min. of reaction, increased rapidly with temperature over the range of 15° to 45°C. There were some varietal differences among the starches in the rate of activity change with temperature. Enzyme activity on Aztec starch appeared to be less sensitive to temperature changes; activity on potato starch was the most sensitive. At 45°C., all three starches were at or near the temperature of maximum glucoamylase activity. At 55°C., the enzyme lost 25% of the activity toward starch hydrolysis that it had at 45°C.

The ratio of enzyme:substrate in this study ranged from 2.5:1 to 5:1, and the extent of starch conversion was relatively insensitive to variations within this range. The starch sample weight was selected to give sufficient absorbance at 250 mμ for accurate measurement of the glucose produced. Bacto glucoamylase was used exclusively in enzyme assay and starch hydrolysis and to reduce the effect of variations in commercial enzyme. Chromatographic analysis of the starch hydrolysates, [illegible], confirmed that glucose was the only product of glucoamylase hydrolysis.

The activity of glucoamylase in a solvent containing 10% DMSO is remarkable and interesting. In this case the properties of the enzyme and the solvent separately and collectively contribute to primary a dissolve and [illegible] system. The

significant change in glucoamylase activity was observed during 4 months' use, and no special precautions to avoid infinite contamination were taken. At 18% DMSO, by volume, DMSO will be in the dihydrate ($DMSO \cdot 2H_2O$) form. The water molecules hydrated to DMSO are more polar than normal water molecules and thus may serve to stabilize the stereochemical configuration of glucoamylase in the preferred active orientation. Collectively, the solvent properties for starch and compatibility with glucoamylase make DMSO a useful solvent for this and other applications.

Automated Reducing Sugar Analysis

The color developed by the reaction of sodium 2,4-dinitrophenolate was measured as indicated in Fig. 1. The details of this procedure will be described in a forthcoming publication[3]. A standard solution of dextrose served to calibrate the chromatogram, and each series of samples contained two dextrose standards, at the first and last samples analyzed, to evaluate the magnitude of any instrumental drift.

The formula to calculate percent recovery data for the automated procedure shown in Table I is:

$$\% \text{ Starch recovery} = \frac{[\text{glucose}]\ 0.9 \times 10^6}{\text{sample weight}\ [100 - \Sigma(\% \text{ nonstarch components})]}$$

where the factor of 0.9 is the accepted factor (11) to account for the molecular weight difference between a starch monomeric unit in a polysaccharide and the glucose produced. The formula to calculate percent recovery by the enzymatic technique is:

$$\% \text{ Starch recovery} = \frac{[\text{glucose}]\ 0.9 \times 4 \times 10^6}{\text{sample weight}\ [100 - \Sigma(\% \text{ nonstarch components})]}$$

and differs only in the dilution factors necessitated by the more sensitive glucose oxidase-peroxidase method. The amount of starch on the "as-received" basis would not include the correction for nonstarch components and would be calculated as the product of glucose produced and the 0.9 factor.

In either formula, the glucose concentration can be corrected for the amount of reducing sugar or glucose initially present in the sample prior to glucoamylolysis. All starch and flour samples were fat-extracted. The sum of the nonstarch components includes water, protein, and ash, and these values were measured by conventional techniques on the fat-extracted sample.

The extraction of these samples with methanol, butanol, and hexane removes components of the sample other than lipids. For flour samples, these components represent about 1% of the original sample weight. This fraction, soluble in methanol/butanol/hexane but insoluble in chloroform, does not contain significant amounts of starch or glucose as measured by the DMSO-glucoamylase and the glucoamylase techniques. The chromatogram suggests a mixture of proteins and carbohydrate materials. Although it is not clear that this

[illegible]

[3] Wilson, D. G., Miller, R. A., Rizzi, A. M., and Robertson, J. G. (to be published).

[illegible] fraction contains all the cellulosic materials or any of the [illegible] components of the fat-extracted samples do not [illegible] with.

Separate experiments to study the important variables in glucose analysis by reaction with 2,4-dinitrophenolate showed that pH, temperature, and the amounts of DMSO, glucoamylase, and citrate must be controlled. The pH of the reaction is controlled by the composition of the color reagent. The temperature is controlled precisely by the Autoanalyzer. The three reagents react with the color reagent, DMSO being the most pronounced color. DMSO-water solutions, without glucose, produced an absorbance maximum at 550 mμ with 2,4-dinitrophenolate which increased as the % DMSO increased from 10 to 90%. Thus, it is very important to avoid conditions which allow DMSO to absorb water during the analysis. All operations were performed in [illegible], and although no special precautions were taken to dry DMSO before use, all possible precautions were employed to maintain the DMSO composition at 90% during dissolution, 36% during hydrolysis, and 10% during analysis. DMSO-water mixtures were freshly prepared before use and were never used for more than 1 day. Glucoamylase and citrate buffer also enhanced the absorbance of 2,4-dinitrophenolate at 550 mμ, but to a lesser extent than DMSO. Glucoamylase and citrate buffer were present in all solutions at fixed concentrations and thus, with equal amount of the DMSO-water composition, the absorbance increases for starch samples were related directly to the glucose formed by glucoamylase.

This work was performed to establish DMSO dissolution conditions for starch and to optimize conditions for glucoamylolysis that provide complete conversion of starch to glucose. The enzymatic colorimetric technique and the glucose-oxidase technique for glucose analysis used here were entirely adequate as described, but other substitutes for glucose analysis may also be compatible with this chemical system and may be used appropriately.

Comparative Results

The gravimetric technique for starch analysis did not produce theoretical (100% recovery) results and for this reason, the polarimetric results for starch recovery shown in Table I were used to assay the validity of this enzymatic technique. The samples were previously extracted by an equivolume mixture of methanol, butanol, and benzene for enzymatic hydrolysis or by absolute methanol chloride in the AACC procedure or ethanol in the AOAC procedure. In the three polarimetric methods, the initial polarimetric results were corrected for the sum of the other nonstarch components (moisture, sucrose, protein, and ash) with the same data as were used in calculating the percent starch recovery for the enzymatic technique. In this form, the results are compared on the same basis, and the agreement between polarimetric and enzymatic results is satisfactory. Without the correction for nonstarch components, the enzymatic and polarimetric results in Table I for corn, wheat, and potato starch will be lower by 18.8, 16.0, and 12.5% respectively. Regardless of the form of the data, the enzymatic results agree with polarimetric results and the percent recovery value of [illegible] Zarembski et al. [3] support this agreement by showing that the [illegible] and enzymatic methods give nearly identical results for starch. The [illegible] problem for the chemistries of

TABLE I. COMPARATIVE DATA OF STARCH ANALYSES EXPRESSED [illegible] RECOVERY OF STARCH

	Type and Analytical Method [illegible][a]			
	Sodium 2,4-Dinitrophenolate		Glucose Oxidase-Peroxidase	
	%	%	%	%
Starches				
Corn (Sigma)	[illegible]	([illegible])[b]	[illegible]	([illegible])
Wheat (Aytex)	[illegible]	([illegible])	[illegible]	([illegible])
Potato (Sigma)	[illegible]	([illegible])	101.7	([illegible])
Short patent wheat flours				
Soft red winter	[illegible]	([illegible])	94.4	([illegible])
White	[illegible]	([illegible])	[illegible]	([illegible])

	Polarimetric[c]			Gravimetric[c] (Munson-Walker)
	AOAC %	AACC %	Ewers[d] %	%
Starches				
Corn (Sigma)	[illegible]	[illegible]	[illegible]	[illegible]
Wheat (Aytex)	[illegible]	[illegible]	[illegible]	[illegible]
Potato (Sigma)	[illegible]	[illegible]	[illegible]	[illegible]
Short patent wheat flours				
Soft red winter	...	...	...	...
White	[illegible]	...	[illegible]	...

[a]Percent starch recovery = $\frac{[\text{glucose}] \times 0.9 \times 10^4}{\text{sample weight } [100 - \Sigma(\% H_2O, \% \text{ protein}, \% \text{ ash})]}$

[b]All values in parentheses corrected for initial reducing sugar content or initial glucose content.

[c]Data corrected for nonstarch components.

[d]Data by commercial laboratory.

corn, wheat, and potato starches on successive days at two sample sizes was 3.1, 2.7, and 2.6% respectively. Our technique is inherently faster than that described in reference 3, because the 1-hr gelatinization at 130°C. is replaced by a 20-min. dissolution in DMSO at 55°C. and the enzymatic reaction time is reduced by a factor of 9. In a routine application for the determination of starch and initial reducing groups, about 15 samples per day can be handled by the automated colorimetric method and about eight per day by the manual glucose oxidase-peroxidase method.

The superiority of the DMSO-glucoamylase technique compared to polarimetry is demonstrated by the two flour samples described in Table I. The nature and level of nonstarch components in these two flour samples introduce error in both the DMSO-glucoamylase and the polarimetric technique. In polarimetry, proteins and nonstarch carbohydrates may introduce error by altering the optical rotation of the dispersion and by producing dispersions which are difficult to filter. In the DMSO-glucoamylase technique, these materials do not appear to alter significantly the [illegible] of glucoamylase for glucosidic linkages, but may introduce

cumulative errors in the result because of the inaccuracies involved in accounting for their presence in the percent recovery calculation.

The 2,4-dinitrophenolate technique and the glucose oxidase-peroxidase technique give nearly identical results on corn, wheat, and potato starch. These two measurement techniques differ to some extent on flour samples and this is probably due to differences in quantity. Maltose and other reducing sugars present at significant levels in flour prior to glucoamylolysis respond differently to 2,4-dinitrophenolate than does the glucose used to calibrate the absorbance-concentration graph. For this reason, the recovery data differ by as much as 9% for 2,4-dinitrophenolate data if the data include correction for the initial reducing sugar content. The glucose oxidase-peroxidase technique is the more specific measurement. Thus, there is no disparity in the correction for initial glucose content.

The extensive application of this basic technique should consider the varietal and processing differences in flour samples, and the conditions for extraction, dissolution, and glucoamylase hydrolysis can then be adjusted, if necessary, to the characteristics of the sample.

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(376) S. J. LIU* and E. M. BOYD

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Adaptation of the gastrointestinal tract to increasing bulk of starch administered orally.

Male albino rats of 140±15 g body weight were given a 60% starch suspension in distilled water by intragastric cannula in increasing doses of from 36 to 456 g starch/kg/day, each dose to 12 to 40 rats with controls given (a) the same volume (60 to 760 ml/kg/day) of distilled water and (b) cannula insertion only. The doses were given in 4 equal parts at intervals of 2.5 hours and this was repeated daily for 14 to 49 days. Water was absorbed from the starch paste in the stomach and upper bowel, starch was converted to a calculus and there followed a considerable hypertrophy of the smooth muscle of the gastrointestinal tract which permitted the administration of increasing daily doses of starch. Autopsy at 14 days revealed a marked, dose-dependent, increase in weight of the stomach and intestines. Enlargement of cardiac stomach but not of intestines occurred in control rats given distilled water. The increase was mainly in dry weight but large doses produced some hydration of gastric tissues. The pyloric stomach was decreased in weight and there was a stress reaction in the adrenal and thymus glands and spleen. Histologically there were minor degenerative changes in the liver and minor capillary-venous congestion of several organs due solely to the large amount of administered water.

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ALLERGY FOR CORN AND ITS DERIVATIVES: EXPERIMENTS WITH A MASKED INGESTION TEST FOR ITS DIAGNOSIS*†

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THIS symposium on food allergy is the outgrowth of recent widespread medical and lay interest aroused by claims[1,2] that cereals and also their derivatives, starches, syrups, sugars,[3] oils, and even their grain alcohols,[4] are important irritants of allergy. The subject entered prominently into hearings before the Federal Food and Drug Administration in 1949, when the advisability of labeling salad dressings, breads, and other edible preparations was discussed from the allergenic angle. At this time, Randolph testified that such manifestations as unexplained headache, fatigue, drowsiness, chilliness, and pulling or aching of the muscles might originate from chronic or masked allergy for such regularly ingested foods as corn, wheat, milk, and eggs.

In anticipation of these Washington hearings, the writer interviewed 10 members of the American Academy of Allergy, from various sections of the country, to learn that only 0.12 per cent of their patients were considered susceptible by clinical standards to kernel or ground corn. No convincing instance of hypersensitivity to ingested oil or sugar could be found, and only a rare case of allergy for starch—one being recalled by Spain and another by Walzer. So great was the disparity between these opinions and those of Rinkel, Randolph, and Rowe, that a search for the underlying reason was instituted. Two steps were taken. First, a canvass was made by questionnaire of specialists in allergy and pediatric allergy to gain a more considered and extensive impression of the frequency of corn allergy. Second, an objective diagnostic procedure was devised whereby individuals suspected of hypersensitivity could be subjected to standard feeding tests with whole corn and subsequently with its derivative starch and syrup. The cornstarch pudding employed for the latter could not be distinguished in appearance, taste, or texture from 2 control puddings made of cane-sweetened starch obtained from tapioca or from arrowroot. The aim was to exclude psychogenic and other extraneous factors. These "blindfold" tests were used by 11 allergists on 25 individuals who gave suggestive histories for susceptibility to corn.

MATERIAL AND METHODS

Ingestion Materials: Mush.—As a check on the validity of the history, a feeding of precooked corn-meal mush (degerminated Quaker corn meal), sweetened with "Frodex," a dried corn syrup, was advocated as a preliminary or a final step in the ingestion studies. This ready-to-serve meal was prepared, as were the puddings, by an independent manufacturer in New York City (National Starch Products Inc., to whose Mr. Vincent Marsilia and Miss Mary Bak-

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which the writer is very grateful for their preparation of 67 packages). The mash consisted of 13.6 Gm. of dry meal to which were added 32.1 Gm. of dried syrup containing dextrose, maltose, and such higher sugars as triose and biose. The corn meal in each feeding carried 180 mg. of protein nitrogen and the syrup added another 2.2 mg. As is the case of the puddings, this test meal was posted in a refrigerated carton to the physician just prior to the arrival of the patient, the request being made through the writer's office. Before the next feeding in the series was prepared, all details of the earlier test were in the writer's hands. Each carton was labeled with an individualized code number, so that the patient, the physician, and the author remained in ignorance as to its contents until the entire series had been completed.

The puddings each contained 10 Gm. of starch. In the case of the tapioca and the arrowroot samples, the sweetener was 20 Gm. of cane sugar, whereas 6.6 Gm. of Frodex could be incorporated into the cornstarch pudding with an equal sweetening effect. As flavoring agents, 0.3 Gm. of salt and 0.3 Gm. of citric acid were included in each pudding, 0.03 Gm. of benzoate of soda being added as preservative. When requested, synthetic wild cherry flavoring was also employed in a volume of 6 drops.

The nitrogen content of the cornstarch pudding was estimated at 4.8 mg. protein nitrogen for the starch and 4.2 mg. for the Frodex, giving a total of 9 mg. In the case of the control puddings, the content of protein in their starches being ⅓ to ⅔ that of cornstarch and the sweetener being used in smaller proportion, the total amount of protein nitrogen in the feeding was about 4.2 mg. Hence, the cornstarch meal possessed over twice the protein of the placebo meals and might have been expected, therefore, to be more allergenic if its activity were dependent on contaminating nitrogen.

Ingestion Procedure.—The routine of Rinkel and Randolph was followed as accurately as possible in its several details, corn and its derivatives being carefully excluded from the diet for at least 4 days preceding any feeding. Tapioca and arrowroot, though more rarely ingested, were also prohibited. Wherever it could be safely recommended, the patient took the suspected allergen for 4 days prior to the period of elimination. He came in fasting, was allowed to rest seated for one-half hour before the start of the experiment. All suggestive signs and symptoms were sought and recorded for the 2-hour test period, as well as for the 2-day period before and after the ingestion. The subjects were also advised to avoid, or at any rate to record, any exposure to drugs, allergens, or other factors which might color the outcome of the test.

FINDINGS

Incidence of Allergy for Whole Corn (Kernel or Meal).—Table I is based on the replies received from 56 physicians who answered the questionnaire. The data have been segregated according to the frequency with which allergy for whole corn was encountered. For 45, the incidence amounted to only 0.16 per cent, or 1 case in 600, among a population of 45,500 individuals. Five additional physicians reported 82 cases in a total of 5,200 patients, giving an incidence 10 times higher and doubling the over-all figure. Another 4 found

59 instances in 1,700, bringing the average to 0.39 per cent for a population of 52,501 subjects.

The findings of the last 4 contributors are individually listed since they differ strikingly from the others. Rinkel, for example, has published a study of 200 patients with suspected hypersensitivity for whom he diagnosed corn as the etiologic factor in 16 per cent. We have taken the liberty of multiplying this population by 5 to make it comparable with those covered by the questionnaire. The same step was taken for Randolph who reported an incidence of 20 per cent in a group of his hypersensitive subjects at the 1949 hearing before the Food and Drug Administration. Rowe's response to the questionnaire was that he encountered 25 to 30 per cent of such cases among 1,200 patients. Finally, Crandall recorded 170 instances among 565 subjects, an incidence of 30 per cent.

It will be noted that the last 4 clinicians make the diagnosis of corn allergy about 100 times more frequently than the average allergist represented by the first group. It is hoped that some possible explanations for this disparity will be found in the results of our experiments with controlled feeding, to be presented below.

TABLE I. INCIDENCE OF CLINICAL ALLERGY FOR WHOLE CORN OR CORN MEAL

ALLERGISTS	PATIENTS OBSERVED	NO. CORN-ALLERGIC	INCIDENCE (%)	CUMULATIVE TOTALS		
				PATIENTS OBSERVED	NO. CORN-ALLERGIC	INCIDENCE (%)
45*	45,573	75	0.16	45,573	75	0.16
5*	5,220	82	1.57	50,793	157	0.30
4*	1,700	59	2.88	52,493	216	0.39
Rinkel†	1,000	160	16.0	53,493	376	0.7
Randolph†	1,000	200	20.0	54,493	576	1.06
Rowe†	1,200	300	25.0	55,693	876	1.57
Crandall†	565	170	30.0	56,258	1,046	1.86

*Members or Fellows of the American Academy of Allergy of whom 15 were certified internists; 2 were certified pediatricians.

†From the August-September, 1949, Hearing before the Federal Food and Drug Administration.

Ingestion Studies.—Table II summarizes the findings of ingestion tests carried out on 25 individuals who gave a history suggestive of allergy for whole corn. The data have been segregated into 3 sections, depending on the clarity of the diagnosis. Group A refers to 13 individuals whose allergy seemed the most certain, confirmation of the history being found by means of ingestion tests with our standard mush meal in 5 instances, with feeding tests devised by other allergists in 5 other cases, and on the basis of convincing histories for the remaining three. Details for the experiments on all but two of the members of this group are to be found in the recorded hearings before the Food and Drug Administration of 1949 as well as in the current issue of this journal under the investigators' names.

The outcome of experiments with these 13 clear-cut instances of corn allergy can be briefly summarized in the following statements. Eleven of the subjects gave entirely negative responses to masked feedings with cornstarch. Of the 2 who reacted to the cornstarch puddings, one (JM) did so on one occasion but failed to do so during an earlier ingestion experiment. The manifestation, consisting of abdominal cramps which occurred 7 hours after the feeding, was of

moderate severity and lasted for 4 hours. The first trial had elicited only a suggestion of "gassy indigestion" after 10 hours. In contrast, the standard mush feeding had provoked marked cramps within one-half hour. Had the pudding test been reproducible, one could have accepted this as an example of cornstarch allergy, attributing the relative delay in symptoms to the lesser amount of protein in the cornstarch as compared with whole corn meal. The lack of reproducibility, however, somewhat weakens one's convictions.

The other instance of cornstarch reaction was much more convincing. Patient MB developed asthma of moderate intensity within 1 hour of receiving a pudding, later identified as containing cornstarch. After 7 days, another such pudding reproduced the manifestation in marked form and a third experiment confirmed the diagnosis of cornstarch allergy. This woman showed unmistakable reactions to whole corn meal, developing prompt contact responses of the mouth and throat as well as immediate severe asthma.

Tapioca sensitivity was noted in one of the 13 patients of Group A, the patient being MB referred to above. She experienced severe asthma within 1 hour after the masked feeding on one occasion and promptly after ingestion on another.

Arrowroot starch caused a moderately severe gastrointestinal disturbance in 1 patient, and mild asthma in another. The first subject was observed by Shulman who reported that diarrhea had occurred in 48 hours and had lasted 2 hours, after she had been given the arrowroot pudding. Rawling's patient felt somewhat nauseated at once and became mildly asthmatic within one-half hour. Both symptoms subsided quickly. The long interval involved in the first instance and the mild, transitory nature of the reaction in the second patient weaken the interpretation of these findings as evidence of allergy to arrowroot.

Of these 5 instances of response to the starch puddings, only 2 are really convincing, those of the patient, MB, who reacted to both corn and tapioca. If so few cases are to be found among a group of patients of such highly selective character, the incidence must indeed be low among the general population. The suitability of the 13 individuals in Group A for the feeding experiments with cornstarch is made evident by their unequivocal clinical sensitivity toward whole corn. Table II reveals that 5 of them developed marked asthma, 7 various degrees of gastrointestinal disturbance, 8 nasal manifestations of allergy, 2 urticaria or atopic eczema, and 1 marked headache. With the possible exception of the latter, all these are classical expressions of allergy. Furthermore, they occurred promptly after the cereal had been eaten, 9 being noted within one-half hour and the rest within 5 hours. Although headache is a borderline manifestation, in the case of Sheldon's patient it was evoked in 30 minutes and was of extreme severity, so that it could be accepted as presumably allergic in origin.

Concerning the other derivatives of corn, none of the 11 individuals tested with syrup, sugar, or oil gave any sign of untoward effect, even on repeated feedings. One patient was also negative to the intravenous administration of dextrose. The involvement of cane sugar can be excluded from consideration since each of the several subjects responding to 1 starch pudding was fully tolerant of the other pudding which contained the same sweetener. This also applies to the flavoring and preservative used in all puddings.

Physician	Patient	Response to Ingestion of Whole Corn: Basis of Diagnosis	Symptoms: Asthma	G.I.	Nasal	Skin	Headache	Onset in Hrs. P.C.	Response to Starches & Corn Products: Corn Starch	Tapioca	Arrowroot	Corn Syrup	Corn Sugar	Corn Oil
Group A: 13 Patients With Excellent Ingestion Result and/or History for Corn Allergy														
Sheldon	1*	Trial		++	++			½	0			0	0	0
Sheldon	2*	Trial			++		+++	½	0			0		0
Sheldon	3*	History	+++					½	0			0		
Sheldon	4*	Trial		+	+			½	0,0			0		
Sheldon	5*	History		+++	++			¾	0			0	0	
Sheldon	6*	History			+++			1	0			0	0	0
Rawling	1*	Trial	+++		+ eye			3	0†		0†		0	0
Casort	KEP†	Mush†		+++		+		¼	0†	0†	0†	0	0	0
Halpin	CH*	Trial	+++	+++		+++		¼	0†				0 Ex.	0
Berston	2*MB	Mush†	+++					¼	0†		0†	0	0	
Cohn	JK	Mush†							+++† +++† +++†	+++† +++†	0†	0	0 0 0	0 0
Shulman	JM	Mush†		+++	+++			3	0†	0†	±†			
Rawling	2	Mush†	+++	+	+			½	±† ++†	0†	++† ±†		0	0
Total	13	5 Trial 5 Mush†	5	7	8	2	1	¼-3	2 pos.	1	2	9 neg.	3 neg.	8 neg.
Group B: 10 Patients With Fair Ingestion Result and/or History for Corn Allergy														
Berston	1*	Mush†			+				0†					
Lazar	PS	Mush†	+++					6	0†	0†	0†			
Black	CB	Mush†	++					12	0†	0†	+†			
Altose	BC	Mush†	+		+			3½	++†	±†	±†			
Altose	JS	Mush†			++	++		24	+++† +++†	+†	0†			
Casort	LO	History	+++	++				chronic	0†	0†	0†			
Casort	MCK	History	+++			++			0†	0†	0†			
Casort	RL*	History	+++	+++				2-3	0	0	0	0		0
Casort	AR	Starch												
Black	WBC	History†	+++			+++		12	+++†					
Total	10	History 5 Mush†	7	2	3	++ 4		12 2-24	0† 3	±† 1	±† 1			
Group C: 2 Patients With Possible History but Negative Ingestion Test														
Loveless	AN	Mush†	0				0		+†	++†	++†			
Loveless	MR	Mush†							++†	0 ±†	0 ±†			

These findings confirmed the claims of Rinkel and Randolph and the observations of Walzer and Spain that cornstarch can act as an ingestant allergen, rather to the surprise of the investigating physicians. The starches of tapioca and probably also of arrowroot can play a similar role. The convincing example found in Berston's patient can be examined in detail in the August-September hearings of the Food and Drug Administration for 1949 and seen in synoptic form in Table III.

TABLE III. INGESTION STUDIES ON ASTHMATIC PATIENT, MB (BERNTON)

MATERIAL INGESTED	AMOUNT FED (GM.)	NO. OF TRIALS	ASTHMA INDUCED	INTERVAL IN MIN.
Corn meal*	15.0	1	+++	several
Dextrose	12.0	3	0	
Cornstarch*	10.0	3	++,+++	5 - 55
Corn oil	30.0	2	0	
Corn syrup	30.0	2	0	
Arrowroot starch*	10.0	3	0	
Potato starch*	10.0	1	0	
Tapioca starch*	10.0	2	+++	15 - 55

*Loveless standard pudding or mush.

The failure of most patients in this group of definite whole corn sensitivity to react to cornstarch can be demonstrated by the case of Halpin. This 27-year-old woman gave a history of immediate edema of lips and tongue as the result of ingesting kernel and ground corn on 2 occasions. Severe urticaria, dyspnea, cough, and wheeze shortly developed and were followed by diarrhea and collapse which required 3 administrations of epinephrine for their control. The standard mush feeding was contraindicated in this severe case. The subject submitted to pudding tests with cornstarch on 2 occasions and with arrowroot on 1, and remained symptom-free. She not only tolerated ingestions of corn syrup, corn oil, and corn sugar but also took the latter by vein equally well. These findings are summarized in Table IV.

The young college student, KE, of Cazort is another example of extreme hypersensitivity toward kernel corn in combination with complete tolerance for large doses of each derivative by mouth. In keeping with his history, mush provoked immediate "tingling, burning, and a thick feeling in the mouth and throat." Severe abdominal cramps and urticaria were present some hours later. On the contrary, no untoward effects followed the taking of masked puddings in which the starches of corn, tapioca, or arrowroot were present. Similarly, corn oil, syrup, and sugar were taken in masked form without incident.

Six other individuals gave equally convincing results when tested by Sheldon with his own types of masked feeding. All these clearly corn-reactive patients proved to be fully tolerant of the starch and syrup of corn, and those given corn sugar and oil were similarly negative. Patient 1, investigated by Rawling with his own method of trial feeding with whole corn, like that of Conn who employed our standard mush, is an acceptable member for Group A in spite of the somewhat delayed appearance of manifestations. Both individuals were given cornstarch pudding, as well as 1 or more control puddings, without significant effect.

TABLE IV. INGESTION STUDIES ON PATIENT C. BY DR. HALPIN

DATE	PRECEDING SYMPTOMS	MATERIAL INGESTED	SYMPTOMS				
			ASTHMA	NAUSEA	DIARRHEA	URTICARIA	EDEMA
1946	0	Corn bread†	onset +++ ¼ hr.	+++	+++	+++ ¼ hr.	+++*
		Corn on cob†	+++ ¼ hr.	+++	+++	+++ ¼ hr.	
1948	0	Glucose 10 % intravenously	0	0	0	0	0
		Corn oil	0	0	0	0	0
		Corn sugar	0	0	0	0	0
		Corn syrup	0	0	0	0	0
7/27/49	0	Cornstarch	0	0	0	0	0
1/10/50	0	Cornstarch	0	0	0	0	0
6/29/49	0	Arrowroot	0	0	0	0	0

*Lips and tongue.
†Small portion of a serving.

TABLE V. INGESTION STUDIES ON PATIENT AN

DATE	PRECEDING SYMPTOMS	MATERIAL INGESTED	SYMPTOMS							
			ASTHMA		HEADACHE		FATIGUE		VERTIGO	
				HRS.P.C.		HRS.P.C.		HRS.P.C.		HRS.P.C.
11/23/49 10:30	0	Mush*	±	¾ - 1¼	0		0		0	
12:40	0 for 1 hr.	Corn on cob	±	¼ - 2¼	0		0		0	
11/18/49	0	Corn starch*	+	1 - 5	0		+++	½ - 4½	++	5½ - 6
11/9/49	Fatigue ++	Arrowroot*	++	3 -	0		0		0	
12/2/49	0	Tapioca*	++	1 - 2½	0		0		0	
1/31/50	0	Tapioca	0		0		0		0	
2/3/50	0	Arrowroot crackers	0		+++	¼ - ½	0		0	
2/8/50 10:00	Asthma ±	Saline 1 c.c.	+	½ - 1¾	+	¼ - 1¾	0		0	
11:45	Asthma ±	Saline 1 c.c.	+	½ -	+++	5 min. - 1	+	1 -	0	

*Lowless standard test meal.

When these 2 experiments are included with those described above for Group A, it is apparent that the large proportion of patients clearly susceptible to whole corn is unquestionably tolerant of its derivative starches and sugars, this being the observation for 11, and possibly 12, of the 13 test subjects.

Group B furnishes less valuable evidence because in general its responses to ingested corn were either mild in degree or delayed in development. Five of its members were diagnosed by ingestion test with our standard mush, whereas the history alone was used as the basis for diagnosis for the other 5. In spite of these shortcomings, some weight may be placed on the negative responses of 7 of the subjects to cornstarch and to such other corn derivatives as were employed. The anomalous situation encountered in the experiments with 2 of the patients, who appeared more reactive to starch than to meal, is worthy of further investigation. The third case listed as positive for cornstarch by the pudding test is difficult to interpret both from the viewpoint of the feeding result and the history. This 30-month-old baby (AR) was suspected of being cornstarch-allergic by its mother and was known to be reactive to both corn and cottonseed flours by inhalation. The only ingestion experiment done was one with cornstarch pudding. Three hours later a few hives had developed on the knee, and by 12 hours the eruption was generalized and mild asthma was present. The latter became so severe by the next morning that the child was hospitalized. It was unfortunate that control and whole corn ingestion studies were refused after this development.

Group C was composed of 2 of the author's patients who deserve brief mention because their symptoms resembled those classed as allergic by Randolph but not generally accepted as such by the profession. These responses were elicited by the derivatives but not by whole corn.

Table V summarizes the findings with An, a woman who was having asthma almost daily which was considered to be caused by several danders, house dust, and foods. After her first ingestion test with arrowroot pudding, she suffered an attack of moderately severe asthma. Nine days later the ingestion of cornstarch pudding was shortly followed by marked fatigue and mild asthma. After their disappearance some 4 hours later, a short spell of vertigo was noted. Her subsequent examination with standard mush and fresh kernel corn failed to duplicate these manifestations aside from a suggestion of mild asthma, which was surprising in view of the fact that larger doses of the hypothetical allergens in corn must have been involved. Interpretation of this woman's reactions was again made difficult by her failure to develop, at home, symptoms of asthma which followed the taking of tapioca in the office. Furthermore, arrowroot appeared to yield asthma of moderate severity 5 hours after she ate a masked pudding under our observation but when she tried arrowroot crackers at home a severe, brief headache was the result. Suspecting by now that factors other than our test meal allergens were operative, we subjected her to a feeding experiment with saline solution which she believed to be an allergenic extract. This gave rise to mild asthma by 30 minutes and to headache almost immediately. Fatigue of light degree was also reported. Without the repeated tests and the saline control, this woman would have been considered allergic to all 3 of the starch puddings, especially if the diagnosis had been guided by the criteria of Rinkel and Randolph.

For comparable reasons, one might have been misled by the initial corn starch reaction of AH. His persistent fatigue was increased as also was his headache by this masked pudding test. Repetition of the ingestion in 2 weeks yielded a negative result except for a suspicion of fatigue. Still a third experiment provoked no complaints whatsoever. These 2 essentially negative responses to cornstarch are consistent with his tolerance of both mush and kernel corn. It was important to the understanding of this case that repeat tests be carried out.

DISCUSSION

A survey of 56 allergists and pediatric allergists throughout the country revealed that for all but a few physicians clinical allergy for whole corn is a rarity which is encountered 100 times less frequently than Rinkel, Randolph, Rowe, and Crandall have reported. Some explanations for this disparity have been uncovered by our experiments with objective feeding tests. It was found that extraneous factors, especially of psychologic origin, can complicate the diagnosis unless the patient is subjected to repeated studies, to masked ingestions and to placebo meals.

Whereas no evidence was found for the allergenicity of corn oil, syrup, or sugar, clinical reactions were, to our surprise, encountered following the controlled ingestion of the several starches employed for these studies. The clinical significance of this type of hypersensitivity would not appear, however, to have the practical importance assigned to it by Randolph and his school. For one thing, its occurrence was rare even among our group of patients who were selected on the basis of their marked allergy for whole corn and who were tested with relatively huge ingestant doses of starch. Randolph has claimed that provocative amounts of starch allergen escape from paper cartons into milk, sauerkraut, and frozen foods. It seems unlikely that our cornstarch-reactive cases would have been affected by such traces. At any rate, the incidence of clinical susceptibility ought to be far lower with such small amounts of this substance than with the 10 Gm. of cornstarch involved in our puddings.

Presumably any allergenicity of starch, syrups, and sugars would be referable to contaminating proteins carried along from the whole cereal into the derivatives. Starch itself would probably not be antigenic in view of its prompt breakdown into smaller molecular substances by enzymes present in all biologic fluids. Furthermore, the proteins of whole corn would also be reduced considerably in activity because of the drastic treatment involved in the preparation of the derivatives, including heating and strong acidification.

Theoretically, bacteria might play a role in contributing antigens to corn derivatives. It is known, for example, that *B. mucosus* is introduced during the preparatory process. In addition, it might be found that this organism like the streptococcus, can synthesize dextrans, which have been shown to possess immunological activity.[7] Before any of these theories can be accepted, it will be necessary to support them by controlled experimental evidence.

It would seem that the chief cause of the disagreement among allergists concerning the frequency of food allergy must relate to the diagnostic criteria employed. The individual feeding procedures of Rinkel and Randolph, as well as

the elimination diets of Rowe[2], were introduced because the cutaneous test was found to be unreliable in this type of hypersensitivity. Although they constitute a step in the right direction, these methods fail to take into account the influence of extraneous factors, particularly those of psychologic origin. Consequently, an abnormally high incidence of positive results would be expected. Furthermore, there has recently been added to the diagnostic list a considerable variety of new symptoms. Although such an increase in the scope of allergy may eventually be found justifiable, this can only be decided after a scientific diagnostic procedure has been devised. To attempt to set up a new method simultaneously with the introduction of new symptomatology is tantamount to testing the validity of one unknown by the use of another. There is, then, an urgent need at the moment for the creation of an adequate diagnostic technique. The adequacy should be firmly established by its application to only those patients whose diagnosis is unequivocal. The members of Group A fulfilled this requirement in the "blindfold" ingestion procedure described in this article, their diagnoses resting on the speed and classical form of their reactions to whole corn. It is true that the psychologic factor was not excluded due to the impossibility of masking the characteristic taste and appearance of the unrefined cereal. However, the mush feeding was only a screening technique to confirm a straightforward history, and the psychologic factor was effectively controlled in the masked ingestion procedure associated with the pudding tests for starch and sugar allergy.

SUMMARY

1. A poll of allergists revealed that clinical symptoms from ingested corn were encountered in only 0.16 per cent of their 45,000 patients. This stands in marked contrast to an incidence of 16 to 30 per cent reported by Rinkel, Randolph, Rowe, and Crandall.

2. "Blindfold" ingestion studies associated with placebo meals were done on 25 patients with histories suggestive of corn allergy. A few instances of reaction to large feedings of the starches of corn, tapioca, and arrowroot were encountered, but no case of susceptibility for corn syrup, sugar, or oil. The symptoms of several patients were found to be psychogenic rather than due to the suspected food.

3. An appeal is made for the introduction of controlled, objective methods in the study of food allergy.

The writer wishes to thank the physicians who cooperated to make this study possible, and to acknowledge aid of Misses C. Spearing and A. Ryan as well as Mrs. H. Rice.

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THE INFLUENCE OF DIETARY CARBOHYDRATES ON THE LIPID PATTERN IN SERUM AND IN ADIPOSE TISSUE

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THERE is evidence to show that both the amount and the type of dietary carbohydrate influences the amount and kind of lipid in the serum, liver and adipose tissue of experimental animals (Macdonald, 1962 *a*). Cohen, Bavly and Poznanski (1961) have suggested that high intakes of dietary sucrose may be responsible for the high incidence of ischaemic heart disease, and many features of the malnutritional syndrome, kwashiorkor, are probably the result of a relative excess of carbohydrate in a protein-deficient diet in children. Dietary carbohydrate may thus have a less benign influence on metabolism than was thought. Men have, therefore, been given diets low in fat with either starch or sucrose as the principal carbohydrate, and the results of these experiments are consistent with the view that lipid metabolism is influenced by the type of carbohydrate consumed.

METHODS

The subjects were seven males aged between 21–41 years, with body weights ranging from 56·8–98·2 kg. The diet consisted of 500 g. of sucrose or raw maize starch daily together with lean meat or fish, green vegetables and water-preserved or raw fruit *ad libitum* and 100 mL of skimmed milk per day. Vitamin tablets were taken daily. The experimental diet was given for 25 days, followed by 25 days on a normal free-choice diet, with a final 25 days on a diet which contained the alternative carbohydrate. The mean daily consumption of protein, fat, carbohydrate and vitamins by each individual is seen in Table 1. Meals were eaten in a metabolic unit twice a day, the carbohydrate intake being divided into six doses and taken in water with lemon juice. As intestinal disturbance prevented the leanest subject from consuming 500 g. of starch per day this was reduced to 450 g. with a similar reduction in his subsequent sucrose intake. Three subjects ate sucrose on the first dietary period and the other four ate starch during their first dietary period.

Each subject was weighed daily, had a venepuncture and a biopsy of adipose tissue (Hirsch, Farquhar, Ahrens, Peterson and Stoffel, 1960) four days after commencing the diet and thereafter at weekly intervals. The biopsy was taken from the outer side of the upper arm. In one subject it was not possible to obtain any adipose tissue. In three subjects the metabolic rate was measured and glucose tolerance tests were carried out on the same day as the venepuncture.

Chemical methods

Serum lipids. The lipid was extracted from the serum by Bloor's method (1925) and the concentrations of (i) total sterol esters, (ii) glycerides, free sterols and free fatty acids and (iii) phospholipids were determined in the extract in duplicate by silicic acid chromatography (Macdonald, 1962 *b*). The percentage of six fatty acids (myristic, palmitic, palmitoleic, stearic, oleic and linoleic) in each of these three fractions was then found, after methylation, by gas-liquid chromatography using 25% Reoplex 400 on 85–100 mesh kieselguhr at 197°C. The total serum cholesterol (Zlatkis, Zak and Boyle, 1953) and phospholipid (Marland and Robison, 1926) concentrations were also determined. Total serum lipid concentration was calculated from the concentrations of serum phospholipid and the percentage of phospholipid in the total serum lipid.

Depot lipid. The extraction was carried out using the method of Hirsch *et al.* (1960) and the percentage of fatty acids determined by gas-liquid chromatography.

Metabolic rate. The oxygen consumption was determined in three subjects from an analysis of the expired air collected in a plastic gas bag and the metabolic rate subsequently calculated.

TABLE 1

Initial weight and mean daily dietary intake of each individual while on the starch and sucrose diets

Subject	Diet	Carbohydrate (g.)	Protein (g.)	Fat (g.)	Starch (g.) or Sucrose (g.)
N.G.	Starch	39	59	27	500
wt. = 71·4 kg.	Sucrose	39	93	40	500
J.J.	Starch	38	81	38	500
wt. = 74·5 kg.	Sucrose	37	109	48	500
I.M.	Starch	40	71	32	500
wt. 73·6 kg.	Sucrose	46	103	43	500
V.M.	Starch	37	82	38	500
wt. 67·3 kg.	Sucrose	38	80	34	500
G.P.	Starch	45	88	42	500
wt. 98·2 kg.	Sucrose	47	122	58	500
C.R.	Starch	39	59	27	500
wt. 84·5 kg.	Sucrose	39	57	26	500
P.S.	Starch	39	59	27	450
wt. = 56·8 kg.	Sucrose	37	59	22	450

Vitamins: (Daily addition) A = 2,500 i.u. C = 15 mg. Riboflavin 15·5 mg. Pyridoxine HCl = 10 mg. Aneurin HCl = 16 mg. D_2 = 300 i.u. Nicotinamide = 37·5 mg.

Glucose tolerance test. Fifty grams glucose in 100 ml. water were given to three subjects followed by half-hourly capillary blood samples. The blood glucose was estimated by the ferricyanide reducing method (Hoffman, 1937).

Serum transaminases. Serum glutamic-oxaloacetic and glutamic-pyruvic transaminases were estimated (Reitman and Frankel, 1957). Creatine phosphokinase was also estimated (Griffiths, 1964).

Results

The only adverse effects noticed by the subjects while on the starch diet were flatus and borborygmi, and symptoms consistent with hypoglycemia were felt on several occasions within half hour of the ingestion of a large quantity of sucrose. These symptoms were epigastric discomfort, aching in the shoulders and down the arms, sweating and anorexia.

The weight changes varied from an increase in 2·3 kg. to a decrease in 1·8 kg. on the sucrose diet with an average increase of 50 g. and from an increase in 1·4 kg. to a decrease in 4·1 kg. on the starch diet with an average loss of 1·1 kg.

Statistical assessments have been made using Student's t test and only probability values less than 0·025 are regarded as significant; lines have been fitted using the method of least squares.

The values for the subjects on the normal diet were calculated from the mean value from samples obtained before the commencement of the experiment together with those obtained weekly during the 25 day interval between the two carbohydrate diets, thus including values which might represent a return to normal, and so tend to mitigate against any differences appearing.

Serum lipids

Total lipid. There was a fall during the starch diet and a rise while on the sucrose diet (Fig. 1).

Lipid fractions. The starch diet produced a fall in the concentration of sterol esters and a fall in the concentration of the phospholipids. While on the sucrose diet there was a rise in the concentration of glycerides (Fig. 1).

Total cholesterol. No change in the level was seen on the sucrose diet but a fall was found on the starch diet (Fig. 1).

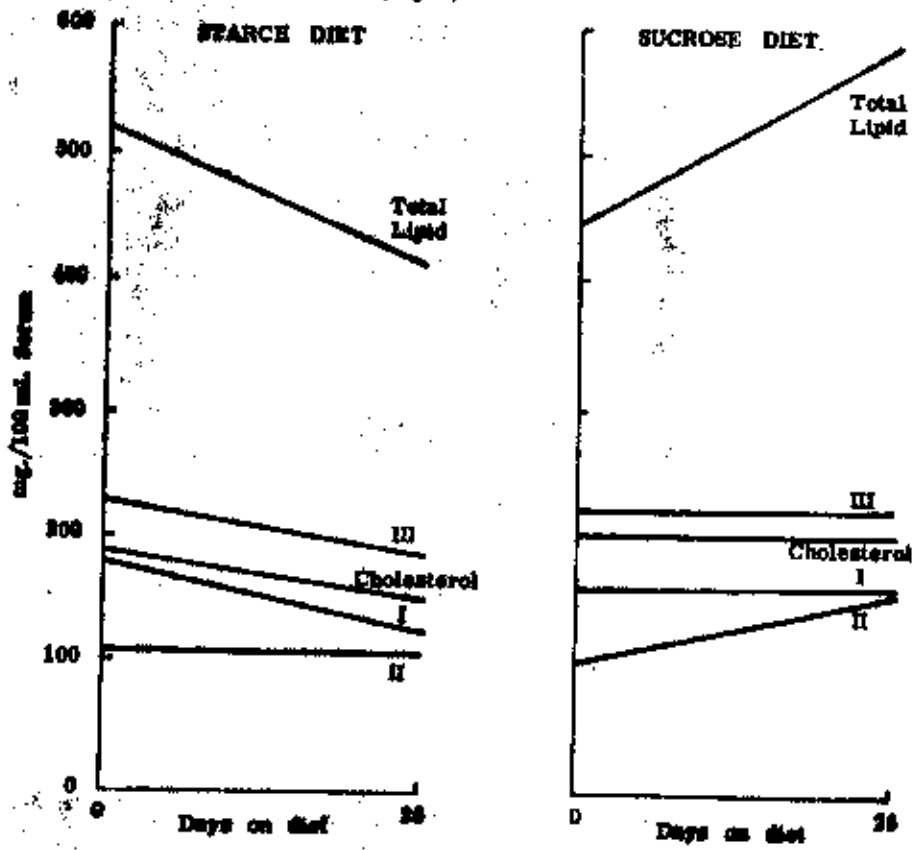

Fig. 1. *Concentration of fasting serum lipid and its various fractions while on the experimental diets.*
I = Sterol esters.
II = Glycerides, free fatty acids and free sterols.
III = Phospholipids.

Fatty acids

Total serum lipid. Both high carbohydrate diets were associated with an increase in the percentages of palmitic and oleic acids and a fall in linoleic acid (Fig. 2). Differences in response to the starch and sucrose diets are seen in the mean percentages of myristic acid which is higher while on the sucrose diet and of stearic acid which is lower while on the starch diet (Table 2).

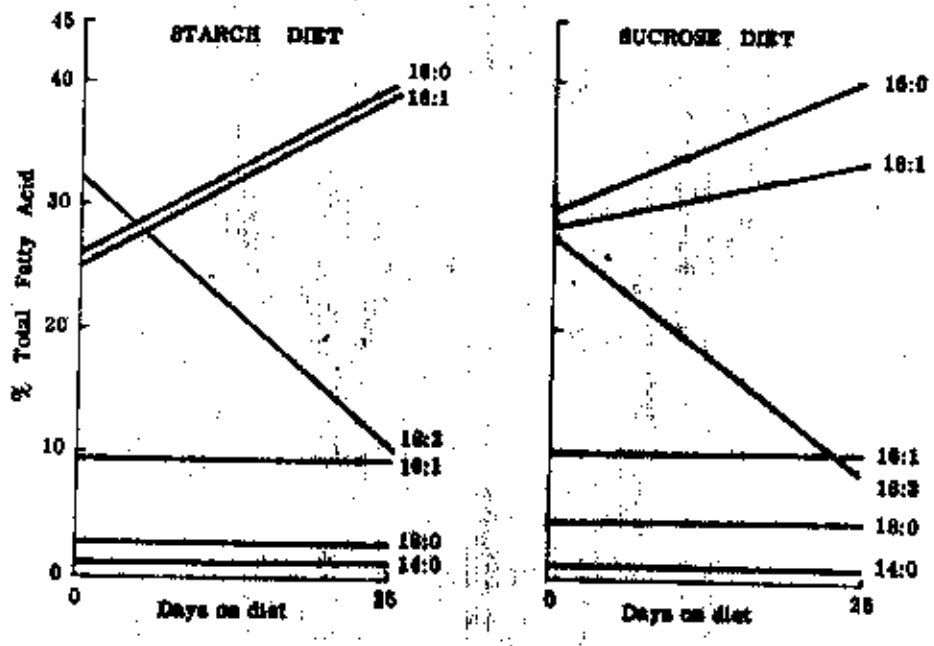

Fig. 2. *Concentrations of various fatty acids in the fasting serum while on the experimental diets.*

TABL

Mean content ($\bar{X}$) of various fatty acids expressed as per cent. of total fatty acid in each class of lipid examined and th

Sample	Diet	n	Myristic 14 : 0			Palmitic 16 : 0			
			$\bar{X}$	b	S.E.	$\bar{X}$	b	S.E.	$\bar{X}$
Total serum lipids	Normal	31	0·3		0·03	29·4		1·72	9·3
	Starch	27	0·4		0·03		+0·57	0·14	9·4
	Sucrose	31	0·6		0·05		+0·44	0·181	10·3
Serum sterol esters	Normal	31	0·7		0·05	16·9		0·92	9·4
	Starch	27	0·6		0·04		+0·58	0·112	11·4
	Sucrose	31		−0·02	0·005	23·4		1·61	
Serum glycerides, etc.	Normal	31	1·3		0·10	35·9		1·28	11·1
	Starch	27	1·2		0·11		+0·60	0·164	11·9
	Sucrose	31	1·1		0·10	41·0		2·07	11·9
Serum phospholipid	Normal	31	0·7		0·05	49·6		2·19	10·0
	Starch	27	0·5		0·04		+0·43	0·20	10·2
	Sucrose	31	0·6		0·06	52·3		2·28	10·7
Adipose tissue	Normal	17	2·1		0·17	24·7		1·31	13·3
	Starch	30	2·0		0·16	23·8		1·24	14·0
	Sucrose	23	1·9		0·12	23·8		0·88	

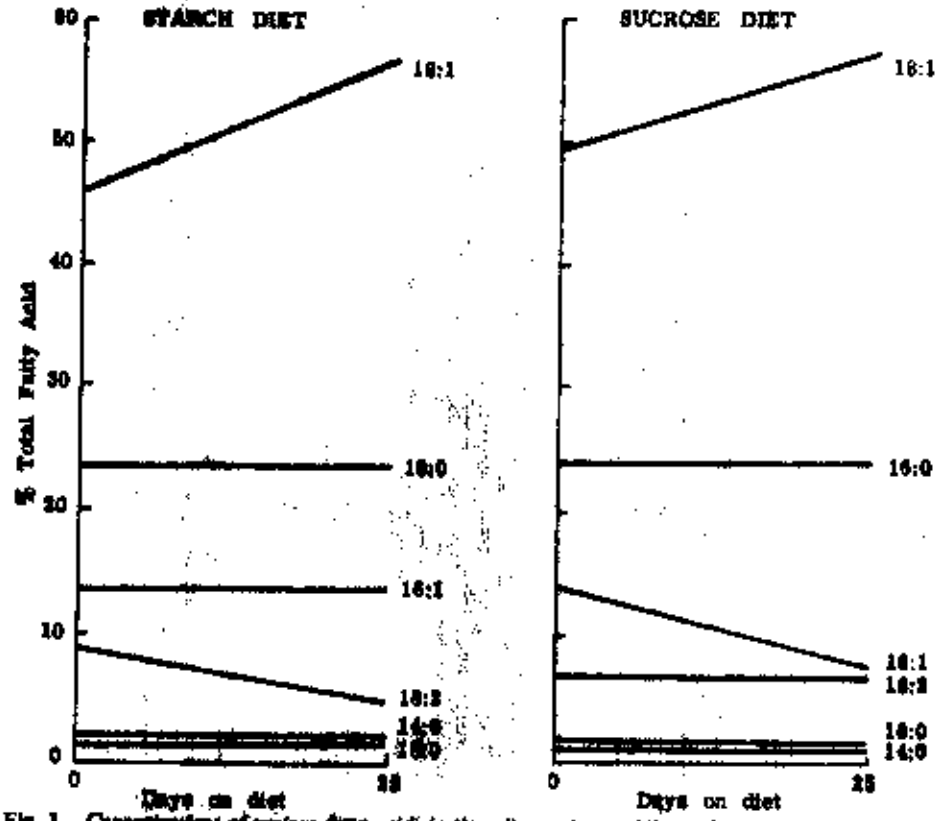

Fig. 3. *Concentrations of various fatty acids in the adipose tissue while on the experimental diets.*

2

slope (b) of the line of per cent. of fatty acid plotted against time on the diet where the P value of the slope is < 0·025.

Palmitoleic 16 : 1			Stearic 18 : 0			Oleic 18 : 1			Linoleic 18 : 2	
b	S.E.	$\bar{X}$	b	S.E.	$\bar{X}$	b	S.E.	$\bar{X}$	b	S.E.
	0·81	10·0		0·33	25·1		0·74	32·0		1·59
	1·01	8·9		0·39		+0·57	0·077		0·98	0·117
	1·30	7·5		0·33		+0·22	0·060		0·75	0·113
	0·70	1·7		0·22	28·6		1·04	42·7		1·38
	0·52	1·3		0·14		[illegible]	0·112		−0·88	0·199
+0·25	0·002	1·9		0·18		[illegible]	0·130		0·85	0·221
	0·87	3·6		0·26	36·7		0·70	11·4		1·21
	0·77	2·2		0·18		−0·41	0·102		−0·29	0·099
	1·17	2·3		0·19		−0·30	0·088	6·6		0·48
	0·77	10·0		0·44	17·2		0·78	12·5		1·26
	0·77	8·9		0·52	16·1		0·82		−0·34	0·103
	0·86	7·5		0·73	21·2		0·88	7·7		0·69
	0·92	2·0		0·21	30·2		1·52	7·7		0·87
	1·03	1·8		0·18		+0·44	0·151		−0·17	0·072
−0·25	0·084	2·6		0·21		+0·33	0·101	7·1		0·70

Serum sterol ester fraction. Both high carbohydrate diets resulted in an increased percentage of palmitic, palmitoleic and oleic acids with a reduction in linoleic acid. As in the total serum lipid the sucrose diet produced an increase in myristic acid and the starch diet resulted in a decrease in stearic acid.

Serum glycerides and free fatty acids. The high carbohydrate diets were again associated with an increased percentage of palmitic acid and a reduction in linoleic acid, but in this fraction in contrast to the previous fraction there is a fall in the oleic acid. The stearic acid fell on both diets.

Serum phospholipid. An increase in palmitic acid and a fall in linoleic acid is seen on both carbohydrate diets. Differences between the effects of the carbohydrate diets are seen in stearic acid which is lower on the sucrose diet and oleic acid which is higher on the sucrose diet.

Adipose tissue

In adipose tissue both carbohydrate diets produced a rise in oleic acid and differences between the effects of the two carbohydrates were seen in the percentages of palmitoleic acid which fell while on the sucrose diet, whereas linoleic acid fell on the starch diet (Fig. 3) and the mean value for stearic acid was significantly lower on the starch diet.

Other findings

There were no significant changes in the metabolic rate or respiratory quotient in the three subjects studied, nor was there any alteration in the pattern of the glucose tolerance curve, but post-prandial glycosuria was seen on the first day on

TABLE 3

Mean concentrations ($\bar{X}$) of serum lipid and of the lipid fractions together with mean values of the serum glutamic-oxaloacetic and glutamic-pyruvic transaminase and the slope (b) of the line of concentration of substance plotted against time on the diet where the P value of the slope is < 0·025

		n	mg./100 ml. serum		
			$\bar{X}$	b	S.E.
Total serum lipid	Normal	31	518		21·4
	Starch	27		−4·3	1·82
	Sucrose	31		+5·5	2·42
Serum sterol esters	Normal	31	176		8·9
	Starch	27		−2·3	0·99
	Sucrose	31	161		12·1
Serum glycerides, free sterols, etc.	Normal	31	112		32·8
	Starch	27	109		21·5
	Sucrose	31		−2·2	0·90
Serum phospholipids	Normal	31	230		24·6
	Starch	27		−1·6	0·63
	Sucrose	31	220		11·9
			Karmen units		
Serum glutamic-oxaloacetic transaminase	Normal	31	29·7		2·92
	Starch	27		−0·67	0·244
	Sucrose	31	27·6		2·64
Serum glutamic-pyruvic transaminase	Normal	31	12·6		1·66
	Starch	27		−0·43	0·143
	Sucrose	31	12·2		1·38

the sucrose diet in some of the subjects. There was a significant rise in the glutamic-oxaloacetic and glutamic-pyruvic transaminases, but no significant alteration in their ratio, while on the starch diet. No change in the transaminases was seen on the sucrose diet, and the serum creatine phosphokinase level did not alter on either diet.

Tables 2 and 3 summarise the findings.

Discussion

The effects of varying the dietary carbohydrate are most significant in the serum lipid which decreased during the starch containing diet, whereas the sucrose diet caused the serum lipid level to rise to values higher than those found on normal free-choice diets. The increase in serum lipids on the sucrose diet is due to the rise in the glyceride fraction, whereas on the starch diet the sterol ester and phospholipid fractions fall, the glyceride level remaining unchanged. Thus, it seems that dietary sucrose may travel along different metabolic pathways to dietary starch.

The composition of the depot fat in animals is known to be altered by increasing the carbohydrate intake, but attempts to verify this in man have not succeeded (Hirsch, 1962). The findings in these six males not only show that dietary carbohydrate can quickly alter the fatty acid pattern but that the effect with starch is different from that with sucrose as seen in the palmitoleic, stearic and linoleic acid percentages. These findings would be in agreement with the many experiments which have shown that adipose tissue has high metabolic activity.

The high carbohydrate : low protein diet leading to kwashiorkor also produces changes in the fatty acid composition of the adipose tissue (Macdonald, 1963) though the changes were not of the same pattern as seen in this investigation on adults whose protein intake was high.

Recently Anderson, Grande, Matsumoto and Keys (1963) have studied the serum lipid response to glucose, sucrose and lactose in the diet and their finding that the phospholipid level follows the cholesterol level is confirmed here. Though starch was not one of the carbohydrates they gave they commented on the marked rise in serum triglyceride on the sucrose diet. Antonis and Bersohn (1961) also noted this but found that after 3–6 months the serum triglycerides had dropped to their previous level: they do not, however, state the nature of the dietary carbohydrate and whether it was changed during the diet. Ahrens, Hirsch, Oette, Farquhar and Stein (1961) stated that fasting lipæmia could be carbohydrate-induced and they found the main increase in the serum lipid fractions was the triglyceride.

The significant rise in the serum transaminases while on the starch diet suggests that tissue breakdown is occurring but that muscle breakdown as a cause seemed unlikely because the creatine phosphokinase showed no elevation. Baron (1960) found raised serum transaminases in children on high carbohydrate : low protein diets and suggested that these changes may be due to hepato-cellular damage. A similar cause may operate in these volunteers on a high starch intake.

These studies can throw no light on the origin of the differences found between the lipid responses to dietary starch and to dietary sucrose. All dietary carbohydrates were formerly believed to be broken down to monosaccharides before absorption could take place, but this is now known to be untrue in man (Dahlquist and Borgström, 1961). The sucrose response might be different from the starch response because the splitting of sucrose leads to fructose formation whereas glucose only is formed from the breakdown of starch. Alternatively, the bacterial flora might be altered in such a way that substances are produced that interfere in lipid or carbohydrate metabolism.

Summary

1. Low-fat diets containing either 500 g. sucrose or 500 g. maize starch were given to seven adult male subjects for periods of 25 days.

2. The high sucrose diet resulted in an increase in serum lipids above normal, mainly in the glyceride fraction, whereas the starch diet produced a fall in serum lipids, mainly evident in the sterol ester and phospholipid fractions.

3. The fatty acid pattern of the various serum lipid fractions also changed and that for starch was different from that for sucrose.

4. The fatty acids in adipose tissue were also affected by the high carbohydrate intake and again the pattern differed between starch and sucrose in the diet.

5. The raised serum transaminases on the starch diet suggested the possibility that liver damage may have occurred while on this diet.

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Effects of isocaloric exchange of dietary sucrose and starch on fasting serum lipids, postprandial insulin secretion and alimentary lipaemia in human subjects

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1. Fasting serum cholesterol and triglyceride, and post prandial insulin secretion and lipaemia were measured in human subjects in a metabolic ward, who were given an ordinary diet (diet 1) in which the sucrose was isocalorically replaced by starch (diet 2) or vice versa. The subjects were nine healthy normolipaemic adult males. In eight of these subjects the effect of sucrose calorie reduction (diet 3) on fasting serum lipids was also studied.

2. When starch replaced sucrose, there were no significant differences in fasting serum lipid concentrations or immunoreactive insulin or in the insulin response and alimentary lipaemia after a standard mixed breakfast.

3. Serum triglyceride concentration fell and cholesterol concentration rose during the period of sucrose (and calorie) restriction.

4. After lunch and supper on the first two diets (when different carbohydrates were given) the lipaemic response was larger and the insulin response smaller after meals containing sucrose.

5. Thus, there was no difference between concentrations of fasting serum lipids when starch replaced sucrose at 23% total calories, but the concentrations of serum triglycerides were higher after individual mixed meals containing sucrose.

6. There were no significant differences in the fatty acid patterns of serum lipids on the different diets.

In a field study we found that fasting serum triglyceride concentrations fell in healthy male volunteers on a sucrose-restricted diet. It seemed, however, that the mechanism for this reduction may have been negative calorie balance in the low-sugar group because they lost weight at the same time (Mann, Truswell, Hendricks & Manning, 1970). The metabolic ward experiments reported here were designed to examine this possibility.

In addition, we hoped to clarify the rather conflicting reports on the effects of different dietary carbohydrates on fasting serum lipids. Some workers have reported elevated serum lipid concentrations in normolipaemic people when sucrose was exchanged for starch (Macdonald & Braithwate, 1964; Macdonald, 1965*a*; Antar & Ohlson, 1965; Hodges, Krehl, Stone & Lopez, 1967; Szanto & Yudkin, 1969; Akinyanju, Qureshi, Salter & Yudkin, 1968; Nestel, Carroll & Havenstein, 1970), while others have reported little or no difference (Keys, Anderson & Grande, 1960; Lees, 1965; Grande, Anderson & Keys, 1965; Macdonald, 1965*b*; Antonis, Iles & Pilkington, 1968; Dunnigan, Fyfe, McKiddie & Crosbie, 1970). However, in only a few studies has sucrose been given in proportions normally eaten (Keys *et al.* 1960; Grande *et al.* 1965; Dunnigan *et al.* 1970).

Szanto & Yudkin (1969) found elevated fasting insulin levels and an abnormal

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insulin response to glucose in some individuals on a diet very high in sucrose (438 g/d). Dunnigan *et al.* (1970) reported no abnormality of insulin response to glucose when comparing diets containing sucrose (20% of total calories) or starch. We have therefore studied the insulin response to the physiological stimulus of a normal meal with diets varying in carbohydrate composition, but containing sucrose in the ordinary range of intake (Walker, Holdsworth & Walker, 1971).

Alimentary lipaemia is cleared more rapidly when glucose rather than sucrose is included in a mixed meal (Mann, Truswell & Pimstone, 1971). It seemed that the greater insulin secretion stimulated by glucose potentiated the clearing factor, adipose tissue lipoprotein lipase. We looked to see whether the same phenomenon occurs following individual meals during the course of the diets in the present experiments. If there were large secretions of insulin on the starch diet, the rate of clearing of endogenous triglyceride could be increased and fasting levels affected.

Finally, we wished to see whether the changes in the fatty acid patterns of serum lipids which some workers have reported on high-carbohydrate diets when dietary sucrose and starch were exchanged (Macdonald & Braithwaite, 1964; Kuo & Bassett, 1965) would be found when carbohydrates were exchanged at ordinary intake levels.

METHODS

Subjects

The subjects were all normolipaemic men aged 30–40 years who had been admitted to hospital earlier for non-metabolic conditions such as cerebral vascular accident and nerve palsy. They had normal reactions to tests of liver function and their erythrocyte sedimentation rates were normal by the time the diets were commenced. They had been on the usual ward diets before admission to the metabolism ward and then, during the first 2 weeks of the adjustment period, were started on either diet 1 or 2 so that each individual's calorie requirement could be determined. Each subject remained in the metabolism ward for 8 weeks. All were ambulant and allowed ordinary ward activities during this period. They received physiotherapy and occupational therapy during their stay in the metabolism ward. Informed consent was obtained from each subject and all co-operated well. Subject 9, however, did not wish to continue in the study after the period on diet 2.

Diets

The subjects were given three diets and each dietary period was of 14 d duration. Diet 1 (see Table 1) was intended to represent a fairly average Western diet in proportions of proximate food constituents and amounts of dietary sucrose (Morris, Marr, Heady, Mills & Pilkington, 1963; Papp, Padilla & Johnson, 1965). In diet 2 the sucrose of diet 1 was isocalorically replaced by two complex carbohydrates, potato and rice, so that the amount of total carbohydrate remained unchanged. Thus the breakfast meal on the two diets was identical. The lunch and supper meals were isocaloric, but at lunch and supper on diet 2, potato and rice respectively, rather than sucrose, were the principal sources of carbohydrate. In diet 3 the total calorie

content of the diet was reduced by eliminating the sucrose from diet 1 and not replacing it with other food constituents.

Minor changes from the values shown in Table 1 were made to suit the individual daily calorie requirement of each volunteer by altering the bread intake. Individual total calorie intakes ranged from 2150 to 2440 kcal (8996–10 209 kJ) per d.

Table 1. *Composition of diet 1, intended to represent an average Western daily diet in proportions of proximate food constituents and amount of dietary sucrose*

		Protein (g)	Fat (g)	Total carbohydrate (g)	Sucrose (g)	Calories (kcal)
Breakfast						
Jungle Oats	300 g*	6·0	3·0	29·1	—	165
Bread	60 g	6·0	1·2	28·8	—	146
Butter	15 g	—	12·0	—	—	114
Milk for day	500 ml	17·5	16·5	24·5	—	325
Clear tea	—	—	—	—	—	—
Mid-morning						
Clear tea	—	—	—	—	—	—
Bread	30 g	3·0	0·6	14·4	—	73
Marmite (yeast extract)	—	—	—	—	—	—
Lunch						
Chicken	100 g†	31·6	3·4	—	—	166
Carrots	100 g‡	0·9	0·2	7·1	—	31
Butter	25 g	—	20·0	—	—	190
Bread	60 g	6·0	1·2	28·8	—	146
Sugar	70 g	—	—	70·0	70·0	266
Clear tea	—	—	—	—	—	—
Mid-afternoon						
Clear tea	—	—	—	—	—	—
Bread	30 g	3·0	0·6	14·4	—	73
Marmite	—	—	—	—	—	—
Supper						
Bread	60 g	6·0	1·2	28·8	—	146
Tomato	100 g§	1·6	—	7·0	—	33
Egg, hard-boiled	(one)	6·5	5·8	0·4	—	81
Butter	10 g	—	8·0	—	—	76
Sugar	70 g	—	—	70·0	70·0	266
Clear tea	—	—	—	—	—	—
Total daily calories (kcal)		—	—	—	—	2297 (9611 kJ)
Quantity of each constituent (g)		88·1	75·7	323·3	140·0	—
Percentage of total daily calories		15	30	55	23	—

* Cooked. † White meat, cooked. ‡ Tinned. § Raw.

Food tables were used to determine the composition of most of the foods used. However, as most of the values in the available tables are not for South African foods and in view of the variable moisture content of food, particular care was taken with the carbohydrate foods involved in the exchange – rice, potato and sugar. Constant carbohydrate sources were used throughout the series of experiments (Tastic rice, Maggi potato flakes and Hulett's white sugar). The moisture content of both rice and potato was checked and remained constant at approximately 9% of undried weight.

The sugar was found to have no appreciable amount of water. The caloric values of these three carbohydrates were determined in duplicate by bomb calorimetry and all were found to be 3·8 kcal/g dry weight. The moisture content of the bread chosen (approximately 40% of undried weight) varied little for 3 d after baking. The moisture content of other breads appeared to vary considerably, which could have caused variation in daily calorie intake. Standard foods were used to make up the remainder of the diet and were the same in all three diets. The same menu was given each day during the dietary period.

A check was made on the daily calorie intake from each diet by homogenizing all the food eaten on one day of each dietary period and then carrying out bomb calorimetry in duplicate on samples of the homogenate. The total calorie contents of diets 1, 2 and 3 measured by this method were 2513, 2498 and 1802 kcal (10514, 10452 and 7540 kJ) per d respectively. For all three diets these values were somewhat higher than the values calculated from food tables – 2297 kcal (9611 kJ) for diets 1 and 2 and 1765 (7384 kJ) for diet 3. However, the agreement between the measured calorie content of diets 1 and 2 was very satisfactory.

I. Macdonald (1970, personal communication) pointed out that for strict accuracy the protein which was present in the rice and potato of diet 2 should have been replaced during the period on diet 1. By Kjeldahl analysis the potato flakes were found to contain 6·88 mg nitrogen/g and the rice 9·79 mg nitrogen/g. This meant that the potato provided 3·1 g protein (N × 6·25) and the rice 4·4 g protein per d. As the protein intake was not marginal, it seemed unlikely that this small amount would be significant; nevertheless the last three subjects were given protein supplements during the period on diet 1 in the form of Casilan (Glaxo), 3·4 g at lunch and 4·6 g at supper.

Diets 1 and 2 were given to the subjects in a random order but diet 3 was always given last. The experiments were all carried out between January and September 1970 in the Groote Schuur Hospital metabolism ward.

Procedures

Subjects were weighed daily under standard conditions on a platform scale throughout the experiment.

Fasting blood samples were taken on days 0, 3, 6, 9, 12, 13 and 14 of each dietary period, day 0 corresponding to day 14 of the previous diet. Serum triglyceride was measured on each of the samples, and serum cholesterol on the last three samples of each dietary period. Fatty acid patterns of serum total lipids and of serum triglyceride were determined on one of the samples from day 12, 13 or 14 of each dietary period, depending upon the day on which most serum was available.

In subjects 4, 5, 7, 8 and 9, patterns of insulin secretion and triglyceride clearance after meals were also studied. On random days during the last 4 d of diets 1 and 2, blood was taken before breakfast, lunch and supper and then again at 0·5 h intervals for 2·5 h after the completion of the meal. Serum triglyceride, serum insulin and blood sugar were measured on each of these samples. These breakfast, lunch and supper experiments were carried out on different days.

Analytical methods

Standard techniques were used for the measurement of serum triglyceride (Van Handel & Zilversmit, 1957; Young & Eastman, 1963), cholesterol (Abell, Levy, Brodie & Kendall, 1952; Anderson & Keys, 1956) and blood sugar (Hoffman, 1937). Serum insulin was assayed by the method described by Morgan & Lazarow (1963). The glycaemic stimulus and lipaemic response to a meal were calculated and expressed as mg-h and the insulin response was microunit-h as previously described from this laboratory (Mann *et al.* 1971).

The fatty acid patterns of serum total lipids and serum triglycerides were determinded by gas–liquid chromatography (James & Martin, 1956) of the methyl esters (Stoffel, Chu & Ahrens, 1959). Triglycerides were first separated by thin-layer chromatography (Wagener, 1965).

Both the Wilcoxon test and Student's *t* test were used to determine the significance of differences between body-weight, serum cholesterol and serum triglyceride concentrations on the different diets. (In fact the same *P* values were obtained by the use of these two different tests.) The significance of differences between lipaemic response, glycaemic stimulus and insulin response to meals on diets 1 and 2 were determined by Student's *t* test.

RESULTS

Body-weight

Mean body-weights were virtually the same at the end of dietary periods 1 and 2 (Table 2). The mean weight for the last 3 d on diet 2 was, however, significantly lower than on diets 1 and 2 ($P < 0.05$ in both instances).

Table 2. *Mean body-weight, serum cholesterol and triglyceride concentrations of the eight subjects (for the last 3 d on each diet) on diets 1, 2 and 3*

	Weight (kg)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)
	Diet 1: basal + sugar		
Mean	59·2	188·8	97·8
Range	47·2–84·3	140–259	67–128
SEM	3·65	14·68	9·95
	Diet 2: basal + starch		
Mean	59·1	187·4	98·4
Range	46·6–84·5	142–228	65–127
SEM	3·65	11·56	9·95
	Diet 3: basal		
Mean	57·8	204·3	84·1
Range	44·7–83·5	162–253	67–107
SEM	4·08	10·56	4·45

Serum triglyceride

There were no significant differences between fasting serum triglyceride concentrations on diets 1 and 2 whether considering daily triglyceride values, mean of all values while on the diets or mean of the last three values while on the two diets

(Table 2). However, the mean for the last 3 d on diet 3 was significantly lower than the mean for the last 3 d on diets 1 ($P < 0.02$) and 2 ($P < 0.05$). The fall in triglyceride concentrations which occurred on diet 3 was confined to those subjects whose fasting serum triglyceride concentrations on diets 1 and 2 were greater than 90 mg/100 ml (Table 3).

Serum cholesterol

There were no significant differences between concentrations on diets 1 and 2 (Table 2) but the mean value was significantly higher at the end of the period on diet 3 ($P < 0.05$).

From the mean values given in Table 4 it appears that replacement of the small amount of protein during the period on diet 1 made no difference to the mean body-weight, or serum cholesterol and trigylceride concentrations on diets 1 and 2.

Table 3. *Mean serum triglyceride concentration (mg/100 ml) (for the last 3 d on each diet) on diets 1, 2 and 3 for each of the subjects*

Subject no.	Diet 1: basal + sugar	Diet 2: basal + starch	Diet 3: basal
1	113	127	96
2	128	125	107
3	67	65	67
4	102	100	83
5	121	116	88
6	91	92	76
7	83	83	82
8	77	79	76
9	163	156	—

Table 4. *Mean values for body-weight and serum lipid concentrations on diets 1 and 2, for the subjects for whom protein of potato and rice was not replaced while on diet 1 (subjects 1–6) and for those for whom it was replaced (subjects 7–9)*

	Diet 1: basal + sugar			Diet 2: basal + starch		
Subjects	Weight (kg)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)	Weight (kg)	Cholesterol (mg/100 ml)	Triglyceride (mg/100 ml)
1–6	61·2	203·2	103·7	61·1	200·5	104·1
7–9	54·0	180·0	107·7	54·1	179·7	106·0

Fatty acid pattern of serum lipids

It can be clearly seen from Table 5 that there were no significant differences between fatty acid patterns of fasting serum triglycerides in dietary periods 1 and 2. The slightly lower myristic and higher linoleic acid concentrations on diet 3 were not statistically significant. Likewise there were no differences in the fatty acid patterns of fasting serum total lipids between the three diets.

Glycaemic stimulus, insulin response and lipaemic response after breakfast, lunch and supper on diets 1 and 2

The glycaemic stimulus, insulin and lipaemic responses to the breakfast, lunch and supper on diets 1 and 2 are shown in Table 6.

After the breakfast on the two different diets (when the stimulus was the same) the total serum insulin responses were almost identical. Similarly, the glycaemic stimulus of the meal and the lipaemic response were similar on the two different diets.

Table 5. *Fatty acid composition (% by weight of total fatty acids) of serum triglycerides of the subjects on diets 1, 2 and 3*

Fatty acid	Diet 1: basal + sugar		Diet 2: basal + starch		Diet 3: basal	
	Mean	SD	Mean	SD	Mean	SD
C14:0	3·4	1·19	3·3	1·13	2·1	0·59
C16:0	31·0	1·60	31·7	1·81	32·1	0·95
C16:1	6·3	1·44	6·4	1·24	6·0	1·55
C18:0	5·0	0·98	5·4	1·18	5·3	0·66
C18:1	43·6	2·43	43·4	3·21	44·3	2·02
C18:2	8·6	1·45	8·8	1·51	9·1	1·02
Others	2·0	1·76	1·1	1·05	1·0	0·72

Table 6. *Glycaemic stimulus, insulin response and lipaemic response of five subjects to each of the meals on diets 1 and 2*

	Glycaemic stimulus (mg-h)*		Insulin response (microunit-h)*		Lipaemic response (mg-h)*	
	Diet 1	Diet 2	Diet 1	Diet 2	Diet 1	Diet 2
Breakfast						
Mean	21·30	23·20	62·30	61·70	18·10	15·50
SEM	2·54	3·14	6·49	5·17	4·66	3·72
Lunch						
Mean	26·80	43·60	49·00	68·70	33·80	23·90
SEM	6·66	5·84	8·44	8·27	7·68	4·87
Supper						
Mean	29·10	31·25	35·90	47·90	7·10	3·60
SEM	4·68	6·98	4·16	14·00	5·47	2·23

* See Mann *et al.* (1971) for methods of calculation.

After lunch, however, on the two different diets when the stimuli differed, the glycaemic stimulus was significantly greater after the starch meal (containing potato) than after the sugar meal ($P < 0·05$) and similarly the total insulin response was significantly greater ($P < 0·001$). Total lipaemic response to the meal was significantly lower after the starch meal than after the meal on the sugar diet ($P < 0·05$). A smaller total lipaemic response reflected the more rapid clearing of ingested triglyceride. This was particularly evident from the individual triglyceride values for each sampling period.* All five subjects in whom these tests were carried out showed similar patterns.

After the supper meal on the two different diets, glycaemic stimulus and insulin response were once again higher after the starch meal than after the sugar meal. This time the differences were not statistically significant; only three of the five subjects showed this pattern. The lipaemic responses to both meals were very small after the supper meal on both diets. Only 10 g butter were given with these meals.

* These results are available from the Editorial Office.

when giving a 500 kcal diet to overweight subjects. On more physiological weight-reducing diets, most investigators have found that weight loss was associated with a fall in serum cholesterol concentration (Walker, Lawry, Love, Mann, Levine & Stare, 1953; Joliffe, Rinzler & Archer, 1962; Greenberg & Elkins, 1964; Galbraith, Connor & Stone, 1966). But in three of the studies the diets had a fairly low total fat content (Walker *et al.* 1953; Joliffe *et al.* 1962; Greenberg *et al.* 1964) and a high proportion of polyunsaturated fat. Other investigators have reported no change in serum cholesterol concentrations on weight-reducing diets (Moore, Fryer, Young & Maynard, 1953; Moore, Young & Maynard, 1954; Caldwell, Watson, Green, Florin, Braun & Bierenbaum, 1963). Two of them gave 80 g fat/d (Moore *et al.* 1953; Moore *et al.* 1954) and the third gave 40 g/d (Caldwell *et al.* 1963). Elevated serum cholesterol concentrations have been reported in anorexia nervosa (Blendis & Crisp, 1968).

In the present experiments total calories were reduced in diet 3, but the amount of fat given (which was nearly all saturated) remained unchanged, so that the proportion of total daily calories provided by fat actually increased from 30 to 39%. It may be that this is an important determinant, but further study is clearly required to determine the cause of the elevated cholesterol levels during this reduced calorie dietary period.

In this study there were no differences in the fatty acid patterns of serum triglycerides and total serum lipids on the different diets. We suggest that differences reported by other workers (Macdonald & Braithwaite, 1964; Kuo & Bassett, 1965) occur only when starch and sucrose are given and exchanged at very high proportions of total calories.

Measurement of the glycaemic stimulus, insulin response and lipaemic response to the meals on the different diets provided interesting results. Unfortunately these tests were carried out on only five of the nine subjects. They had to be carried out on selected subjects whose veins were not too difficult to puncture since a large number of venepunctures were required.

Fasting serum insulin concentrations did not differ significantly on diets 1 and 2, thus confirming the results of Dunnigan *et al.* (1970). Glycaemic stimulus, insulin response and lipaemic response to the breakfasts were virtually identical on the two different diets. The actual meal was the same on the two diets, thus showing that these responses to a physiological stimulus (such as breakfast) do not differ significantly when a diet containing an ordinary amount of sucrose is compared with one containing complex carbohydrates.

A different pattern was observed after lunch and supper when the effects of potato and rice were compared with those of sucrose. From our earlier study (Mann *et al.* 1971) and the results of other workers (Grodsky, Batts, Bennett, Vcella, McWilliams & Smith, 1963; Swan, Davidson & Albrink, 1966), it was expected that higher concentrations of serum insulin might be found after the complex carbohydrates, since these consist of polymers of glucose. After digestion, therefore, they would be expected to be stronger stimulators of insulin secretion than an isocaloric amount of sucrose, which is digested to glucose and fructose – the latter a very poor insulin stimulator. After the midday meals, when potato was compared with sucrose, this was in fact found to be

The insulin response seemed to be related to the glycaemic stimulus in that there was no significant difference between ratios of insulin response to glycaemic stimulus on either diet when different glycaemic stimuli were obtained with different meals. The mean ratios were 2·48 (±0·38) on diet 1 and 2·21 (±0·29) on diet 2. Fasting serum insulin concentrations did not differ significantly on diets 1 and 2 (3·6±0·68 and 4·0±0·71 microunits per ml respectively).

DISCUSSION

This study has shown that, when sucrose (in amounts commonly eaten) was isocalorically replaced by complex carbohydrates, there was no significant change in the fasting serum lipid levels of normolipaemic adult males. Failure to have replaced, during the period on diet 1, the small quantity of protein present in the rice and potato of diet 2 for some subjects appears to have made no difference to fasting serum cholesterol and triglyceride concentrations. However, when the sucrose was eliminated from the diet and the 532 kcal (2226 kJ) therefrom not replaced, certain changes were noted. Mean serum triglyceride concentrations had started to fall by the 9th day after the commencement of this reduced-caloric diet. The mean triglyceride concentrations for the last 3 d on diet 3 were significantly lower than on the other two diets. This change in serum triglyceride only took place in those subjects whose fasting levels were greater than 90 mg/100 ml. On the other hand, serum cholesterol concentrations for the last 3 d were significantly increased on the reduced-calorie diet when compared with the values on diets 1 and 2, between which they did not differ significantly. These serum lipid changes coincided with the significant weight reduction which occurred during the third dietary period.

The fall in serum triglyceride concentration on diet 3, after values had remained unchanged on diets 1 and 2, supports the suggestion that changes in calorie balance can explain the observed differences in fasting serum lipids when complex carbohydrate replaces sucrose (Antonis *et al.* 1968; Porte, Bierman & Bagdade, 1966). Several of the subjects in the present study felt that they were being given too much to eat when switched from diet 1 to diet 2. Starch-containing foods are more bulky than sucrose and it is probably difficult for an individual voluntarily to replace all his dietary sucrose with starch.

It has been claimed that substitution of sucrose for starch causes elevation of serum lipids when the dietary fat is saturated (Stoffel *et al.* 1959; Macdonald, 1967; Antar, Little, Lucas, Buckley & Caima, 1970). However, in the present experiments most of the fat (provided as butter) was saturated, yet fasting serum lipids were not affected by sucrose, possibly because the sucrose was given in more physiological amounts.

The increase in serum cholesterol during the third dietary period was unexpected. Several workers have demonstrated elevated serum cholesterol concentrations during acute starvation (Bloom, Azar & Clark, 1966; Ende, 1960), with the highest values 72 and 96 h after the commencement of starvation. Others (Kaminen & Miettinen, 1963) found no significant increase in serum cholesterol over the same time-interval

10. Significantly higher insulin concentrations were found on the starch diet. As in our previous study (Mann *et al.* 1971), the degree of lipaemia was significantly smaller after the starch meals when the insulin response was greater. This smaller lipaemic response after the starch meal appeared to be due to more rapid clearing of ingested fat, resulting from the higher levels of serum insulin, which potentiates clearing factor, lipoprotein lipase (Kessler, 1962, 1963; Nikkilä, 1969).

As in the previous experiment (Mann *et al.* 1971), the differences in insulin response appeared to be due to differences in glycaemic stimuli in that there was no significant difference between the ratios of insulin response to glycaemic stimulus on the two different diets when different stimuli were obtained.

At the evening meals with rice and sucrose, the mean values for glycaemic stimuli and insulin responses were once again higher after the starch meal than after the sucrose, but only three of the five subjects showed this pattern and the differences were not statistically significant. Lipaemia was very small after supper on both diets, because only 10 g butter were given.

It appears therefore that the differences in insulin secretion and triglyceride clearing after meals which occur when starch replaces sucrose in the diet are not sufficient to alter fasting serum lipid concentrations and fatty acid patterns when the sucrose is given at levels ordinarily consumed.

We are very grateful to the volunteers for their co-operation. Mrs E. Manning assisted with the preparation of the diets. Mr R. M. Stegen of the Department of Mechanical Engineering, University of Cape Town, was responsible for the bomb calorimetry. We wish to thank Dr B. L. Pimstone for serum insulin determinations and Professor W. P. U. Jackson for determinations of blood sugars. Technical assistance was provided by Mrs D. Hendricks and Messrs S. Hendricks and M. Samuels. Financial support was received from the South African Medical Research Council, U.S. Public Health Service Grant AM-03995 and the South African Sugar Association.

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FURTHER STUDIES ON AMYLASE BIOSYNTHESIS BY PANCREAS OF RATS FED ON A STARCH-RICH OR A CASEIN-RICH DIET.

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Previous investigations (Reboud *et al*, 1962. Ben Abdeljlil *et al*, 1963) have shown that the specific activities of amylase and proteolytic enzymes are markedly different in pancreas and pancreatic juices of rats fed on a starch-rich or a casein-rich diet (rats G or F). It is of interest to know whether these variations are associated with a change in the activity or in the amounts of the enzymes concerned and, in the latter case, whether the rate of biosynthesis of these enzymes has been altered by the diet. The following experiments indicate that different amounts of amylase are actually present in pancreas of the two groups of animals and that the relative rates of C^{14}-valine incorporation into pure amylase also are different.

1. Purification of Rat pancreatic Amylase.

The left part of Fig. 1 gives the chromatographic diagram of rat pancreatic juice on DEAE-cellulose at pH 8.0. Amylase emerges quantitatively in the break-through peak with ribonuclease and lipase. The second step of the purification procedure is founded upon the selective precipitation on a micro scale of the complex amylase-glycogen (Lovtrup and Schramm 1962)

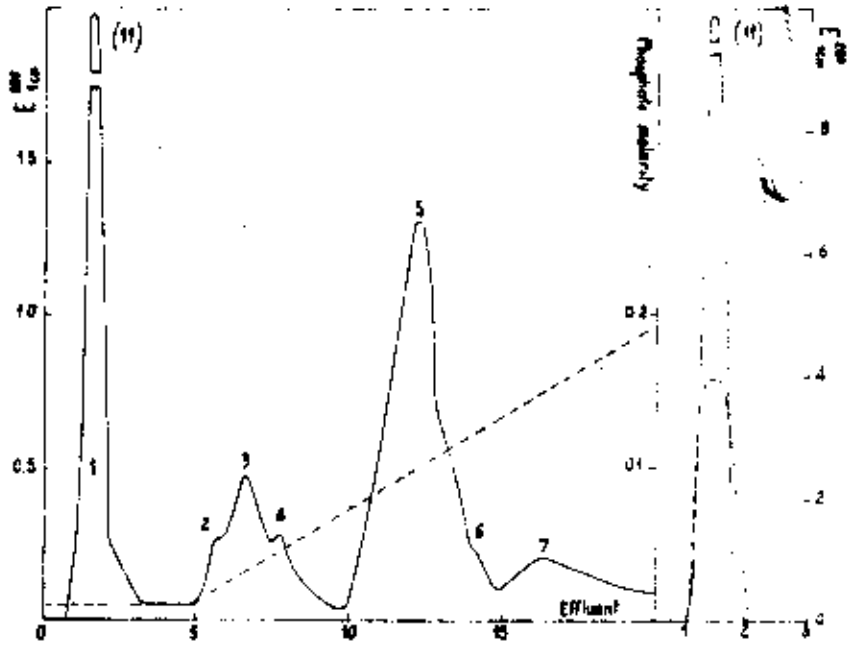

Fig. 1 : Chromatography of Rat pancreatic juice on DEAE-cellulose at pH 8.0.
The column is equilibrated with a 0.005 M phosphate buffer pH 8.0 containing DFP 10^{-4} M and eluted by a linear concentration gradient. On the left, complete diagram of juice G with one cationic peak (amylase, ribonuclease and lipase) and six anionic peaks ((2) unidentified, (3) carboxypeptidase B, (4) carboxypeptidase A, (5) chymotrypsinogen and trypsinogen, (6) procarboxypeptidase A, (7) procarboxypeptidase B). On the right, cationic peaks obtained with the same amount of proteins from juice G (solid line) and juice P (dotted line). The volume of the affluent is given in number of retention volumes of the column.

A pancreas G or P is homogenized in 9 volumes of 0.1 Phosphate buffer pH 8.0 containing DFP 10^{-3} M. The clear supernatant (105,000 g during 20 min.) is dialysed twice against 0.005 M phosphate pH 8.0 during 2 h. and passed through a 12 x 0.9 cm DEAE-cellulose column equilibrated with the same buffer. The break-through peak is dialysed overnight against a 0.02 M phosphate buffer pH 6.9 containing NaCl 0.006 M. The concentration is adjusted to 1.000 amylase units/ml. Ethanol (10 %) and glycogen

(4 mg per ml) are added. The precipitate is spun down, washed twice with a small volume of the buffer containing 10 % ethanol and finally dissolved in the buffer. In spite of the presence of glycogen, the preparations thus obtained from pancreas G and P may for all practical purposes be considered as pure. Their specific activities (amylase units per mg protein) are similar to the maximal value found for porcine pancreatic amylase (Fisher and de Mantmollin, 1951 ; Loyter and Schramm, 1962). By hydrazinolysis, they exclusively give rise to lysine as COOH-terminal residue*. The yield is almost quantitative.

The amount of pure enzyme obtained from each pancreas can be evaluated by determining the number of amylase units in the final solution, or its protein content by colorimetry or spectrophotometry. On the average, this amount is found to be 3 times higher with pancreas G than with the same weight of pancreas P. Moreover, the right part of Fig. 1 shows that the area of the break-through peak obtained by chromatography is nearly twice as large with juice G as with juice P for the same weight of total proteins in the juice. Since the peak contains, as judged by its specific activity, about 95 % amylase in the first case and 75 % in the second, the amylase amounts are again found in the ratio 3 : 1.

2. <u>Rate of Valine incorporation into pure Amylase by Pancreas G and P.</u>

20 μC of DL-C^{14}-valine (5 mC/mmole) are injected intraperitoneally into rats G and P. 15 min. later, the pancreas

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* These observations further suggest that pancreas G and P form

of each animal is excised and homogenized. Pure amylase (see above) and total proteins (Schneider, 1945) are prepared from the homogenate. Radioactivity (counts/min.) is determined in both preparations and calculated for the total amounts obtainable from 1 g fresh pancreas. The whole process is separately carried out on 10 pancreas G and 10 pancreas P. Average values and their ratios are given in Table I. Specific radioactivities (counts/min./mg) of amylase and total proteins are not indicated in the Table since, under the conditions used, they vary noticeably within the same group of animals. These variations may be due, at least partly, to dilution of newly synthesized molecules by different amounts of pre-existent material.

Table I

Incorporation of radioactive Valine into Amylase and total Proteins by Pancreas G or P during 15 min.

Diet	Radioactivity			Amylase specific activity (units per mg protein) in homogenates.
	Amylase	Total Proteins	Ratio	
Starch-rich (Pancreas G)	63,720	197,055	0.320 ± 0.006	150
Casein-rich (Pancreas P)	8,600	111,350	0.081 ± 0.007	51

Ratios similar to these of Table I are obtained when animals are killed 10, 15 or 25 min. after valine injection. Since

these ratios are independant from the respective size of the valine pool in pancreas G and P, they indicate that, for the same incorporation into total proteins, pancreas G incorporates about 4 times as much valine into amylase as pancreas P. If the assumption is made that the valine content is about the same in rat pancreatic amylase and total proteins, and that no amylase is being destroyed or exported during the assays, the ratios of Table I further suggest that, for 100 molecules of total proteins, pancreas G synthesizes 30 molecules of amylase and pancreas P, only 8. These values are in fair agreement with the specific activity and weight ratios determined above for amylase in pancreas G and P. They also agree with the fact that amylase represents about 25 % of the total proteins in pancreas G and 8 % in pancreas P.

Absolute rates of amylase biosynthesis in pancreas have not yet been determined. However, all results so far obtained are consistent with the view that a starch-rich diet induces in rat pancreas a more active biosynthesis of amylase than a casein-rich diet.

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COMMUNICATION TO THE EDITOR
Gelatinization of Starch during Cooking of Spaghetti

TO THE EDITOR:

As part of a study to determine the nature of starch in spaghetti at various stages of processing, an examination was made of the degree of gelatinization of the starch at various stages of cooking. Starch granules were examined under polarized light to determine the ratio of birefringent to nonbirefringent granules as an indication of degree of gelatinization (1).

To obtain an indication of whether protein content and durum variety affected the results, the samples used contained 8 and 17% protein and were made from two varieties, Leeds and Tunisian. These samples had previously been used by Matsuo et al. (2) to study the effect of protein level on cooking quality. Five-gram samples were cooked in 150 ml. of boiling tap water, with a portion of the sample being removed after 5 min. of cooking, and the remainder being cooked to *al dente* as determined by the Braibanti technique (3). In every case the samples were rinsed in cold water immediately after cooking. For control purposes, samples of the uncooked spaghetti were also soaked in water at room temperature for 4 hr., then examined microscopically.

For microscopic examination, samples were pressed between two Plexiglas plates and wet mounts made of a full-width section of the pressed strand. In the case of sample portions cooked 5 min., squeezing them between the plates revealed two or three areas or zones of cooking. Each of these zones was dissected out of the sample and examined separately under the microscope. All samples were examined under polarized light at 10X and 40X magnifications.

Microscopic examination of starch extracted from the control samples revealed almost no loss of birefringence, suggesting that virtually no gelatinization had taken place during soaking. As shown in Fig. 1, samples cooked for 5 min. showed zones of cooking. Unlike the high-protein samples which showed three zones, the low-protein samples showed only two. The outer zone for all samples showed

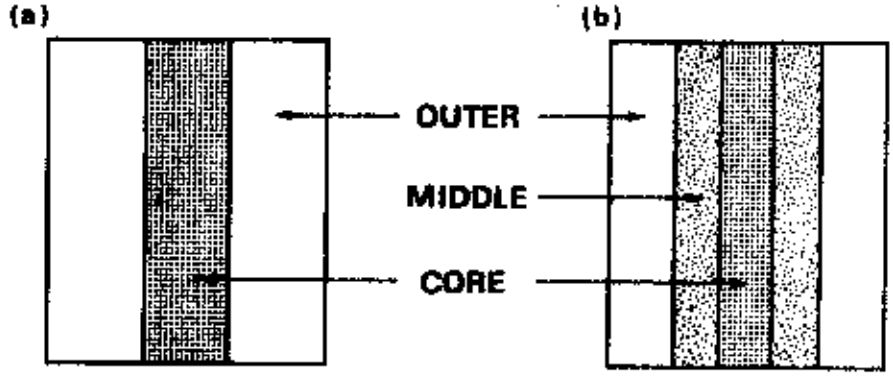

Fig. 1. Zones of cooking visible when the samples are pressed between Plexiglas plates after 5 min. of cooking. a, Low-protein (8%) sample; b, high-protein (17%) sample.

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almost total loss of birefringence while the inner core showed very little evidence of gelatinization. The middle zone in the high-protein samples showed intermediate loss of birefringence. After the samples had been cooked to *al dente*, loss of birefringence was almost total and no separate zones of cooking were observed.

These results suggest that gelatinization of starch takes place gradually during cooking of spaghetti. The presence and nature of the zones observed after 5 min. cooking suggest that gelatinization takes place in an inward direction and that water penetration, and hence cooking, is more rapid at lower protein levels. This latter suggestion is supported by the fact that the high-protein sample took 1.5 min. longer to cook than the low-protein sample for pasta made from the Tunisian durum. However, samples made from Leeds required the same amount of cooking time to reach *al dente*, regardless of the protein content. Use of a larger number of samples in future work should help to clarify this particular finding. In addition, it appears that although protein quality and quantity are thought to affect the cooking quality of pasta, the end point in cooking is determined by examination of the starch.

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MEDICAL INTELLIGENCE

PAROTID ENLARGEMENT RESULTING FROM EXCESSIVE INGESTION OF STARCH*

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BILATERAL, noninflammatory enlargement of the parotid glands has been described in association with a variety of nutritional disturbances. For many years it has frequently been reported among the acutely or chronically malnourished peoples of Europe, Africa and Asia. Although the condition has not been as thoroughly investigated in the United States, Rothbell and Duggan,[1,2] in reviewing the world literature, also reported its presence in 50 of their own patients, most of whom were known to have either liver disease, diabetes mellitus, obesity or combinations of these states. They considered its significance much the same as that of fatty infiltration of the liver, "representing a disturbance of nutrition or metabolism without defining the disturbance."

The patient described below is unique in that the parotid enlargement developed as the result of a diet composed almost entirely of laundry starch. Although this particular dietary aberration is rare in the northern United States, it has been commonly reported among the Negroes of the South, particularly among pregnant women.

CASE REPORT

M.W., a 36-year-old Negro housewife, was admitted to the New York Hospital because of swollen parotid glands. She had been in excellent health until approximately 8 months previously, when she noted the appearance of bilateral painless swellings at the angles of the jaw. This had remained

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essentially unchanged until admission. In association with it, she had noted excessive salivation to such an extent that she often awoke to find her pillow wet. She had no other complaints and otherwise felt quite well. There was no history of alcoholism, liver disease or diabetes mellitus.

The past history was significant in that she had been eating large amounts of laundry starch, a habit she had acquired during her last pregnancy, 3 years before admission. She was unable to break herself of the habit and had begun consuming increasingly large amounts. In the year before admission she had been eating a full box of starch daily. It had become her principal dietary constituent, and was supplemented only by an occasional sandwich or piece of meat.

Physical examination revealed a well developed, apparently well nourished woman with bilateral, diffuse, nontender enlargement of the parotid glands. Stensen's duct on the right side appeared slightly hypertrophied although the left side was normal. The submaxillary and sublingual glands were not enlarged. Lacrimation appeared normal, but salivation was excessive. There was no hepatomegaly, spider angiomas, ascites or edema.

The hemoglobin was 10.0 gm. per 100 ml., and the hematocrit 33 per cent. The red blood cells were hypochromic and microcytic. The fasting blood sugar was 73 mg. per 100 ml., with a normal glucose tolerance curve. The bromsulfalein retention was 2.9 per cent in 45 minutes; the total protein was 7.1 gm. per 100 ml. (albumin of 4.4 gm. and globulin of 2.7 gm.). The serum amylase was 185 units, and the salivary amylase was 147 units. Other normal laboratory findings included the blood urea nitrogen, serum calcium, phosphorus, alkaline phosphatase, bilirubin, cephalin flocculation, lipase and casoteme. An electrocardiogram was normal. X-ray films of the chest were unremarkable, with no evidence of sarcoidosis.

A sialogram of the left parotid gland was normal. Biopsy of the right parotid gland (Fig. 1) showed histologically unremarkable glandular tissue, separated by delicate fibrous connective-tissue septums and trabeculae, with minimal ductal dilatation and lymphocytic infiltration. There was no necrosis, granuloma formation or foreign-body giant cells.

The final impression was that the parotid enlargement was the result of the starch ingestion and that other known causes of Mikulicz's syndrome (for example, malignant lymphoma, Boeck's sarcoid, tuberculosis, leukemia and Sjögren's syndrome) had been excluded as diagnostic possibilities. The patient was discharged from the hospital with instructions to discontinue the starch eating and to resume a balanced diet. Although she was unable to eliminate the starch entirely, she did limit it to an occasional indulgence. She adopted a more normal dietary pattern, and within 2 months, the parotid enlargement had virtually disappeared.

Discussion

Ferguson and Keaton[8] have described the common practice among Caribbean and southern Negroes of ingesting large amounts of clay and laundry starch during pregnancy. In 1 study 25 per cent of pregnant women in Mississippi admitted to eating clay, and 39 per cent said they had eaten laundry starch. The only explanation offered is that it is one of the so-called "cravings" of pregnancy and a custom that has been passed down through the generations. Although the practice usually ends with the pregnancy, many women continue to eat the starch long afterward. However, it is most unusual for the clay or starch to become the principal dietary constituent, as in the present case.

As far as is known this patient is the first in whom parotid enlargement developed with this excessive ingestion of starch. However, the literature describes parallel situations. Isolated cases of parotid enlargement have been associated with other bizarre dietary histories. For example, the condition has been described in a man who lived principally on biscuits made of flour and baking soda and in another man who ate nothing but white potatoes.[4]

Several theories about the etiology of this condition have been put forth. Some authors believe that it is a response to "work" from ingestion of a high-carbohydrate diet. Several observations have lent support to this theory. It is known, for example, that parotid enlargement may be one of the first signs of improvement in the treatment of children with kwashiorkor.[5] German soldiers returning from Russian war camps acquired the condition only after several weeks of large food intake, often totaling as much as 7000 calories per day. Bonnin et al.[6] presented histologic evidence of hypersecretion and a high rate of amylase production in a group of cirrhotic patients with parotid enlargement.

On the other hand, protein deprivation and hypoproteinemia are commonly found in the background

Figure 1. *Photomicrograph of the Biopsy Specimen from the Parotid Gland (X100).*

There is normal glandular architecture, with minimal ductal dilatation and periductal lymphocytic infiltration.

of these patients. The diversity of dietary deficiencies has led to the supposition that the parotid change is a nonspecific result of malnutrition.[2]

The patient under discussion is interesting in that she had no signs of malnutrition, except, perhaps, for the iron-deficiency anemia. She had no evidence of diabetes mellitus, liver disease, avitaminosis or hypoproteinemia. The history of ptyalism appears to support the theory of excessive carbohydrate stimulation. Pavlov remarked upon the adaptability or "purposeful character" of the salivary reflex.[7] Both the quantity and the nature of the salivary juice are adapted to the particular substance initiating the reflex. For example, if a piece of meat is fed to a dog, it is quickly swallowed. The saliva serves as a lubricant. Accordingly, a highly viscous juice, rich in mucin, is produced. On the other hand, a diet rich

in carbohydrate will cause the dog to produce a saliva that is relatively richer in ptyalin and poorer in mucin, a characteristic of parotid saliva. It may readily be assumed that a daily diet of laundry starch could have initiated considerable stimulus to the parotid glands, with resultant enlargement on the basis of "work hypertrophy."

It need not be assumed that the etiology of parotid enlargement is invariably the same in cases of starvation, dietary aberrations, obesity, cirrhosis and diabetes mellitus. One can merely state that the abnormality in this particular patient is related to her extraordinary dietary pattern. The value of obtaining a complete nutritional history is clearly demonstrated. One should be alerted to the possible presence of the condition when other "starch eaters" are encountered.

Summary

A patient is described in whom noninflammatory parotid enlargement developed on a diet of laundry starch. Reference is made to the relation of other nutritional disturbances to this syndrome. A work-hypertrophy mechanism is suspected in the present case.

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Comparison of the lipid compositions of the aortic lesions in rabbits given different atherogenic diets

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1. The compositions of the lipids in the atheromatous lesions of rabbits have been studied in an experiment in which two groups of rabbits (twelve/group) were given different atherogenic diets for a period of 38 weeks. One group of rabbits was given a diet in which 35 % of the total calories was derived from butterfat and the other group was given a diet in which 48 % of the total calories was derived from starch.

2. At the end of the feeding period the rabbits were killed and the atheromatous plaques were dissected out from the aortic intima of each rabbit. The lipid compositions of the aortic lesions were determined and the results compared with the compositions of the plasma lipids.

3. In the lipids of the aortic lesions in the rabbits given the high-butterfat diet, the proportions of cholesterol and cholesterol esters were higher and the proportion of triglycerides was lower than in the lipids of the aortic lesions in the rabbits given the high-starch diet. Irrespective of dietary treatment, free cholesterol constituted a higher proportion of the lesion lipids than it did of the plasma lipids.

4. In the rabbits on either dietary treatment, the lesion cholesterol esters contained higher concentrations of oleic acid and lower concentrations of linoleic acid than did the plasma cholesterol esters. In the lesion phospholipids there were higher concentrations of palmitic, palmitoleic, stearic and oleic acids and lower concentrations of linoleic and arachidonic acids than in the plasma phospholipids.

5. In both groups of rabbits the phospholipids of the aortic lesions contained higher proportions of cephalin and sphingomyelin and lower proportions of lecithin than did the plasma phospholipids.

6. The results of this investigation are discussed in relation to the possible origins of the lipids in the aortic lesions of the experimental rabbits.

The work of Moore & Kon (1963) and Moore & Williams (1964*b*) has shown that rabbits develop aortic atherosis when given semi-purified diets containing no added cholesterol but in which the major proportion of the total calories is supplied either as butterfat or starch. The histological appearances of the atheromatous lesions in the aortas of the rabbits given the high-butterfat diet or the high-starch diet have been described by Moore & Williams (1964*b*). These atheromatous lesions resemble the early aortic lesions observed in man (Constantinides, Booth & Carlson, 1960). When examined by ordinary light, histological sections of the aortic lesions obtained from the rabbits on either dietary treatment appeared similar with respect to the intensity of the Sudan IV stain that had been taken up by the lipid deposits. This indicated that similar amounts of total lipid had been deposited in the aortic lesions of the rabbits given either of the two atherogenic diets. However, when the sections were examined by polarized light, the concentration of birefringent material in the aortic lesions of the rabbits given the high-butterfat diet appeared considerably greater than that in the aortic lesions of the rabbits given the high-starch diet (Moore, unpublished observations). Although such observations must be interpreted with extreme caution, it

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seemed possible that in the aortic lesions of the rabbits given the high-butterfat diet cholesterol constituted a higher proportion of the total lipid than it did in the aortic lesions of the rabbits given the high-starch diet. To investigate this possibility the compositions of the lipids extracted from the aortic lesions of rabbits given the high-butterfat diet or high-starch diet have been determined. The results of this study are now reported together with a comparison of the compositions of the lesion and plasma lipids obtained from rabbits on either of the two dietary treatments.

EXPERIMENTAL

Rabbits, diets and experimental procedure

Twenty-four male New Zealand White rabbits of one strain were obtained at the age of 6 months from a local breeder. On arrival at the laboratory, the animals were housed in individual metal metabolism cages and were given a commercial rabbit diet for a short period until they became accustomed to their surroundings. The rabbits were then randomly divided into two groups of twelve each and the commercial diet was gradually replaced by the two experimental diets during a 4-week period by which time all the animals were readily consuming the experimental diets. The experimental diets were given to the animals for a period of 38 weeks. Throughout the whole experiment, the rabbits were given food and water *ad lib.*

The percentage composition of the high-butterfat diet was: butterfat 20, wheat starch (Starch Products Ltd, Slough, Bucks.) 16·3, sucrose 10, casein (lactic acid casein; Glaxo Research Ltd, Greenford, Middx.) 25·0, Solkafloc (grade BW 40; Johnsen, Jorgensen and Wettre Ltd, London, EC4) 19·0, methyl cellulose (Celacol M.450; J. M. Steel and Co. Ltd, London, WC2) 1·0, potassium acetate 2·5, magnesium oxide 0·5, sodium chloride 0·7, choline chloride 0·5, salt mixture 4·0 and vitamin mixture 0·5. The percentage composition of the high-starch diet was: maize oil 0·4, wheat starch 48·1, sucrose 8·1, casein 20·2, Solkafloc 15·4, methyl cellulose 0·8, potassium acetate 2·0, magnesium oxide 0·4, sodium chloride 0·6, choline chloride 0·4, salt mixture 3·2 and vitamin mixture 0·4. No cholesterol was added to either of the two experimental diets. The methods of preparing the butterfat and pelleting the diets have been given by Moore & Williams (1964*b*), as have the compositions of the salt and vitamin mixtures. As explained by Moore & Williams (1964*b*), these two experimental diets were designed so that the ratio of linoleic acid to gross energy was the same for each diet.

At the end of the feeding period the animals were killed by a blow on the head and as rapidly as possible thereafter, the aorta, extending to the point of division into the two common iliac arteries, was removed from each rabbit.

Treatment of tissues and methods of analysis

The aortas were cut open longitudinally and the intimal surfaces were washed briefly with 0·9% (w/v) sodium chloride solution. The intimal layer, including the internal elastic membrane, was removed from each aorta and the atheromatous plaques were dissected out from the normal intimal tissues. To obtain sufficient lipid

for analysis, the atheromatous plaques obtained from the aortas of the rabbits on each dietary treatment were pooled into four samples so that each pooled sample contained the atheromatous plaques obtained from three rabbits.

The lipids were extracted from the pooled samples of atheromatous lesions by the method of Folch, Lees & Stanley (1957). The methods used for the fractionation and analysis of cholesterol, cholesterol esters, triglycerides, unesterified fatty acids and total phospholipids in the eight samples of lesion lipids have been given in detail by Moore (1962), Moore & Doran (1962) and Moore & Williams (1966*a*). When these analyses were complete, the remainder of the four lipid extracts obtained from the aortic lesions of the rabbits on each dietary treatment were combined so that the lesion phospholipids could be examined in greater detail. The phospholipids in the two combined extracts were fractionated and analysed by the methods described by Noble & Moore (1965). The fatty-acid compositions of the various lipid fractions were determined as described by Moore & Williams (1963, 1964*a*).

RESULTS

As observed in previous experiments (Moore & Kon, 1963; Moore & Williams, 1964*b*), the degree of aortic atherosis in the rabbits given the high-butterfat diet was the same as that in the rabbits given the high-starch diet. In each group of rabbits the aortic lesions were confined to the intima, and histological examination revealed the presence of extensive amounts of lipid deposited between the internal elastic membrane and the endothelial surface. This lipid material appeared to be present either extracellularly as fine droplets scattered throughout the ground substance or intracellularly as accumulations in lipid-laden phagocytes. The atheromatous lesions in both groups of rabbits appeared identical when sections were examined under ordinary light, but when sections were examined under polarized light the lesions in the aortas of the rabbits given the high-butterfat diet appeared to contain higher concentrations of birefringent material.

In Tables 1–5 the results for the various lipid components of the aortic lesions obtained from the rabbits on each dietary treatment are given as the means and standard errors of the means of four pooled samples of lesion lipids. For comparison, mean values for the corresponding lipid components of the plasma of rabbits given either the high-butterfat diet or the high-starch diet are also presented in Tables 1–5. It must be emphasized, however, that these mean values for the plasma lipids were obtained from previous experiments, the detailed results of which have appeared in earlier publications (Moore & Williams, 1964*c*, *d*, 1965, 1966*a*, *b*). Nevertheless, comparison of the compositions of the plasma and lesion lipids seemed justified since, in the previous experiments and in the one now reported, the compositions of the corresponding experimental diets were identical, as were the breed and strain of experimental rabbits, the age of the animals at the beginning of the experiment and the duration of the feeding period. Moreover, it has been observed in the author's laboratory that the composition of the plasma lipids of rabbits given a particular diet does not differ appreciably from experiment to experiment. The mean concentrations

of total lipid and total cholesterol in the plasma of the rabbits given the high-butterfat diet were 451 and 151 mg/100 ml respectively. The mean concentrations of total lipid and total cholesterol in the plasma of the rabbits given the high-starch diet were 262 and 85·4 mg/100 ml respectively. The values for the plasma lipids of the rabbits given the high-butterfat diet are means derived from a group of thirteen animals, whereas the corresponding values for the rabbits given the high-starch diet are means derived from a group of twelve animals.

Table 1. *Compositions* of the lipids present in the aortic lesions† and plasma‡ of rabbits given different atherogenic diets*

Constituent	Butterfat diet		Starch diet	
	Aortic lesions	Plasma	Aortic lesions	Plasma
Free cholesterol	19·0 ± 0·89	8·6	12·5 ± 1·14	9·6
Cholesterol esters	34·0 ± 1·45	38·9	24·5 ± 1·10	37·4
Triglycerides	11·8 ± 1·46	14·6	28·3 ± 0·97	22·6
Unesterified fatty acids	4·8 ± 0·52	3·6	3·9 ± 0·73	3·8
Phospholipids	30·6 ± 2·73	34·3	30·9 ± 0·76	26·7

* Expressed as percentages of the total lipid.
† Mean values with their standard errors.
‡ Values taken from Moore & Williams (1964*b*, *c*).

The compositions of the lipids isolated from the aortic lesions and the plasma of rabbits given the high-butterfat diet or the high-starch diet are presented in Table 1. Cholesterol and cholesterol esters together accounted for over 50 % of the lesion lipids obtained from the aortas of the rabbits given the high-butterfat diet but for only 37 % of the lesion lipids obtained from the aortas of the rabbits given the high-starch diet. The proportion of triglycerides in the lipids of the aortic lesions of the rabbits given the high-starch diet was considerably greater than that in the lipids of the aortic lesions of the rabbits given the high-butterfat diet. It should be noted that the proportion of triglycerides in the total plasma lipids of the rabbits given the high-starch diet was also higher than that in the plasma lipids of the rabbits given the high-butterfat diet. The lipids extracted from the aortic lesions in both groups of rabbits contained the same proportion of total phospholipids and similar proportions of unesterified fatty acids. In the rabbits on both dietary treatments about 70 % of the plasma cholesterol occurred as cholesterol esters, whereas only about 50 % of the lesion cholesterol was present in the esterified form. Irrespective of dietary treatment, free cholesterol constituted a higher proportion of the lesion lipids than it did of the plasma lipids.

The fatty-acid compositions of the cholesterol ester fractions present in the aortic lesions and in the plasma of rabbits given either of the two atherogenic diets are presented in Table 2 in which, and elsewhere in this paper, the shorthand designation suggested by Farquhar, Insull, Rosen, Stoffel & Ahrens (1959) is used to denote individual fatty acids. The fatty acids listed in Table 2 (and in Tables 3, 4 and 5) accounted for about 95 % of the total fatty acids present. The remaining 5 % consisted of trace concentrations of fatty acids that were tentatively identified as 15:0, 17:0,

17:0 br, 18:3, 20:1, 20:2 and 20:3. There was a broad similarity in the fatty-acid compositions of the cholesterol esters in the aortic lesions of the two groups of rabbits and, as in the plasma cholesterol esters, oleic acid was the major constituent fatty acid. However, in the rabbits on each dietary treatment, the concentrations of oleic acid in the lesion cholesterol esters were greater than the concentrations of this fatty acid in the plasma cholesterol esters. On the other hand, the concentrations of linoleic acid in the lesion cholesterol esters were less than the concentrations of this dienoic acid in the plasma cholesterol esters.

Table 2. *Fatty-acid compositions* of the cholesterol esters isolated from the aortic lesions† and plasma of rabbits given different atherogenic diets*

Fatty acid	Butterfat diet		Starch diet	
	Aortic lesions	Plasma	Aortic lesions	Plasma
16:0	16·9 ± 1·40	18·1	16·9 ± 0·62	17·9
16:1	3·2 ± 0·32	3·5	5·1 ± 0·32	6·7
18:0	6·3 ± 0·41	5·0	4·7 ± 0·37	3·8
18:1	61·6 ± 1·69	50·6	55·4 ± 0·44	45·8
18:2	5·8 ± 0·45	16·1	11·8 ± 0·41	20·0
20:4	0·9 ± 0·26	0·9	1·0 ± 0·15	0·9

* Weight percentages of the total fatty acids.
† Mean values with their standard errors.

Table 3. *Fatty-acid compositions* of the triglycerides isolated from the aortic lesions† and plasma of rabbits given different atherogenic diets*

Fatty acid	Butterfat diet		Starch diet	
	Aortic lesions	Plasma	Aortic lesions	Plasma
14:0	6·5 ± 0·56	6·5	2·7 ± 0·22	2·7
16:0	31·0 ± 0·57	31·9	38·2 ± 1·27	35·6
16:1	2·8 ± 0·33	2·9	5·7 ± 0·68	6·9
18:0	9·7 ± 0·04	7·8	8·3 ± 0·47	6·3
18:1	31·3 ± 0·41	28·6	33·2 ± 0·90	33·6
18:2	12·5 ± 0·75	14·4	8·4 ± 0·28	9·9
20:4	0·5 ± 0·14	1·0	0·3 ± 0·11	0·4

* Weight percentages of the total fatty acids.
† Mean values with their standard errors.

The fatty-acid compositions of the triglycerides isolated from the aortic lesions a from the plasma of rabbits given the different atherogenic diets are shown in Table There was an overall similarity in the fatty-acid compositions of the lesion and plas triglycerides but, in the rabbits given either the high-butterfat diet or the high-star diet, there was a suggestion that the lesion triglycerides contained slightly higl concentrations of stearic and slightly lower concentrations of linoleic acid than did th plasma triglycerides. There was also an overall similarity in the compositions of the unesterified fatty acids present in the aortic lesions and plasma of the rabbits on either dietary treatment, but the unesterified fatty acids in the aortic lesions appeared to

contain somewhat lower concentrations of palmitic acid and somewhat higher concentrations of oleic acid (Table 4).

The fatty-acid compositions of the total phospholipids present in the aortic lesions and plasma of rabbits given the high-butterfat diet or the high-starch diet are presented in Table 5. In the rabbits on either dietary treatment, saturated fatty acids accounted for over 60% of the total fatty acids present in the lesion phospholipids but for only about 44% of the total fatty acids present in the plasma phospholipids. In the lesion phospholipids there were higher concentrations of palmitic, palmitoleic,

Table 4. *Compositions* of the unesterified fatty acids present in the aortic lesions† and plasma of rabbits given different atherogenic diets*

Fatty acid	Butterfat diet		Starch diet	
	Aortic lesions	Plasma	Aortic lesions	Plasma
14:0	5·8 ± 0·73	5·0	4·2 ± 0·21	3·6
16:0	28·1 ± 0·98	34·2	30·0 ± 0·61	35·8
16:1	2·9 ± 0·43	2·7	5·7 ± 0·49	5·3
18:0	14·9 ± 0·40	14·9	13·1 ± 0·81	11·7
18:1	35·1 ± 1·23	29·1	33·3 ± 0·47	29·7
18:2	7·6 ± 0·46	8·5	8·1 ± 0·35	8·0
20:4	0·3 ± 0·04	0·2	0·5 ± 0·06	0·7

* Weight percentages of the total fatty acids.
† Mean values with their standard errors.

Table 5. *Fatty-acid compositions* of the total phospholipids isolated from the aortic lesions† and plasma of rabbits given different atherogenic diets*

Fatty acid	Butterfat diet		Starch diet	
	Aortic lesions	Plasma	Aortic lesions	Plasma
16:0	39·2 ± 2·30	25·8	36·2 ± 1·86	26·7
16:1	2·6 ± 0·27	0·8	3·4 ± 0·24	1·5
18:0	24·3 ± 1·88	18·3	25·4 ± 1·97	17·2
18:1	18·2 ± 1·05	25·9	18·9 ± 1·24	23·9
18:2	9·6 ± 1·02	22·2	9·5 ± 0·96	21·5
20:4	2·0 ± 0·16	3·3	2·1 ± 0·31	3·5

* Weight percentages of the total fatty acids.
† Mean values with their standard errors.

Table 6. *Compositions* of the phospholipids present in the aortic lesions and plasma of rabbits given different atherogenic diets*

Phospholipid	Butterfat diet		Starch diet	
	Aortic lesions	Plasma	Aortic lesions	Plasma
Cephalin	21·2	9·7	16·6	7·1
Lecithin	33·1	68·8	29·7	65·1
Sphingomyelin	36·4	12·9	43·3	16·2
Lysolecithin	9·3	8·6	10·3	11·5

* Expressed as percentages of the total phospholipids.

stearic and oleic acids and lower concentrations of linoleic and arachidonic acids than in the plasma phospholipids.

Since the values for the individual phospholipids present in the aortic lesions and plasma were, in each instance, obtained only from a single pooled sample of lesion lipid, the results given in Table 6 must be interpreted with some caution. Nevertheless, the results were reasonably consistent and it would appear that the lesion phospholipids contained markedly higher proportions of cephalin and sphingomyelin and lower proportions of lecithin than did the plasma phospholipids.

DISCUSSION

The fact that the lipids extracted from the aortic lesions of the rabbits given the high-starch diet contained a higher proportion of triglycerides and lower proportions of cholesterol and cholesterol esters than did the lipids extracted from the aortic lesions of the rabbits given the high-butterfat diet (Table 1) is consistent with the earlier observations (Moore, unpublished) on the differences in the amounts of birefringent material in the histological sections of the aortic lesions in rabbits given either of these two atherogenic diets. The results for the lipid composition of the aortic lesions of the rabbits given the high-butterfat diet (Table 1) were closely similar to those obtained by Böttcher, Woodford, Boelsma-van Houte & van Gent (1959) and Böttcher, Boelsma-van Houte, ter Haar Romeny-Wachter, Woodford & van Gent (1960) for the lipids extracted from human atheromatous aortas. Böttcher, Boelsma-van Houte *et al.* (1960) also showed that, in man, the proportion of triglycerides in the lipids of atheromatous coronary arteries was considerably greater than that in the lipids of atheromatous aortas. It is of interest to note that the lipid composition of the aortic lesions of the rabbits given the high-starch diet was almost identical to that reported by Böttcher, Boelsma-van Houte *et al.* (1960) for atheromatous coronary arteries in man. The results for the plasma and lesion lipids of rabbits (Table 1) are also in agreement with the results of Buck & Rossiter (1951) who showed that the ratio of free cholesterol to esterified cholesterol in the atheromatous lesions of human aortas was markedly higher than in the corresponding ratio in human plasma. In rabbits given a commercial diet supplemented with cholesterol and maize oil or with cholesterol and olive oil, Swell, Law & Treadwell (1962) found that the ratios of free cholesterol to esterified cholesterol in the lipids of the aortas were higher than the corresponding ratios in the serum lipids.

The results in Table 2 are in agreement with those of Zilversmit, Sweeley & Newburgh (1961) and Evrard, van den Bosch, de Somer & Joossens (1962) who found that the plasma cholesterol esters of rabbits given a diet supplemented with cholesterol contained higher concentrations of linoleic acid and lower concentrations of oleic acid than did the cholesterol esters of the aortas. Somewhat different results were reported by Swell, Law, Schools & Treadwell (1961) and Swell *et al.* (1962). Although these investigators found similar concentrations of oleic acid in the cholesterol esters of the liver and aortas of rabbits given diets supplemented with cholesterol, they noted that the serum cholesterol esters contained higher concentrations of linoleic acid and lower concentrations of palmitic acid than did the aortic cholesterol esters. The

differences in fatty-acid composition of the cholesterol esters in the plasma and aortic lesions of the rabbit (Table 2) are similar to the differences that have been reported by certain authorities to occur between the composition of the cholesterol esters in the serum or plasma and atheromatous lesions in man. For instance, Swell, Field, Schools & Treadwell (1960*a*, *b*) compared the fatty-acid compositions of the cholesterol esters obtained from the serum and from advanced lesions of the abdominal aortas of elderly male subjects with atherosclerosis and found that the lesion cholesterol esters contained higher concentrations of oleic acid and lower concentrations of linoleic acid than did the serum cholesterol esters. These findings of Swell *et al.* (1960*a*, *b*) are consistent with the earlier observations of Luddy, Barford, Riemenschneider & Evans (1958), Tuna, Reckers & Frantz (1958) and Lewis (1958). On the other hand, Wright, Pitt & Morton (1959) could find no differences in the concentrations of oleic acid in the cholesterol esters present in the plasma and aortic lesions of man. Nevertheless, Wright *et al.* (1959) did report that the plasma cholesterol esters contained higher concentrations of linoleic acid and lower concentrations of saturated fatty acids than did the lesion cholesterol esters. According to Lawrie (1963), there were no differences in the fatty-acid compositions of the cholesterol esters isolated from atherosclerotic plaques and from three different serum lipoprotein fractions in man. However, the plaque lipids analysed by Lawrie (1963) were obtained from fairly advanced lesions and Böttcher, Woodford, ter Haar Romeny-Wachter, Boelsma-van Houte & van Gent (1960) have shown that the concentration of linoleic acid in the aortic cholesterol esters of man increases with the degree of atherosclerotic involvement. It should be noted that there was no accumulation of trienoic acids in the cholesterol esters obtained from the aortic lesions of the rabbits on either dietary treatment (Table 2). Smith (1962) has reported an increased trienoic:tetraenoic acid ratio in the cholesterol esters of early atheromatous lesions in man. Although this observation suggests that there might have been a localized deficiency of essential fatty acids in the degenerating tissues the work of Geer & Malcom (1965) has shown that the trienoic acid that occurs in the cholesterol esters of atheromatous lesions in the human thoracic aorta is $\Delta^{8,11,14}$-eicosatrienoic acid and not $\Delta^{5,8,11}$-eicosatrienoic acid (the fatty acid that accumulates in tissues deficient in essential fatty acids). Since $\Delta^{8,11,14}$-eicosatrienoic acid is an intermediate in the synthesis of arachidonic acid from linoleic acid, it is not surprising that this trienoic acid did not accumulate in the lesion cholesterol esters of the rabbit. The enzyme system that synthesizes arachidonic acid from linoleic acid does not appear to be particularly active in rabbit tissues (Moore & Williams, 1964*c*, *d*, 1965).

The results for the plasma and lesion triglycerides of the experimental rabbits (Table 3) are consistent with the results reported by other investigators. In experiments with groups of rabbits given various diets supplemented with cholesterol Swell *et al.* (1961) and Swell *et al.* (1962) found that, for the animals on each dietary treatment, the fatty-acid composition of the serum triglycerides was almost identical to that of the triglycerides obtained from the atheromatous aortas. Böttcher, Woodford *et al.* (1960) compared their results for the triglycerides isolated from human atheromatous aortas with those obtained by Schrade, Böhle & Biegler (1959) for the plasma triglycerides of patients with atherosclerosis and noted a close similarity in the fatty

acid compositions of the plasma and aortic triglycerides. In agreement with these findings, Lawrie (1963) reported that the fatty-acid composition of the triglycerides obtained from the atherosclerotic plaques of human aortas was very similar to the fatty-acid composition of the triglycerides obtained from the various lipoprotein fractions in human serum.

In agreement with the results given in Table 5, Swell *et al.* (1961) have shown that, in rabbits given diets supplemented with cholesterol, the phospholipids of the aortic lesions contained higher concentrations of saturated fatty acids and lower concentrations of linoleic acid than did the serum phospholipids. These observations may be partly explained by the fact that the proportion of sphingomyelin in the lesion lipids was higher than that in the plasma lipids (Table 6). High concentrations of sphingomyelin have been reported to occur in the phospholipids of aortic lesions in the rabbit (Zilversmit *et al.* 1961) and in man (Weinhouse & Hirsch, 1940; Buck & Rossiter, 1951; Smith, 1959; Böttcher & van Gent, 1961). Zilversmit *et al.* (1961) have reported that the sphingomyelin isolated from the intimal lesions of rabbits given diets supplemented with cholesterol contains particularly high concentrations of palmitic acid and low concentrations of linoleic acid. Furthermore, the work of Zilversmit *et al.* (1961) has shown that the lecithin of the intimal lesions of rabbit aortas contained higher concentrations of saturated fatty acids and lower concentrations of linoleic acid than did the plasma lecithin.

Although there is considerable evidence from experiments with rabbits that the major proportion of the cholesterol present in atheromatous plaques is derived from the plasma rather than from synthesis in the tissues of the aorta (Biggs & Kritchevsky, 1951; Drury & Swell, 1960; Schwenk & Stevens, 1960), it is clear that a simple infiltration of blood lipids into the aortic intima cannot account for the results presented in Tables 1 and 2. However, the differences in the ratios of free cholesterol to esterified cholesterol in the plasma and lesions are in line with the experiments of Newman, McCandless & Zilversmit (1961) and Newman & Zilversmit (1962) which indicated that, in the rabbit, the rate of entry of free cholesterol from the plasma into the aortic intima was greater than that of esterified cholesterol. In spite of the similarities in the fatty-acid compositions of the plasma and lesions triglycerides (Table 3) and the fact that triglycerides constituted a higher proportion of the total lipid in both the plasma and the aortic lesions of the rabbits given the high-starch diet, the isotope experiments of Newman *et al.* (1961) would seem to indicate that the triglycerides present in the atheromatous aortas of rabbits given diets supplemented with cholesterol are synthesized *in situ* and are not derived from the plasma. A higher rate of triglyceride synthesis in the aortic lesions of the rabbits given the high-starch diet would be in accord with the many reports (e.g. Masoro, Chaikoff, Chernick & Felts, 1950) of enhanced fatty-acid synthesis in the tissues of various experimental animals given diets in which a high proportion of the total calories was derived from carbohydrate. Zilversmit, Shore & Ackerman (1954), Shore, Zilversmit and Ackerman (1955) and Newman *et al.* (1961) have shown that the phospholipids in the aortic lesions of the cholesterol-fed rabbit were derived mainly from synthesis in the aortic intima: only a small proportion of the lesion phospholipids was derived from the plasma. The

marked differences in the fatty-acid compositions of the total phospholipids (Table 5) and in the proportions of the individual phospholipids (Table 6) in the plasma and lesions of the experimental rabbits would thus appear to be a result of the differences in the metabolic origins of the plasma and lesion phospholipids.

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The effect of diet on the composition and positional distribution of the fatty acids in the triglycerides obtained from the adipose tissues of rabbits

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1. Five groups of male rabbits (six to eight per group) were given *ad lib.*, for a period of 6 months, diets that contained high levels of maize oil, butter, butterfat, hydrogenated coconut oil or starch. At the end of this period the rabbits were killed and the fatty acid compositions of the triglycerides obtained from the subcutaneous and perinephric adipose tissues were determined.

2. Irrespective of whether there had been a net gain or a net loss of adipose tissue during the feeding period, the fatty acid compositions of the diets were reflected to some extent in the fatty acid compositions of the adipose tissue triglycerides. The concentration of linoleic acid was unexpectedly high in the adipose tissue triglycerides of the rabbits given the diet containing hydrogenated coconut oil.

3. Significant subcutaneous–perinephric differences in fatty acid composition were invariably negative for lauric, myristic, palmitic and palmitoleic acids and positive for stearic, oleic, linoleic and linolenic acids.

4. Within each group of rabbits, the liver triglycerides contained higher concentrations of lauric, myristic, palmitic and palmitoleic acids and lower concentrations of stearic and oleic acids than did the adipose tissue triglycerides.

5. There was no marked preferential esterification of individual fatty acids in the α,α'- or β-positions of the triglycerides in the adipose tissues of the experimental rabbits. Only in the rabbits given the high-maize oil diet was there any differential distribution of linoleic acid between the α,α'- and β-positions. In this group of rabbits linoleic acid occurred in higher concentrations in the α,α'-position in both types of adipose tissue triglycerides.

In a previous publication, Moore & Williams (1964*a*) described the effects of diets containing high levels of maize oil, butter, butterfat, hydrogenated coconut oil or starch on the concentrations of cholesterol in the plasma, liver, heart and kidneys and on the degree of aortic atherosis in rabbits. This study has now been extended by a detailed investigation of the effects of these different diets on the fatty acid compositions of the triglycerides isolated from the adipose tissues of the same experimental rabbits. The results of this investigation are now reported together with a comparison of the fatty acid compositions of the triglycerides of the liver and adipose tissues.

EXPERIMENTAL

Rabbits, diets and experimental procedure

Forty male New Zealand White rabbits, obtained at the age of 6 months from a local breeder, were divided into five groups of eight each. The rabbits were housed in individual cages and were given food and water *ad lib.* The basal diet consisted (parts

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by weight) of starch 10·3, sucrose 10, casein 23·0, wheat straw (ground to pass through a 2 mm sieve) 19·0, methyl cellulose 1·0, potassium acetate 2·5, magnesium oxide [illegible], sodium chloride 0·7, choline chloride 0·3, salt mixture 4·0 and vitamin mixture 0·[illegible] making 80 parts in all. The compositions of the salt and vitamin mixture have been given by Moore & Williams (1964*a*). To 80 parts of this basal diet were added: for group 1, 20 parts maize oil; for group 2, 24 parts butter; for group 3, 20 parts butterfat; for group 4, 20 parts hydrogenated coconut oil; and for group 5, 1 part maize oil and 41·0 parts starch. The high-starch diet given to the rabbits in group 5 was devised so that the linoleic acid : gross energy ratio was the same as that of the diet containing 20 % butterfat given to the rabbits of group 3 (Moore & Williams, 1964*a*). The fatty

Table 1. *Fatty acid compositions (g/100 g diet) of the experimental diets*

Acid	Group 1 (high-maize oil diet)	Group 2 (butter diet)	Group 3 (butterfat diet)	Group 4 (coconut oil diet)	Group 5 (high-starch diet)
Lauric	0·03	0·18	0·18	0·09	0·22
Myristic	0·09	1·90	1·71	1·15	0·07
Palmitic	2·00	5·31	3·61	2·77	0·18
Palmitoleic	0·06	0·35	0·34	0·07	0·01
Stearic	0·27	4·14	[illegible]	3·44	0·03
Oleic	4·34	5·72	4·94	6·34	0·22
Linoleic + linolenic	10·61	0·53	0·57	[illegible]	0·44

acid compositions of the experimental diets are given in Table 1. These diets were given to the rabbits for a period of 9 months. Certain of the animals died during the experiment and the numbers that survived in each group are given in Table 2. At the end of the 9-month period, the animals were killed and the livers and samples of subcutaneous and perinephric adipose tissues were taken for analysis. The samples of subcutaneous adipose tissues were taken from the interscapular region.

A further six male New Zealand White rabbits (group 6) were obtained at the age of 6 months from the same local breeder. It was ascertained that, up to this age, these rabbits had been given the same commercial diet as had the animals of the previous consignment. On arrival at the laboratory, these six animals were killed and samples of subcutaneous and perinephric adipose tissues were taken for analysis. The mean weight (kg, ± SEM) of the rabbits in group 6 was 3·32 ± 0·18.

Extraction of lipids and methods of analysis

The lipids were extracted from the livers and adipose tissues by the method of Folch, Lees & Stanley (1957). To separate the triglycerides from the remaining lipid fractions, portions of the purified lipid extracts were then chromatographed on columns of silicic acid as described by Moore & Doran (1962). The fatty acid compositions of the triglycerides were then determined by the procedure given in detail by Moore & Williams (1964*b*). The fatty acid compositions of the adipose tissue triglycerides were determined on the samples obtained from each of the animals in groups 1–6, whereas the fatty acid compositions of the liver triglycerides were de-

determined on pooled samples obtained from each group (1–5) of rabbits. The positional distributions of the various fatty acids in the triglycerides extracted from each type of adipose tissue were determined on pooled samples of triglycerides obtained from each group (1–5) of rabbits. The technique of determining the distribution of the various fatty acids between the α,α'- and β-positions of the triglycerides was that of Coleman (1961).

Statistical analysis

Differences between dietary treatments. Within each fatty acid and type of adipose tissue, the range of concentration percentages was fairly small and an analysis of variance was carried out on the observed values to examine dietary differences. The tests of significance were based on the multiple-range test (Duncan, 1955).

Differences between tissues. For individual fatty acids, analyses of variance between the concentrations in the triglycerides of the subcutaneous and perinephric adipose tissues provided tests of group and of overall average differences.

Table 2. *Numbers of rabbits in each group and weights* (kg) of the rabbits at the beginning and end of the experiment*

Group no.	No. of rabbits group	Wt at beginning of expt	Wt at end of expt
1 (high-maize oil diet)	8	3·65 ± 0·23	[illegible] ± 0·16
2 (butter diet)	7	3·12 ± 0·27	3·68 ± 0·24
3 (butterfat diet)	6	3·63 ± 0·20	3·97 ± 0·23
4 (coconut oil diet)	6	3·87 ± 0·05	3·15 ± 0·11
5 (high-starch diet)	6	3·59 ± 0·14	3·93 ± 0·13

* Mean values with their standard errors.

RESULTS

With the exception of the rabbits given the diet containing hydrogenated coconut oil (group 4), all of the animals gained weight during the experiment (Table 2). The rabbits in group 4 lost weight progressively and this loss in weight was reflected by the smaller amounts of adipose tissue that were found in the animals in this group at the end of the experiment.

The fatty acid compositions of the triglycerides obtained from the subcutaneous and perinephric adipose tissues of the rabbits on the various dietary treatments are given in Tables 3 and 4. Lauric acid was found only in low concentrations in the adipose tissue triglycerides of all the experimental rabbits including those in group 4 that were given the diet containing hydrogenated coconut oil. However, the concentration of lauric acid in both the subcutaneous and perinephric triglycerides of the rabbits in group 4 was significantly higher than that in the corresponding triglycerides of the rabbits in the other groups (Tables 3 and 4). Higher levels of myristic acid were present in the diets containing butter, butterfat or hydrogenated coconut oil (Table 1) and the concentrations of myristic acid in the adipose tissue triglycerides of the rabbits in groups 2, 3 and 4 tended to be higher than those observed for the rabbits in groups 1 and 5, but, even in the rabbits of group 4, myristic acid did not constitute more than

10% of the fatty acids in the adipose tissue triglycerides. In both the subcutaneous and perinephric adipose tissues, palmitic acid accounted for about 25% of the triglyceride fatty acids in groups 2, 3, 4 and 5, but the concentration of palmitic acid in the adipose tissue triglycerides of the rabbits in group 1 was significantly lower than that found for all the other groups of rabbits. Palmitoleic acid occurred in significantly higher concentrations in the adipose tissue triglycerides of the rabbits in group 5 given the high-starch diet. The concentration of stearic acid in the subcutaneous trigly-

Table 3. *Fatty acid compositions (weight percentages of the total) of the triglycerides extracted from the subcutaneous adipose tissues of rabbits on the various dietary treatments*

(Mean values and standard errors of single observations are given)

Acid	Group 1 (high-maize oil diet)	Group 2 (butter diet)	Group 3 (butter-fat diet)	Group 4 (coconut oil diet)	Group 5 (high-starch diet)	SE (28 df)	Significance of differences*
Lauric	0·1	0·2	0·2	1·2	0·1	0·25	1 5 3 2 4
Myristic	0·8	5·5	4·7	5·7	2·6	0·77	1 5 3 2 4
Palmitic	12·8	24·2	25·2	23·9	25·0	2·52	1 4 2 5 3
Palmitoleic	2·1	4·0	4·6	2·6	8·0	1·26	1 4 2 3 5
Stearic	3·8	7·3	7·4	8·8	6·1	1·06	1 5 2 3 4
Oleic	32·4	43·4	41·5	30·3	41·2	2·80	4 1 5 3 2
Linoleic	45·4	7·8	11·4	22·4	14·3	3·02	2 3 5 4 1
Linolenic	0·6	0·9	1·2	1·8	0·6	0·39	1 5 2 3 4

The number of rabbits in each group is given in Table 2.

* The mean values differ significantly ($P < 0·01$) except when the group numbers share a common underlining.

Table 4. *Fatty acid compositions (weight percentages of the total) of the triglycerides extracted from the perinephric adipose tissues of rabbits on the various dietary treatments*

(Mean values and standard errors of single observations are given)

Acid	Group 1 (high-maize oil diet)	Group 2 (butter diet)	Group 3 (butter-fat diet)	Group 4 (coconut oil diet)	Group 5 (high-starch diet)	SE (28 df)	Significance of differences*
Lauric	0·2	0·3	0·3	2·4	0·2	0·89	1 5 2 3 4
Myristic	1·1	6·0	4·8	9·0	2·7	1·92	1 5 3 2 4
Palmitic	18·2	27·9	25·9	25·1	27·1	3·29	1 4 3 5 2
Palmitoleic	3·2	3·8	4·4	3·2	9·2	1·31	1 4 2 3 5
Stearic	2·8	7·5	7·2	8·3	4·4	1·27	1 5 3 2 4
Oleic	28·9	43·6	42·9	29·5	37·4	3·30	1 4 5 3 2
Linoleic	45·4	7·1	10·5	20·1	15·3	3·51	2 3 5 4 1
Linolenic	0·5	0·8	0·9	1·1	0·5	0·17	1 5 2 3 4

The number of rabbits in each group is given in Table 2.

* The mean values differ significantly ($P < 0·01$) except when the group numbers share a common underlining.

...rides of the rabbits in group 1 was significantly lower than that observed in the other groups of rabbits. Similar differences in the concentration of stearic acid occurred in the perinephric triglycerides, but the value obtained for the rabbits in group 1 did not differ significantly from that obtained for the rabbits in group 5. Oleic acid was the major fatty acid present in the triglycerides of both the subcutaneous and perinephric adipose tissues of the rabbits in groups 2, 3 and 5, but the triglycerides in the corresponding tissues of the rabbits in groups 1 and 4 contained significantly lower concentrations of oleic acid. The higher concentration of linoleic acid in the adipose tissue triglycerides of the rabbits in group 1 was undoubtedly a reflection of the high level of linoleic acid in the diet given to this group of rabbits. However, a most unexpected finding was the relatively high concentration of linoleic acid in the diet of the adipose tissue triglycerides of the rabbits in group 4 that were given the diet containing the lowest concentration (0·17 %) of linoleic acid.

Table 5. *Differences between fatty acid compositions (weight percentages of the total) of the triglycerides extracted from the subcutaneous and from the perinephric adipose tissues of rabbits on the various dietary treatments*

(Mean differences (subcutaneous–perinephric) and standard errors of single differences are given)

Acid	Group 1 (high-maize oil diet) (8)	Group 2 (butter diet) (7)	Group 3 (butterfat diet) (6)	Group 4 (coconut oil diet) (6)	Group 5 (high-starch diet) (6)	Groups 1–5 (all diets) (33)	SE (28 df)
Lauric	−0·1	−0·1	−0·1	−1·0**	−0·1	−0·3	0·87
Myristic	−0·3	−0·5	−0·1	−3·3***	−0·1	−0·8**	1·49
Palmitic	−5·4**	−3·7*	−0·7	−1·2	−2·1	−2·8**	4·43
Palmitoleic	−1·1***	0·2	0·2	−0·6	−1·2**	−0·5***	0·82
Stearic	1·0*	−0·2	0·2	0·5	1·7**	0·6**	1·39
Oleic	3·5*	−0·2	−1·4	0·8	5·8*	1·4*	3·86
Linoleic	0·0	0·7	0·9	2·3*	−1·0	0·5	2·57
Linolenic	0·1	0·1	0·3	0·7***	0·1	0·2**	0·45

Figures in parentheses are the numbers of rabbits.
* $0·05 > P > 0·01$; ** $0·01 > P > 0·001$; *** $0·001 > P$.

The results in Table 5 show that significant subcutaneous–perinephric differences were invariably negative for lauric, myristic, palmitic and palmitoleic acids and positive for stearic, oleic, linoleic and linolenic acids. The non-significant differences generally showed supporting negative or positive trends within each group of fatty acids.

The fatty acid compositions of the pooled samples of liver triglycerides obtained from the five groups of rabbits are given in Table 6. The effects of diet on the fatty acid composition of the liver triglycerides followed a pattern similar to that observed for the adipose tissue triglycerides. However, within each group, there were marked differences between the fatty acid compositions of the liver and adipose tissue triglycerides. In general, the liver triglycerides contained higher concentrations of lauric, myristic, palmitic and palmitoleic acids and lower concentrations of stearic and oleic acids than did the adipose tissue triglycerides. The high levels of lauric and myristic

acids in the diets given to the rabbits in groups 2, 3 and 4 were reflected to a great extent in the fatty acid compositions of the liver triglycerides. This was particularly noticeable in the rabbits in group 4 where the concentrations of lauric and myristic acids in the liver triglycerides were respectively 6 and 4 times greater than the corresponding concentrations of these fatty acids in the adipose tissue triglycerides. It should be noted that in the rabbits of group 4 the concentration of linoleic acid in the adipose tissue triglycerides was considerably greater than that in the liver triglycerides.

Table 6. *Fatty acid compositions (weight percentages of the total) of the liver triglycerides of rabbits on the various dietary treatments*

(Values are for pooled samples obtained from each group)

Acid	Group 1 (high-maize oil diet)	Group 2 (butter diet)	Group 3 (butterfat diet)	Group 4 (coconut oil diet)	Group 5 (high-starch diet)
Lauric	0·8	0·6	0·8	7·2	0·4
Myristic	4·5	8·7	11·8	23·4	6·2
Palmitic	26·8	37·5	38·8	31·8	40·8
Palmitoleic	2·4	5·2	5·7	3·8	9·5
Stearic	2·4	5·1	3·8	4·6	3·2
Oleic	19·4	32·6	29·4	20·6	25·6
Linoleic – linolenic	41·1	9·6	6·9	5·6	10·8
Arachidonic	0·6	0·5	0·5	0·4	1·2

The compositions of the fatty acids in the α,α'- and β-position of the pooled samples of subcutaneous or perinephric triglycerides obtained from the five groups of rabbits are given in Tables 7 and 8. These results show that in the adipose tissue triglycerides of the rabbit there is no marked preferential esterification of individual fatty acids in either the β- or the α,α'-positions in the triglyceride molecules. In all groups and in both types of adipose tissue triglycerides there was a tendency for myristic acid to be esterified preferentially in the β-position. Higher concentrations of palmitic acid were observed in the β-positions of the subcutaneous and perinephric triglycerides only in the rabbits of group 1, but higher concentrations of palmitoleic acid were found in the β-positions of the subcutaneous and perinephric triglycerides in the rabbits of both groups 1 and 5. Only in the rabbits given the diet containing 20 % maize oil was there any differential distribution of linoleic acid between the α,α'- and β-positions. In this group of rabbits, linoleic acid occurred in higher concentrations in the α,α'-position in both types of adipose tissue triglycerides.

The fatty acid compositions of the subcutaneous and perinephric triglycerides obtained from the sixth group of rabbits that were given a commercial diet and were killed at 6 months of age are given in Table 9. It should be noted that the concentrations of linoleic acid in the triglycerides of both types of adipose tissues were similar to the concentrations of linoleic acid in the adipose tissue triglycerides of the rabbits given the diet containing hydrogenated coconut oil (group 4, Tables 3 and 4).

Table 7. *Compositions (weight percentages of the total) of the total fatty acids and of the fatty acids in the α,α'- and β-positions of the triglycerides obtained from the subcutaneous adipose tissues of rabbits on the various dietary treatments*

(Values are for pooled samples obtained from each group)

Acid	Group 1 (high-maize oil diet)			Group 2 (butter diet)			Group 3 (butterfat diet)			Group 4 (coconut oil diet)			Group 5 (high-starch diet)		
	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position
Lauric	—	—	—	—	—		—	—	—	1·9	2·2	1·2	—	—	—
Myristic	1·2	0·2	3·2	3·9	4·8	9·6	6·2	4·4	9·8	7·9	7·4	8·9	3·7	3·9	5·3
Palmitic	15·9	14·7	18·4	29·5	30·2	28·1	29·9	30·7	28·3	26·8	28·2	24·0	31·2	32·6	28·4
Palmitoleic	2·2	1·6	3·5	3·6	3·5	3·8	3·4	3·4	3·4	2·3	2·0	2·9	8·1	6·2	11·8
Stearic	3·4	3·3	3·6	7·1	8·5	4·2	6·3	7·6	4·5	7·2	7·3	7·0	5·9	5·9	5·9
Oleic	35·3	36·5	32·9	40·6	39·8	42·1	40·8	41·5	39·4	32·0	30·7	33·5	38·5	39·2	37·1
Linoleic + linolenic	40·9	43·2	36·3	8·2	7·9	8·8	9·8	9·2	10·9	22·0	22·6	21·2	10·6	10·8	10·1

Table 8. *Compositions (weight percentages of the total) of the total fatty acids and of the fatty acids in the α,α'- and β-positions of the triglycerides obtained from the perinephric adipose tissues of rabbits on the various dietary treatments*

(Values are for pooled samples obtained from each group)

Acid	Group 1 (high-maize oil diet)			Group 2 (butter diet)			Group 3 (butterfat diet)			Group 4 (coconut oil diet)			Group 5 (high-starch diet)		
	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position	Total	α,α'-Position	β-Position
Lauric	—		—	—	—	—				2·0	2·4	1·4		—	—
Myristic	1·1	0·4	2·5	4·6	2·8	8·2	6·0	4·8	8·4	12·9	12·5	13·7	2·7	2·1	3·9
Palmitic	18·1	13·8	27·0	28·9	28·5	29·2	26·9	26·5	27·7	26·2	27·0	24·6	31·0	34·0	32·3
Palmitoleic	2·7	1·0	6·1	3·9	4·0	3·7	4·2	4·2	4·2	2·8	2·4	3·6	8·0	6·2	11·1
Stearic	3·7	4·1	6·5	7·2	8·4	4·8	6·3	6·8	5·2	7·6	7·7	7·4	1·6	1·4	5·0
Oleic	32·3	33·2	30·5	40·2	40·4	39·7	40·4	41·1	39·0	28·3	38·8	27·2	37·1	38·0	35·3
Linoleic + linolenic	11·5	48·5	27·5	9·8	10·2	9·0	12·1	12·3	11·7	19·4	19·2	19·1	11·5	12·0	10·0

Table 9. *Fatty acid compositions (weight percentages of the total) of the triglycerides extracted from the subcutaneous and perinephric adipose tissues of six rabbits given commercial diet (group 6)*

(Mean values are given with their standard errors)

Acid	Subcutaneous	Perinephric
Lauric	0·2 ± 0·03	0·2 ± 0·04
Myristic	1·0 ± 0·2	1·5 ± 0·4
Palmitic	20·0 ± 1·6	20·6 ± 1·1
Palmitoleic	4·6 ± 0·2	4·9 ± 0·3
Stearic	5·2 ± 0·6	5·4 ± 0·4
Oleic	31·0 ± 1·4	29·1 ± 0·8
Linoleic	24·7 ± 1·4	25·3 ± 0·2
Linolenic	1·8 ± 0·2	1·9 ± 0·3

DISCUSSION

According to the work of Schoenheimer & Rittenberg (1936) depot fat exists in a state of dynamic equilibrium with other body fat and even when there is no net gain or net loss of depot fat, the synthesis and degradation of adipose tissue triglycerides occur continuously. Whether there is a net gain or net loss of depot fat will depend on the relative rates of the synthesis and degradation of adipose tissue triglycerides. It must be remembered that the rabbits in group 4 (given the diet containing hydrogenated coconut oil) lost weight continuously during the feeding experiment and at the end of the experiment the rabbits in this group contained noticeably less adipose tissue than did the rabbits in group 6. It seems reasonable to assume, therefore, that in the rabbits of group 4, the rate of degradation of adipose tissue triglycerides was always in excess of the rate of synthesis. On the other hand, the rabbits in groups 1, 2, 3 and 5 gained weight during the feeding period and at the end of this period contained appreciably more adipose tissue than did the rabbits in group 6. It is also reasonable to assume that in the rabbits in groups 1, 2, 3 and 5 the rate of degradation of the adipose tissue triglycerides was always less than the rate of synthesis. Thus, the influence of different dietary fatty acids on the fatty acid composition of the adipose tissue triglycerides was observed to some extent in all of the rabbits that had been given the high-fat diets (groups 1–4) irrespective of whether there had been a net gain or a net loss of adipose tissue during the feeding experiment. For instance, higher concentrations of myristic acid were found in the adipose tissue triglycerides of the rabbits given the diets containing butter, butterfat or hydrogenated coconut oil and higher concentrations of linoleic acid were found in the adipose tissue triglycerides of the rabbits given the diet containing 20% maize oil. Nevertheless, if a state of dynamic equilibrium exists between depot fat and other body fats it is difficult to explain why the adipose tissue triglycerides of the rabbits in group 4 contained such relatively high concentrations of linoleic acid. This group of rabbits had been given for 9 months a diet low in this essential fatty acid yet the linoleic acid in the adipose tissue triglycerides (Tables 3 and 4) was about four times the concentration of this acid in the liver triglycerides (Table 6). Moreover, linoleic acid was found to constitute only 9 and 13% respectively of the fatty acids in the liver cholesteryl esters and phospholipids

the rabbits in group 4. Continual degradation and re-synthesis of triglycerides in the adipose tissues coupled with a low intake of dietary linoleic acid and low concentrations of this essential fatty acid in the various lipid fractions of other tissues might have been expected to give rise to a progressive decrease in the concentration of linoleic acid in the adipose tissue triglycerides of the rabbits in group 4 during the feeding experiments. If it is assumed that the fatty acid composition of the adipose tissue triglycerides of the rabbits in group 4 at the beginning of the feeding period was similar to that of the adipose tissue triglycerides of the rabbits in group 6, then it is evident that the concentration of linoleic acid in the adipose tissue triglycerides of the rabbits in group 4 did not change appreciably during the feeding period. Since there is some evidence from experiments with mice that the fatty acids that occupy the β-position of adipose tissue triglycerides are metabolized more slowly than those that occupy the α,α'-position (Tove, 1961), it seemed possible that the linoleic acid that remained in relatively high concentrations in the adipose tissue triglycerides of the rabbits in group 4 might be esterified predominantly in the β-position. However, this was subsequently shown not to be so (Tables 7 and 8). It is clear that further studies are needed on the mechanisms that control the specificities of the uptake and release of different fatty acids by adipose tissues.

The results in Tables 7 and 8 are in broad agreement with the report of Coleman (1961) that there was little or no specificity in the distribution of the different fatty acids between the α,α'- and β-positions of the triglycerides in the adipose tissues of the rabbit. These results for the adipose tissues of the rabbit are thus in contrast to those that have been obtained for the adipose tissue triglycerides of other animals. For instance, in the adipose tissue triglycerides of the pig there is a marked preferential esterification of palmitic acid in the β-position, whereas the C_{18} saturated and unsaturated fatty acids occur predominantly in the α-position (Coleman, 1961; Garton & Duncan, 1965). In the adipose tissue triglycerides of ruminants, palmitic and stearic acids are preferentially esterified in the α,α'-position, whereas the C_{18} unsaturated fatty acids are preferentially esterified in the β-position (Garton & Duncan, 1965). According to the work of Perkins (1964), the positional distribution of the different fatty acids in the adipose tissue triglycerides of the rat is similar to that found in the adipose tissue triglycerides of ruminants. However, Tove (1961) has shown that the positional distribution of the various fatty acids in the adipose tissue triglycerides of the mouse depends to some extent on the fatty acid composition of the diet. Tove (1961) found that when mice were given normal diets, linoleic acid was predominantly esterified in the β-position of the adipose tissue triglycerides, but when the mice were given diets containing high levels of linoleic acid then the linoleic acid was predominantly esterified in the α,α'-positions of the adipose tissue triglycerides. In this respect, it is of interest that in the rabbits of group 1 given the diet containing the highest levels of linoleic acid, the concentration of the fatty acid in the α,α'-position was greater than that in the β-position of both types of adipose tissue triglycerides.

In the pig, the subcutaneous triglycerides contain higher concentrations of oleic acid and lower concentrations of palmitic acid than do the perinephric triglycerides (Hilditch & Peachy, 1940). This difference in fatty acid composition is thought to be

due to the different temperatures at the site of fat deposition (Henriques & Hansen 1901). Although it might be expected that the temperature differential between the exterior and interior regions of the body of the rabbit would be less than that between the exterior and interior regions of the body of the pig, it is of interest to note that the results in Table 5 support those obtained with pigs by Hilditch & Pedelty (1940).

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Influence of the early introduction of edible starch on the behavior of the seric amylases and on the growth of premature babies

by G. C. Mussa, M. L. Colombo and G. Ferrari

The amylases, enzymes which hydrolyze starch, attack glycogen and some dextrins up to the formation of polysaccharides, are normally present in the blood, though the mechanism which regulates their circulation is not well known. The principal sources of amylases are the liver, the pancreas, and the salivary glands. Electrophoretic studies have shown that about 3/4 of the seric amylases are of hepatic origin and is connected with the albumin fraction, while the est is of pancreatic origin and is connected with the globulin fraction.

It is important to note that the amylase diminishes in the blood when there is an increased utilization of carbohydrates, and it increases, even under normal conditions, when the utilization diminishes.

It is known that this enzyme is absent in newborn babies and appears to a greater extent in premature babies, generally in about two months and is quantitatively determinable with the methods available to us only after three months, and attains a content similar to that present in the adult which varies between 80 and 150 Somogyi U./100 cc after one year. Though there are individual variations, the content of amylase tends to be constant in time.

The object of the present study was to see if the early administration of starches in premature babies is capable of stimulating the organism to produce the enzyme which is capable of metabolizing these substances without causing unpleasant side effects.

To this end we determined the steric amylase, before starting the experiments, in a group of 12 premature healthy babies ranging in age from 11 to 40 days and repeated the determination on the 20th day from the administration of cream of rice added to the normal diet (cow's milk) in an amount of

2%, and after 2 and 8 days from the suspension of this treatment. For the determination of the seric amylase we used the Somogyi method, which is widely used both for the practicality of the application and for the reliability of its results. As known, this method is based on the determination of the reducing sugars (maltose) produced from the hydrolysis of the starch under standard conditions.

The results obtained in the present studies are compiled in table 1.

An examination of the table shows that in no case was it possible to demonstrate the presence of the seric amylase prior to the administration of starch.

After 20 days from the administration of cream of rice, we again repeated the determinations and we succeeded in finding the amylase in all subjects, except two, namely case no. 6 and 9.

The values varied from 18.4 S.U. (case no. 7) to 35.5 S.U. As it can be seen, these values are clearly lower than those normally present in healthy adult subjects (80 - 150 S.U.). In addition, we have to stress that in another group of healthy premature babies of the same age, which were used as controls, and with a diet free of starch, the seric amylase could not be determined.

After 48 hours from the suspension of the cream of rice diet, the seric amylase showed in general minor fluctuations with regard to the data obtained before, and while it remained unchanged in some cases (no. 2, 11 and 12), in other cases it was slightly reduced (no. 3, 8 and 10) and in others it was moderately increased (no. 1, 4, 5 and 7).

As in the preceding determinations, it was absent in case no. 6 and 9.

The determinations made after 8 days from the suspension of the cream of rice diet furnished interesting data, in fact in all cases the seric amylase was more or less substantially increased.

In case no. 6 too, where the preceding determination had been negative, we found 32.7 S.I. seric amylase per 100 cc 8 days after the suspension of the cream of rice diet.

The only case where this enzyme was always absent was case no. 9. The increase of this enzyme observed in the serum 8 days after the suspension of the food was due to the fact that the influence of the liver on the seric amylase content is related to the metabolism of the carbohydrates, that is, more amylase is used up by the liver and less tends to enter the blood.

With regard to the body weight in the group of babies subjected to the early administration of cream of rice, we found a daily weight increase of 40.1% with regard to the group of subjects used as controls.

In no case did the early administration of cream of rice result in disorders of the gastrointestinal tract.

From the results obtained in the present studies of the influence which the early administration of starch has on the behavior of the seric amylase and on the growth of premature babies we can draw the following conclusions:

1. The early administration of cream of rice in a concentration of 2% results after 20 days of treatment in 10 out of 12 cases examined in the appearance of seric amylase, though at a lower rate than in the healthy adult subject; after 48 hours from the suspension the values of the seric amylase showed minor fluctuations with regard to the data obtained before, while in the determinations made 8 days after the suspension of cream of rice it was more or less substantially increased in all cases.

2. The early administration of cream of rice resulted in an acceleration of the body weight of 40.1% relative to that of the controls. After the suspension of cream of rice, the daily increase returned to the limits observed in the controls.

3. Subjects examined had tolerated the starch well and did not show any disorder of the digestive apparatus.

It seems thus evident from those findings that the early administration of edible starch in premature subjects, also in the neonatal state, finds an organism capable of producing the enzymes suitable for its metabolism and hence for its utilization for flexible purposes without causing any unpleasant side effects.

(Translated by Carl Demrick Associates, Inc/IE/t)

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Istituto di Puericultura dell'Università di Torino.
Direttore Prof. M. Gonzato Sandrucci

Influenza della precoce introduzione di amido alimentare sul comportamento dell'amilasi serica e sull'accrescimento dell'immaturo

G. C. Mussa M. L. Colombo G. Ferrari

Le amilasi, enzimi che idrolizzano l'amido, attaccano il glicogeno ed alcune destrine fino alla formazione di polisaccaridi, normalmente sono presenti nel sangue, pur non essendo ancora ben noto il meccanismo che regola il loro passaggio in circolo. Le principali sorgenti di amilasi sono il fegato, il pancreas e le ghiandole salivari. Ricerche elettroforetiche hanno dimostrato che per circa i tre quarti l'amilasi serica è di origine epatica ed è legata alla frazione albuminica, mentre la rimanente è d'origine pancreatica e salivare ed è legata alla frazione globulinica.

È importante ricordare che l'amilasi nel sangue diminuisce quando vi è una aumentata utilizzazione dei carboidrati ed aumenta, anche in condizioni normali, quando vi è una diminuita utilizzazione.

È noto che questo enzima è assente nei neonati ed a maggior ragione in quelli prematuri, compare in genere verso i due mesi, ma è quantitativamente dosabile con le metodiche a nostra disposizione soltanto dopo i 3 mesi e solo verso l'anno raggiunge un tasso analogo a quello presente nell'adulto che oscilla tra 80-150 U. Somogyi/100 cc. Pur essendoci variazioni individuali, in ogni individuo il tasso di amilasi tende a mantenersi costante nel tempo.

Scopo della presente ricerca è stato quello di vedere se la precoce somministrazione di amidi in neonati immaturi prematuri è in grado di stimolare l'organismo a produrre l'enzima capace di metabolizzare queste sostanze senza provocare fenomeni secondari spiacevoli.

A tal fine in un gruppo di 12 neonati immaturi prematuri sani di età compresa tra gli 11 ed i 40 giorni di vita abbiamo determinato l'amilasi serica prima di iniziare gli esperimenti ed abbiamo ripetuto il dosaggio al 20° giorno dalla somministrazione di crema di riso aggiunta all'alimento abituale (latte vaccino) nella dose del 2 %, e dopo 2 ed 8 giorni dalla sospensione di questo trattamento. Per la determinazione dell'amilasi serica ci siamo valsi del metodo Somogyi, che è il più usato sia per la praticità di applicazione sia per l'attendibilità dei risultati. Come è noto questo metodo è basato sul dosaggio degli zuccheri riducenti (maltosio) prodotti dall'idrolisi dell'amido in condizioni standard.

I risultati ottenuti nelle presenti ricerche sono stati riportati nella tabella I.

Dalla disamina della tabella è possibile vedere che in nessun caso siamo riusciti ad evidenziare l'amilasi serica prima d'iniziare la somministrazione di amido.

Dopo 20 giorni dalla somministrazione della crema di riso, abbiamo nuovamente ripetuto le determinazioni e siamo riusciti a dosare l'amilasi serica in

TABELLA I. — *Valori di amilasi serica espressi in Unità Somogyi/100 cc.*

Caso n.	Nome	P.N.N.	Età inizio somministrazione C.R.	Controllo	Dopo 20 giorni somministraz. C.R.	Dopo 48 ore sospesa C.R.	Dopo 8 giorni sospesa C.R.
1)	G. Lia	1.100	25	—	33,33	35,2	45,71
2)	B. F. Anna Maria	1.700	35	—	33,33	33	45
3)	B. F. Davide	1.600	35	—	28,5	22,2	45
4)	B. F. Roberto	2.280	35	—	28,5	37,4	52,1
5)	D. F. Fortunata	2.000	35	—	22,6	25,8	34,32
6)	M. Luisa	2.070	22	—	—	—	32,7
7)	V. Aurelia	1.980	22	—	18,4	25	28,3
8)	L. Piero	1.920	11	—	21,44	20,3	25
9)	P. Marilena	1.650	18	—	—	—	—
10)	P. Rosa	2.080	20	—	35,5	32	39,5
11)	V. [illegible]	2.020	40	—	28	28	33,4
12)	D. M. Roberto	1.780	28	—	28,5	28	41,3

tutti i soggetti tranne due e precisamente i casi n. 6 e 9.

I valori sono oscillati da 18,4 U.S. (caso 7) a 45,5 U.S. (caso 10). Come si vede questi valori sono nettamente inferiori a quelli normalmente presenti nei soggetti adulti sani (80-150 U.S.). Inoltre riteniamo sottolineare che, in un altro gruppo di immaturi-prematuri sani di pari età tenuti come controllo e con una dieta priva di amido, l'amilasi serica è risultata indosabile.

Dopo 48 ore dalla sospensione della crema di riso l'amilasi serica ha presentato in generale scarse oscillazioni rispetto ai dati ottenuti in precedenza e mentre in alcuni casi è rimasta invariata (caso 2, 11 e 12) in altri si è ridotta ma di poco (caso 3, 8 e 10) ed in altri ancora è modicamente aumentata (caso 1, 4, 5 e 7).

Come nelle determinazioni precedenti nei casi 6 e 9 è risultata assente.

Le determinazioni eseguite dopo 8 giorni dalla sospensione della crema di riso hanno fornito un dato interessante, infatti in tutti i casi l'amilasi serica è risultata aumentata in maniera più o meno sensibile.

Anche nel caso 6 in cui le determinazioni precedenti avevano dato esito negativo, dopo 8 giorni dalla sospensione della crema di riso l'amilasi serica è risultata di 32,7 U.S./100 cc.

L'unico caso in cui questo enzima è sempre risultato indosabile è stato il caso 9. L'aumento di questo enzima osservato nel siero 8 giorni dopo la sospensione dell'alimento è dovuto al fatto che l'influenza del fegato sul tasso dell'amilasi serica è in rapporto al metabolismo dei carboidrati, ossia più amilasi viene utilizzata dal fegato e meno tende ad entrare nel sangue.

Per quanto riguarda il comportamento del peso corporeo nel gruppo dei bambini sottoposti a precoce somministrazione di crema di riso si è avuto un incremento ponderale giornaliero del 40,1 % superiore al gruppo dei soggetti tenuti come controllo.

In nessun caso la precoce somministrazione di crema di riso ha determinato disturbi a carico dell'apparato gastroenterico.

Dall'insieme dei risultati conseguiti nelle presenti ricerche emerge l'influenza esercitata dalla precoce introduzione di amido sul comportamento dell'amilasi serica e sull'accrescimento dell'immaturo: riteniamo di poter trarre le seguenti conclusioni.

1) la precoce somministrazione di crema di riso alla concentrazione del 2 % ha determinato dopo 20 giorni di trattamento in 10 sui 12 casi esaminati la comparsa dell'amilasi serica, sebbene ad un tasso inferiore a quello del soggetto adulto sano; dopo 48 ore dalla sospensione i valori dell'amilasi serica hanno presentato scarse oscillazioni rispetto ai dati ottenuti in precedenza, mentre invece nelle determinazioni eseguite dopo 8 giorni dalla sospensione della crema di riso sono aumentati in tutti i casi in modo più o meno sensibile;

2) la precoce somministrazione di crema di riso ha fatto riscontrare un'accelerazione sull'accrescimento corporeo del 40,1 % superiore a quello dei controlli. Dopo la sospensione della crema di riso l'aumento giornaliero è ritornato nei limiti osservati nei controlli;

3) tutti i soggetti esaminati hanno tollerato bene l'alimento e non hanno presentato nessun disturbo a carico dell'apparato digerente.

Da quanto esposto appare quindi evidente che la precoce somministrazione di amido alimentare in soggetti immaturi-prematuri anche in periodo neonatale, trova un organismo capace di produrre gli enzimi atti al suo metabolismo e quindi alla sua utilizzazione a scopo plastico senza determinare fenomeni collaterali spiacevoli.

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CHOLESTEREMIA OF RABBITS FED CARBOHYDRATE DIETS*

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During the past decades much attention has been paid to the role of lipids, especially cholesterol, in atherogenesis. Only in recent years has it been pointed out that the blood cholesterol level is influenced not only by dietary lipids but also by dietary proteins and carbohydrates. The number of references to carbohydrates affecting blood cholesterol and lipoproteins is still limited.

Portman and co-workers (1) observed that the blood cholesterol level of 4 cebus monkeys decreased when dietary sucrose was replaced by corn starch, but increased when sucrose was substituted for starch. Cheng-Chun and Herman (2) noted a twofold to threefold rise in the concentration of blood cholesterol and a tenfold rise of liver cholesterol in rats fed a diet containing 15 per cent sucrose. Grant and Fahrenbach (3) fed sucrose and glucose monohydrate to a series of chicks; blood cholesterol levels were similar. On addition of cholesterol to these diets, birds fed sucrose had blood cholesterol levels twice those of birds fed glucose. Later, these authors (4) modified and expanded their experiments, with little change in results. In a series of rabbits fed for sixty days with a diet containing 50 per cent sugar, the average concentration of blood cholesterol was 1015 mg. per 100 ml. after glucose feeding, and 1539 mg. per 100 ml. after sucrose feeding. Nath and associates (5) found that diets containing sucrose, dextrin or anhydrous glucose caused similar degrees of hypercholesteremia.

Adams *et al.* (6) observed hypercholesteremia in rats fed glucose. Van Handel and Zilversmit (7) stated that rabbits fed a sucrose diet exhibited lower serum cholesterol levels than rabbits fed liquid oil, hydrogenated oil or wheat germ oil. Wells and Cooper (8) reported absorption of 40.4 per cent of cholesterol administered to rats fed sucrose, and 66.2 per cent absorption when rats were fed lactose instead of sucrose.

Attempts to explain these divergent observations resulted in several new experimental designs. Kritchevsky and co-workers (9) investigated the effect of sugars on the turnover of cholesterol-4-C^{14} in chickens, and concluded that glucose is utilized faster than sucrose. Addition of chlortetracycline to the diet caused an increase of blood cholesterol in glucose-fed birds to the level observed in sucrose-fed birds, but did not alter the level in chickens fed a sucrose diet.

Portman *et al.* (10) implicated cholate in the hypercholesteremic action of carbohydrates. In rats fed corn starch or sucrose alone, blood cholesterol concentrations averaged 106 mg. and 124 mg. per 100 ml., respectively; with cholate added, the corresponding figures were 257 mg. and 378 mg. per 100 ml. Glucose

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and cholate caused blood cholesterol concentration to rise to 361 mg. per 100 ml., and fructose and cholate resulted in a level of 411 mg. per 100 ml. These diets also contained 8 per cent of corn oil. According to Filios and co-workers (11), after hypercholesterolemia had been induced in rats by a diet of sucrose, corn starch and cholate, a short-term change to a starch diet resulted in a reduction of the blood cholesterol level, but prolonged starch feeding had no effect. Adams *et al.* (6) failed to observe any decrease in the concentration of blood cholesterol in rats fed a starch diet.

Since these observations lack uniformity, and explanation of experimental results is not satisfactory, a broad study of carbohydrate diets in rabbits was undertaken.

MATERIALS AND METHODS

White buck rabbits of a New Zealand breed were used. Their average weight was 1500 Gm., with ±20 Gm. variation. In the first trial, 10 rabbits were used in each of the 30 series of the study. Since the observations contradicted reports of many investigators who studied the influence of carbohydrates on the blood cholesterol level, the whole study was repeated during a second year and again during a third year, each time using the same total number of animals.

The arrangement of the experiment was as follows:

Series	First Feeding Period (2 wks.)	Second Feeding Period (2 wks.)	Third Feeding Period (4 wks.)
I	Basal diet	Basal diet	Basal diet
II	Cholesterol	Basal diet	Basal diet
III*	Carbohydrates	Basal diet	Basal diet
IV*	Cholesterol	Carbohydrates	Basal diet
V*	Cholesterol and carbohydrates	Basal diet	Basal diet
VI*	Cholesterol and carbohydrates	Carbohydrates	Basal diet

* Series III–VI each contained 7 carbohydrate groups—dextrose, fructose, maltose, sucrose, rice, corn starch, and potato starch. The basal diet was Purina rabbit chow.

Cholesterol (powder, Merck) was fed in a daily dose of 2 Gm. The carbohydrates were each fed in a daily amount of 20 grams. Dextrose (anhydrous powder, Baker), fructose (powder, Eastman Organic Chemicals), maltose (powder, Matheson, Coleman & Bell), sucrose (crystals, Baker), rice (white, uncooked, Sultana brand, A&P), corn starch (Knighthood brand, Reeves, Parvin & Co.), and potato starch (Samuel Zuckerman & Co., Philadelphia, Pa.) were used. Sugars were dissolved, and other substances were suspended in water. The water was poured over 20 Gm. of rabbit chow (pellets, Purina), and the pellets were dried. Rice was mixed with the pellets. Only after we had made sure that all the treated feed had been consumed were the rabbits given additional chow without restriction.

Blood was drawn prior to the experimental diet, after the first two-week feeding period, after the second two-week feeding period, and four more times at weekly intervals while the rabbits were fed chow only. All blood samples were analyzed for total cholesterol, total protein, glucose, and urea nitrogen. The Autoanalyzer (Technicon Co.) was used for these assays. The animals were weighed before the start of the study, after two and four weeks, and at the end of the observation period. Autopsies were performed. The liver was weighed and liver cholesterol was determined. The aorta was grossly stained with Oil Red O and then processed for microscopic examination.

OBSERVATIONS

The rabbits consumed the diets reluctantly. Rice disappeared from the feeding dish faster than other carbohydrates. After two to three days the animals got used to the sugar-coated pellets and ate better. They never became used to the starches and many stubborn, starving rabbits had to be replaced by more cooperative animals. Cholesterol was consumed fairly readily and its addition to carbohydrate diets did not influence food consumption.

Rabbits with abnormal blood cholesterol values were replaced, in order to establish a uniform population. In the total study, the extremes for the initial blood cholesterol values were 81 and 110 mg. per 100 ml., but 90 per cent of all values were between 85 and 101 mg., and 86 per cent were between 91 and 99 mg. The average was 91 mg. per 100 ml. To facilitate evaluation of results, all pre-experimental levels were increased to 100 mg. per 100 ml. and all subsequent values were proportioned accordingly.

The average pre-experimental glucose level was 85 mg. per 100 ml. of blood, with a range of 81 to 107 mg. Total serum protein ranged from 5.1 to 6.3 Gm. per 100 ml., with an average of 5.72 Gm. Blood urea nitrogen ranged from 9 to 13 mg. per 100 ml., with an average of 12 mg.

All rabbits gained weight during the eight-week observation period. Rabbits fed sucrose gained least—215 Gm. on the average. Rabbits fed corn or potato starch gained most—1,000 Gm. on the average. The average weight gain for the other series was 650 Gm.; the animals fed maltose gained more weight than others.

The weight of the liver increased in all animals fed cholesterol, and more so in rabbits fed cholesterol and carbohydrates. The increase in liver weight paralleled the increase in liver cholesterol. Compared with normal-controls (Series I), the liver cholesterol of rabbits fed cholesterol (Series II) was 30 per cent higher, and the liver cholesterol of rabbits fed carbohydrates at any time (Series III–VI) was, on the average, 40 times higher.

Aortic atherosclerotic plaques were not found in rabbits whose blood cholesterol level was less than 400 mg. per 100 ml. In animals with higher blood cholesterol levels, the number of patches roughly paralleled the degree and duration of hypercholesteremia.

The concentration of blood sugar was not influenced by the various diets. Fluctuations were within normal limits. In rabbits receiving cholesterol and carbohydrates for the first two weeks, carbohydrates for the next two weeks and chow for the following four weeks (Series VI), the blood urea nitrogen level was elevated during the fourth and fifth weeks of the period. It increased up to 21 mg. per 100 ml. but declined along with the cholesterol level.

In all but the control series (Series I), the serum total protein curve paralleled the blood cholesterol curve. At the peak, protein concentration ranged between 7.5 and 8.8 Gm. per 100 ml. reflecting the height of the cholesterol concentration.

Detailed tabulating of chemical results seems superfluous, except for the

TABLE I

Blood Total Cholesterol Levels (mg. per 100 ml.) in Rabbits Before and During Experimental Diets

SERIES	RÉGIMEN		INITIAL LEVEL	AFTER 2 WEEKS	AFTER 4 WEEKS	AFTER 5 WEEKS	AFTER 6 WEEKS	AFTER 7 WEEKS	AFTER 8 WEEKS
I	8 WEEKS, B		100	105	100	98	97	101	100
II	2 WEEKS, CH 6 " B		100	512	397	267	99	101	103
III	2 WEEKS, CBH * 6 " B		100	99	95	100	106	100	101
IV	2 WEEKS, CH 2 " CBH 4 " B								
		D	100	510	333	392	172	81	105
		F	100	590	375	350	225	105	120
		M	100	500	422	357	250	138	115
		S	100	501	363	215	130	110	110
		R	100	453	233	169	175	117	108
		C	100	478	508	264	203	134	130
		P	100	439	311	303	227	176	131
V	2 WEEKS, CH " sCBH 6 " B								
		D	100	305	311	290	203	137	101
		F	100	267	175	300	222	100	101
		M	100	500	308	280	250	178	102
		S	100	308	408	175	183	178	102
		R	100	308	408	175	183	100	108
		C	100	250	408	167	183	100	108
		P	100	313	408	408	217	213	108
VI	2 WEEKS, CH sCBH 2 " CBH 4 " B								
		D	100	568	670	515	302	220	115
		F	100	500	193	220	200	141	130
		M	100	375	308	242	150	110	100
		S	100	308	308	115	117	98	98
		R	100	314	408	167	183	117	108
		C	100	281	408	267	183	132	108
		P	100	612	738	393	338	403	308

* D, F, M, S, R, C, P

DIETS ... B ... BASAL DIET
CH ... CHOLESTEROL
CBH ... CARBOHYDRATES

D ... DEXTROSE,
F ... FRUCTOSE,
M ... MALTOSE,
S ... SUCROSE,
R ... RICE,
C ... CORN STARCH,
P ... POTATO STARCH

cholesterol determinations. Average blood cholesterol levels are presented in Table I and in Figure 1. Each value in the table represents the average of 30 determinations.

BLOOD CHOLESTEROL DATA

Series I. Blood cholesterol concentration remained within the normal range throughout the eight-week observation period. When plotted, the averages lay almost on a straight line.

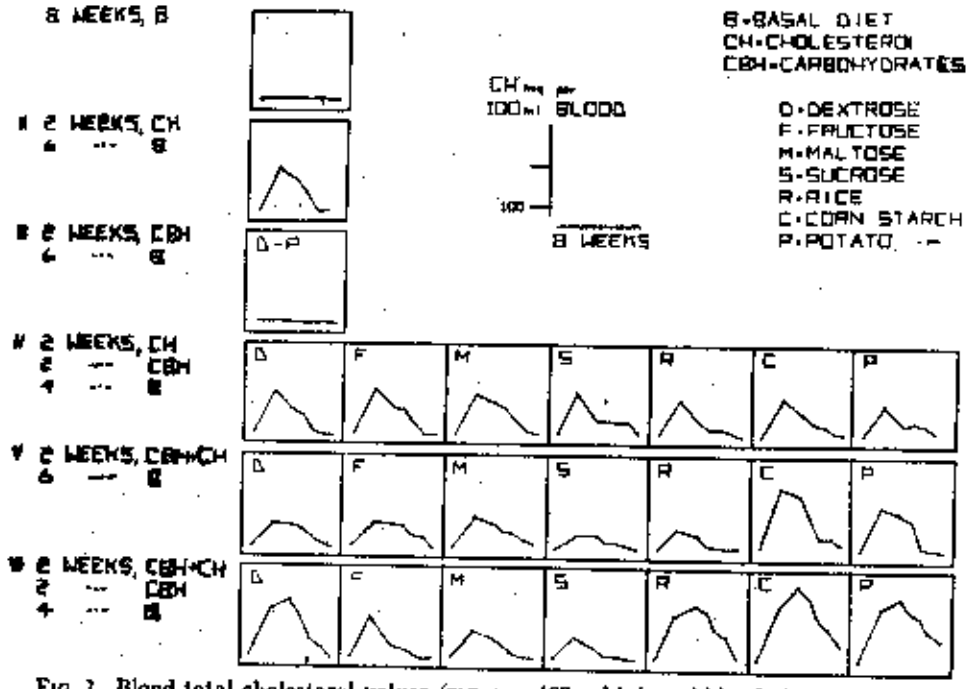

FIG. 1. Blood total cholesterol values (mg. per 100 ml.) in rabbits before and during experimental diets.

Series II. After feeding 2 Gm. of cholesterol daily for fourteen days, blood cholesterol concentration increased fivefold. Two weeks after cessation of cholesterol feeding, the blood level had decreased by 25 per cent and within two additional weeks it equaled the pre-experimental value.

Series III. In the rabbits maintained for two weeks with 7 different carbohydrate diets, the blood cholesterol level was similar to that in the control animals receiving the basal diet of chow (Series I).

Series IV. After two weeks of cholesterol feeding the blood cholesterol concentration rose to 484 mg. per 100 ml. (range, 410 mg. to 540 mg.). A downward trend started promptly after discontinuation of cholesterol feeding and, regardless of the type of carbohydrate administered, the cholesterol level had dropped by 34 per cent within the two-week period. After the next two weeks, the average level was still twice the normal—204 mg. per 100 ml. After another two weeks, at the end of the eight-week period, it was 116 mg. per 100 ml.

The slope of the curve was not the same for all 7 groups. The final level for rabbits fed corn starch was 130 mg. per 100 ml., and for rabbits fed potato starch, 131 mg. per 100 ml., although the maximal values for these two groups were lower than the peak values for 4 out of the other 5 groups in the series.

Series V. The average peak level of cholesterol for rabbits fed cholesterol and corn starch was 701 mg. per 100 ml., and for rabbits fed cholesterol and potato starch it was 513 mg. per 100 ml. For the other 5 groups, the peaks were lower than for animals fed cholesterol alone (Series II, average, 512 mg.). The values were 390 mg. for maltose, 308 mg. for dextrose, 286 mg. for rice,

213 mg. for fructose and 207 mg. for sucrose. For the whole series, the peak value was 378 mg. per 100 ml. Without the values for the two starch-fed groups, the peak was 278 mg. per 100 ml.

Two weeks after termination of these diets a few cholesterol values declined, but only moderately. Most values remained the same or increased. The average level for Series V was 302 mg. per 100 ml. (the previous one had been 378 mg.). One week later, the levels were still fairly high. At the end of the sixth week they were comparable to those in rabbits fed carbohydrates after the cholesterol diet (Series IV). At the end of the observation period, blood cholesterol levels were normal except in the rabbits previously fed maltose or corn starch (140 mg. and 165 mg. per 100 ml., respectively).

In this series, the blood cholesterol concentration in rabbits fed cholesterol-sucrose reached, after two weeks, 207 mg. per 100 ml. After another two weeks of the basal diet, it was 238 mg per 100 ml., and it became normal during the subsequent three weeks.

Series VI. Although the first two-week period of this experiment was similar to that in Series V, the response of the animals was different. It resembled the reaction of rabbits fed cholesterol alone (Series III). The blood cholesterol level for the series rose to an average of 502 mg. per 100 ml. (in Series III it was 512 mg.). Differences between the 7 groups of the series were not as pronounced as in Series V. In rabbits fed cholesterol and corn starch for two weeks, the blood cholesterol level was 615 mg. per 100 ml., and in rabbits fed potato starch it was 612 mg. per 100 ml. The combined corresponding level for the other 5 groups was 451 mg. per 100 ml. As with Series V, in rabbits fed cholesterol-sucrose, the blood cholesterol level reached only 302 mg. per 100 ml.

After the second feeding period, the blood cholesterol concentration was 511 mg. per 100 ml. (the preceding value was 502 mg.) for the whole series. This high average was caused by a sharp increase in 4 groups of the series. The blood cholesterol levels in rabbits fed first cholesterol and corn starch, and then corn starch alone, were 615 mg. and 850 mg. per 100 ml. respectively; the absolute increase was 205 mg., and the relative increase was 30 per cent. Absolute increases of 126, 118 and 102 mg., and relative ones of 20, 19 and 18 per cent, were observed in rabbits fed rice, potato starch, and dextrose, respectively. In the remaining 3 groups of the series, the decline of the blood cholesterol curve was prompt and was comparable to that in other experiments (Series III, IV).

The blood cholesterol curve for rabbits fed cholesterol-sucrose and then sucrose alone, differed markedly from those for all the other groups. The peak was 302 mg. per 100 ml. after the first two weeks of the diet, 201 mg. two weeks later, and well within normal limits three weeks thereafter.

At the end of the observation period the concentration of blood cholesterol in rabbits fed dextrose was 115 mg. per 100 ml., in spite of a rise from 568 mg. to 670 mg. during the second feeding period. The adjustment was also good for rabbits fed rice; in spite of a rise from 514 mg. to 640 mg. during the second

period, the final value was 131 mg. per 100 ml. Terminal values were normal for animals fed sucrose or maltose. Although the blood cholesterol level of rabbits fed fructose dropped rapidly after the end of the first two weeks of the diet, it was still fairly high (150 mg.) at the time of the terminal observation. After eight weeks, the blood cholesterol concentration was still 275 mg. per 100 ml. in rabbits fed corn starch, and 300 mg. per 100 ml. in rabbits fed potato starch.

DISCUSSION

The following hypotheses have been advanced to explain the different effects of various carbohydrates on the blood cholesterol level: *a)* Sugars affect the appetite, and thus food consumption and body weight. Investigations have shown that rats fed starch ate more than rats fed sucrose (12). Rabbits fed lactose ate more than those fed sucrose (13). In our experiments, rabbits fed sucrose gained least, and rabbits fed starches gained most although they consumed less food. *b)* Gastric secretion and the emptying time of the stomach are influenced by sugars. Oral or intravenous glucose can cause delay in the evacuation of the stomach, and thus delay of fat resorption in rats (14). *c)* Biliary and fecal cholic acid may vary with the type of carbohydrate diet. Sucrose and glucose decrease cholic acid in the bile and feces of rats, but corn starch does not affect this acid (15). *d)* Bile flow is influenced by sugars. Galactose and lactose, both with OH groups on C_4 and C_6, are inhibitors of bile flow in rats, whereas fructose and glucose have no such effect (16). In dogs with a biliary fistula, sucrose depresses the secretion of bile acid in the fistula (17). *e)* The proportion of bile acid to cholesterol may be modified by sugars. Retention of cholesterol during a period of sucrose diet has caused gall stone formation in hamsters (18). *f)* The absorption of cholesterol is influenced by dietary carbohydrates. In chicks, cholesterol resorption is favored by sucrose rather than glucose (3). In rabbits, lactose enhances resorption more than sucrose does (13). In rats, four different carbohydrates mixed with cholate generally induced hypercholesteremia; corn starch was the least effective, glucose and sucrose were stronger and about equal in action, and fructose was the most potent (10). *g)* Carbohydrates alter the reaction of the contents of the cecum. On feeding 40 per cent lactose to rats for only five days the pH of the cecal contents can be decreased (8); no such change in reaction occurs in rabbits (13). *h)* The intestinal microflora may be altered by dietary carbohydrates. Aureomycin seems to have a secondary effect on glucose metabolism since it enhances hypercholesteremia in chicks fed glucose and cholesterol but not in chicks fed sucrose and cholesterol (9). *i)* The change in pH due to sugars depresses the intestinal microflora which normally converts cholesterol to coprostanol. At a pH of 5.8 the cecal flora is inhibited; at the same time liver cholesterol is increased, cholesterol absorption is promoted and coprostanol formation is diminished (8). *j)* Carbohydrates contribute to the formation of triglycerides of fatty acid and other substances; some have a nitrogen-sparing effect. The faster rise of amino nitrogen after ingestion of

fructose than after ingestion of glucose points to a faster rate of metabolism of fructose (19).

It seems generally accepted that various carbohydrates do not alter the normal blood cholesterol level, and that the blood cholesterol level is influenced in different ways by various cholesterol-carbohydrate diets. Hypercholesteremic animals are affected variously by carbohydrate diets. Although most authors seem to single out sucrose as a potent hypercholesteremic agent, there is no uniformity in their reports. In our studies, sucrose retarded the development of hypercholesteremia; other sugars had little or no influence on the blood cholesterol level, and starches enhanced it. Comparison of experiments is almost impossible since different carbohydrates were fed in different quantities for different periods of time to different animal species. In addition, the total calorie intake, the amount and type of the non-carbohydrate components of diets, and many other factors varied in the studies discussed in the literature. It has been pointed out that results will vary with the length of dietary periods (11). Our experiments were of short duration. It has been demonstrated that addition of protein to the diet lessens the effect of sugars on the concentration of blood cholesterol (16), and that calcium alters cholesterol absorption and counteracts the effect of lactose (8). Moreover, the cholesterol-promoting or cholesterol-lowering effect may depend upon the purity or degree of hydration of a hexose (5).

A single factor or multiple factors may account for the different reactions to the combined cholesterol-carbohydrate diets. Many factors have been mentioned but others could be added. Consideration should be given to inhibition of an intestinal enzyme system by certain carbohydrates or by some microorganisms influenced by the diets. In our experiments, there was a fairly close correlation between the gain in body weight and the blood cholesterol response. Animals fed starches gained more weight, had heavier livers, higher and more persistent blood cholesterol levels and more aortic plaques than did rabbits fed rice or hexoses. The delay in clearing of the blood in rabbits fed carbohydrate in addition to cholesterol, or fed carbohydrates continuously after the combined carbohydrate-cholesterol dietary period, may have been largely due to competition within the liver. This organ, overloaded with lipid and cholesterol, had an additional burden caused by an exaggerated carbohydrate intake.

It is questionable which, if any, of these observations on rabbits have validity for other species, especially man. However, it is well known that more members of populations receiving high starch diets are overweight, have hypercholesteremia and have advanced atherosclerosis than members of populations whose staple diet is rice. In some areas of the world many persons, especially in low-income groups, consume large quantities of sucrose, yet their weight is lower, their blood cholesterol levels are lower, and the development of atherogenesis is slower with fewer clinical complications. Detailed scientific investigation of such populations is warranted. It seems clear that the concentration of blood cholesterol depends as much on the level of carbohydrate and protein consumption as on the level of fat and cholesterol consumption.

SUMMARY

The feeding of 7 different carbohydrate diets (dextrose, fructose, maltose, sucrose, rice, corn starch, and potato starch) to normocholesteremic rabbits had no effect on the concentration of blood cholesterol.

Carbohydrates administered to hypercholesteremic rabbits caused a fairly uniform delay in the rate at which the blood cholesterol level returned to pre-experimental values, compared with the rate in the control series.

Combined cholesterol-carbohydrate feeding had varied effects. Potato starch, and particularly corn starch, enhanced the degree of hypercholesteremia. A cholesterol-rice diet or a cholesterol-dextrose diet had an effect comparable to that of cholesterol administration. In rabbits fed cholesterol and maltose, the blood cholesterol level rose less than in the control series. A combined cholesterol-sucrose diet resulted in only a moderate elevation of the blood cholesterol level.

The reasons for the varied effects of carbohydrate diets on the concentration of blood cholesterol in rabbits are discussed. Practical applications for man are suggested.

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Studies on the Utilization of Various Starches and Sugars in the Rat

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ABSTRACT Various types of dietary carbohydrates were fed to growing rats for 28 days with either casein or wheat gluten as the source of dietary protein. Unmodified potato starch and potato amylose resulted in increases in body weight gains and enlarged ceca when casein supplied a dietary protein level of 6%. The same effect was shown when wheat gluten was the dietary protein at either a 9 or 15% protein level. These 2 sources of carbohydrate gave relatively poor weight gains and protein efficiency values when the casein level of the diet was increased to 15% protein. Unmodified corn, wheat, rice, tapioca, and amioca starches, as well as maltose, corn amylopectin, and potato amylopectin gave normal body weight gain values, protein efficiency ratios and ceca weights at both the high and low dietary protein levels. Lactose, unmodified arrowroot starch, fructose, and dextrose gave inferior body weight gains and protein efficiency values. The prevalance of diarrhea and an enlargement of the ceca gave evidence that commercial samples of corn dextrin and pregelatinized amioca starch were not as well utilized as unmodified corn starch or unmodified amioca starch.

Recently there has been an increased interest in the biological utilization of carbohydrates because of the development of new starch products for the food industry. During the past several years, amylose has been produced in large quantities from both corn starch and potato starch. Many new pregelatinized starch products have been developed, also, with special functional properties for food use.

It has been claimed that starches from various sources and modified starches produce varying growth responses in the rat. Booher et al. (1) carried out an extensive study in which it was shown that unmodified potato, arrowroot, and sago starches were not completely available to the growing rat, whereas the starches from wheat, corn, waxy maize, rice, and cassava were well-utilized for rat growth. It was also shown that commercial samples of hydrochloric acid-modified and hypochlorite-oxidized wheat starch performed as well as unmodified wheat starch. Commercially modified corn starch products were shown by Whistler and Belfort (2) to give body weight gains in rats which were similar to those obtained from feeding unmodified corn starch. However, these workers also showed that a very highly oxidized, laboratory-prepared starch gave poor body weight gains.

In vitro studies with amylase enzymes have indicated that whole starches of various types, and starch fractions are digested at different rates. Waxy maize starch was hydrolyzed more slowly than unfractionated corn starch, and much more slowly than the linear fraction from corn starch according to Caldwell and Adams (3); Sandstedt et al. (4) found that the resistance of high amylose corn starch products to digestibility by α-amylase was not directly associated with the amylose content of the starches, and Borchers (5) reported that high amylose corn starch gave digestibility values of 66 to 77% in the rat, which were much lower than the value of 95% given by normal corn starch.

Various dietary carbohydrates have been reported to affect protein metabolism. Thus, Harper et al. (6) showed that at low dietary protein levels, dextrin gives better rat growth than sucrose and fructose, and glucose gives a growth rate between that of dextrin and sucrose. When sucrose was the source of dietary carbohydrate in low protein diets, from 2 to 3% more protein was required to support rat growth equivalent to that obtained from

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a similar diet in which corn starch was the source of dietary carbohydrate (7). Chang (8) found that raw potato starch, cooked potato starch, dextrin, sucrose, corn starch, and glucose had different effects on the availability of lysine in wheat gluten diets.

The purpose of this study was to compare the protein efficiency values, and rat growth data obtained from feeding a series of unmodified starches, pregelatinized starches, disaccharides, and monosaccharides, as well as several amylose and amylopectin products.

EXPERIMENTAL

Groups of 10 rats each were fed semipurified diets containing various sources of dietary carbohydrate. The animals were Wistar strain males weighing from 50 to 55 g at the beginning of the assays. The animals were housed in individual raised-bottom wire cages in an air conditioned room kept at 75° ± 2°F. The diets contained 4% USP salts XIV, 10% corn oil, either casein[2] or wheat gluten[3] to supply protein, ample vitamins and 2% cellulose. No adjustments were made to equalize the moisture contents of the diets because previous experiments had shown that the addition of water to diets containing high levels of some sugars resulted in a poor diet texture. The maximal moisture content of the diets used in these studies was 12%.

The 15% protein diets contained 66% of each carbohydrate. Casein was also fed at 6% protein, and wheat gluten at 9% protein. These latter diets contained 77 and 73%, respectively, of the carbohydrates being evaluated. Reagent grade sugars were used as sources of fructose, maltose, dextrose, sucrose and galactose. The pregelatinized starches were obtained from various manufacturers, and therefore were subjected to different processing conditions. The amylose and amylopectin samples were also from commercial sources and probably contained small amounts of the respective branched-chain and linear fractions.

Three different rat assays were carried out to study the nutritional effects of dietary carbohydrate. At the termination of the first rat study, it was noted that the ceca of some of the animals were greatly enlarged and engorged with food. This condition would give erroneous body weight gain values in some of the test groups. Therefore, at the termination of the assays the ceca were removed and weighed so that corrections might be made for the large amounts of incompletely digested food that remained in this portion of the gastrointestinal tract. In the first assay, ceca were weighed from at least 6 animals in most test groups. In the other 2 assays all the ceca were weighed except where the ceca burst due to extreme enlargement. The cecal weights were not deducted from the body weight gains given in the tables since they were not available from all the animals in each assay.

The body weight gains, with and without the cecal weights, and the protein efficiency values were analyzed statistically by analysis of variance technique as described by Federer (9). The variation of animals within each diet was calculated. No differences in these variances could be detected among diets within an assay. Hence a weighted average variance was used for each assay as the measure of animal to animal variation. Such an average variance is more precise than the variance of a single diet because the average variance is based on more observations. Also, because it is based on more observations, the average variance permits more sensitive tests of differences among diets.

The 95% confidence limits in the tables show ± 2 standard deviations of the average, based on the averaged variance of each assay.

RESULTS AND DISCUSSION

First study with casein as dietary protein. A large selection of starches and sugars was included in the first study to determine the effects of dietary carbohydrate on the utilization of protein. As indicated in table 1 there was considerable variation in the protein efficiency values obtained from feeding different carbohydrates to weanling rats. Of the unmodified

[2] A.N.R.C. Casein, Sheffield Chemical Company, Norwich, New York.
[3] Pro 80 Wheat Gluten, General Mills, Minneapolis, Minnesota.

TABLE 1

Summary of data from first 28-day study with casein as the dietary protein

Carbohydrate source	6% Dietary protein level				15% Dietary protein level			
	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]
	g		g	g	g		g	g
Unmodified starches								
Corn	46.3 ± 7.1[3]	2.43 ± 0.36[3]	290	1.3(9)	143 ± 15.0[3]	2.42 ± 0.20[3]	361	1.4(10)
Potato	64.5 ± 7.5[4]	2.54 ± 0.37	375	9.5(7)	101 ± 15.0[4]	1.65 ± 0.20[4]	362	6.4(9)
Wheat	42.9 ± 7.1	2.27 ± 0.36	287	1.3(6)	142 ± 15.0	2.47 ± 0.20	350	1.0(7)
Arrowroot	33.1 ± 7.5[4]	1.56 ± 0.37[4]	312	5.3(10)	111 ± 15.0[4]	2.09 ± 0.20[4]	315	4.3(7)
Rice	41.9 ± 7.1	2.22 ± 0.36	285	1.3(7)	132 ± 15.0	2.27 ± 0.20	352	1.0(7)
Tapioca	37.0 ± 7.1	2.21 ± 0.36	251	1.5(7)	146 ± 15.0	2.33 ± 0.20	376	0.9(7)
Pregelatinized starches								
Potato	40.8 ± 7.5	2.62 ± 0.37	249	1.3(6)	131 ± 15.0	2.30 ± 0.20	359	1.2(10)
Arrowroot	35.1 ± 7.5[4]	2.17 ± 0.37	259	1.6(9)	118 ± 15.0[4]	2.30 ± 0.20	329	1.2(8)
Tapioca	42.2 ± 7.1	2.15 ± 0.36	315	1.2(7)	126 ± 15.0	2.21 ± 0.20	363	1.1(7)
Amioca	39.4 ± 7.1	2.33 ± 0.36	259	4.4(9)	115 ± 15.0[4]	2.03 ± 0.20[4]	348	2.8(8)
Starch derivatives								
Corn dextrin	42.6 ± 7.1	2.15 ± 0.36	314	2.8(7)	126 ± 15.0	1.97 ± 0.20[4]	401	2.2(7)
Potato amylose	62.7 ± 7.1[4]	2.49 ± 0.36	377	4.8(9)	124 ± 15.0	2.04 ± 0.20[4]	367	4.3(8)
Potato amylopectin	49.4 ± 7.1	2.65 ± 0.36	282	2.1(10)	120 ± 15.0[4]	2.37 ± 0.20	308	2.8(9)
Disaccharides								
Sucrose	27.2 ± 7.1[4]	1.57 ± 0.36[4]	285	1.2(7)	131 ± 15.0	2.42 ± 0.20	353	1.1(7)
Maltose	38.8 ± 7.1	2.16 ± 0.36	294	1.1(6)	149 ± 15.0	2.57 ± 0.20	376	1.0(7)
Lactose	1.2 ± 10.1[4]	0.13 ± 0.50[4]	157	14.4(1)	48.2 ± 21.2[4]	1.10 ± 0.28[4]	287	— (0)
Lactose-dextrose (50:50)	13.3 ± 7.1[4]	0.86 ± 0.36[4]	241	6.5(6)	102 ± 15.0[4]	2.06 ± 0.20[4]	316	4.1(7)
Monosaccharides								
Fructose	24.8 ± 7.5[4]	1.16 ± 0.37[4]	352	1.4(6)	110 ± 15.0[4]	2.00 ± 0.20[4]	357	1.5(7)
Galactose-dextrose	47.8 ± 7.1	2.29 ± 0.36	335	1.6(6)	122 ± 15.0	1.93 ± 0.20[4]	405	1.3(7)
Dextrose	37.4 ± 7.1	1.62 ± 0.36[4]	368	1.8(7)	115 ± 15.0[4]	1.95 ± 0.20[4]	364	1.3(7)

[1] Mean values based on 10 animals group where no mortality occurred. Five animals died when fed the lactose diets at both protein levels and one death occurred with the unmodified potato starch, pregelatinized potato starch, unmodified arrowroot starch and fructose, all at the 6% protein level.

[2] Mean value with number of animals in parentheses. These include both the cecum and its contents.

[3] Mean and 95% confidence limit on the mean.

[4] Means are significantly different at the 5% significance level when compared to those obtained with unmodified corn starch.

tarches, potato and arrowroot gave enlarged ceca at both the 6 and 15% protein levels and poor body weight gains at the higher protein level when casein was the dietary protein. At the lower protein level, feeding of unmodified potato starch resulted in significantly better body weight gains than those produced by the other dietary carbohydrates, and a high protein efficiency value was obtained. This superiority in weight gain from the diet containing potato starch was also evident when the cecal weights were deducted from the terminal body weights. The animals that were fed the unmodified potato starch diet consumed much more food than the other groups in this study. On the other hand, arrowroot gave a very poor protein efficiency value at the 6% protein level, and unmodified potato starch gave an unusually poor protein efficiency value at the 15% protein level. The unmodified corn, wheat, rice, and tapioca starches all gave good growth and protein efficiency values at both the high and low dietary protein levels. No evidence of enlarged ceca were noted from these starches.

Throughout these studies unmodified corn starch was used for comparisons because this carbohydrate has been used frequently as a "normal" control in the literature. In the series of pregelatinized starches which were tested at both protein levels, the arrowroot product gave growth values which were significantly lower than those obtained from unmodified corn starch. Feeding of pregelatinized amioca starch in a diet containing 15% protein resulted in significantly poorer growth and protein efficiency values when compared to corn starch in the 15% protein diets. This pregelatinized starch is a commercial product made from waxy maize corn starch. The diet containing pregelatinized amioca starch gave a diarrhea throughout the test and resulted in ceca which were enlarged as compared to the cecal weights obtained from the animals fed the other pregelatinized starches. Also, the corn dextrin diets caused a slight diarrhea. This processed starch gave ceca which were about twice the weight of those obtained with unmodified corn starch. The protein efficiency value obtained from the corn dextrin diet was also significantly poorer than the value obtained from unmodified corn starch when the dietary level of protein was 15% for both diets.

A commercial sample of potato amylose gave significantly better body weight gain and protein efficiency values at the lower casein levels when compared with the responses given by either corn dextrin or corn starch. However, as shown with the unmodified potato starch, this superiority by the amylose product was not evident when the protein level was increased to 15%. The potato amylose also resulted in enlarged ceca at both levels of dietary protein. Potato amylopectin supported normal body weight gains at the low protein level and slightly poorer body weight gains than corn starch at the higher protein level. Very good protein efficiency values were evident at both protein levels with the amylopectin. The amylopectin diets gave ceca which were slightly enlarged, but smaller than those observed in the animals fed the amylose. These data indicate that the amylopectin fraction of potato starch was better utilized than the amylose fraction at both dietary protein levels.

Of the lower molecular weight sugars, lactose produced poor weight gains, even when diluted with an equal part of dextrose. When lactose was fed as the sole source of carbohydrate there was a 50% mortality at both dietary protein levels. This was to be expected since it is known that lactose is poorly utilized by the rat at high dietary levels. Maltose and sucrose gave good growth and protein efficiency values at the 15% dietary protein level, whereas the sucrose diet gave significantly poorer body weight gains and protein efficiency when compared with unmodified corn starch at the 6% dietary protein level. Fructose or dextrose as the only source of carbohydrate gave relatively poor body weight gain and protein efficiency ratings. These values for fructose at the 6% dietary protein level were the lowest of any of the carbohydrates evaluated, with the exception of lactose.

The fructose diets resulted in somewhat larger livers, on a unit body weight basis, than the other diets. The sucrose diets also produced slightly larger livers than

the other carbohydrates (table 2). The data in the same table do not indicate that the larger livers were related to a high fat content. However, at the low casein level the sucrose diet resulted in slightly greater liver fat values than most of the diets. Most of the liver fat values were in the normal range for these levels of dietary protein with the possible exceptions of the low liver fat values obtained from feeding lactose at both protein levels, and unmodified arrowroot starch at the 6% dietary protein level. The 14.1% liver fat value obtained from the maltose diet with the higher casein level was higher than expected. Usually diets producing good protein efficiency values have liver fat values in the range of 9 to 12% on a dry basis. The animals fed sucrose, fructose, dextrose or lactose had a very "unthrifty" appearance which was especially apparent in those fed dextrose at the 15% dietary protein level.

During the last 2 weeks of the 28-day study the apparent gross digestibility of some diets was determined by a quantitative collection of the feces from 6 animals at each protein level. The average digestibility value obtained from the potato amylose was 91%, and the unmodified potato starch gave a value of 82%. The potato amylopectin, pregelatinized potato starch, pregelatinized amioca starch, and unmodified potato starch gave values of 97 to 98%. This was a further indication that the amylose, and unmodified potato starch were not completely digested.

Second study with casein as dietary protein. A second rat study was carried out to obtain data on the utilization of the amylose and amylopectin fractions of corn starch. A high amylose corn starch sample containing 55% amylose[4] and a potato amylose product from a different commercial source than the one evaluated in the first study were also included.

The data in table 3 show that the second potato amylose sample also resulted in a significantly greater body weight gain and a higher protein efficiency value at the low dietary protein level when compared with the unmodified corn starch. At both protein levels the corn amylose resulted in smaller ceca than the potato

[4] Amylon, National Starch and Chemical Corporation, New York City.

TABLE 2

Summary of liver weights and liver fat contents from first casein series[1]

Dietary carbohydrate	Liver weight: 100 g body wt		Liver fat content on dry basis[2]	
	6% Protein level	15% Protein level	6% Protein level	15% Protein level
	g	g	%	%
Unmodified potato starch	3.7	4.2	9.4	9.8
Unmodified arrowroot starch	3.5	3.8	6.2	8.5
Unmodified tapioca starch	3.8	4.0	9.4	10.7
Unmodified wheat starch	3.8	3.6	8.2	9.4
Unmodified rice starch	3.7	3.9	8.0	9.4
Unmodified corn starch	3.8	3.9	8.2	11.1
Pregelatinized amioca starch	3.8	4.3	8.1	8.4
Pregelatinized potato starch	4.0	3.9	10.4	8.7
Pregelatinized tapioca starch	4.1	4.3	12.0	8.7
Pregelatinized arrowroot starch	4.1	3.9	9.3	8.1
Corn dextrin	4.0	3.6	8.7	7.8
Potato amylose	3.6	3.8	9.0	9.2
Potato amylopectin	3.9	4.3	11.8	10.6
Sucrose	5.0	4.7	12.3	9.5
Maltose	4.1	3.9	10.0	14.1
Lactose	3.8	4.2	6.5	4.0
Lactose-dextrose (50:50)	3.9	4.2	6.7	11.4
Fructose	5.7	5.2	10.3	7.6
Galactose-dextrose	4.3	4.5	10.0	14.1
Dextrose	3.9	3.9	10.0	11.9

[1] Average liver weights determined from all the animals surviving in each group.
[2] Average liver fat data calculated from the livers which were pooled and dried before extraction with ethyl ether

TABLE 3

Summary of data from second 28-day rat study with casein as the dietary protein

Carbohydrate source	6% Dietary protein level				15% Dietary protein level			
	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[1]	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[1]
	g		g	g	g		g	g
Unmodified corn starch	51.3 ± 6.2[a]	2.95 ± 0.20[a]	260	1.7(10)	162 ± 20.2[a]	2.74 ± 0.32[a]	355	1.1(8)
Unmodified amioca starch	46.4 ± 6.2	2.58 ± 0.20	269	1.8(9)	150 ± 18.1	2.67 ± 0.28	336	1.2(10)
Pregelatinized amioca starch	42.9 ± 6.2[a]	2.90 ± 0.20	250	4.0(10)	119 ± 18.1[a]	2.60 ± 0.28	291	2.4(10)
High amylose corn starch	49.4 ± 6.2	2.55 ± 0.20	296	6.5(4)	124 ± 19.0[a]	2.38 ± 0.30[a]	350	4.5(9)
Corn amylopectin	48.6 ± 6.2	3.15 ± 0.20	258	1.6(9)	128 ± 18.1[a]	2.71 ± 0.28	301	1.1(10)
Corn amylose	52.7 ± 6.2	3.09 ± 0.20	262	4.1(10)	151 ± 18.1	2.60 ± 0.28	354	3.4(7)
Potato amylose no. 2	73.2 ± 6.24	3.25 ± 0.20	332	5.6(10)	142 ± 18.1	2.46 ± 0.28	342	4.1(10)

[1] Mean values based on an average of 10 animals/group except 8 animals in the corn starch group and 9 in the high amylose corn starch group when the protein level was 15% and 8 animals in the high amylose corn starch group when the protein level was 6%.

[a] Mean value with the number of animals in parentheses. These include both the cecum and its contents.

[a] Mean and 95% confidence limit on the mean.

[a] Means are significantly different at the 5% significance level when compared to those obtained with unmodified corn starch.

amylose. The corn amylose did not result in the large weight gains and food intakes at the lower dietary protein level as shown by both the unmodified potato starch and the potato amylose in the 2 studies where casein was the protein source. This suggests that the corn amylose may have been better utilized than the potato amylose product at the low dietary protein level. The high amylose corn starch gave the largest ceca in this series of diets; however, the enlarged ceca were not accompanied by increases in body weight gains at the lower level of protein intake. When the 15% protein diets were compared, the high amylose corn starch produced significantly poorer body weight gains and protein efficiency than the unmodified corn starch.

The corn amylopectin gave relatively poorer body weight gains at the higher dietary protein level. This appeared to be due to a depression in food intake since the protein efficiency values and ceca sizes were normal at both levels of dietary protein.

In this second study the pregelatinized amioca starch gave larger ceca and significantly poorer body weight gains than either the unmodified amioca starch or the unmodified corn starch in both the high and low protein diets. This pregelatinized starch product resulted in a diarrhea which was not observed in the animals fed the unmodified amioca starch. These data indicate that the processing conditions used in manufacturing the pregelatinized amioca starch could have been responsible for the different responses obtained. Moinuddin and Lee (10) reported that the addition of cellobiose, a poorly digested carbohydrate, to a high protein casein diet also resulted in diarrhea, poor body weight gains, and enlarged ceca in the rat.

Wheat gluten as the dietary protein. A third rat assay included a series of carbohydrates with wheat gluten as the protein source at 9 and 15% dietary protein levels. The 9% protein level was fed in this series since wheat gluten is a protein of relatively poor quality and it was doubtful whether the animals would grow on a level of 6% protein.

The data in table 4 show that unmodified potato and arrowroot starches gave

TABLE 4

Summary of 28-day data with wheat gluten as the dietary protein

Carbohydrate source	8% Dietary protein level				15% Dietary protein level			
	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]	Body wt gain[1]	Protein efficiency[1]	Dry food consumed[1]	Cecum wt/100 g body wt[2]
	g		g	g	g		g	g
Unmodified starches								
Potato	15.1 ± 3.2[3,4]	0.41 ± 0.12[3]	358	7.2(9)	48.1 ± 5.1[3,4]	0.66 ± 0.11[3]	431	6.8(9)
Arrowroot	7.4 ± 3.0	0.19 ± 0.12[4]	384	4.9(10)	23.9 ± 5.1	0.38 ± 0.11[4]	383	5.3(9)
Corn	10.4 ± 3.0	0.36 ± 0.12	286	1.4(10)	29.1 ± 4.8	0.57 ± 0.10	331	0.9(10)
Modified starches and starch derivatives								
Pregelatinized potato starch	14.1 ± 3.0	0.54 ± 0.12[4]	284	1.2(10)	32.3 ± 5.1	0.70 ± 0.11	290	1.0(10)
Pregelatinized tapioca starch	10.5 ± 3.0	0.40 ± 0.12	277	3.7(10)	26.9 ± 4.8	0.65 ± 0.10	260	3.1(10)
Pregelatinized arrowroot starch	11.3 ± 3.0	0.49 ± 0.12	249	1.8(10)	26.5 ± 4.8	0.51 ± 0.10	310	1.3(10)
Potato amylose	17.8 ± 3.2[4]	0.71 ± 0.12[4]	253	5.6(10)	50.4 ± 4.8[4]	0.90 ± 0.10[4]	344	5.0(10)
Potato amylopectin	13.1 ± 3.0	0.50 ± 0.12	260	1.8(10)	37.1 ± 4.8[4]	0.80 ± 0.10[4]	283	1.6(10)
Corn dextrin	10.8 ± 3.0	0.38 ± 0.12	331	2.5(10)	36.1 ± 4.8	0.71 ± 0.10	323	2.4(10)
High dextrose dextrin	11.1 ± 3.0	0.42 ± 0.12	293	1.4(10)	32.5 ± 4.8	0.72 ± 0.10	291	1.1(10)
Di- and monosaccharides								
Maltose	8.6 ± 3.2	0.39 ± 0.12	238	1.6(9)	30.0 ± 4.8	0.68 ± 0.10	298	1.0(10)
Sucrose	5.2 ± 3.0	0.26 ± 0.12	211	1.6(10)	27.5 ± 4.8	0.71 ± 0.10	256	1.1(10)
Dextrose-fructose (50:50)	9.6 ± 3.4	0.35 ± 0.13	281	1.4(10)	31.2 ± 5.1	0.59 ± 0.11	357	1.0(9)
Dextrose	13.0 ± 3.0	0.45 ± 0.12	315	1.4(10)	30.5 ± 4.8	0.57 ± 0.10	347	1.0(10)

[1] Mean values based on 10 animals/group with the exception of 9 animals in the groups fed unmodified potato starch, pregelatinized potato starch, unmodified arrowroot starch and dextrose-fructose in the 15% protein diets; also 9 animals in the group fed amylose, unmodified potato starch, and maltose and 8 animals from the diet containing dextrose-fructose in the 8% protein diets.

[2] Mean value with number of animals in parentheses. These include both the cecum and its contents.

[3] Mean and 95% confidence limit on the mean.

[4] Values are significantly different at the 5% significance level when compared to those obtained with unmodified corn starch.

enlarged ceca and greater food intakes than the other carbohydrates included in the study. The sucrose diet resulted in poor food consumption which was below that obtained from feeding a mixture of fructose and dextrose at the levels equivalent to their levels in sucrose. At the 15% dietary protein level, potato amylose and unmodified potato starch gave significantly better body weight gains than the unmodified corn starch, even when the cecal weights were deducted from the terminal body weights. This is similar to the data obtained at the 6% dietary protein level with casein as the source of protein. At both wheat gluten protein levels, the amylose gave significantly better protein efficiency values than unmodified corn starch.

The unmodified arrowroot starch did not result in large body weight gains in spite of the presence of greatly enlarged ceca. In fact, at both protein levels the protein efficiency values obtained from the unmodified arrowroot starch were significantly lower than the corresponding values obtained from unmodified corn starch. Another similarity to the first study was the cecal enlargement noted in the rats fed either the pregelatinized amioca starch or corn dextrin. The dextrin product containing the higher dextrose level, which was equivalent to 29% reducing sugars as dextrose, gave normal ceca. This again gives an indication that the process used in making the corn dextrin with the lower dextrose content resulted in a product which was more resistant to digestion. The low dextrose corn dextrin used in these studies had been prepared from corn starch which was heated in the presence of "mineral acid." In a recent publication, Leach and Schoch (11) have shown that an 80-fluidity corn starch which had been treated with acid was more resistant to solubilization by dimethyl sulfoxide than unmodified corn starch. These authors noted that poorly soluble starches were resistant to digestion by α-amylose.

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THE DIGESTIBILITY OF HIGH-AMYLOSE CORN STARCHES COMPARED TO THAT OF OTHER STARCHES. THE APPARENT EFFECT OF THE *ae* GENE ON SUSCEPTIBILITY TO AMYLASE ACTION[1]

RUDOLPH M. SANDSTEDT, DONNA STRAHAN, S. UEDA,[2] AND R. C. ABBOT

ABSTRACT

There is much variation between the raw starches in their susceptibility to the action of pancreatic alpha-amylase. In general the resistance to digestion was not directly associated with amylose content. A 40% amylose starch from corn was the most susceptible, quite similar to waxy corn starch with less than 5% amylose. Wrinkled-pea starch with 65% amylose was intermediate in digestibility, whereas a 61% amylose corn starch was highly resistant but not as resistant as potato starch with 22% amylose. Among the high-amylose corn starches, those from corn homozygous for either the *du* or *su*$_2$ gene, or both, were highly susceptible to digestion whereas those from corn homozygous for the *ae* gene alone or in combination with *su*$_2$ or *du* were resistant to digestion. A high-amylose corn having a starch highly resistant to enzyme action proved to be highly resistant to digestion when fed to rats and chicks, confirming the *in vitro* laboratory digestions.

High-amylose corn starch is one of the promising starches on the industrial horizon. At present, corn yielding starch with 60 to 65% amylose is being grown under contract for industrial research purposes, and an intense breeding program is in progress designed to increase the amylose content. The present industrial goal is the production of a hybrid corn with good agronomic characteristics which will yield starch with 80% or more amylose.

In the course of studies on the structure of starch granules, the susceptibility of various raw starches to enzyme action was investigated. It was noted that a high-amylose corn starch (Amylin with an amylose content of 55 to 60%) was much more resistant to the digestive enzymes than were the other cereal starches. This was unexpected, since the raw cereal starches, in comparison to the resistant raw "root" starches, were all thought to be readily susceptible to digestion in the animal digestive tract (3). Such indigestibility could be important, since, if at some time it were produced in excess of industrial requirements, it would normally be diverted into feed channels. Accordingly, a number of high-amylose corn starches varying in amylose content from 35 to 67% and, for feeding purposes, a corn yielding a starch with 63% amylose were obtained for further study.

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Laboratory Methods and Materials

Digestibility of Raw Starch. The method used was a modification of that described by Gates and Sandstedt (5). Essentially it consisted of a 20-hour digestion of 0.4 g. of raw starch in 15 ml. of a solution containing 30 mg. calcium chloride, 30 mg. gelatin, 1 ml. chloroform and pancreatin (to give 25 SKB units of alpha-amylase) at 30°C. and at pH 6.5 with continuous shaking. The undigested residue was filtered, dried, and weighed. The loss in weight expressed as percent of the starch is designated as the digestibility of the starch. However, under these conditions the accumulated end-products of digestion are present during the digestion and, in cases of high digestibility, these end-products may materially inhibit enzyme action. The spread in digestibility accordingly may be wider than that shown.

Determination of Alpha-Amylase. The alpha-amylase content of the enzymes was determined by the SKB method (12), modified by using the glass color-standard as suggested by Redfern (10) and the "beta-amylase for analytical purposes" from the Wallerstein Laboratories.

Digestibility of Starch in Ground Corn. The method was essentially determination of the CO_2 pressure produced by fermentation of sugars obtained from pancreatic digestion of the starch in the ground corn. A 2-g. sample of the corn, ground in a Wiley mill to pass a 1-mm. sieve, was placed in a 50-ml. beaker, mixed with 10 ml. water containing 160 mg. pancreatin (200 SKB units of pancreatic alpha-amylase) and 0.3 g. of baker's compressed yeast. The beaker and contents were placed in a pressuremeter (11) and allowed to ferment for 20 hours at 30°C. The CO_2 pressure (mm. Hg) produced was read at suitable intervals and plotted against time. The curve obtained was corrected for a blank representing fermentable sugars present in the grain, or in some cases possibly representing damaged starch (13). This blank was obtained by extrapolating to zero time a straight line drawn from the points representing the pressures obtained at 2 hours and at 8 hours.

Factors for the Conversion of CO_2 Pressures (mm. Hg) to mg. Maltose. A 500-mg. sample of maltose monohydrate was mixed with the substance under study (ground corn, starch, gelatinized starch, etc.) plus all ingredients that were used in the regular fermentation except the enzyme (pancreatin, malt, or other). This mixture was fermented for 20 hours. A blank containing no maltose or enzyme was also fermented. The pressure obtained from the maltose fermentation was corrected for the blank. Then 500 divided by the corrected pressure

= maltose per mm. pressure. This factor may vary according to the yeast nutrients and activators present with the fermenting maltose mixture (2).

Starches. All starches were unmodified. The 38-, 40-, 47-, 54-, 57-, and 61% -amylose samples were prepared in the Nebraska Laboratory from corn furnished by H. H. Kramer of Purdue University and the 63% -amylose starch from a double-cross hybrid corn furnished by American Maize-Products Co. The other starches were furnished by National Starch Products Co. and American Maize Co. The information concerning the amylose content and the genetic composition of the high-amylose starch samples was furnished by the individuals who supplied the samples.

Pancreatin. Fisher Scientific Co. Assayed for alpha-amylase content.

Results and Discussion

The data presented in Fig. 1 compare the susceptibility to digestion by pancreatin of a number of raw starches. The values given are averages of closely agreeing duplicates.

"Root" Starches. It is often stated that the so-called "root" starches (tubers, bulbs, enlarged root stocks, corms, and rhizomes) are exceedingly difficult to digest. The data of Fig. 1 indicate that there was considerable variation in resistance to enzyme action among these starches, canna being only 3% digested and potato 5%, but cassava was ten times higher. To be certain that this was representative of cassava starches, three samples obtained from different sources over widely separated periods of time (about 10 years) were analyzed; digestibilities of 53, 55, and 58% were obtained. Cassava starch has an amylose content of about 18% and potato starch about 22%.

Cereal Starches. The cereal starches were exceedingly variable, the widest variations being in the maize starches. On arranging the maize starches in the order of increasing amylose content, it was noted that digestibility apparently was not directly related to amylose content. The waxy corn starch with less than 5% amylose was highly susceptible, but so were the samples with intermediate amylose content (36, 38, 40, and 47%). The 40% -amylose sample was the most susceptible of the entire series of starches. Yet with greater increases in amylose content (48% and higher amylose), digestibility dropped drastically.

Genetic Composition and Susceptibility to Amylase Action. The genetic composition and interaction of the genes of high-amylose corn have been thoroughly reviewed by Kramer, Pfohler, and Whistler (7). Unfortunately only a few high-amylose samples with known genetic composition were available for this particular study, but it

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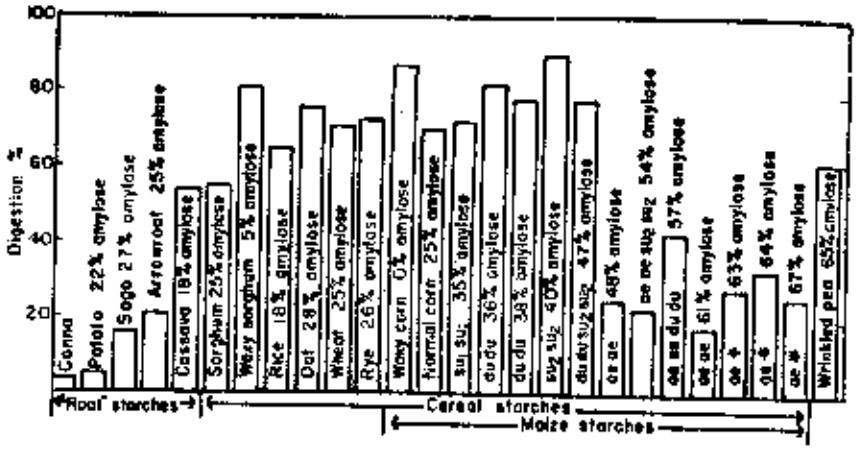

Fig. 1. A comparison of the susceptibility of various starches to pancreatic digestion. Asterisk denotes unknown genetic composition.

appears from the data obtained (Fig. 1) that the gene *ae* may be associated with both high amylose and high resistance to enzyme action. On the other hand, the genes *du* and su_2, though associated with an intermediate amylose, seem also to be associated with high susceptibility to digestion. The samples homozygous for the *du* and su_2 genes (*du du* and $su_2 su_2$) and their combination, *du du* $su_2 su_2$, were highly susceptible, the $su_2 su_2$ sample being the most susceptible. The sample with 61% amylose, homozygous for the *ae* gene, was the most resistant of the corn samples. The samples homozygous for *ae* and *du* (*ae ae du du*) or *ae* and su_2 (*ae ae* su_2 su_2) were also low in digestibility. If the resistance to digestion is controlled by the *ae* gene and susceptibility by the *du* or su_2 genes, the *ae* gene would appear to exert an epistatic effect on the *du* or su_2 genes, thereby giving not only high amylose but also high resistance to digestion. The only information available concerning the genetic composition of the samples with 63, 64, and 67% amylose is that they contained the *ae* gene.

Susceptibility vs. Amylose Content. Comparisons of other data of Fig. 1 including the noncereal starches give further evidence that amylose content, in itself, is not the factor that determines digestibility. Potato and normal corn starches have approximately the same amylose content but were widely different in digestibility, whereas wrinkled-pea, with an amylose content of about 65% (6), was relatively susceptible if compared to normal corn but highly susceptible if compared to potato. The reasons for differences in susceptibility to enzyme action are still obscure; the answer probably lies in the

structure of the starch granules, i.e., in differences in the bonding between the starch molecules; and possibly, also in anomalous linkages within the molecules.

Digestibility of Starches in Ground Whole Corn. Since starch is not fed to animals as purified starch but in the grain, it is of interest to show whether the differences in digestibility of starches will also be shown in digestions of ground corn or whether the differences would be masked by the difficulty of digesting larger pieces of endosperm.

Since the method used for the study of the purified starches could not be used for the digestion of starches mixed with other insoluble materials, a fermentation method for determining the sugars produced by digestion appeared to be the best method available (9,11). This method has an advantage in that the sugars produced are fermented and thus do not inhibit amylase action. Also, pressure readings may be taken at intervals enabling curves to be drawn showing the change in rate of digestion with time. It has the disadvantage, when pancreatin with its pH optimum of about 6.5 is used, of a decreasing pH with time; however, the pH change did not seem to cause a decrease in rate of digestion. Maltose ferments rapidly in the presence of ground corn, indicating that the corn furnishes an adequate supply of yeast nutrients and activators (2).

Figure 2 shows data obtained from a fermentation study of the digestibility of the starch in normal ground corn compared to that in high-amylose (63% -amylose) corn.

The steep slope in the curve for the high-amylose corn during the first 2 hours probably indicates the presence of a small amount of fermentable sugar. This fermentable sugar is to be expected since both the *du* and su_2 genes induce the formation of a "sugary" endosperm (7,8). A straight line running through the 2-hour and 8-hour pressure points extrapolated back to zero-time gives a correction of 90 mm. pressure for this fermentable material. The dashed curve is the curve corrected for this blank. There is no correction for the normal corn; in this case the extrapolated line runs through zero-time at the origin and there is no break in the curve at approximately 2 hours.

The slopes of the curves indicate the relative rates of digestion. The ratios of the pressure at any given time give the relative digestibility of the starches. From 2 to 10 hours the digestion on the high-amylose corn was 40% of that of the normal corn; after this time the digestion on the normal corn was slowing down slightly more than that on the high-amylose corn; accordingly, at 20 hours the high-amylose corn was 44% of the normal corn. According to the data of

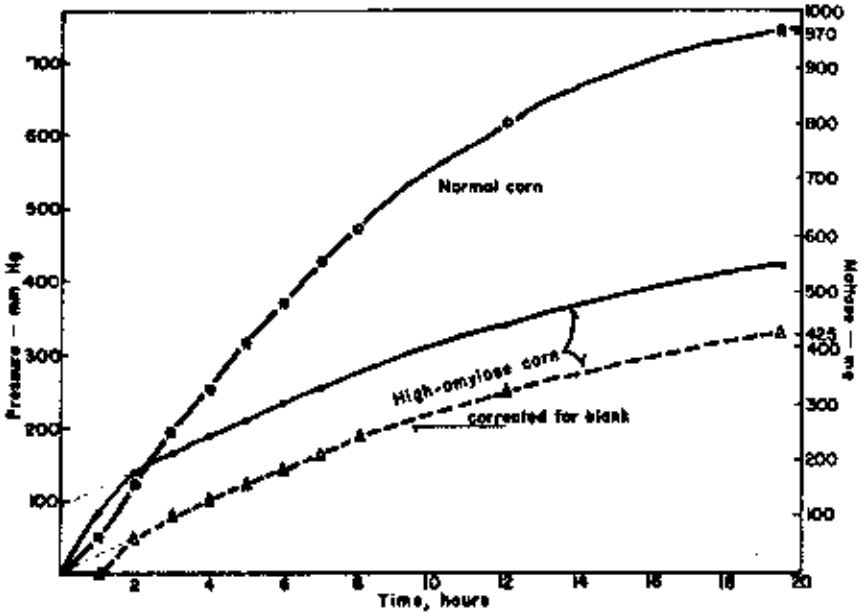

Fig. 2. A comparison of the digestibility of a normal ground corn with that of a 63% -amylose corn.

Fig. 1, the 63% -amylose starch was 37% as digestible as the normal. Considering the wide differences in the mechanical condition of the starches in these two materials (starch vs. ground corn), this may be considered excellent agreement.

In Vivo Studies

The *in vitro* digestibility of the starch contained in the 63% -amylose corn compared to that of normal corn has been substantiated by a study of the comparative utilization of these starches in the ground grains by growing rats and chicks.

Rat-Feeding Trials. Under the direction of R. L. Borchers [4], growing rats were fed equivalent diets containing 75% ground corn: normal corn versus 63% -amylose corn [4]. The feed consumption was held at the same level for all rats.

The most dramatic evidence of the relative digestibilities of the starches in the two diets is presented in Fig. 3. This picture compares the 24-hour accumulation of feces from two rats, A on normal corn, B on high-amylose corn. No starch was notable on microscopic examination of the feces from normal corn; this would be expected, since the fragments of granules remaining after 95

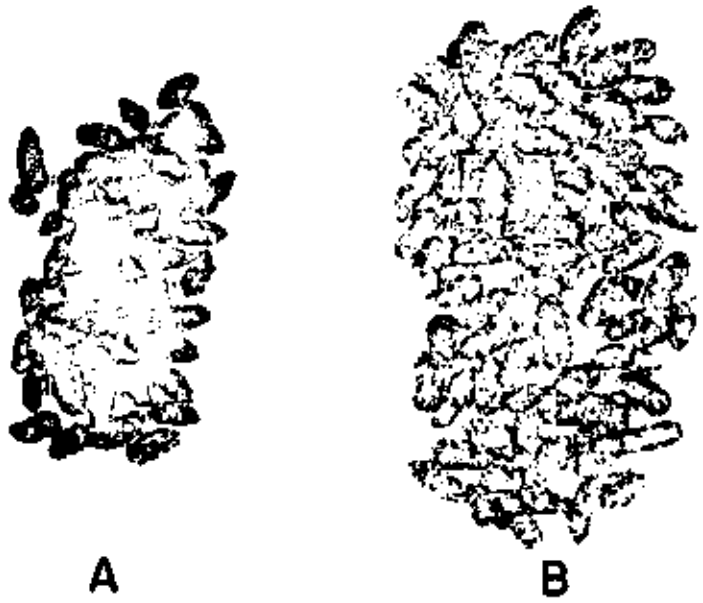

Fig. 3. Visual evidence of the resistance to digestion by rats of a 63% -amylose corn compared to that of normal corn. The 24-hour feces from: A, a rat on a diet containing 75% of normal corn; B, a rat on a diet containing 75% of 63% -amylose corn.

to 98% digestion (3,4) would be difficult to recognize among the other residues. On the other hand, the feces from the high-amylose corn diet were loaded with intact starch granules. The average daily weight (dry basis) of feces from normal corn was 0.76 g., whereas from high-amylose corn it was 1.97 g., or 2.6 times as much. Assuming that the extra weight of the feces from the high-amylose diet was due to undigested starch and that undigested starch was practically absent from feces from the normal corn, there would be 61% starch in the high-amylose feces.

Borchers' data (4) indicate incomplete digestion of the high-amylose corn starch by rats, but a much more complete digestion than under the conditions for *in vitro* digestions in the laboratory.

Chick Feeding. Evidence similar to that obtained from rat feeding also was obtained by C. W. Ackerson (1) from chick feeding. In this feeding trial, the first week's accumulation of feces from a lot of 15 chicks on normal corn weighed 621 g. (air-dry) as compared to 1,231 g. (air dry) from the lot of 15 chicks on the high-amylose diet, or 1.9 times as much. The visual comparison (Fig. 4) is not as dramatic as the comparison on the rats, since chickens excrete large quantities of uric acid which is white; this obscures the difference due to the white

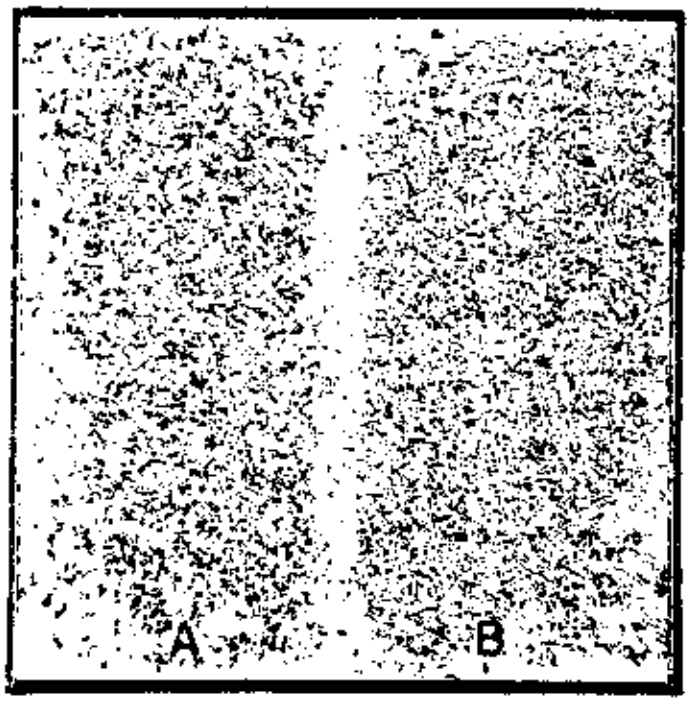

Fig. 4. The digestion by chicks of normal vs. 63% -amylose corn. One week's accumulated feces from a lot of 15 chicks on a diet containing: A, 60% normal corn; B, 60% of 63% amylose corn.

undigested starch. Microscopic examination showed no starch in the sample from the normal corn but much in the sample from high-amylose corn. Again assuming the entire extra weight of the feces from high-amylose corn to be due to undigested starch, the high-amylose sample was 58% starch.

Conclusions

The digestibility of raw corn starch is not directly associated with amylose content; however, the genes involved in producing high-amylose starch in corn are apparently also associated with the digestibility of the starch. According to the data at hand, the su_2 and *du* genes produce susceptibility, whereas the *ae* gene, which seems necessary for the production of high amylose in combination with desirable agronomic characteristics, produces starch highly resistant to enzyme action. Accordingly, high-amylose corn, which is desirable for industrial purposes, will probably contain the *ae* gene and therefore may be resistant to digestion.

Acknowledgments

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Seronde '69
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Influence of Dietary Carbohydrate on Duodenal Lesions in the Mouse [1,2]

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ABSTRACT A complete purified diet which promotes normal growth in rats and mice, and in which glucose is the only carbohydrate, increases the incidence and severity of avillous hyperplasia, a lesion of the duodenal mucosa occasionally encountered in stock-fed mice. The lesion is a focal thickening, composed of tubules derived from the crypts of Lieberkühn, and villi are absent. A further increase in incidence and severity of the lesion occurs in pantothenic acid deficiency, and chronic ulceration may then be superimposed upon it. Sucrose, substituted for glucose in the complete purified diet, causes a moderate decrease in incidence of the lesion. Cornstarch substituted for glucose causes a marked decrease in incidence. In neither case is severity significantly affected. In the pantothenate-deficient diet, cornstarch substituted for glucose reduces incidence of the lesion to approximately that produced by the glucose-containing complete diet. Severity, though markedly reduced by cornstarch in the pantothenate-deficient diet, still remains well above levels for the complete diet. The incidence of chronic ulcer in the starch-containing pantothenate-deficient diet fell from 30% to 19%, but this was not statistically significant.

Focal avillous hyperplasia of the crypts of Lieberkühn is the first of two lesions to be discussed in this report (1). It consists of grossly visible thickened plaques in the proximal duodenal mucosa. These lack villi, and microscopically are composed of large numbers of parallel tubules extending from the level of the muscularis mucosae to the surface. The second lesion is the deep chronic ulcer of pantothenic acid deficiency (2), believed to develop only in foci of avillous hyperplasia. Both these lesions are illustrated in figure 1. Avillous hyperplasia occurs to an inconspicuous extent in mice fed a natural foodstuffs stock diet. Its incidence and severity is far greater if a complete purified diet, containing 70.9% glucose, is fed in place of the stock diet; and a further marked increase occurs if the purified diet is deficient in pantothenic acid.

This aggravation of avillous hyperplasia by our complete purified diet, which long experience had demonstrated to be satisfactory in all other respects, provoked a search for an explanation; and, after certain other factors had been tested and eliminated, the following experiments were undertaken in which the type of carbohydrate was varied.

MATERIALS AND METHODS

Mice of the Princeton strain (2) were used exclusively. The composition of the purified diets is given in tables 1 to 3. The natural foodstuffs stock diet [3] contained 23% protein derived principally from skim milk powder, fish meal, and soybean oil meal; 4% fat; and about 60% carbohydrate, presumed to be mostly (cooked) starches. The pantothenic acid-deficient diet (designated 2517) was identical to the purified diet (2520), except for the omission of that vitamin.

All animals were fed ad libitum. They had access to the stock diet prior to complete weaning at 21 days, and were placed on experiment when males had attained a body weight of at least 14 g, and females 12 g, at about 4 weeks of age. At this time they were transferred from enamel pans containing pine shavings to wire-mesh-bottomed cages. For the first week, the purified diets were modified by mixing them with 25% of powdered stock diet to improve their acceptance and to reduce the number of spontaneous casualties

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[1] Supported in part by Red Acre Farm, Inc., and in part by Mrs. Christian A. Herter.
[2] Presented in part at FASEB meeting, Atlantic City, New Jersey, April 16, 1969. Federation Proc., 28: 625, 1969 (abstract).
[3] Hemlock Hollow, Wayne, New Jersey.

TABLE 1

Composition of purified diets

	g/kg
Casein (vitamin free) [1]	180.0
Cottonseed oil	48.0
Glucose, sucrose, or raw cornstarch	709.0
Bone ash	25.0
Wesson salt mixture, modified [2]	12.0
Vitamin supplement [3]	20.0
l-Cystine	2.0
Choline-Cl	1.0
Cystoine-HCl (as antioxidant)	1.0
Tocopherol, carotene, and vitamin D_2 in cottonseed oil [4]	2.0

[1] Purchased from Whitson Products, New York, New York.
[2] Ingredients listed in table 2.
[3] Ingredients listed in table 3.
[4] Alpha-tocopherol acetate, 50 mg; vitamin A as carotene, 15,000 IU; vitamin D_2, 1000 IU.

TABLE 2

Composition of modified (3) Wesson salt mixture

	g/kg diet
NaCl	3.6
KCl	4.3
$MgSO_4$	3.3
$FeSO_4$ "dried"	0.57
	mg/kg diet
NaF	21.0
ZnO	12.0
$MnSO_4 \cdot H_2O$	16.1
Cu_2O	4.0
$AlK(SO_4)_2 \cdot 12H_2O$	3.3
KI	1.8

TABLE 3

Composition of vitamin supplement

	mg/kg diet
Thiamine·HCl	10.0
Pyridoxine·HCl	10.0
Riboflavin	20.0
Niacin	40.0
Calcium pantothenate [1]	40.0
Pteroyl glutamic acid	2.0
Biotin	0.20
Vitamin B_{12}	0.012
Inositol	200.0
Para-aminobenzoic acid	100.0

[1] Omitted from diet 2517.

during the first few days. Such deaths could generally be attributed to starvation. Thereafter, the experimental diets were fed undiluted. Both sexes were used, and were caged separately, five or six to a cage (18 by 20 by 20 cm).

In experiment 1, 53 mice were fed the complete purified diet, modified by substituting sucrose for glucose. There were 44 controls. In experiment 2, 61 mice received the diet prepared with cornstarch instead of glucose, and were compared with 57 controls. In experiment 3, 62 mice were fed the pantothenic acid-deficient diet, modified with cornstarch. Seventy controls received the glucose-containing pantothenate-deficient diet.

After 4 weeks on the undiluted diets, animals were anesthetized with ether, opened, and the duodenums were injected with buffered (pH 7.3) formalin via the stomach. Following this, the specimens were removed, opened and pinned out under additional formalin for completion of fixation and for observation. Lesions were measured with a micrometer eyepiece in a dissecting microscope.

RESULTS

Villous hyperplasia. In the complete diet (2520), a highly significant reduction in incidence of lesions occurred as the complexity of dietary carbohydrates was increased (table 4). The average area of lesions was not significantly changed.

In the pantothenate-deficient diet (2517), when glucose was replaced by starch, the enhancing effect of the deficiency upon incidence and average area of lesions was eliminated for the former, and much reduced for the latter.

Fig. 1 Longitudinal section through the distal pylorus (right), and the proximal duodenum (left), of a pantothenate-deficient mouse. The large mucosal discontinuity in the center of the photograph is a deep chronic ulcer (U) which has penetrated all layers of the duodenum, and provoked marked edema, inflammation, and scarring of the underlying pancreas with its supporting connective tissue. To the left of the ulcer, the duodenal mucosa has lost nearly all of its customary villated character, and become thickened, due to marked overgrowth (hyperplasia) of the crypts of Lieberkühn (AH). The mucosa of the pylorus (PY) bears a superficial resemblance to the duodenal mucosa, at this magnification, but is actually not involved in the disease process. Chloroazole black E × 48. (Photograph by Leo Goodman, Mallory Institute of Pathology.)

Figure 1

TABLE 4

Incidence and area of lesions; complete diets (2520), and pantothenate-deficient diets (2517)

Diet	Incidence	Area				
		Avg [1]	se	df	t	P
	%	mm²				
2520 (Glucose)	91.0	2.48	0.54	—	—	—
2520 (Sucrose)	56.7	3.42	0.76	—	—	—
Comparison	$\chi^2 = 14.093$	—	—	68	1.038	0.4-0.2
2520 (Glucose)	86.0	2.48	0.66	—	—	—
2520 (Starch)	11.5	1.29	0.43	—	—	—
Comparison	$\chi^2 = 65.575$	—	—	51	0.983	0.4-0.2
2517 (Glucose)	97.0	16.38	1.29	—	—	—
2517 (Starch)	82.0	6.25	0.94	—	—	—
Comparison	χ^2 8.213	—	—	117	6.012	< 0.001

[1] Averages include only affected animals.

Ulcer. The incidence of chronic duodenal ulcer in mice fed the glucose-containing pantothenate-deficient diet was 30%. When cornstarch was substituted for glucose, the incidence fell to 18%, but this was not statistically significant (χ^2 = 2.71). When figures in the control series were supplemented with 56 additional cases taken from previous experiments in which the cornstarch modification had not been fed, the value for χ^2 rose to 2.95—still remaining below the level of significance.

DISCUSSION

Focal avillous hyperplasia of the proximal duodenal mucosa is shown to be affected by the type of carbohydrate ingested. An influence of this sort has not, to our knowledge, been previously reported, and the mechanism is unknown. Logically, we presume it to be related, directly or indirectly, to the nature of the chyme whose composition would vary according to whether a starch or glucose diet had been fed. In the former instance it is unlikely that more than traces of glucose would be present in the proximal duodenum, while distally, rapid absorption of the glucose gradually released by digestion, would prevent any marked accumulation (4). In the case of a high-glucose diet, an abundant and relatively concentrated glucose solution would strike the duodenal mucosa, affecting it perhaps directly through its pH or osmotic properties, or indirectly by fostering abnormal bacterial or protozoan inhabitants. Differential metabolic effects produced by the feeding of various carbohydrates are abundantly documented (5), but we have found no reported link between any of these and the morphology of the intestinal mucosa.

Avillous hyperplasia closely resembles a nonspecific lesion which has been encountered clinically and experimentally under a variety of conditions (6). In their descriptions, investigators generally have put greater emphasis upon the absence of villi than upon subvillous changes; and the lesion has been most frequently referred to as "villus atrophy." Because villus cells are passive as far as growth is concerned, and because the crypts from which they arise are so manifestly altered, we feel that our emphasis is justified. In the course of the present investigation, a carbohydrate effect has been recognized and it has become possible to separate it from that of pantothenic acid deficiency. This removes the carbohydrate effect, at least provisionally, from our main line of investigation of chronic duodenal ulceration, although the phenomenon remains of interest in itself.

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Shaw '72
Bib No. 1356

Caries in Rats with Starch as the Only Dietary Carbohydrate

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Starch replacement for all monosaccharides or disaccharides in cariogenic diets has resulted in major reductions or prevention of caries in rodents (W. G. SHAFER, *Science* **110**: 143-144, 1949; G. FROSHEIT, P. H. KEYES, and R. H. LARSON, *J Nutr* **93**:65-76, 1967). However, in experiments in this laboratory (unpublished results) some rats maintained on diets with corn starch as the only carbohydrate developed extensive carious lesions in the occlusal sulci and on the smooth surfaces. An experiment was conducted to determine if continuous use of corn starch as the only dietary carbohydrate for several generations leads to a greater frequency of rats with carious lesions and higher caries activity. The rats were descendants of stock obtained 20 years ago from the Wistar Institute's mutant albino strain. Throughout life rats were fed diet 2718, Harvard cariogenic diet 2700 in which all sucrose had been replaced by corn starch (J. H. SHAW, *J Nutr* **88**:419-426, 1966). Breeders were selected from each generation on the basis of grossly observable carious lesions. The remaining rats were killed to evaluate the extent of the carious process at 84 days of age in the first four generations, and at 150 days in the fifth through seventh generations. None of the rats, breeders or experimentals, throughout the seven consecutive generations consumed any monosaccharides or disaccharides. The indigenous flora of these rats was not modified by inoculation with known cariogenic flora. Comparable populations were maintained for the first four generations on diets that contained only sucrose or glucose as the carbohydrate.

Offspring of parents fed the starch diet grew less rapidly and were subject to higher neonatal mortality than offspring of parents fed the same diet with sucrose or glucose as the carbohydrate. The results for carious lesions in the occlusal sulci and on the smooth surfaces are shown in the table. Throughout the seven generations the percentage of cariesfree rats was lower and the extent of carious lesions at both types of sites in the molars was higher than would be expected from previously published data where starch was the only carbohydrate. Although the breeders were selected to emphasize the development of carious lesions, considerable variation was observed in the expression of caries from generation to generation. No trend was observed toward a higher frequency or more rapid progression of the lesions as a result of genetic selection. Among comparable rats fed sucrose or glucose only, an occasional rat was cariesfree. For the 69 rats fed the sucrose diet, caries activity in the occlusal sulci was 34.1+ ± 1.9+ and on the smooth surfaces it was 5.0+ ± 0.8+. For the 79 rats fed the glucose diet, caries activity in the occlusal sulci was 26.6+ ± 1.8+ and on the smooth surfaces it was 4.4+ ± 0.6+.

Typical carious lesions in a rat of the sixth generation are shown in the illustration.

Typical carious lesions in sixth generation rat.

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Additional information available on request to authors.

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TABLE
INFLUENCE OF STARCH DIET ON CARIES ACTIVITY

Generation	Age	No. of rats	Carious Lesions in Occlusal Sulci: Percentage of Cariesfree Rats	Carious Lesions in Occlusal Sulci: Extent of Carious Lesions	Carious Lesions on Smooth Surfaces: Percentage of Cariesfree Rats	Carious Lesions on Smooth Surfaces: Extent of Carious Lesions
1	84	8	37.5	12.4+ (5.1+)	12.5	10.1+ (2.6+)
2	84	5	60.0	10.6+ (3.0+)	0	5.9+ (1.5+)
3	84	6	66.7	2.2+ (1.5+)	50.0	0.7+ (0.4+)
4	84	12	33.3	10.4+ (3.3+)	33.3	2.1+ (0.6+)
5	150	26	73.1	5.5+ (2.4+)	46.2	3.1+ (1.0+)
6	150	27	22.2	12.0+ (4.8+)	18.5	8.3+ (1.4+)
7	150	47	38.3	16.7+ (2.9+)	25.5	5.7+ (0.8+)

Silverman '66
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Ann. Intern. Med. 64: 842-846, 1966.

Bilateral Parotid Enlargement and Starch Ingestion

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THE FINDING of bilateral, asymptomatic parotid gland enlargement associated with prolonged starch ingestion has been reported once in the literature. In that case, Merkatz (1) described a 36-year-old Negro female chronic starch eater whose dietary history showed minimal protein intake. The suggested primary relationship between starch ingestion and parotid enlargement might be subject to criticism since parotid swelling has been attributed alone to various states of malnutrition. Recently a patient with chronic starch ingestion and bilateral parotid enlargement came to our attention. This patient had adequate nutritional intake. This case is reported to strengthen the proposed etiologic relationship between starch eating and parotid swelling. The differential diagnosis and theories of parotid enlargement are reviewed. Some comments are added concerning sociologic aspects of geophagy.

CASE REPORT

A 29-year-old Negro female was admitted to University Hospital June 2, 1964, for evaluation of hypertension, anemia, and bilateral, painless parotid gland enlargement (Figure 1). Enlargement of the right parotid gland had been noticed in the medical clinic in 1961 and was thought to be related to an abscessed tooth. In May, 1964, bilateral parotid swelling was observed. Past history included mumps parotitis as a child, toxemia during pregnancy, and extreme nervousness. An anemia as low as 8.4 g of hemoglobin/100 ml had been noted since 1961, but the etiology was not investigated. Alcohol ingestion was minimal and infrequent. A dietary history, obtained by the medical clinic dietitian, showed an estimated daily minimal protein consumption of 30 g. Positive physical findings included mild obesity of 190 lbs blood pressure of 150/100 mm Hg, and bilateral, diffuse, nontender parotid swelling. Tear formation was possible, and saliva was present. There was no lymphadenopathy or hepatosplenomegaly. Pertinent laboratory data included a hematocrit reading of 37%, hemoglobin of 10.8 g/100 ml, serum amylase of 352 units (normal, 200 units or less), serum iron 15 μg/100 ml with an iron binding capacity of 413 μg/100 ml. The alpha-2 globulin was elevated to 13.2% (control serum value 6.4%) on serum protein electrophoresis. Normal laboratory data included direct and total serum bilirubin, phosphorus, blood urea nitrogen, creatinine, vanillylmandelic acid, latex fixation, lupus erythematosus and sickle cell preparations, and bleeding and clotting times. Serum potassium on two occasions was 3.8 and 4.0 mEq/liter. An intravenous pyelogram, upper gastrointestinal series, cholecystogram, and electrocardiogram were normal. Sialograms showed bilateral parotid gland enlargement without evidence of abnormal ductal configuration. Slit lamp ophthalmoscopy was normal. A mumps complement fixation test was negative. Purified protein derivative intermediate skin test was nonreactive. Bone marrow examination was normal for all elements except slight megakaryocytic hyperplasia. Further evaluation of her anemia was not accomplished.

The patient refused parotid biopsy and was discharged undiagnosed. Several months after discharge, the additional information was elicited that she was a starch eater. For as long as the patient could remember, she had ingested about ½ lb of Argo laundry starch daily. This habit began as a child in Georgia when she observed people eating clay, dirt, and starch. She began to eat red clay dirt, and starch, eventually consuming only starch in addition to her usual diet. Starch was especially craved during pregnancy, at nighttime, and when ever nervous. She ate starch because she liked the "crackling feel" in her mouth. Her children are all starch eaters, and her youngest daughter eats handfuls of baking powder. An aunt eats newspapers.

Since discharge from the hospital, the parotid swelling has remained unchanged though the patient has abstained from eating laundry starch. However, close questioning reveals that she has been substituting potato chips for the laundry starch. On oral iron therapy her hemoglobin has risen to 13 g/100 ml with a serum iron of 125 μg/100 ml and iron binding capacity of 515 μg/100 ml. A punch biopsy of the right parotid gland on November 10, 1964, when the woman was an

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war famine of 1918, swollen parotids were found in Polish children (26). German prisoners in Russia in World War II also exhibited the same findings (27). Parotid enlargement is common in malnourished Africans (5). The exact dietary deficiency is unclear; however, protein deficiency seems to be paramount (28). Rats fed a protein-free diet containing adequate calories, vitamins, and minerals develop parotid edema and swelling (29). Other deficiencies, including vitamin B_2 and vitamin A have been suggested (7, 8). Rats on a test diet lacking vitamin A develop parotid enlargement, which subsides once vitamin A is added (8). Biopsy of these parotid glands before the addition of vitamin A showed changes similar to that in humans with parotid swelling related to malnourishment.

An alternate theory proposes parotid gland hypertrophy in response to hyperstimulation (2, 4, 5, 26, 30). Chronic alcoholics have an increased salivary volume and amylase content (31). War prisoners who were rapidly refed after imprisonment developed parotid gland enlargement (30). An early clinical sign of improvement in kwashiorkor is parotid swelling (5). The salivary reflex is known to be adaptable to the type of stimulation. For example, Pavlov noticed that meat fed to a dog elicits saliva that is highly viscous and rich in mucin, whereas a diet rich in carbohydrate stimulates saliva rich in amylase and poor in mucin (32).

Biopsies of the enlarged parotids in many of these conditions have shown a varied microscopic picture including normal gland, hypertrophy of secreting cells with increased number of zymogen granules (17, 33, 34), increased intralobular fat (17, 33, 34), increased individual cells packed with zymogen granules (34), and enlargement of individual tubules or acini crowded with secretory granules (2, 5, 8). Zymogen granules in the serous cells of the normal parotid gland are in large numbers in the resting gland and depleted when stimulated (32).

In our patient, the history of excessive starch ingestion, a good nutritional intake, and cells depleted of zymogen granules suggests an etiology of work hypertrophy.

Iron deficiency anemia was observed in the starch-eating patient reported by Merkatz (1) and in the subject of this report. Though it is tempting to suggest a causal relationship between starch ingestion and anemia, no data have appeared in the literature supporting this viewpoint. Recent study by Mengel (35) of a large series of starch eaters showed no statistical relationship to the occurrence of iron deficiency anemia.

Though dirt, clay, and starch eating have received only scant attention in the medical literature, the practice is widespread among southern Negro women. In a random hospital study of 331 Negro females, 27% ate clay and 11% ate starch (36). A study of 207 school children in Mississippi revealed that 25% had eaten dirt within the last 16 days (37). In some health clinics, 75% of the women eat starch and 50% eat clay (38). The practice is said to be uncommon and almost unknown in men. In our obstetric clinic, 44 (25.5%) of 172 consecutive patients had eaten starch at some time in their life (39). Thirty-nine (88.6%) of the starch eaters were Negro. Of the total group, 15 (8.7%) had eaten starch within the past 6 months. The clinic population studied was 51% Negro. A survey of 302 general medical clinic patients, a much older group of men and women, demonstrated that 75 (24.8%) had eaten clay, starch, or dirt at some time in the past, though very few were doing so presently (39). A surprising finding was that 18 (24%) of this group were male in contrast to previous studies. Sixty-seven (89.3%) of the 75 were Negro. The medical clinic population was 58% Negro.

The clay chosen is either white or red with the white preferred because of its smoother quality (40, 41). The taste is said to be sour and puckerish. Stream and erosion banks are the favorite sources (36). Many take pride in their clay hole and jealously guard it. Connoisseurs are known to walk many miles for the right kind and often have relatives send them clay when they move to unappetizing clay areas of the north (37). If the desired clay cannot be procured, these people complain of being worried, upset, and feeling terrible.

The starch chosen is invariably Argo laundry starch, which also contains small amounts of protein, ash, fiber, and iron (36). Quantities up to 2 lb daily have been known to be consumed. Reasons given by these people for eating starch include "helping pregnancy," "relieving nau-

sea," "preventing dizziness," "helping babies," "relieving headaches," and a strong fear that the unborn will be marked if they abstain (37, 38). Other motivations suggested include unconscious endeavor to compensate for certain nutritional deficiencies, pleasure of chewing, social approval, religious ceremony, superstition, hunger pains, and, in the old plantation days, as a deliberate attempt to get sick (37, 38, 40, 42). In an 1811 treatise quoted by Cragin (43) entitled "Practical Rules for the Management and Medical Treatment of Negro Slaves in the Sugar Colonies, by a Professional Planter," it was pointed out that "Negroes labouring under any great depression of mind from the rigorous treatment of their master or from any other cause, addict themselves singularly to the eating of dirt." Speaking to this in 1835, Cragin (43) reported of "cachexia africana" in which the essential symptom described was a "universally depraved appetite and an ungovernable determination to the eating of dirt. The only appreciable signs of mental activity exhibited during the course of the disease are the crafty and cunning plans that the patient most subtly matures and as stealthily executes to procure his desired repast. This consists usually of charcoal, chalk, dried mortar, mud, clay, sand, rotten wood, shreds of paper, and hair which they pick from their own heads until they are bald."

Treatment today in stopping this insatiable craving is no better than in 1835 when it was noted that "neither promises nor threats nor yet the confinement of the legs and hands in stocks and manacles exert the least influence. A metallic mask or mouth piece, secured by a lock, is the principal means of security. If left for a moment to themselves, or does this effect a cure or save the life of the patient."

Summary

A woman is presented with bilateral, nontender parotid gland enlargement and a history of starch ingestion. It is noteworthy that this developed in a woman with good nutritional intake. Though the mechanism of the parotid swelling is uncertain, this was felt to represent an example of parotid gland work hypertrophy due to chronic starch ingestion. Some experimental and clinical evidence of parotid gland enlargement associated with both malnutrition and stimulation is reviewed. The frequency of starch ingestion in several population groups is mentioned, and a study of our clinics is reported to show that the practice is not limited to the southern Negro woman. Some of the social and psychological aspects of starch, clay, and dirt eating are discussed.

Summario in Interlingua

Es presentate le caso de un femina con bilateral, nondypersensibile allargamento del glandula parotidic e le antecedente del ingestion de amylo de prolongate continuitate. Es notabile que le condition se disveloppava in un femina con bon habitudes nutritional. Ben que le mechanismo del tumescentia del parotide es incerte, il es opinate que le presente caso representa un exemplo de hypertrophia del glandula parotidic per excesso de travalio in consequentia de un chronic ingestion de amylo. Es revistate certe datos experimental e clinic relative a allargamento parotidic associate tanto con malnutrition como etiam con stimulation. Es mentionate le frequentia de un forte ingestion de amylo in plure gruppos del population. Un studio es reportate, effectuate al clinicas del autores, indicante que le practica non es restringite a feminas negre al sud del Statos Unite. Es commentate varie aspectos social e psychologic del ingestion de amylo, de argilla, e de terra.

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FOOD COMPOSITION

Determination of Starch by Glucose Oxidase and Polarographic Measurement

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A method for the determination of starch in foodstuffs is reported. Interferences which are sometimes encountered with titrimetric or spectrophotometric determinative steps are avoided. In the method, the starch is hydrolyzed to glucose and the glucose is selectively oxidized in the presence of glucose oxidase. The rate of oxygen absorption for the oxidation reaction is measured and related to the starch content of the foodstuff. Results are presented for wheat and potato flours, which are shown to contain 71 and 59% starch, respectively. The method is specific, accurate, simple, and rapid and is applicable to foodstuffs containing interfering substances which often preclude analysis by other means.

The conventional methods of starch determination are based on the development of a color with iodine (qualitative and quantitative methods), on selective precipitation with alcohol and weighing of the precipitate (1), on determination of optical rotation (2), and on acidic (3) or enzymatic (4) hydrolysis followed by titration (5) or spectrophotometric determination (4) of the glucose produced (quantitative methods).

Most of the above methods do not effectively deal with certain interfering substances present in some foodstuffs (e.g., coloring, compounds which are only slightly soluble in water) and food colors which contain or are derived from carbohydrates (e.g., caramel, plant extract). These substances interfere with the spectrophotometric examination or the end point of the titration.

Turner (6) described a method for the quantitative determination of starch by extraction of soluble sugars with aqueous alcohol. The starch was then hydrolyzed with hydrochloric acid and the glucose formed was determined by a colorimetric reaction based on oxidation with glucose oxidase in the presence of a chromagen. For colored or turbid materials, however, this method is not accurate, since the blank used in the spectrophotometric determination is too high.

A method has been developed in our laboratory for the determination of the presence of starch or dextrin in foodstuffs without titration or spectrophotometric analysis. The method is based on the acidic hydrolysis of starch or dextrin to glucose and the subsequent determination of the glucose produced. The glucose is oxidized in the presence of glucose oxidase. The quantity of oxygen required to oxidize the glucose is determined polarographically with an oxygen electrode in an oxygen monitor apparatus. This method is accurate, simple, and rapid.

METHOD

Reagents and Apparatus

(a) *Glucose oxidase solution.*—Dissolve 1 g glucose oxidase (Servs, W. Germany) in 100 ml 0.2*M* phosphate buffer (pH 7.0). Store at 4°C. Enzyme loses part of its activity after 4 days.

(b) *Glucose standard solutions.*—Dissolve 200 mg anhydrous D(+)-glucose, $[\alpha]_{546}^{20.5b}$ +63±2° (Fluka AG, Buchs SG, Switzerland) in 10 ml distilled water. Dilute this solution to prepare working solutions containing 0.25, 0.5, 1.0, 1.5, 2.0, 5.0, 10.0, and 20 mg glucose/ml.

(c) *Dialysis apparatus.*—1½" Diameter dialysis tubing (Will Scientific, Inc., Rochester, N.Y.) in 5 L plastic tanks. Run stream of tap water through tanks from bottom to top at 1 L/hr.

(d) *Oxygen monitor apparatus.*—Capable of determining rate of disappearance of oxygen in solution. Model 53 (259) vi oxygen monitor (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio), consisting of Clark-type polarographic electrode, 4 sample chambers with magnetic stirrers and constant temperature bath, and recorder, is satisfactory. Calibrate apparatus with 3 ml water previously equilibrated with room air. Solution contains 1.5.5 µl O_2 at 20°C. Adjust scale of monitor to read 100% at this concentration. Sensitivity of monitor is ±0.3 µl O_2.

Preparation of Starch Extract

Finely grind (dry) or thoroughly pound (moist) 10 g sample. Slowly add, with gentle stirring, enough distilled water to make smooth mass (generally ca

2–10× sample weight). Heat mixture 10 min on boiling water bath, stirring constantly. Filter mixture and repeat extraction 3 times. Combine 4 filtrates. Dialyze solution overnight against flowing tap water.

Acid Hydrolysis of Starch Extract

Prepare 100 ml 2.5% solution of dialyzed sample extract in water. Maintain solution at 50°C while stirring magnetically 1 hr. Add 1.25 ml concentrated H_2SO_4 and stir again. Divide solution into ten 1 ml portions and place over boiling water bath. Remove first set of duplicate portions after 2 hr and subsequently remove duplicate portions at 4, 6, 8, and 10 hr. After hydrolysis is complete, bring pH of each solution to 7.0 with 1 N NaOH and dilute solution to 50 ml with water. Use aliquots of these solutions for glucose determination.

Standard Curve

Proceed as follows for each glucose standard solution: Pipet 2.5 ml glucose standard solution into cell of oxygen monitor apparatus. Add 0.5 ml glucose oxidase solution and measure oxygen absorption rate at 25°C. Under these conditions, 3 ml solution contains 15.5 μl free oxygen.

Plot oxygen absorption rate (μl O_2/ml/min) vs. glucose concentration (mg/ml).

Determination

Pipet 2.5 ml neutralized starch hydrolysate solution into cell of oxygen monitor apparatus and proceed as described under *Standard Curve*, beginning "Add 0.5 ml glucose oxidase solution. . . ." Determine glucose concentration from standard curve.

Results and Discussion

Glucose Determinations

Solutions of various concentrations of glucose were analyzed for glucose content by the method described above. The oxygen absorption rate of solutions containing from 0.25 to 20 mg glucose/ml was found to be linear with glucose concentration.

Corn starch solution was hydrolyzed under acid conditions as described above. Aliquots were analyzed at various time intervals. As can be seen in Table 1, hydrolysis is complete after 5 hr of heating with sulfuric acid. Thereafter, the oxygen absorption rate in the presence of glucose oxidase enzyme does not increase.

According to the standard curve, an oxygen absorption rate of 4.0 μl O_2/ml/min is equivalent to 2.1 mg glucose/ml. Therefore, the concentration of the initial starch solution is 1.89 mg/ml, which is exactly equal to the concentration of the solution taken. The same result was obtained by the Benedict method (7) for reducing sugars.

Table 1. Glucose determination in 0.18% starch solution after acid hydrolysis

Hydrolysis Time, hr	Absorption Rate, μl O_2/ml/min
1	1.5
2	3.1
3	3.5
4	3.9
5	4.0
6	4.0

The sensitivity of the above method for free glucose is 0.25 mg/ml, which represents 0.225 mg starch/ml. This method is suitable for detecting the presence of as little as 2% starch in colored foodstuffs. Starch can be detected in solutions containing at least 0.2% starch after concentration of the neutralized solutions by vacuum evaporation to 1/10 volume.

Determination of Starch in Flours and Powders

Solutions of various concentrations of wheat and potato flours were prepared. The starch was hydrolyzed in acid and its concentration was determined by glucose oxidation. At the same time, determination according to the Benedict method (7) for reducing sugars was made. The results are presented in Tables 1 and 2. From the data in Table 2, it is seen that after 8 hr of acidic hydrolysis under the above conditions, the starch present in wheat flour is completely changed into glucose. The Benedict and glucose oxidase methods gave the same values. The results show that wheat flour contains 71% starch. Table 2 also shows that potato starch is completely hydrolyzed after 2 hr acidic hydrolysis and that potato flour contains 59% starch.

Starch Determination in the Presence of Dyes

Starch solutions (1%) containing various food dyes were analyzed by the above procedure with pure starch solution as control; after hydrolysis solutions were diluted 5-fold with water. Results are given in Table 3. The maximum relative deviation compared with the control solution is ±2%.

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Table 2. Glucose determination in solutions of wheat and potato flours after acidic hydrolysis

Hydrolysis Time, hr	Reducing Sugars,[a] mg/ml	Absorption Rate,[b] µl O_2/ml/min	Starch Concn,[c] mg/ml
	3.07 mg Wheat Flour/ml		
2	1.68	3.4	1.51
4	2.10	4.0	1.89
6	2.40	4.2	2.16
8	2.42	4.5	2.18
10	2.42	4.5	2.18
	3.2 mg Potato Flour/ml		
2	2.1	4.0	1.89
4	2.1	4.0	1.89
6	2.1	4.0	1.89
8	2.1	4.0	1.89

[a] According to Benedict method (7).
[b] Determination catalyzed by glucose oxidase.
[c] Expressed as equivalents of reducing sugars.

Specificity

The method of analysis presented here is highly specific for free glucose, since the glucose oxidase enzyme acts only on glucose. Therefore, no other sugars interfere. Free glucose, if present, is eliminated by dialysis, while larger molecules such as starch (MW = 40,000-180,000) are retained.

In the determination of dextrin, dialysis does not differentiate between glucose and dextrin molecules. In this case, therefore, free glucose must be determined before and after starch hydrolysis. The amount of glucose determined before hydrolysis is subtracted from the amount after hydrolysis and the result is equal to the glucose derived from the hydrolysis of dextrin.

Table 3. Starch determination in presence of food dyes

Sample[a]	Absorption Rate, µl O_2/ml/min	Starch Concn, mg/ml
Control	4.15	1.96
0.5% w/v Carmine	4.15	1.96
0.5% Ponceau 4R	4.10	1.94
0.5% Erythrosine	4.15	1.96
0.5% Tartrazine	4.20	2.16
0.5% Sunset yellow	4.20	2.16
0.1% Caramel	4.75	2.25
0.1% Caramel (without starch)	0.65	0.34

[a] Control is 0.2% commercial starch solution (1% starch solution diluted 5-fold after hydrolysis). Other samples were prepared by adding various dyes to 1% starch solutions, which were diluted 5-fold after hydrolysis (0.2% starch).

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PARIETAL (CONTACT) DIGESTION

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For more than 100 years it has been generally supposed that digestion in the higher animals takes place as follows:

The food passes into the cavity of the digestive tract, becomes finely divided and mixed with the secretions of various glands so as to form a chyme. In the chyme, under the influence of various enzymes the food becomes broken down into nonspecific constituent elements capable of being absorbed.

This view, although generally accepted, involves certain serious contradictions. In particular, in spite of great advances in our knowledge of the physiology of digestion and the chemistry of enzymes, until now it has not been possible to reproduce in vitro those rapid breakdown processes which occur in vivo.

In attempting to understand the conditions which govern digestive hydrolysis in the living animal, we have studied the hydrolysis of starch by amylase in the presence of small pieces of intestine. During the course of the work we obtained results which indicated that cavity digestion (throughout the whole mass of chyme) is not the only or even the main mechanism which brings about the breakdown and absorption of food, and that in addition there are other very important and hitherto unknown processes which we have referred to collectively as parietal or contact digestion.

METHOD

We have studied the effect of small portions of living rat small intestine having a surface of serosa of 1-1.5 cm^2 on the hydrolysis of starch by amylase in vitro.

The rate of hydrolysis was determined photometrically in terms of the amount of starch destroyed, using the Smith-Row method and incubating for 30 minutes at 38° in a phosphate buffer at pH 7.5 containing 0.85% NaCl, or in Ringer's solution; the results obtained by both methods were almost identical.

In the first set of experiments we compared the activity of 1 ml of diluted pancreatic amylase, the activity of a piece of small intestine in 1 ml of phosphate buffer, and the activity of 1 ml of amylase to which a piece of small intestine was added during the period of incubation with starch.

In subsequent experiments we used the "trichloracetic" models of the esophagus, stomach, and small and large intestines.

These were prepared as follows: The animal was decapitated and portions of intestine, stomach, etc., rapidly removed and washed several times in physiological saline cooled to 3-5°. The portions were then transferred for 1½-2 hours to a 10% solution of trichloracetic acid (TCA). After being treated for two days with TCA, the intestine was washed in several changes of Ringer at a temperature of approximately 0°.

RESULTS

It can be seen from Fig. 1 that in the presence of a portion of living intestine, the rate of enzymatic hydrolysis of starch increases, so that it becomes considerably greater than the summed effects of the amylase solution and the intestine when incubated separately with the standard amount of starch. This effect cannot be ascribed exclusively to the activity of the living structures of the intestine or to the presence in the gut of particular enzymes, because the pieces of intestine previously killed by treatment with 10% TCA increase the activity of the amylase, although they themselves have no enzymatic activity.

As can be seen from Table 1, the presence of pieces of dead intestine caused a marked increase in enzymatic activity.

The following explanation of the phenomenon may be advanced: The intestine contains substances which pass into solution and increase the action of

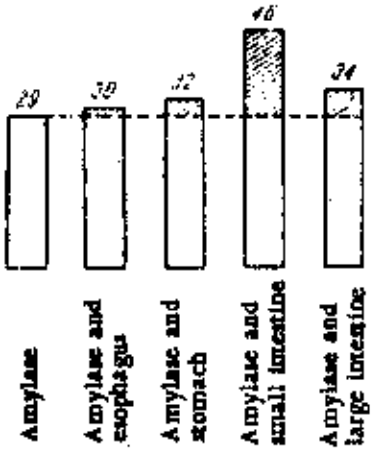

Fig. 2. Effect of equal areas of esophagus, stomach, small and large intestine (treated with TCA) on the activity of pancreatic amylase.

whereby parietal hydrolysis represents the chief supply of absorbable products and takes place directly on the surface of the villi constitutes extremely favorable conditions for absorption. A rough estimate based on our figures shows that the concentration of the starch breakdown products in the immediate neighborhood of the intestinal wall ought to be several times greater than in the chyme. Possibly absorption is not determined by the concentration gradient between chyme and blood, but by that between the parietal layer and the blood.

Because the enzymatic layer on the intestinal surface is related not only to the hydrolysis of food but also to its absorption it ought perhaps to be referred to as the "digestive-transport" layer.

We have found that the adsorbed enzymes are quite firmly fixed to the intestinal surface, and only pass back into solution with difficulty. This indicates that the classical view of the enzymes moving with the chyme along the intestine where they quite soon enter the large intestine to be further broken down or voided to the outside, needs to be refined considerably.

It is usual to suppose that the action of enzymes is limited to the particular portion of chyme into which they have passed when secreted, and that the digestive enzymes pass quite rapidly out of the small intestine together with the chyme, so that at each new act of digestion there ought to be a complete renewal of the enzymes which bring about the breakdown of food. We consider that as a result of adsorption quite a stable enzymatic layer is formed. The result is that the enzymes act on the whole of the food passing past a given section of intestine, and not only on one particular portion of chyme. It is still more important, however, that thanks to the parietal digestion the useful life (if we may call it that) of the digestive enzymes is increased several times. By being adsorbed it is likely that they do not take part merely in one but in several digestive acts.

Probably owing to the presence of two hydrolytic mechanisms (cavity and parietal) and through the interaction of these two mechanisms conditions are established for very effective action on the food. Here, probably the first stages of digestion are brought about chiefly in the lumen (the molecules of the enzymes being oriented with respect to the large portions of substrate), and subsequently hydrolysis takes place on the surface of the villi (the substrate molecules being orientated with respect to the fixed molecules of the enzyme).

Adsorption makes it possible for a particular enzymatic molecule to come into contact with many portions of chyme moving past a given section of intestine. The time of action of the enzymes in the digestive tract is thus increased, and the loss of enzyme is reduced. Finally, the features of parietal digestion as described establish extremely favorable conditions for absorption.

I. P. Pavlov compared the digestive system to a highly perfected chemical factory. The part played in modern chemical engineering by catalysts absorbed on to surfaces helps us to understand the significance of

TABLE 2 Effect on Activity of Incubating a TCA Model of Intestine in an Amylase Solution

Number of experiment	Amylase activity of hydrolyzed starch, %			
	original amylase activity	original intestinal activity	amylase activity after incubating with intestine	intestinal activity after incubating with amylase
1	47	0	47	20
2	47	0	44.5	25.5
3	63	0	56	19
4	59	0	57	20
5	46	0	40	29

amylase, or alternatively the increase in amylolytic activity results from the fact that the intestine exerts an influence on the kinetics of the enzymatic reaction by presenting an active porous surface.

We have shown that the addition of aqueous or saline extracts of small intestine to the amylase cause no appreciable effect on the hydrolysis of starch as is shown by comparing it with a control mixture containing the same amount of enzyme but no intestinal extract.

It can be deduced from these experiments that the increase in amylolytic breakdown of starch caused by adding intestine is not due to the presence in it of substances activating amylase.

If we suppose that under the conditions of our experiments there is an increase in the activity of the amylase in solution, then it would be expected that first incubating the amylase with pieces of intestine would cause an increased hydrolysis of starch when the latter was added after the intestine had been removed (by analogy with the activation of trypsinogen by enterokinase). However, it can be seen from Table 2, that after incubating with pieces of intestine there is never any increase in amylase activity, but in most cases there is some reduction. These results do not accord with the hypothesis that substances activating amylase pass from the intestine into the solution, or that the enzymatic activity of amylase in the solution is increased.

From this table it can be seen that after incubating with amylase, the intestine acquires a considerable enzymatic activity several times greater than the loss of the enzyme in the original amylase solution. The experiments demonstrate that a certain amount of amylase must be adsorbed onto the surface of the intestine in such a way as to increase the activity of the adsorbed enzyme considerably.

It is usually supposed that the epithelium of the small intestine performs the functions both of absorption and secretion. No experimental study has been made of the problem of how the large surface area affects the course of the enzymatic reactions in the

TABLE 1 Effect of TCA Model of the Small Intestine on Amylase Activity

Number of experiment	Amylolytic activity of hydrolyzed starch, %		
	amylase	intestine	amylase and intestine
1	28	0	46
2	31	0	52
3	19	0	34
4	12	0	20
5	47	0	57

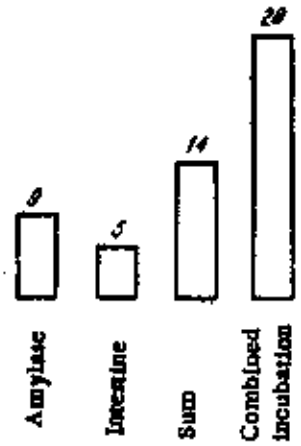

Fig. 1. Effect of a piece of living intestine on the breakdown of starch by amylase. The activity is shown as a percentage of the substrate hydrolyzed. From left to right: activity of a preparation of pancreatic amylase, innate amylolytic activity of the strip of intestine, some of the two activities, activity shown during combined incubation with amylase and intestine.

lumen. Nevertheless it is well known that the rate of enzymatic processes on a surface is very different from that occurring in a fluid medium.

Our results indicate that the large surface of the small intestine performs a further important function — that of reconstituting a living porous adsorbant. Under normal conditions, part of the digestive enzymes remain in solution, and being about digestion in the lumen, and until now this has been considered the only mechanism responsible for the breakdown of food, while the remaining part of the enzymes are adsorbed on to the surface of the mucosa where they bring about contact or parietal digestion.

Evidently, parietal digestion will be best developed in the small intestine. We have compared the increase in the amylolytic breakdown of starch under the influences of pieces of mucosa from the esophagus, stomach, and small and large intestines (experiments on models previously treated with TCA). It can be seen from Fig. 2, that the enhancing effect is comparatively small for pieces of stomach and large intestine, negligible for fragments of esophagus, but well shown for fragments of small intestine.

This differentiation of the digestive tract does not arise by chance. We have compared the rates of breakdown of soluble (partially hydrolyzed) amylose and insoluble amylose by the enzyme in solution and the enzyme adsorbed onto intestine. It was found that the larger amylose molecules are more intensely attacked by the dissolved enzyme, while the smaller molecules (previously partially hydrolyzed) on the contrary were more rapidly broken down in the parietal layer by the adsorbed enzymes.

Evidently, the very first stages in digestion take place chiefly in the lumen of the digestive tract, and subsequently on the surface of the intestine. This view agreed well with the results given previously which showed that parietal digestion occurs chiefly in the small intestine, i.e., where the greatest amount of hydrolyzed substrate is to be found. The arrangement

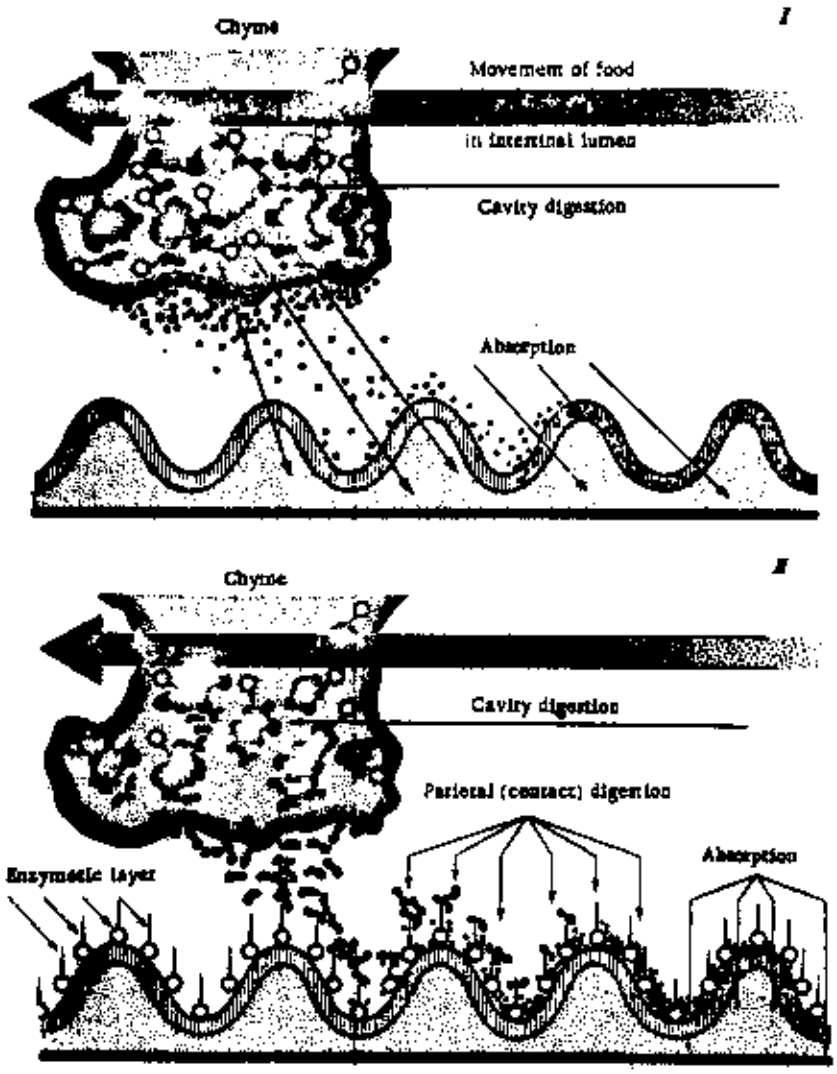

Fig. 3. General scheme of digestion. I) as usually accepted; II) taking into account parietal hydrolysis.

contact digestion in the activity of the living factory which brings about the breakdown and assimilation of food.

The last diagram (Fig. 3) shows the general course of digestion and indicates how cavity and parietal (contact) hydrolyses take place. Above, the usually accepted scheme for digestion is illustrated.

SUMMARY

The evidence presented shows that in higher animals cavity digestion is not the only or even the principal mechanism of breakdown and assimilation of food, but that along with it there is a system of hitherto unknown processes which we here refer to as parietal or contact digestion. The latter occurs under the influence of enzymes adsorbed onto the surface of the mucosa which is concerned in the hydrolysis and absorption of food; a number of specific features of this process are described.

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Primary Portal Transport of Persorbed Starch Granules from the Intestinal Wall

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Under the heading PERSORPTION we have examined within the last few years the phenomenon by which solid, undissolved food *particles* enter the body from the intestinal lumen as a side-effect of the normal digestive process.

Whereas with *absorption* dissolved substances of molecular magnitude are taken up by the epithelial cell of the small intestine, uptake of particles during *persorption* is a process which occurs in a paracellular manner. The transport of solid food particles previously has been observed in individual experiments [1, 2, 3]. We observed this phenomenon more thoroughly with a team of coworkers.

By means of animal experiments we were able to demonstrate the transport mechanism of solid particles through the epithelial cell layer: model particles suitable for persorption enter the space between the epithelial cells and are transported in a basal direction in the subepithelial region. Mechanical and functional factors play an essential role in this process: the motility of the intestinal mucosa is of particular importance for the persorption process. Only in the deeper mucosal layers was it possible to find the model particles in the lumen of lymph or blood vessels. Primary transport

from the intestinal wall occurred via the portal system as well as via the chylus.

As to the location of the persorption process: persorption is possible wherever a single-layer-epithelium covers the mucosa of the intestinal tract; most frequently it can be observed in the region of the desquamation zones of the epithelium.

For quantitative observations of the persorption phenomenon we used starch granules of various origins. They are especially well suited for this demonstration as larger amounts of them can be orally administered and because they are readily demonstrable in the body fluids.

In previous tests we were able to observe by the method subsequently described that unchanged starch granules can be demonstrated in the peripheral blood only a few minutes after the ingestion of starch. For example, if 200 g of potato starch are administered to young trial subjects, the number of persorbed starch granules demonstrable in the blood reaches its first peak 10 min after ingestion. A second peak can be observed approximately 90 min later. 90 min after the ingestion of e.g. 200 g of rolled oats or wheat flakes it was possible to demonstrate approximately 60 starch granules per 10 ml of venous blood. This means — assuming the circulating blood volume to be 3000 ml — that at this time approximately 18 000 starch granules circulate in the blood. The number of starch granules in the blood does not differ very much, if 200 g of corn starch containing 48 billions of granules, or 200 g of potato starch containing 2.4 billions of granules were ingested. These test series, however, demonstrate significant differences if 100 g, 200 g or 300 g of the respective starch types were ingested. Similar observations were made after the ingestion of cookies, cakes and biscuits. Such pastries made of short-cake contain considerably fewer starch granules suitable for persorption per 200 g than e.g. 200 g of corn starch. Nevertheless, the number of starch granules circulating in the blood after the ingestion of cookies or short-cake is not significantly lower. With administration of grain in the shredded form (e.g. shredded wheat) one might discuss the mechanical stimulation of the mucosa as the possible cause for the relatively high rate of persorption. Prostigmine increases persorption, whereas atropine decreases it.

Definition of the Problem

In our previous examinations we always found persorbed starch granules in the thoracic duct at first. Initially, this led us to assume a primary transport of persorbed particles via the chylus. However, during quantitative tests to determine the rate of persorption we soon found that orally ingested starch granules

could be demonstrated in the venous blood after only 1 to 2 min. These observations suggested rapid removal from the intestinal wall via the circulatory system.

The subsequent experiments aimed at finding out whether there was a difference in the number of persorbed starch granules in the portal vein and in the arterial blood if starch was previously administered orally.

Material and Methods

Test Animals

Nine female and male dogs of medium weight with an average weight of 17 kg were fed 250 g of potato starch suspended in milk or meat broth after they had been fasting for 36 h. 120 min later the test animals were anesthetized by intravenous injection of an average dose of 2 g of sodium hexobarbital. The femoral artery was exposed after the dog had been correctly positioned. After laparotomy the splenic vessels were ligated. At intervals 10 ml of blood was simultaneously taken from the femoral artery and from the portal vein. These blood samples were repeated 9 times from 130 to 210 min after feeding. The blood was examined by the subsequent technique and the number of potato starch granules contained in 10 ml of blood was determined.

Method Used for the Quantitative Determination of the Number of Starch Granules in the Blood

For each 10 ml blood sample one must have available:

a) a test tube containing 9 ml of distilled water and 0.5 ml of acetic acid and closed with a rubber stopper;

b) a centrifuge tube with a rubber stopper;

c) a larger amount of a solution of distilled water/acetic acid in the ratio of 2 : 1.

Exactly 10 ml of blood are removed and are put into the test tube containing 9 ml of water and 0.5 ml of acetic acid. The test tube is then shaken without creating foam. Approximately two-thirds of the test tube content is poured into a centrifuge tube. A small amount of water/acetic acid mixture is added. It is centrifuged for 15 min at 3000 rpm. Approximately two-thirds of the centrifuge tube is then carefully poured off and discarded. Once again a small amount of water/acetic acid mixture is added to the residue in the centrifuge tube and it is agitated once more. The residue in the test tube is put into the centrifuge tube, it is centrifuged once again and the supernatant is poured off. Irrigation fluid from the test tube is added to the sediment which has been formed. The water/acetic acid mixture is used several times for irrigation. The sediment of the centrifuge tube is then shaken with this irrigation fluid and is then centrifuged again. The centrifuged fluid is now clear. After pouring off the supernatant a brown sediment remains; this sediment is examined under the light microscope on slides with a cover glass.

If the search for starch granules in the blood sediment is performed with the normal optical arrangement, 3 drops of Lugol's solution are added to the sediment prior to the examination. Lugol's solution must also be heated previously in a water bath. Iodine is not added if the examination is carried out in a polarized light.

It is essential in this search that absolutely the *entire* sediment is examined. For this reason it must be stirred and removed with a metal loop and a glass-rod. The last residue of the sediment as well as the residues adhering to the wall of the centrifuge tube must be rinsed again with a drop of water and must be removed, as particularly this last residue of the bottom sediment layer still contains starch granules. With some effort and experience it is possible to collect even the last residue of the sediment concentrate and to examine it. These examinations are very time-consuming and can only be reasonably exact if all the prerequisites stated above are fulfilled.

Results

When blood taken simultaneously is from the portal vein and the femoral artery after feeding 250 g of potato starch, the portal vein blood always contained definitely more starch granules per 10 ml than did the arterial blood (fig. 1). The arithmetic mean, both of portal vein blood and of arterial blood is shown in the form of a time-graph. A test shows that the ratio of the mean value of the potato starch granules in the portal vein blood and in the arterial blood is constant and independent of time. (Homogenicity test using the x^2-test of *E. Weber*, 1964.)

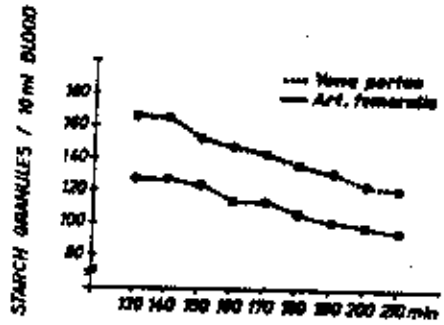

Fig. 1. Number of potato starch granules per 10 ml of blood: determination in the arterial blood and in the portal vein blood after feeding of 250 g of potato starch to dogs (n = 9).

	$-x^2$	degrees of freedom	α
Portal vein/femoral artery	0.47	8	99%

The ratio of the potato starch granules from the portal vein system to the potato starch granules in the arterial blood is as follows:

	mean value	S.d. deviation
Portal vein (A)/femoral artery (B)	1.29	± 0.02

The probability that the value for (A)/(B) lies within the stated range is 98.8%. The percentage difference between portal vein blood and arterial blood is 29.9 ± 1.0% related to the number of potato starch granules. This is valid for the measuring range of 130–210 min after oral administration of 250 g of potato starch under the test conditions stated previously.

Discussion

After feeding 250 g potato starch to dogs the portal vein blood definitely contains more starch granules than the arterial blood. This proves a primary *portal* transport of persorbed potato starch granules. (In similar test arrangements not described in this report we found that after intravenous administration of starch the portal vein blood definitely contained fewer starch granules than the arterial blood.) Thus orally administered starch granules are not only removed from the intestinal wall through lymph vessels (as has been observed previously), but are also primarily removed via blood vessels. We must admit that in histological sections it was possible to observe occasional starch granules in the lumen of blood vessels of the intestinal wall. This observation, however, cannot be used as proof for portal transport: it is possible that such a starch granule found in the vessel lumen was first removed via the chylus and only reached its present location after one passage through the circulatory system. Only the constant difference of the number of starch granules in the two circulatory systems could be used as proof of the existence of a primary portal transport of persorbed starch granules from the intestinal wall.

Summary

The portal vein blood of dogs fed with potato starch contains 29% more starch granules than the arterial blood. This difference is regarded as proof of a primary *portal* transport of persorbed potato starch granules. It has already been proved histologically that, in addition to this, primary transport of persorbed starch granules also occurs via the chylus.

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ESOPHAGOGASTRIC ULCERS IN SWINE FED DIETS HIGH IN CORNSTARCH*

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GASTRIC ulcers have been recognized in swine for many years (McIntosh[9] 1897); however, they were seldom diagnosed and rarely described in the literature before 1945. Subsequently, numerous clinical reports and experimental studies have been reported on the subject. It has been observed that esophagogastric ulceration is occurring with increasing frequency in swine in the United States and in Europe (Curtin[2] and Muggenburg[12]). There has not been a satisfactory explanation for such an increase although many possible etiologic factors have been proposed and studied.

Several attempts have been made to clarify the pathogenesis of porcine esophagogastric ulcers by devising methods to reproduce the condition experimentally. These approaches have produced valuable information on the ulcerogenic roles of diet, gastric acidity, environmental stress, and infectious organisms.

Frequently, the etiology of a disease process has been clarified by observing and reporting the various conditions

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†The data are from a thesis submitted by the senior author to the Graduate School, University of Illinois, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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under which it occurs. Thus various investigators have noted that swine seem more likely to develop ulcers when confined and fed pelleted diets than when raised on pasture and fed unheated, unpelleted diets (Gamble, *et al.*[4]).

The studies reported here were prompted by the unexpected occurrence of esophagogastric ulcers in 21 of 31 swine which had recently been placed on a nutritional study. Ten of the swine died of gastric hemorrhage. The case was particularly interesting because of the nutritional conditions under which the ulcers developed and the infrequency of gastric ulcers in swine raised on this farm under conditions which were identical except for diet. The incident was studied in some detail because the diets used in these experiments had not previously been reported as ulcerogenic for swine. Furthermore, the diets appeared to offer a new means for reproducing esophagogastric ulcers in swine and thus provided an opportunity to study the ulcerogenic process in this species.

MATERIALS AND METHODS

In the initial nutritional study, weanling pigs from four litters of the same genetic background were randomly assigned to four treatment groups. There were seven pigs in group 1 and eight pigs in each of groups 2, 3, and 4. Pigs in group 4 received a 16 percent protein diet until they weighed approximately 15 kg and then switched to a 12 percent protein ration until the experiment was terminated. The diets used in this portion of the study are shown in table I. The cornstarch in each ration was pearl starch grits, a granular, raw cornstarch product containing 7 to 10 percent gelatinized cornstarch. The feeding period was 80 days.

A second experiment was conducted to compare the ulcerogenic properties of raw cornstarch flour and partially gelatinized pearl starch grits when incorporated at high levels in swine rations. Four castrated males and four females were selected from each of two litters (Duroc and Duroc × Yorkshire) of 8-week-old pigs. Each of the four rations was fed to one male and one female from each litter throughout the experimental period. Pigs were hand-fed twice daily rather than *ad libitum* to prevent wide variations in feed intake between

treatment groups. Each feeding consisted initially of 0.45 kg. This quantity was gradually and uniformly increased during the course of the 35 day feeding period.

In both experiments, pigs were housed individually in pens having concrete floors and separated by solid partitions. Each pen contained an automatic waterer.

TABLE 1

Composition of diets

	Calculated percent crude protein				
	0.0	5.0	10.0	16.0	12.0
Yellow corn	—	3.76	7.52	12.03	9.02
Soybean meal (50%)	—	9.40	18.80	30.08	22.56
Corn starch[a]	89.51	76.75	63.69	48.35	58.75
Dicalcium phosphate	2.80	2.40	2.10	1.61	1.85
Ground limestone	—	0.20	0.40	0.51	0.53
Corn oil	3.00	3.00	3.00	3.00	3.00
Cellulose[b]	3.00	3.00	3.00	3.00	3.00
Trace mineral salt[c]	0.70	0.50	0.50	0.50	0.50
Vitamin mix[d]	0.20	0.20	0.20	0.20	0.20
Antibiotic[e]	0.10	0.10	0.10	0.10	0.10
K_2CO_3	0.56	0.56	0.56	0.56	0.56
MgCO	0.13	0.13	0.13	0.13	0.13
	100.00	100.00	100.00	100.00	100.00

[a]Products of A. E. Staley Mfg. Co., Decatur, Ill. Corn starch grits used in experiment 1; both grits and flour used in experiment 2.

[b]Pure wood cellulose.

[c]Contained in percent, 0.8 zinc, 0.1 manganese, 0.[illegible] iron, 0.048 copper, 0.011 iodine, and 0.022 cobalt.

[d]Contained per kg: 3.03 million USP units vitamin A, 303,000 USP units vitamin D, 1.0 g riboflavin, 5.0 g calcium pantothenate, 10.0 g niacin, 110 g choline chloride, and 17 mg vitamin B_{12}.

[e]Contained 15.0 g streptomycin (as streptomycin sulfate) and 5.0 procaine penicillin per pound.

Following the 80 and 35 day feeding periods, surviving pigs were killed by exsanguination within 2 hours after leaving their pens. This avoided the period of starvation and shipping stress to which pigs are often subjected enroute to market. In the second experiment, final live weights were recorded immediately before slaughter. As soon as the viscera were removed, the stomachs were opened along their greater curva-

tures, flushed with cold tap water and examined for ulceration. A sample of mucosa was taken from the cardiac and pyloric regions of each stomach and dried overnight at 75 C for determination of tissue mucus by the hexosamine method (Robert *et al.*[9]).

RESULTS AND DISCUSSION

Several swine became acutely ill and died of bleeding esophagogastric ulcers within 3 weeks after the initial experiment began. During the 80 day feeding period, 10 of the original 31 pigs died of hemorrhage associated with gastric ulcers. Postmortem examination revealed esophagogastric ulcers in the stomachs of 11 pigs which survived the entire feeding period. Distribution of the 21 pigs with ulcers among the four treatment groups is summarized in table II. There was excessive cornification of the *pars esophagu* in 28 of the stomachs.

TABLE II

Summary of esophagogastric ulceration and death occurrence — initial experiment

	% dietary protein			
	0	5	10	16-12*
No. pigs	7	8	8	8
Total ulcers	6	8	6	1
Deaths from ulcers	4	4	1	1
Excess cornification	4	8	8	8
Ulcers in survivors	2	4	5	0

*A 16 percent protein diet was fed until the pigs averaged about 45 kg in weight; then the protein level was reduced to 12 percent.

Results of the second experiment comparing ulcerogenic effects of partially heat-treated cornstarch (pearl starch grits) and raw cornstarch flour are presented in table III. Blood appeared in the feces of several pigs within 10 days after the start of the experiment and appeared irregularly in the feces of 5 of the 16 pigs during the 35 day feeding period. There were no deaths during the feeding period.

Esophagogastric ulcers were observed at postmortem

examination in 10 of the 16 pigs used in this study. There was an ulcer in the stomach of each of the five pigs previously observed to pass blood in the feces. All four pigs which received the 10 percent protein ration containing pearl starch grits developed gastric ulcers. However, at least one pig in each of the remaining three groups also developed a gastric ulcer.

Although gastric ulcers occurred in both litters, Chi-square comparison revealed a significantly greater ($P < 0.05$) number of ulcers in the Duroc × Yorkshire litter.

Results of tissue hexosamine determinations on the cardiac and pyloric mucosa of the 16 stomachs show (table III) that hexosamine concentrations ranged from 1.11 to 3.38 mg per 100 g of dried mucosa. There were no significant differences ($P > 0.05$) in mucosal hexosamine concentrations in corresponding portions of gastric mucosa between litters.

The ulcers were 1 to 3 inches in diameter, gray or brown, and roughened with necrotic tags of tissue and cellular debris. The margins of the ulcers were raised and sharply delineated beause of grossly visible submucosal edema. Lesions were confined to the nonglandular stratified squamous epithelium of the *pars esophaga* surrounding the esophageal orifice. In each nonulcerated stomach, there was some degree of excessive cornification of the *pars esophaga*.

Results of the initial study suggested that high starch diets, particularly those containing 0, 5, or 10 percent protein, were ulcerogenic for growing swine. Previous studies had indicated that diets containing gelatinized whole corn (Perry, *et al.*[1]) or gelatinized corn endosperm (Nawer, *et al.*[2]) were ulcerogenic for swine. Therefore, the partially gelatinized cornstarch, supplied as pearl starch grits, was suspected as the ulcerogenic fraction of the diets used in experiment 1 of the present study.

The second experiment was designed to confirm the ulcerogenic property of high starch diets and to determine whether gelatinized cornstarch was the ulcerogenic factor. There was no significant difference in ulcerogenic activities between cornstarch flour and pearl starch grits. In both ex-

TABLE III

Weight gain, ulcer incidence, and gastric mucosal hexosamine concentration in two litters of swine fed high starch diets for 25 day

Diet	No. of [illegible]	Weight gain/day Mean ± SE	No. ulcers	Mg. hexosamine ± SE/100 g dried mucosa Pyloric	Cardiac
10% protein Starch grits	4	9.8 ± 1.7	1	1.51 ± 0.12	1.62 ± 0.22
10% protein Starch flour	4	11.8 ± 2.0	3	1.21 ± 0.15	1.74 ± 0.11
16% protein Starch grits	4	10.5 ± 1.2	1	1.77 ± 0.17	1.89 ± 0.18
16% protein Starch flour	4	13.6 ± 2.8	2	1.73 ± 0.10	2.01 ± 0.17

periments, the highest incidence of ulceration occurred in swine receiving low protein, high cornstarch rations.

There was a significant difference ($P < 0.05$) in ulcer occurrence between the two litters used in the second experiment. The different genetic backgrounds of the litters may be responsible for this finding. Data of Mahan *et al.*[11] also suggest that some strains of swine are more susceptible to esophagogastric ulcers than others.

Seven of the eight pigs fed a 10 percent protein, high starch ration developed gastric ulcers within a 35 day period in the second experiment. The appearance of blood in the feces of several pigs suggested that ulcers may have begun to develop as soon as 10 days after the beginning of the experiment. In the initial study, six of eight swine fed the 10 percent protein ration that contained starch grits also developed gastric ulcers during the 80-day-feeding period.

Peptic ulcers have been recorded in man and experimental animals after intervals of total starvation (Anonymous[1]). Weech and Paige[17] found gastric ulcers in 12 of 22 normal dogs fed a protein-deficient ration for 90 days. Although a 10 percent protein ration is inadequate for young swine, it is unlikely that protein deficiency alone accounted for the ulcers observed in these experiments. There was excessive cornification of the *pars esophagea* in all the pigs in experiment 1 which received the 16 to 12 percent protein rations. One pig in this group developed a gastric ulcer. However, dietary proteins do exert a buffering action on gastric contents, and low protein rations may thus favor ulcerogenesis because they allow relatively rapid intragastric acidification after a meal.

There was no apparent relationship between presence of gastric ulcers and rate of gain during the 35 day feeding period. This finding is in agreement with work reported by Mahan *et al.*[11] However, the result is somewhat difficult to understand because gastric ulceration and subsequent scarring had produced severe stenosis of the esophagus in several pigs. Esophageal stenosis, which is sometimes associated with field cases of gastric ulcer in swine, might be expected to induce vomiting and limit feed consumption and rate of gain.

Stimulation of excessive gastric acid secretion and reduction of gastric mucus production are theoretical means by which peptic ulceration of the stomach may be produced. Robert *et al.* (1963) found that gastric ulcers appeared in starved rats when the mucus content (hexosamine method) of gastric tissues decreased. In the present study, esophagogastric ulceration in swine produced by high starch rations was unaccompanied by significant changes in hexosamine content of the cardiac or pyloric gastric mucosa. Tissue hexosamine values obtained in swine were similar to those reported by Robert, Bayer, and Nezamis[sic] for rat tissues. Because there was no significant reduction of hexosamine values in ulcerated swine stomachs, high starch rations did not appear to cause esophagogastric ulceration by weakening the defensive mucous barrier of the adjacent glandular gastric mucosa.

The mechanism by which ulcerogenic rations produce esophagogastric ulcers in swine is unknown. Although mucus content of gastric mucosa appeared unaffected by the various experimental conditions, high starch rations may act to stimulate hypersecretion of hydrochloric acid directly by contact with the gastric mucosa or indirectly by slowing gastric emptying. Draystedt believes that in man, delayed gastric emptying, gastric hypomotility, and prolonged contact between food and the antral mucosa result in sustained gastrin release, gastric hyperacidity, and eventual peptic ulceration. The porcine stomach normally empties slowly (Hanawalt, *et al.*) and may thus be particularly susceptible to antral hypofunction.

Hay *et al.*[sic] first described the experimental production of gastric and duodenal ulcers with repository histamine injections. They studied a number of laboratory and domestic animal species including three small pigs. Huber and Wallin (1965) employed 16 swine weighing 13 to 55 kg and established a technic of starvation plus repository histamine injections to produce esophagogastric ulcers within 72 hours. Moggerslung *et al.*[sic] performed similar studies and agreed that daily injections of repository histamine produced esophagogastric ulcers in swine.

Esophagogastric ulcers have also been produced by subjecting swine to close restraint for 24 hours (LeBars, *et al.*[8]), feeding "gelatinized" whole corn (Perry, *et al.*[14]) or feeding finely ground raw corn (Mahan, *et al.*[10]). Gamble, *et al.*[4] reported that the incidence of ulcers was greater on pelleted than on meal rations and greater in confinement than on pasture, but addition of a buffering agent ($NaHCO_3$) had no apparent effect. Reese *et al.*[15] reported that moderate or high levels of oats in the rations reduced incidence of ulcers. Feeding low protein, high cornstarch rations appears to be another technic in a growing list of methods for producing esophagogastric ulceration in swine.

SUMMARY

Esophagogastric ulcers developed in 31 of 47 young swine which were fed diets containing 0 to 16 percent protein and 48 to 89 percent cornstarch. High cornstarch rations containing either raw cornstarch flour or partially heat-treated (gelatinized) cornstarch were equally ulcerogenic. There was no apparent relationship between the presence of gastric ulcers and rate of gain in body weight during a 35 day feeding period. Ulcers were not accompanied by significant changes in mucus content (hexosamine method) of the cardiac or pyloric gastric mucosa.

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Warshauer '66
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Starch-Eater's Anemia

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Here is a condition, probably not rare, which possibly receives little attention in the taking of the history in colored women who have anemia.

NUTRITIONAL ANEMIA is seldom encountered in this country since adequate dietary products are plentiful and available. Iron-deficiency anemia occurs more frequently in women as a result of the hemoglobin lost in menstrual flow and the demands of pregnancy. When the diet is totally lacking in iron, the degree of anemia will be correspondingly more severe.

Recently, several instances of severe hypochromic anemia have been observed in Negro women whose diets have consisted chiefly of corn starch, as used for laundering clothes. This curious predeliction deserves emphasis since most physicians are quite ignorant of it. Sage[1] investigated this habit among patients in the obstetric outpatient clinic of Temple University Medical Center. His subjects, all Negro women, had an average hemoglobin of 8.7 Gm. The more starch they ate, the lower the hemoglobin. Five of the 21 ate one or more one pound (453.5 Gm.) boxes a day. Edwards and her associates[2] reviewed the clay and starch eating habits of 86 women attending obstetric clinics in Tuskegee, Alabama. Forty-two ate clay, 23 ate starch and 6 ate both. They seemed to have a psychologic compulsion to eat this material. Clay was used in small amounts, rather like an appetizer or dessert, while starch was used frequently in such quantity as to constitute a main course. Ferguson and Keaton[3] made a dietary study of 361 pregnant women in rural Mississippi and found that 39% ate a varying amount of starch. If Negroes alone were considered, the incidence would have been 41% for starch and 27% for clay. The quantity of starch eaten varied from a few lumps to 3 one-half pound (226.8 Gm.) boxes a day, while that of clay ranged from a tablespoonful to a cupful. The husbands of none of the patients ate these substances. These authors also observed unusually low average hemoglobin in the same group.

The ingestion of corn starch is not confined to pregnant women. Indeed, the most severe effects have been found in nonpregnant ones. Allan and Woodruff[4] reported a gastrolith composed of starch in an 18 year old housemaid. Merkatz[5] reported a case of enlargement of the parotid glands associated with starch-eating. The patient had nutritional deficiency because she lived on one box of starch a day and an occasional sandwich. Neither of these patients had severe anemia, the hemoglobin being 10.5 and 10.0 Gm./100 ml. respectively.

The following two cases illustrate the severe anemia which may occur in starch-eaters.

Case 1. A 42 year old Negro woman entered the hospital on April 30, 1965, because of weakness, indigestion, nausea, mild diarrhea, and edema of the ankles. She had been ill about 4 months. There had never been observed bleeding from the gastrointestinal tract, and the menses were normal. Two years previously she had a similar illness for which she had been treated in another hospital and had been given 6 blood transfusions. For several years she had eaten 2 boxes of starch a day. (One of her sisters ate as much as 4 boxes of starch a day.)

Physical examination disclosed only marked pallor of the mucous membranes. The B.P. was 140/80 mm. Hg. (Pelvic examination disclosed a small, anteriorly placed uterus; rectal examination was negative.)

Laboratory data: Hgb. was 4.5 Gm./100 ml., R.B.C. count 2.46 million, HCT. 18%, MCHC 25, MCV 76 cu. μ, and MCH. 19 $\mu\mu$; reticulocytes 0.6%, leukocytes and platelets were normal. Bone marrow showed erythroid hyperplasia and the absence of stainable iron.

While in the hospital, she was treated with 0.3 Gm. ferrous sulfate t.i.d. and a single intramuscular injection of iron-dextran complex (Imferon) 8 ml. Following discharge from the hospital on May 7, 1965, ferrous sulfate was continued with one more injection of iron dextran complex, 4 ml. Hemoglobin determinations were May 8, 4.2 Gm.; May 19, 5.9; June 5, 9.1; June 26, 14.0; and Aug. 7, 16.0 Gm.

Treatment was then discontinued; she was advised to eat a conventional diet without added corn starch.

Case 2. A Negro woman was first seen in 1955 at the age of 38. She had had 5 children, the first at the age of 18 and the last when she was 27 years old. Dur-

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ing her last pregnancy she had begun to eat clay and starch. She was then living in Spartanburg, South Carolina, but shortly thereafter moved to Wilmington, North Carolina, after which she continued to eat starch, but stopped eating clay since the kind she liked was no longer available.

The desire to eat starch persisted after the last pregnancy and became greater as time passed. Her minimum daily intake was 4 to 5 one-half pound boxes, and at times she had eaten as much as 7 boxes. She used Argo Gloss Starch, which she purchased wholesale by the case, and often was joined by a companion at "mealtime" who also liked starch.

At about the age of 55 she consulted a gynecologist because of excessive menstrual flow. He found the pelvic examination to be quite normal. She was referred to me because of anemia, the Hgb. being 8 Gm./100 ml. Planned studies were not done because she failed to return for them, though she had been given an apparently adequate amount of ferrous sulfate. She returned on Nov. 9, 1959, complaining of nausea and shortness of breath, and admitted eating 2 or 3 boxes of starch a day.

Physical examination was negative except for pallor and obesity. The weight was 198 lbs. (90 kg.), ht. 65 inches (165 cm.). Hgb. was found to be 5 Gm.; the stained smear showed hypochromia and microcytosis.

Two weeks later, after treatment with iron, the Hgb. was 9.2 Gm. She was again lost from observation, returning on Sept. 19, 1960, with a Hgb. of 3.8 Gm. Hospitalization was urgently advised and she was admitted the same day. During the interval between visits her diet had consisted almost exclusively of corn starch.

Blood studies in the hospital were as follows: R.B.C. count 3.3 million, HCT. 23%, Hgb. 5.5 Gm., reticulocyte count 9%, and marked anisocytosis and hypochromia were noted on smear. Bone marrow showed no stainable iron and erythroid hyperplasia.

The patient was treated with iron by mouth and improved rapidly. She was discharged Sept. 22, 1960. On Dec. 17, 1960, the Hgb. was 12.2 Gm.

By Dec. 19, 1961, she again had severe anemia, with a Hgb. of 3.4 Gm. The next available record is of Feb. 28, 1963, when she was found to have palpable uterine fibroid tumors. The Hgb. had dropped to 2.7 Gm. Treatment was under the direction of a surgeon who gave her 4 transfusions of blood as well as iron, but was unable to convince her of the need for a hysterectomy. This was done, however, in October of that year by another surgeon after the Hgb. had fallen to 3.1 Gm. and the R.B.C. count to 1,940,000. Eight units of blood returned the blood to normal, following which the uterus was removed. It weighed 200 Gm. and contained many fibroid tumors which were suspected to be the cause of abnormal bleeding.

She was last examined on Sept. 7, 1965. Her weight was 225 lbs. (101 kg.), B.P. was 150/100 mm.Hg. and the Hgb. 14.4 Gm. She had lost all desire for starch after the hysterectomy and had eaten none since.

Discussion

Starch-eating appears to be confined to Negro women; I have not observed this habit in men nor in Caucasians of either sex. To estimate the incidence of starch-eating in the local population, I questioned all patients in my private practice for a period of several days. The white persons professed ignorance and surprise at the question, while almost all the Negroes were familiar with the habit. They had either eaten starch themselves or were acquainted with someone who did. One offered the information that a friend ate a box a day; many just nibbled starch; not infrequently they had friends who consumed several boxes a day. My cook stated that she knew a person who bought starch in five-pound boxes and ate that much daily. Another patient, the operator of a laundry, commented that the Negro women employed by him would take a handful of starch from the bin and eat it while working. He never saw a man eat starch, and after he changed to a liquid preparation he saw no more starch-eating. One patient, a former starch-eater, admitted that some years previously she had been hospitalized for anemia and was given 3 blood transfusions. Her physician advised her to stop eating starch and she has since given up the habit.

The starch eaten by these patients was that used for starching clothes, and marketed under the trade name of Argo Gloss Starch (Corn Products Company, Argo, Ill.). It is chemically the same as food starch, but differs in being in lumps rather than in powder form. Sage[1] calculated that one pound of starch yielded 1,590 calories, being composed of 86% carbohydrate and 14% water. Other materials, including iron, were present in negligible amounts. Therefore, the starch-eater in effect consumes a 100% carbohydrate diet without iron. The anemia develops through iron-loss by menstrual attrition, and if abnormally excessive bleeding occurs, anemia develops more quickly, at times becoming very severe. I was impressed by the rather general association of obesity with the anemia, and when a fat Negro woman had a pale tongue, starch-eating could be predicted with a fair degree of certainty.

Treatment of this malady consists of the use of iron by mouth, for which ferrous sulfate is quite satisfactory. If there is need for urgency, parenteral iron can be used. Transfusions are rarely needed even if the hemoglobin is as low as 4 Gm. per 100 ml., since

the response to iron medication is sufficiently rapid to remedy the condition.

Summary

The frequent occurrence of iron-deficiency anemia in Negro women addicted to the eating of corn starch is described and two examples are cited.

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Past history, review of systems, and family history were noncontributory. The patient was taking only aspirin 0.6 to 2 Gm. per day prior to admission.

Examination revealed an acutely and chronically ill man with P. of 104, B.P. 140/90, and T. 100°. Both knees, the left sternoclavicular, right temperomandibular, right elbow, and left ankle joints were hot, swollen, tender and erythematous. The posterior pharynx was injected, but no exudate noted. No subcutaneous nodules or skin rash were present. The lungs were clear, but unfortunately no chest film was obtained.

Admission laboratory data revealed the following: HCT. 33%, Hgb. 11.2 Gm., W.B.C. count 19,270 with 85 P.M.N., 8 bands, and 7 lymphocytes. Fasting blood sugar, BUN., VDRL slide test, and serum uric acid, were normal on admission. Latex fixation was positive 1:320 and E.S.R. was 51 mm. per hour uncorrected. Total protein was 6.95 Gm. with 2.7 of albumin and 4.25 of globulin. Admission urinalysis contained 4+ sugar, but was otherwise normal. EKG. showed old anteroseptal infarct.

The admission diagnosis was acute rheumatoid arthritis and the patient was placed on aspirin but failed to respond. ACTH. 40 units daily I.M. was then added, also without measurable patient response. Approximately 2 weeks after onset of illness, the joints were aspirated and purulent fluid was obtained from the knees and the right elbow. Pneumococci were present on Gram's stain and culture of the fluid. Treatment with large doses of penicillin was begun. Aspiration of knee, elbow, and ankle with instillation of aqueous penicillin were then performed, and the patient slowly became afebrile, but has subsequently shown marked destruction of both knee joints and the right elbow joint. As might be expected, the peripheral blood and synovial fluid, white blood cell counts rapidly return to normal.

Discussion

Review of the literature discloses that the introduction of penicillin for treatment of pneumococcal disease initiated a rapid decline in the incidence of pneumococcal arthritis.[1,2] Since 1950, the English literature has contained reports of 9 cases of pneumococcal arthritis in adults, one of which specified RA as being present prior to the onset of suppurative arthritis. Infectious arthritis from all causes is still quite prevalent, and recent articles have stressed the frequency of *S. aureus* as an etiologic organism.[3] With an ageing population, the concomitant increase in chronic disease in general and arthritis in particular, may be expected to contribute to a rising rate of suppurative arthritis superimposed on other chronic disease.

Diabetes mellitus, alcoholism, preexisting arthritis (noninfectious), respiratory tract infections, steroid treatment (oral and/or intra-articular), trauma, surgical procedures (particularly to the genitourinary tract), and in fact any debilitating disease predispose to infectious arthritis.[4] It is probable, but not proved, that in this case the pneumococcal infection began in the respiratory tract. The case reported here was seen at New Orleans V.A. Hospital, but a review of 7 years' records at this hospital and at New Orleans Charity Hospital failed to reveal a single positive synovial fluid culture for pneumococci.

Pneumococcal arthritis can be diagnosed with certainty only by identification of the organism in the synovial fluid, which is usually not difficult if the diagnosis is considered. The well-known classical signs and symptoms of infectious arthritis may be absent or minimal in the patient treated with steroids, or in the debilitated patient, and x ray examination does not provide pathognomonic early diagnostic signs.

Summary

A case of pneumococcal arthritis complicating rheumatoid arthritis is reported and attention called to the problem of suppurative arthritis complicating rheumatoid arthritis and other chronic disease states. The necessity for considering infectious arthritis in a patient with rheumatoid disease, and for routine examination of the synovial fluid is pointed out.

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Nutritional Value of Chemically Modified Corn Starches

Abstract. Male and female weanling rats were fed daily 5 g of a balanced diet supplemented with 1 or 2 g of corn starch, oxidized corn starch, corn starch phosphate, or hydroxyethyl corn starch. Commercially modified corn starches produced the same weight gain as normal corn starch during 21 days of feeding. In contrast to commercial starches, a very highly oxidized laboratory-prepared starch produced a lower weight gain during the same period.

Since increasing amounts of modified starch are being produced to meet requirements for industrial and food uses, it seemed desirable to determine whether slightly modified corn starch might be somewhat more digestible and give higher caloric value than unmodified corn starch.

The nutritional value of starches has been examined by several investigators. Booher *et al.* (*1*) showed that wheat, rice, corn, and waxy maize starches are more digestible in rats than the starches of arrowroot, white potato, and sago palm. Sakurai *et al.* (*2*) found that raw or cooked starches from most cereal grains are effective in producing growth in rats when they are fed as 75 percent of a balanced diet. White potato starch, ball-milled for 250 hours, is equally effective in producing growth, but raw untreated white potato starch is less effective. Jelinek *et al.* (*3*) stated that raw white potato starch is a poor nutritional carbohydrate when fed as 73.6 percent of the diet of weanling rats, but that autoclaved, modified, or ground potato starch is more fully utilized. Booher *et al.* (*1*) showed that modification which produces hydration of, changes the chemical nature of, or disrupts the starch granule makes the carbohydrate a better food substance.

The feeding procedure used was that designed by E. E. Rice *et al.* (*4*) which is based on the theory that food is used for energy to the greatest extent until minimum energy requirements are met. It has been established that the growth of weanling rats varies in proportion to the metabolically available energy of a food when a minimal diet is fed.

Corn starches used as diet supplements were commercial products: corn starch oxidized by 6 percent (wt./wt.) of chlorine, intrinsic viscosity 0.25, carboxyl content 20 meq/100 g of starch; corn starch oxidized by 2.5 percent (wt./wt.) of chlorine, intrinsic viscosity 0.45, carboxyl content 7 meq/100 g of starch; hydroxyethyl corn starch, 0.11 degree of substitution; corn starch phosphate, 0.5 to 0.9 degree of substitution (two samples); and a laboratory sample of corn starch oxidized by 2 equivalents of hypochlorite per D-glucose unit, 43.2 percent (wt./wt.) of chlorine. This very highly oxidized noncommercial starch was prepared only to determine whether

Table 1. Weights gained by rats in 21 days on different types of starch. D.S., degree of substitution.

Type of corn starch	Supplement level: 1 g	Supplement level: 2 g
	Weight gain (g)	
Group 1		
Corn starch	33.7	41.4
a) Corn starch oxidized by 6 percent (wt./wt.) chlorine	33.6	45.0
b) Hydroxyethyl corn starch, 0.11 D.S.	31.6	42.3
c) Corn starch oxidized by 2 eq of hypochlorite, 43.2 percent (wt./wt.) chlorine	18.9	29.7
Group 2		
Corn starch	32.8	43.2
d) Corn starch oxidized by 2.5 percent (wt./wt.) chlorine	35.2	46.8
e) Corn starch phosphate, 0.5 to 0.9 D.S.	36.5	43.5
f) Corn starch phosphate, 0.5 to 0.9 D.S.	33.8	45.2

any effect in weight gain could be produced by feeding a seriously overoxidized product.

Weanling rats (Wistar-Purdue strain) were fed daily 5 g of a highly nutritious basal diet (*5*) supplemented by 1 or 2 g per day of the starch products under investigation. The basal diet (5 g/day) was sufficient to produce a weight gain of 4 to 8 g per week during 4 weeks.

The experiment was conducted in two parts, in each of which nine diets were fed. These were as follows: one basal diet of 5 g/day; four supplemented diets containing 5 g of basal diet and 1 g of a starch product, or a total of 6 g/day; and four supplemented diets containing the basal diet and 2 g of a starch product, or a total of 7 g/day.

The nine diets were arranged in a randomized incomplete block design (*6*), and each diet was replicated four times. Equal numbers of male and female weanling rats were grouped (three litter mates per block, three blocks per replication), individually caged, and fed 5.0 ± 0.1 g per day for 7 days. Water was freely supplied. At the end of this period the animals were weighed and either continued on the basal diet or changed to diets composed of basal diet mixed with 1 or 2 g of starch product supplement. Animals were weighed again after 3, 7, 14, and 21 days of supplementation.

Responses to supplementation were so uniform that 7-day weights reflected the increased caloric intake as definitely as weights at 14 days or 21 days. The weight gains at 21 days for the rats in the two groups of the experiment are presented in Table 1. The data for each group were analyzed in a 4 by 2 factorial design: four types of starch fed at two levels. This anaylsis permitted evaluation of the effect of the amount of supplement, the type of supplement, and the interaction of type and amount.

All the commercial starches produced weight gains similar to corn starch (Table 1). However, starch *c*, heavily oxidized and degraded by 2 equivalents of hypochlorite per D-glucose unit (43.2 percent, wt./wt., of chlorine) had a low nutritional value since the average weight gain produced by feeding this starch was significantly lower than all the others. The average weight gains produced by supplements of 1 and 2 g of this starch were not very much different from that produced by the basal diet (a gain of 26.7 g during 21 days).

The average weight gain produced by all 2-g supplemented diets was significantly greater than that produced by 1-g supplemented diets, the difference being about 10 g per rat in 21 days. There was no interaction between the amount of supplement and type of starch; this is shown by the fact that doubling the amount of supplement increased the gain about equally for all the starch products. There was close agreement between the weight gains reported for corn starch in groups 1 and 2. This similarity allowed a comparison of weight gain between products in the two groups. Heavily oxidized noncommercial starch (*c*) induced diarrhea after the 2nd day of supplementation. Hydroxyethyl starch produced a mild diarrhea.

Autopsies performed on one rat from each of the 2-g supplemented diets disclosed that rats fed heavily oxidized starch *c* had a marked dilation of the colon. The other animals appeared to be normal (*7*).

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government and industry combine efforts to clear

Food Starches—Modified

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For many years, the food industry has used a wide variety of starches in the processing of numerous fabricated foods. Unmodified starches from different botanical origins, as well as modifications of those starches, were commonly used to thicken and produce desirable textures in foods. The first official review of such starches occurred at the hearings held in 1948 by the Food and Drug Administration to receive testimony regarding proposed standards for salad dressings. These various starches were included in the salad dressing standards under the term "food starch."

Although these starches had wide usage in a variety of fabricated foods, the starch industry had to establish the status of such food starches under the Food Additives Amendment of 1958. The starch industry regarded all such starches — unmodified or modified — as starch; and technical information available to the industry, and their long history of use, showed them to be safe. On the other hand, the FDA, after reviewing the situation, decided that only certain starches could be regarded by them as *generally recognized as safe*, and not "food additives" under the amendment. All others had to be covered by food additive regulations.

Manufacturers of those starches which the FDA did not regard as generally recognized as safe, individually and cooperatively through the Corn Industries Research Foundation, filed food additive petitions requesting that a suitable regulation be issued for the various products in order to clarify their status. This involved the collection of considerable manufacturing, technical, and usage information made available by the industry so that FDA officials would have necessary facts to support appropriate regulations. By way of illustration then, this paper will review the information which was submitted by the industry for a long-established type of modified starch in order to support its safe usage in foods without limitation.

Sodium hypochlorite has been used for many years to bleach starch. The use of small amounts of hypochlorite for this purpose was generally recognized as safe, but the FDA wanted more information about its safety when larger amounts were used to produce food starches with different viscosity and other physical characteristics. Some time ago, these modified starches were frequently referred to as "chlorinated" starches, but in recent years the term "oxidized" has been used more freely to indicate starches prepared in this way. Such starches are manufactured by a number of suppliers and represent one of the more readily available starches.

Procedures and Data

These types of "oxidized" starch are produced by treating a starch-water suspension at room temperature with not more than 0.055 pounds of chlorine as sodium hypochlorite per pound of dry starch until a desired viscosity is obtained. The process is normally carried out under alkaline conditions maintained with sodium hydroxide. The excess hypochlorite in the starch-water suspension is neutralized at the end of the process by reaction with hydrogen peroxide, sodium bisulfite, hydrochloric acid, or sulfuric acid. The neutralized product is dewatered on a washing filter using maximum wash water. It is then reslurried and rewashed using maximum wash water to remove residual reaction products prior to the final filtration and drying. Any residue remaining from the process would be sodium chloride and/or sodium sulfate. The amount of sulfur dioxide remaining in the starch is less than 0.015 percent.

Although these starches were referred to as "chlorinated" starch, the process does not produce any chlorination of the starch molecule, even though a chlorine-containing compound (hypochlorite) is used. Some years ago, it was thought that hypochlorite oxidized the hydroxyl on the sixth carbon of the anhydroglucose unit to a carboxyl. Later, starch chemists learned that other carbons of the anhydroglucose unit were attacked by hypochlorite. More recently, Whistler (3) and his co-workers showed that starches prepared with hypochlorite, as indicated, are oxidized to a substantial extent at the second and third carbons with cleavage of the anhydroglucose unit and the formation of carboxyl groups. There is very little of this even with the most highly-treated, commercial types since the amount of oxidation of the starch molecule, expressed as carboxyl groups, does not exceed 1%. It should be emphasized that these starches should not be confused with the very highly "oxidized" starches

(Continued on page 54)

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studied by Whistler and Belfort (4), in which eight times as much hypochlorite was used to prepare "a seriously over-oxidized product." Their results with commercially "oxidized" starches agree quite well with those presented here.

Commercial samples of two "oxidized" corn starches (supplied by National Starch and Chemical Corp.) were studied[1] by Shuman Chemical Laboratory, Inc. to determine whether they were as digestible as normal corn starch. Willstatter's (5) method of determining amylolytic activity was adapted to measure digestibility by using an excess of enzyme and a limited amount of substrate. This method measures reducing groups by reaction with iodine in alkaline solution. One percent solutions of starch were used in the tests and the solutions were prepared by dispersing the starch in cold water, boiling for five minutes, cooling and diluting to volume.

These same starches were tested for digestion by saliva, using the same general type of procedure as that used with pancreatin. However, pH 5.6 acetate buffer was substituted for a pH 6.8 phosphate buffer used with pancreatin. The saliva was collected from three people, pooled, diluted with an equal volume of water, and filtered through filter paper covered with a layer of Celite. Two milliliters of this saliva filtrate were used in digestion studies with 250 g. of starch. Maltose equivalents were determined to measure the extent of digestibility. The values shown in Table I represent the amount of digestion after thirty minutes. The rates of digestion were of the same order as the quantitative values. Although the "oxidized" starches digested slightly more slowly and less completely (10-15%) by pancreatin than regular corn starch, there was little difference, if any, in the rate or extent of digestion with saliva.

Additional data were submitted by the A. E. Staley Manufacturing Co.[2] to the FDA as part of the cooperative Corn Industries Research Foundation effort, to compare the digestibility and caloric value of unmodified corn starch. The test used was that developed by Rice (2), with modifications suggested by Kline and Friedman of the FDA.

Table I. Results of a Test to Determine the Extent of Digestibility (after 30 minutes) of Saliva and Pancreatin on Regular and Oxidized Corn Starch

Corn Starch	Chlorine %	Carboxyl %	Digestibility Pancreatin %	Digestibility Saliva %
Regular	0	0	100	100
Slightly Oxidized	2.5	0.42	84.8	98.6
Moderately Oxidized	5.0	0.84	88.4	97.6

In these tests, the rats were housed in randomly spaced metal cages at a constant temperature of 78°F. (± 5°). At the termination of the ten-day test period, they were etherized and autopsied in the usual manner. The adult animals were fed a normal, basal, test diet with up to 62% of the starch added as a sole source of carbohydrate. No gross pathological conditions were observed throughout the test. The liver, kidney, heart and spleen weights increased in direct proportion to the daily caloric intakes, and in accordance with normal expectancy. The "oxidized" starches demonstrated caloric values equal to or greater than that of unmodified corn starch in spite of slight, apparent reductions in their relative digestibilities. Table II summarizes these results.

Previously Booher (1) and coworkers had demonstrated that commercial "oxidized" wheat starch made with hypochlorite has the same digestibility as regular wheat starch and unmodified corn starch. They also found that different starches, even unmodified starches, had different rates of digestibility. On the other hand, if all of the starches were tested in gelatinized form, the rates and extent of digestibility were essentially equivalent.

Table II. Results of a 10-Day Test Diet on Rats to Determine Digestibility and Caloric Values of Regular and Modified Starch

Corn Starch	Chlorine %	Carboxyl %	Digestibility %	Caloric Value
Regular	0	0	100	100
Lightly Oxidized	3.9	0.57	97.0	114.7
Moderately Oxidized	4.5	0.80	96.9	99.6
Moderately Oxidized	5.5	0.90	96.5	110.9

Discussion

Petitions covering other modified starches not regarded by the FDA as generally recognized as safe contained equivalent information and data in order to show that such starches were safe for use generally in foods. These starch products were nutritionally —on the basic of caloric values, digestibility and feeding studies— the equivalent of the unmodified starch from which they were prepared. They were shown to be, for all practical purposes, essentially starch because the changes in their molecular construction, if any, were slight and only amounted to a few percent. Moreover, all of these modified starches had the general characteristics of starch and were used and thought of as starch. These basic principles and views, reported in the official salad dressing hearings, have guided the starch industry in preparing food starches during the past fifteen years.

It should be noted that a distinction was made at the salad dressing hearings between modified food starch types and those chemical derivatives of starch. The food starch modification processes were intended to produce, at most, mild molecular changes for particular physical or texture-producing properties, rather than chemical reaction products of starch containing substantial amounts of substituted groups designed to produce basically different properties and nutritional values as compared to starch.

The first Food Additives Regulation for modified starch covered a number of processes, but it was confusing in several respects. The term "modified starch" was too broad and did not indicate that the product was for use in foods as was considered desirable. The regulation did not cover a number of modified starches which were regarded as generally recognized as safe. This presented a

[1] Personal communication.
[2] Personal communication.

labeling problem for users of food starches. Moreover, certain processes and combinations of processes for modifying starch were not covered in the first regulation. For these reasons, petitions were submitted to amend the regulation in order to have the additional processes included, and to cover all modified food starches under one regulation that would simplify listing as ingredients used in fabricated foods.

The amended regulation, 121.1031 for food starch — modified, was published in the Federal Register on January 9, 1962. Although this regulation was more complete and did classify the starches as "Food Starch—Modified," there are some details which still need to be clarified and revised. Discussions with the FDA have clarified the secondary labeling question for these starches. They have advised that: "For the purpose of compliance with the requirements of Section 403 (i) (2) whenever any of the modified food starches subject to regulation 121.1031 are used as ingredients in fabricated foods we will consider the name 'food starch—modified' or 'modified food starch' as satisfactory." In addition, the Meat Inspection Division of the Department of Agriculture has coordinated their requirements for these starches with those of the FDA. The M.I.D. advises that: "Food starches—modified, covered by Food Additives Regulation 121.1031, are acceptable to this Department for use in meat-food products, where starches are permitted; and labels for commodities containing such substances should be designed with ingredients statements which refer to their presence as either 'Food Starch—Modified' or 'Modified Food Starch'."

Thus a wide variety of modified food starches have been recognized as safe, or shown to be safe, under the provisions of the Food Additives Amendment for use generally in the fabrication of foods. The question of how these starches should be listed in ingredient statements has been clarified and simplified.

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REPRODUCTIVE CAPACITY OF FEMALE RATS AS AFFECTED BY KINDS OF CARBOHYDRATES IN THE RATION [1]

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INTRODUCTION

When experimental rats used in a study of galactose metabolism [2] failed to reproduce normally, it was decided to determine the character of the abnormality and also, if possible, the relation of the carbohydrate in the ration to the reproductive capacity of the rat.

LITERATURE

The effects on reproduction of moderate amounts of sucrose and lactose in the rations of rats have been reported by several workers. Mitchell (*6*) [3] found that lactose, when constituting not more than 30 percent of the ration or 50 percent of the total carbohydrate in the ration, did not interfere with growth or well-being. Skinner, Van Donk, and Steenbock (*8*) found that ovarian function, as measured by length and regularity of oestrus cycles, was improved by the addition of 10 percent of sucrose to a ration of mineralized milk. Keil, Keil, and Nelson (*4*), however, reported interference with reproduction as a result of the addition of 5 percent of sucrose to a differently mineralized milk ration.

Evans (*3*) stated that a lack of vitamin E has no effect on the ova or ovarian tissue. In a comparison of vitamin E deficiency with that of vitamins A and B, he stated that vitamin A deficiency is characterized by a continuous cornified smear.

Smith and Engle (*10*) found that anterior pituitary transplants stimulated the ovaries of immature and mature rats, producing an excess of follicles. Ovulation occurred in the young animals. In older animals there were some follicular cysts, and there was also some luteinization. The cysts were sometimes twice the size of normal follicles. Engle and Smith (*2*), in a study of corpus luteum formation in pituitary-stimulated animals, found that follicles did not rupture, but formed cysts which transformed directly into corpora lutea. Sometimes eggs were found "trapped" as a result of luteinization of the follicular wall. Smith (*9*) observed that hypophysectomy of the rat resulted in a regression of the ovaries. If corpora lutea were present, they persisted for a long time. Transplants of anterior pituitary stimulated these regressed ovaries to renewed activity but follicular and lutein cysts resulted. Casida and Hellbaum (*1*), using 21- to 25-day-old rats, produced large follicles and

[1] Received for publication Apr. 14, 1936; issued November 1936. Contribution no. 205 from the Department of Chemistry and no. 349 from the Department of Animal Husbandry, Kansas Agricultural Experiment Station.

[2] These animals were under the care of G. N. Woodruff of Kansas State College.

[3] Reference is made by number (italic) to Literature Cited, p. 532.

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corpora lutea by injections of extracts from adrenal glands of horses. They obtained ovulation in about 50 percent of the treated animals. They made no attempt to determine whether this effect was direct or indirect. Moore and Price (7) concluded from their work that the only gonad-stimulating hormones are hypophyseal or hypophysealike substances.

MATERIALS AND METHODS

The rations fed were designed to be adequate in every known respect. One group of rations contained a minimum amount of galactose while the other group contained lactose which provided a liberal supply of readily available galactose.

The regular experimental rations (designated according to the characteristic carbohydrate) are shown in table 1.

TABLE 1.—*Composition of experimental rations*

Food product	Sucrose	Lactose	Starch
	Percent	*Percent*	*Percent*
Yeast	5	5	4
Osborne and Mendel salt mixture	4	4	5
Cod-liver oil	1	1	1
Casein	20	20	20
Butter oil	20	20	20
Starch	30	30	51
Sucrose	20		
Lactose		20	

Commercial lactose was used for all but one group of lactose-fed rats. The substitution of Mallinkrodt's "analytical reagent" lactose for this group produced no change in the results. No further attempt was made to differentiate lactose from factors which might be associated with it as the important constituent of the ration.

These rations were fed to many groups of rats during a period of more than 2 years. The early groups included several females for which the reproductive record is not complete enough to justify detailed description but which furnished the incentive for the detailed records set forth in this paper.

Some of the rats were mated in order to test fertility. Ages at which the vaginas opened were determined. Microscopic observations were made on the ovaries of some animals, and the uteri of these animals were examined macroscopically. Serial sections of the ovaries were made for microscopic study. In some cases one ovary and part of one horn of the uterus were removed by laparotomy. Since little difference was noted between the remaining ovaries of these animals and ovaries from other animals receiving the same feed, they have been included in the appropriate age and feed groups.

OBSERVATIONS

Seven animals fed lactose, five fed sucrose, and two fed starch were kept with fertile males for 40 days or until pregnancy was detected. During the time of this test all the animals were changed from the experimental rations to the regular stock ration. The results are presented in table 2.

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	105	6	All died within 3 days.
84b	do	79	102	7	Normal.
90b	do	79	105	5	Do.
121c	do	50	80	4	1 died, 3 normal.
125c	do	56	93	8	Normal.
129d	do	48	85	8	Do.
1	Sucrose	60		0	
2	do	70		0	
3	do	70	111	3	2 died, 1 weakly.
87b	do	79		0	
119c	do	56	80		Abortion.
123c	Starch	66	94	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 90 days without producing another litter.

As table 2 shows, each lactose- and starch-fed animal reproduced. All these litters, except possibly one born to a lactose-fed female, were normal. The one sucrose-fed female which produced a live litter was afterward sterile.

The age at which the vagina opened and the type of vaginal smears were determined for 17 sucrose-, 4 starch-, 6 stock-, and 18 lactose-fed animals. The results are presented in table 3. The sucrose-fed

TABLE 3.—*Effect of feeding various carbohydrates on age at which vaginas of rats opened, and types of smears secured*

Ration fed	Animals	Age at which vagina opened: Youngest	Age at which vagina opened: Oldest	Age at which vagina opened: Average	Standard deviation	Vaginal smear record
	Number	*Days*	*Days*	*Days*		
Sucrose	17	32	51	38	4.8	1-3 cycles, then diestrum
Starch	4	46	53	49	3.8	Not observed.
Stock	6	51	60	55	3.4	Normal cycles.
Lactose	18	45	64	59	6.7	Do.

animals showed sexual activity, as measured by the age at which the vaginas opened, earlier than any of the other groups. There is considerably less than 1 chance per 1,000 that this difference is due to chance. The smears of the sucrose-fed animals, however, showed only a few oestrus cycles. The lactose-fed animals were slightly late in reaching sexual maturity but showed normal cyclic changes in the smears. The starch- and stock-fed groups consisted of only a few animals, but these showed no abnormalities. They closely resembled the lactose group.

The observations made on the ovaries of the animals fed sucrose and those fed lactose are shown according to age groups in table 4.

TABLE 4.—*Effect of feeding sucrose and lactose in relation to microscopic observations of the ovaries of rats in various age classes*

Animals (number)	Age	Carbohydrate fed	Corpora lutea	Luteinization of follicles	Atresia [1]
	Days				
3	37	Sucrose	+	+	−
2	37	do	+	+	−
1	37	Lactose	−	−	+
5	44-47	Sucrose	+	+	+
2	44-47	Lactose	−	+	+
10	64-72	Sucrose	+	+	+
10	64-72	Lactose	[2] +	−	+
8	64-72	do	−	−	+

[1] Atresia indicates that follicles are so degenerated that they should soon disappear.
[2] 3 animals in this group had fresh rupture points in some of the follicles. Eggs were found in the Fallopian tubes of 2.

Photomicrographs of typical sections from ovaries of sucrose- and lactose-fed animals of each age group are shown in plate 1, *A* to *F*. These sections all have the same magnification, but they do not represent the relative sizes of the ovaries from different animals.

The presence of luteinized follicles in the sucrose-fed animals of all age groups would account for the lack of atretic disappearance of follicles. Luteinization of immature follicles would also explain the failure of the follicles to rupture.

As table 4 shows, the sucrose-fed animals were precocious. Corpora lutea were found in the ovaries of some of these animals at 37 days of age (pl. 1, *A*). In sharp contrast to this, the lactose-fed animal of this age was practically in an infantile sexual condition. Small follicles only were present in the ovary (pl. 1, *B*).

At 44 to 47 days of age similar differences existed as in the 37-day-old animals but they were even more striking. The sucrose-fed animals showed many more corpora lutea, luteinization of the larger follicles, and only a few small follicles (pl. 1, *C*). There was some indication that the follicles were being transformed into corpora lutea without a rupture occurring; at least no rupture points were observed. Some of the large luteinized follicles contained eggs that were degenerating. The lactose-fed animals, on the other hand, contained ovaries with follicles of all sizes (pl. 1, *D*). There was no luteinization of follicles among lactose-fed animals.

In the age group 64 to 72 days, many large corpora lutea were found in the ovaries of the sucrose-fed animals. There were no cyclic ages of corpora lutea, and no small follicles were present. Some cysts due to luteinization of the large follicles were found, but none of the corpora lutea showed signs of degenerating as would be expected if normal cycles were occurring (pl. 1, *E*). There was considerable variation among the lactose-fed animals. Some had not yet ovulated, showing some indication of retardation. In most cases, however, fresh corpora lutea and normal follicles of different sizes were found (pl. 1, *F*). In some of the lactose-fed animals the rupture points were visible, and eggs were found in the tubes of two animals. More than one age of corpora lutea was observed in the ovaries of some animals. There was no luteinization of follicles.

A, Left ovary from a 25-day-old sucrose-fed rat, showing large corpora lutea (*cl*) and small luteinized follicles (*lf*). *B*, Left ovary from a 25-day-old lactose-fed rat, showing many small follicles (*f*). There is no luteinization of any follicles. Some atresia is present. *C*, Right ovary from the same animal as *A*, at 45 days of age. A sucrose-fed rat, showing large corpora lutea (*cl*) and luteinized follicles (*lf*). No normal-appearing follicles of any size. *D*, Right ovary from the same animal as *B*, at 45 days of age. A lactose-fed rat, showing follicles of different sizes (*f*). No corpora lutea and no luteinization of follicles. *E*, Ovary from 70-day-old sucrose-fed rat, showing many corpora lutea (*cl*) and luteinized follicles, some containing degenerating eggs (*de*). Some follicles are so heavily luteinized that cysts have resulted (*fc*). *F*, Ovary from 70-day-old lactose-fed rat, showing follicles of different sizes (*f*) and recent corpora lutea with blood clots (*rcl*). There is no luteinization of follicles.

The uteri of the sucrose-fed animals of all age groups were generally larger than those of the corresponding lactose-fed animals. Some were distended with fluid and some were highly vascular.

Ovaries of three lactose-fed animals were observed about 30 days after the animals had given birth to litters. The normal cyclic ages of corpora lutea were present. Ovaries of four other animals aged about 200 days were also observed. These had been on a sucrose ration until about 70 days of age. Afterward they had been on the regular stock ration. The ovaries were made up almost entirely of masses of corpora lutea and follicular cysts. No evidence of cycles was seen, and there was indication of the cysts transforming into corpora lutea.

DISCUSSION

With our present knowledge of nutrition it is difficult to understand how the replacement of either the 20 percent lactose, or 20 of the maximum 50 percent starch, with 20 percent sucrose in the ration could have produced the observed sexual precocity and luteinization of follicles. A few instances have been reported in which sucrose in the ration was associated with abnormal reproduction. The observed abnormalities, however, were not linked with either abnormal ovarian structure nor with any pituitary disturbances. The absence of galactose might possibly be associated with the results produced by the sucrose ration. Sorensen and Haugaard (*11*) have shown that casein contains a small amount of galactose. It is possible that the additional cornstarch contained sufficient galactose to meet the need and make possible the observed relations of the three rations studied. It might, on the other hand, be assumed that the sucrose affected some gland which secreted a gonad-stimulating substance. Since most work indicates that the pituitary is the only gland which could have produced this effect, there is the possibility that the sucrose either directly or indirectly affected the activity of the pituitary.

The results obtained were similar to those produced by pituitary implants (*10*). One difference, however, is that no excessive number of follicles or corpora lutea were present. The luteinization of follicles and formation of cysts and the persistence of the corpora lutea were more like the results produced by implanting pituitaries into hypophysectomized rats (*9*). The transformation of follicular cysts into corpora lutea is similar to results obtained by Engle and Smith (*2*). The similarity of the ovarian abnormalities in the writers' animals to defects noted by other workers when the pituitary function was altered is strong evidence that some pituitary disturbance existed.

The possibility that a vitamin deficiency might have brought about the abnormalities noted is very small. The symptoms observed were not the characteristic symptoms of any vitamin deficiency. The smear record and ovarian observations are far from indicating a deficiency of either vitamin A or E.

When the mixture of purified amino acids recently announced (*5*) becomes available as a substitute for proteins, it may be practical to determine more definitely what characteristics of the carbohydrates studied are associated with the results secured in this investigation.

SUMMARY

In this study of the relation of the carbohydrate in the ration to the reproductive capacity of rats, three carbohydrates were fed, sucrose, lactose, and starch.

The sucrose-containing ration was found to be inadequate for normal reproduction in rats, even when replaced during adult life by a normal ration.

Rats receiving the sucrose ration were precocious in sexual development.

Extreme luteinization of follicles and persistence of corpora lutea were characteristic of the ovaries of the sucrose-fed animals. This adequately explains the failure of these animals to reproduce.

The ovarian abnormalities of the sucrose-fed animals indicate pituitary disturbances.

Animals fed the lactose- and starch-containing rations reproduced normally and had normal ovarian structures.

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Utilization and Toxicity of Dialdehyde- and Dicarboxyl-Starches.* (25380)

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The modified physical and taste characteristics of oxidized starch have suggested the possibility of use in foods. A preliminary study of effects on the rat is presented here. Anhydroglucose units of starch can be oxidized by periodic acid to dialdehydes(1). The reaction is stoichiometric so that the number of glucose units thus oxidized can be regulated by amount of periodic acid used. An electrolytic procedure which regenerates the periodic acid as it is used up has recently been introduced(2). Further oxidation by chlorous acid quantitatively converts the aldehyde groups to carboxyl groups(3). The reactions are represented in Fig. 1.

Materials. Oxidized cornstarches were obtained from Northern Regional Research Lab., Peoria, Ill. Four dialdehyde starches had 0.05, 2, 40 and 100% of glucose units oxidized. These will be referred to as DAS-.05, -2, -40, and -100, respectively. The dicarboxyl starch had 5% of the glucose units oxidized (DCS-5). *Methods. Acute toxicity* tests were not performed with DCS, because some animals in the subacute study were ingesting daily 16-17 g/kg without apparent ill effects. Acute oral toxicity was studied with DAS-100. The material was dissolved with a minimum of NaOH, neutralized immediately with HCl and given to young adult rats (2 males and 5 females) at 1 g/kg. One male rat died after 11 days with multiple lung abscesses, presumably not due to the material. The others remained alive and healthy during 2 weeks observation. The basal diet for subacute toxicity experiments consisted of corn meal, 51.5; casein, 11.5; linseed oil cake meal, 10; alfalfa meal, 2; cod liver oil, 3; bone ash, 1.5; NaCl, 0.5; and cornstarch, 20%. Experimental diets were made by replacing an equal amount of corn starch with the desired amount of the appropriate oxidized starch. The animals were 30-day-old male rats, except for the DAS-0.05 test, where females were used. They were caged in groups of 4 to 6 animals, with water and food available *ad lib.* Feeding of diets continued for 90 to 100 days except when marked toxicity or limited supply forced earlier termination (lack of sufficient DAS-0.05 ended the feeding of 20% diet after 41 days). Concentrations of oxidized starches ranged from 1 to 20% of the diet for each of the DAS products, and from 0.2 to 20% for DCS-5.

* Histopathological examinations were done by Dr. Wm. E. Ribelin.

† Agric. Research Service, U. S. Dept. Agric.

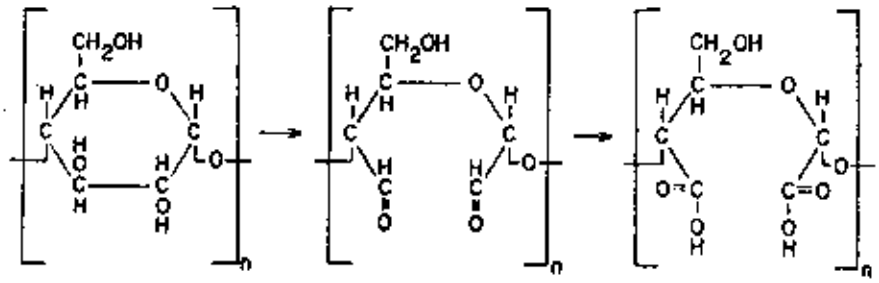

FIG. 1. Oxidation of anhydroglucose units of starch.

Results. Continued feeding of diets containing 10 and 20% of DAS-100 led to early death. Concentrations of 5 and 2%, and probably 1%, inhibited growth. DAS-40 permitted normal growth at dietary level of 1%, inhibited growth at 5%, very poor growth at 10%, and early death at 20%. Feeding of DAS-2 and -0.05 permitted normal growth at all levels including 20%. Low food intake accompanied poor growth; in no case was excessive food intake noted. Growth and appearance were normal with DCS-5, but food intake seemed excessive. This will be discussed below.

No gross abnormalities which could be attributed to the diets, were seen at autopsy. Histopathological examination of tissues showed testicular atrophy in rats receiving DAS-100 and -40. In spite of finding testicular atrophy in one control, it appears very probable that DAS-100 produces this atrophy in sexually mature rats in concentrations of 1% or more of the diet. There was an unusual multinucleated cell formation within testicular tubules. Diets containing DAS-40 produced various degrees of testicular atrophy in sexually mature rats in concentrations of 5% or more. Formation of multinucleated cells was not as conspicuous in this group. No significant microscopic abnormalities were detected in any of the DCS-5 animals.

Although growth rates were uniform between groups of DCS animals, there was from the start an apparently much greater food intake for rats receiving the 20% diet than for those on control diet (13.2 g vs 11.2 g/rat/day at the end of experiment), and possibly rats receiving the 2% diet ate more (11.7 g). To check on the possibility of food wastage, the following experiment was tried. Six male rats 90 days old were placed in individual false-bottom cages, with facilities for collection of feces and wasted food. Half received control diet and half the 20% DCS-5 diet. Food wastage was minimal, showing that rejection of objectionable food was not a factor in the results. Body weights, food intake, and weights of air-dried feces were obtained for individual animals at 3- or 4-day intervals for 2 weeks. Growth was fairly uniform. Food intakes and fecal outputs are shown in the Table. It seems clear that the animals were not utilizing the DCS diet as efficiently as that made with unmodified cornstarch. The greater food intake, however, furnished sufficient calories to maintain normal growth.

This poorer utilization evidenced by greater food intake and fecal output suggested that digestion of oxidized starch might be incomplete. This led to the following qualitative test. Boiled cornstarch and DCS solutions (1%) were mixed with saliva. Aliquots of the digestion mixtures were tested at intervals with dilute I-in-KI and with Benedict's qualitative sugar reagent. Both starches were digested to the point of no color with iodine, although rate of digestion seemed to be faster for cornstarch. Probably the reduction of

TABLE I. Effect of DCS-5 on Food Intake and Fecal Output.

Diet	Food intake, g/rat/day	Feces, g/rat/day	Feces / 100 Food intake
Control	10.57	1.04	9.8
	11.21	1.06	9.5
	11.86	1.09	9.2
Avg	11.21	1.06	9.5
DCS-5	13.36	1.54	11.5
	11.29	1.26	11.2
	13.14	1.56	11.9
Avg	12.60	1.45	11.5

Benedict's reagent was faster and perhaps more complete with the cornstarch-saliva mixture.

Summary. When 40% or more of anhydroglucose units of starch are oxidized to dialdehydes, the material is toxic to rats, as manifested by poor growth, increased mortality and testicular damage. No acute toxicity was noted. No toxicity occurred when 2% or less of the units were oxidized, even at levels of 20% of diet. Starch further oxidized to contain 5% of glucose units in the dicarboxyl form, produced no toxic symptoms with as much as 20% of diet consisting of this modified starch, but food intake was increased and utilization decreased, perhaps due to faulty digestion of the dicarboxyl starch.

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WHO '72
Bib No. 1680

World Health Organization; Food Additives Series 1:7, 23-60. 1972.

TOXICOLOGICAL EVALUATION: MODIFIED STARCHES[1]

	Evaluation[2]	Monograph	Specification
Oxidized starches	Not limited	Yes	Yes
Starch acetate	Not limited	Yes	Yes
Hydroxypropyl starch	Not limited	Yes	Yes
Hydroxypropyl distarch glycerol	Not limited	Yes	Yes
Distarch phosphate	Not limited	Yes	Yes
Phosphated distarch phosphate	Not limited	Yes	Yes
Acetylated distarch phosphate	Not limited	Yes	Yes
Distarch glycerol	Not limited	Yes	Yes
Acetylated distarch glycerol	Not limited	Yes	Yes
Acetylated distarch adipate	Not limited	Yes	Yes
Hydroxypropyl distarch phosphate	Not acceptable	Yes	Yes[3]

[1] For results of evaluation on other modified starches see Annex 4, Thirteenth Report, Wld Hlth Org. techn. Rep. Ser., No. 445.

[2] Use limited by good manufacturing practice; temporary acceptance.

[3] Tentative specification.

OXIDIZED STARCHES

Biological data

Whatever oxidizing agent is used only minor modifications of the starch molecule are achieved in normal manufacturing practice. These are equivalent to the introduction of 1 per cent. w/w of carboxyl (-COOH) or 0.5 per cent. w/w of keto (-CO) groups or, 3.6 carboxyl and 2.9 carbonyl groups per 100 glucopyranose units. No chlorine is introduced into the molecule and the final products usually contain only residues of sodium chloride, sodium sulfate and sulfur dioxide.

Biochemical aspects

Early comparative experiments pointed to inhibition of amylolysis (Tremolières et al., 1959). In vitro digestibility by pancreatin or saliva was used to compare slightly and highly oxidized corn starch with unmodified corn starch and a reference starch. Maltose production after a fixed interval of enzyme action was taken as a measure of digestibility. The oxidized starch was 10-15 per cent. less digestible by pancreatin than unmodified starch but there was no obvious difference as regards salivary digestion (Shuman, C. C. & Mertz, E. T., 1959). The digestibility of oxidized wheat starch (conditions not stated) was examined in rats by matched-feeding techniques using the modified starch as the sole source of carbohydrate at a level of 63.7 per cent. (dry basis) of the diet. The degree of assimilation by and the general effects on groups of 6 rats over a feeding period of 28 days were assessed from consideration of body-weight changes, faecal residues, digestibility coefficients for starch and post-mortem appearance of the animals and their gastrointestinal tracts. The digestibility coefficients were calculated from the starch content of ingested food and residues found in faeces and post-mortem gastrointestinal contents. Body-weight gain and digestibility coefficients were practically indistinguishable from those obtained for wheat starch or corn starch. Nothing abnormal was noted on post-mortem examination (Booher et al., 1959).

Other studies, in 3 groups of 3 rats each, used corn starch oxidized with 3.9 per cent., 4.5 per cent., or 5.5 per cent., hypochlorite calculated as chlorine. This corresponds to the introduction of 0.57 per cent., (2.04 COOH groups per 100 glucopyranose units), 0.8 per cent., (2.86 COOH groups per 100 glucopyranose units) and 0.9 per cent. (3.57 COOH groups per 100 glucopyranose units) carboxyl groups. To 5 g basal diet were added 1, 2 or 4 g modified or control starch and this diet was fed to rats for 10 days. Comparison of digestibilities showed an apparent decrease with increasing oxidation but no effect on caloric values.

No tissue damage was associated with the diarrhoea and caecal enlargements observed in groups receiving 2 g or 4 g starch in their feed. Liver, kidney, heart and spleen weights were normal. Diarrhoea and caecal enlargement are known to occur in rats fed starches of poor digestibility or other carbohydrates (White, 1963).

The digestibility of oxidized starches at levels of 2.5 per cent., 6 per cent. and 13.2 per cent. calculated as chlorine, equivalent to a carboxyl content of 0.32 per cent. (1.15 COOH per 100 glucopyranose units), 0.9 per cent. (3.81 COOH per 100 glucopyranose units) or 1.46 per cent. (5.23 COOH per 100 glucopyranose units), was studied in groups of 6 male and 6 female rats. The animals were kept for 7 days on 5 g basal diet and then given either 1 g or 2 g starch supplements for 21 days. Poor weight gain with diarrhoea were noted only with the highly oxidized material at both dietary levels. One rat from each of the high dietary level groups was examined. Marked caecal dilatation was seen only in animals fed the heavily oxidized starch. It is to be noted that this very highly oxidized starch is a commercially unacceptable product (Whistler & Belfort, 1961).

Short-term studies

Rat

Starch treated at a level of 0.375 per cent. chlorine was fed to weanling albino rats at 70 per cent. of their diet for 10 weeks with corn starch as control. Feeding was either unrestricted or by paired-feeding technique. No toxic effects were noted. No details of this work, carried out in 1944-5, were available (Garton Sons & Co. Ltd., 1967).

Long-term studies

None supplied.

Comments

The digestibility of hypochlorite-oxidized starch has been investigated in vivo and found to be similar to that of unmodified starch. The longest of the short-term studies reported extended only over 10 weeks and was carried out with an inadequately defined sample. Studies with highly oxidized starches (1.4 per cent. or more carboxyl groups) are not applicable because these products are not commercially acceptable. Provided the chemical change is limited to the introduction of no more than 1 carboxyl group per 25 anhydro-glucose units, the biological effects of the modified starch do not appear to be deleterious.

EVALUATION

Temporarily not limited.*

Further work required by 1973

Ninety-day studies in 2 species.

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* Except for good manufacturing practice.

STARCH ACETATE

Biological data

Esterification is achieved by using either acetic anhydride (up to 8 per cent.) or vinyl acetate (up to 7.5 per cent.). A maximum of 2.5 per cent. acetyl groups is introduced corresponding to a maximum degree of substitution of 0.1.

Biochemical aspects

The digestibility of acetylated starches was measured by the biochemical oxygen demand of incubated samples. As the acetyl contents increased so the BOD values decreased and, in parallel, the digestibility. Starch acetates containing 2.5 per cent. acetyl groups are only 93.7 per cent. as digestible as native starch (Turner, 1961). Digestibility by fungal amyloglucosidase was shown to be 68-81 per cent. of that of native starch (Turner, 1961; Kruger, 1970). The digestibility of starch acetate (containing 1.98 per cent. acetyl groups by pancreatin and porcine mucosal enzymes was found to be 90 per cent. of that of the unmodified starch (Leegwater, 1971). Caloric value was determined in groups of 10 male rats fed for 4 weeks a diet supplemented with graded doses of 0, 1.5 g, 3.0 g, 4.5 g and 6.0 g dextrose (equivalent to 0, 6, 12, 18 and 24 calories). The dose response curve was used to estimate the caloric value of supplements of 3 g and 4.5 g of acetylated (1.8 per cent. acetyl) or native starch. No significant difference was found between the starch samples with regard to caloric value (Oser, 1961).

Short-term studies

Rat

Groups of 10 male rats were fed for 28 days diets containing 60 per cent. of various starch acetates (the degree of acetylation varied from 0, 1.24, 2, 2.56 to 3.25 per cent.). Weight gain was reduced in groups receiving starch acetates with more than 2 per cent. acetylation but feed efficiency remained unaffected. Diarrhoea occurred at 2 per cent. and higher degrees of acetylation and there was noticeable caecal enlargement at the same levels. No tissue damage or inflammation were noted in association with the diarrhoea (Turner, 1961).

In another experiment potato starch acetate (acetylated to 1.2[illegible] per cent.) was fed for 13 weeks to groups of 10 male and 10 female rats at levels of 5, 15 and 45 per cent. of the diet. The 5 per cent. level was fed for only 4 weeks. No animal died. Growth rate and haematological findings were not significantly affected. The

relative weights of liver, kidney, adrenal, pituitary and thyroid showed some significant differences compared with controls, being generally lower except for male thyroids. Male caecal weights were higher than controls and distended caeca were seen at the 15 per cent. and 45 per cent. dietary levels. No histopathological changes due to starch acetate were seen (Feron et al., 1967).

In a further experiment starch acetate (acetylated to 1.98 per cent.) was fed to groups of 10 male and 10 female rats for 8 weeks at dietary levels of 25 per cent. and 50 per cent. No effects were noted on growth and body-weight. Water content of faeces and faecal production, as measured by dry matter content, showed no consistent effects but there was a tendency towards increased faecal dry matter at the 50 per cent. dietary level in both sexes. No diarrhoea was observed at any dietary level. Caecal weight and caecal enlargement occurred in a dose-related manner in all treatment groups. However, histological examination revealed no abnormality of the caeca examined (de Groot & Spanjers, 1970).

Man

Twelve volunteers consumed on each of 4 consecutive days 60 g starch acetate with 1.98 per cent. acetyl content. No effect was noted on frequency and amount of faeces, faecal water or lactic acid content. No other adverse effects were noticed (Pieters et al., 1971).

Long-term studies

Rat

Groups of 30 male and 30 female weanling rats were fed on diets containing 0, 5, 10 and 30 per cent. of starch acetate (acetylated to 1.98 per cent.) for 2 years. No significant differences were observed with respect to behaviour, general health and mortality. Growth and food consumption were essentially similar to those of the controls. Production of faeces during weeks 11 and 12 showed no dose-related differences among the various groups. Haematology, serum chemistry and serum enzymes as well as urinalysis showed no effects related to the administration of the test material. Among organ weights only the caecal weight of male rats showed a dose-related increase at 10 per cent. and higher levels and the caecal weight of female rats was increased at the 30 per cent. level compared with controls. No other significant changes were noted which could be ascribed to the test substance. The histological examination is still incomplete (Til et al., 1971a).

Reproduction studies

Rat

A three generation study was performed using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations by mating at week 12 and 20 after weaning. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10 per cent. of the diet consisted of starch modified with 5 per cent. acetic anhydride (degree of substitutions 0.079). No adverse effects were noted regarding health, behaviour, mortality, body-weights, fertility, litter size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were increased. Gross pathology of the F_{3b} generation revealed that kidney weights in females differed in a statistically significant way from controls but histopathology is still underway (Til et al., 1971b).

Special studies

Groups of 10 male and 10 female rats were given 25 per cent. and 50 per cent. of starch acetate (acetylated to 1.98 per cent.) in a low residue diet for 7 days. Thereafter 4 per cent. cellulose was added for a further 3 days. Body-weights were slightly reduced in both sexes at the 50 per cent. level after 7 days. Faecal dry matter was increased in all test groups but not in a dose-related manner. Slight diarrhoea occurred only at the 50 per cent. level in both sexes and this was unaffected by the feeding of additional cellulose in the diet. No loss of hair was noted (de Groot & Spanjers, 1970).

Comments

The feeding studies with rats did not show any deleterious effects. The available evidence for the group of modified starches considered suggests that caecal enlargement without associated histopathological changes is of little toxicological significance. Several short-term studies, a two-year study and a reproduction study in rats are now available for evaluation.

EVALUATION

Temporarily not limited.*

Further work required by 1973

Histological report to complete the two-year rat study.

Histological report to complete the reproduction study.

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* Except for good manufacturing practice.

HYDROXYPROPYL STARCH

Modification is carried out by propylene oxide at levels up to 25 per cent. and the resultant starch is usually lightly oxidized, bleached or acid modified after etherification. Substitution may amount to a maximum of 40 ether linkages per 100 glucopyranose units if 25 per cent. propylene oxide is used, and 4-6 ether linkages per 100 glucopyranose units if 5 per cent. propylene oxide is used.

Biological data

Biochemical aspects

In vitro digestibility by pancreatin was estimated by comparing the amount of reducing material liberated with that formed from native wheat starch. No significant difference could be detected between low (1 in 10) and high (4 in 10) substituted starches compared with unmodified starch (Kay & Calandra, 1962). In contrast the digestibility by pancreatin was found to decrease with increased substitution degree. At 0.04 degree the digestibility was 80 per cent. of that of unmodified starch (Leegwater & Luten, 1971). Corn starch treated with propylene oxide -2-C^{14} to produce hydroxypropyl starch (degree of substitution 0.12) was given to a male rat by gavage. Ninety-two per cent. of the radioactivity was excreted in the faeces and 3.6 per cent. in the urine over the next 50 hours. The urinary activity was probably derived from propyleneglycol in the test material. Further investigation of the degradation products in the faeces revealed hydroxypropyl maltose to be the major metabolite *in vitro* and *in vivo* (Leegwater, 1971).

Short-term studies

Rat

Groups of 10 male and 10 female rats were fed for 90 days diets containing 0, 2, 5, 10 and 25 per cent. of highly modified starch (25 per cent. propylene oxide) and 25 per cent. unmodified starch. No systemic toxicity was noted. There were no adverse effects regarding mortality, urinalysis or haematology at any level. There was slight reduction in growth rate at the highest dietary level with lower food utilization and without an equivalent increase in food consumption. Mild diarrhoea occurred at 25 per cent. dietary level. No adverse effects occurred at any other level. At autopsy there were no significant differences in the organ weights of liver, kidney, spleen, gonad, heart or brain. The observed increased ratios at 25 per cent. dietary level for liver/body-weight and kidney/body-weight were due to the relatively lower body-weight.

Gross and histological examination of all major tissues revealed no abnormalities due to the feeding of highly modified starch (Kay & Calandra, 1961). In another experiment groups of 10 male and 10 female rats were fed for 90 days on diets containing 0, 5, 15 and 45 per cent. of low modified starch (5 per cent. propylene oxide). Haematological findings at 12 weeks were comparable for all groups. Body-weights did not differ significantly from controls but were consistently lower in male rats only. Feed efficiency was similar in all groups. Caecal enlargement was seen at the 45 per cent. and very slightly at the 15 per cent. level. No histological abnormalities were detected in any major organs, which were due to the test substance. The enlarged caeca showed no evidence of inflammation or changes in the muscular coat (Feron et al., 1967).

Long-term studies

None available.

SUPPLEMENTARY INFORMATION

Biological data on propylene chlorohydrin

Biochemical aspects

Propylene chlorohydrin was identified as the residue formed in foods fumigated with propylene oxide (Wesley et al., 1965). The chlorohydrin is formed by the reaction of the epoxide with the chloride of food. Previously, it had been believed that propylene glycol, formed by reaction with water in the food, was the residue. Propylene chlorohydrin is also formed in starches modified by hydroxypropylation. Both propylene chlorohydrin isomers have been identified in fumigated foods (Ragelis et al., 1966).

When volatilization was precluded, a combination of high temperature and prolonged time in cooking did not appreciably alter the propylene chlorohydrin content of food, but when volatilization was possible, the chlorohydrin content was reduced 50 per cent. by cooking (Wesley et al., 1965). When propylene chlorohydrin was added to a standard ground laboratory rat diet, 20 minutes of mixing in an open mixer at room temperature resulted in a 65 per cent. decrease in the propylene chlorohydrin content (USFDA, 1966).

Acute toxicity

Animal	Route	LD_{50} (mg/kg body-weight)	Reference
rat	oral	218	USFDA, 1969
dog	oral	150 mg/kg - no deaths 200 mg/kg - 1/7 deaths 250, 300 mg/kg - 6/6 deaths	USFDA, 1969

Short-term toxicity

Rat

Groups of 10 male and 10 female five-week-old rats were fed, for 25 weeks, diets to which propylene chlorohydrin had been added. The planned dietary levels were 0, 1000, 2500, 5000 and 10 000 ppm, but analysis of the 10 000 ppm diet after mixing in the test compound (open mixer, 20 minute mixing time, room temperature) showed an actual concentration of 3568 ppm or 35 per cent. of the planned level. The 2-chloro isomer constituted 27 per cent. of the total found. The actual level in this diet, after 7 days exposure to laboratory conditions, was reduced to 838 ppm, with 32 per cent. of the 2-chloro isomer, or less than 10 per cent. of the planned concentration. Weight gain in both sexes on the 5000 and 10 000 ppm levels was depressed. The depression was slight in the males on the 5000 ppm level and both groups of females and moderate in the males on the 10 000 ppm level. Food consumption was slightly decreased in these groups but food efficiency was normal. The average liver and kidney weights of the males and the liver weight of the females on the 10 000 ppm level were decreased but the organ weight/body-weight ratios were normal. The decreased spleen weights and spleen/body-weight ratios in the males and other minor organ weight variations appeared to be unrelated to the treatment. No effects on haematological values, mortality, or gross or microscopic lesions in the tissues were observed (USFDA, 1969).

Propylene chlorohydrin was administered to groups of 10 male and 10 female eight-week old rats by stomach tube in doses of 0, 25, 50, 75 and 100 mg/kg/day for 22 weeks. The dose for the high level was increased from 100 mg/kg to 150 mg/kg in the eleventh week, to 200 mg/kg in the fourteenth week, and to 250 mg/kg in the sixteenth week. Doses of 200 mg/kg and less did not increase mortality. All the rats on the high level were dead by the nineteenth week with all but one of the deaths occurring between the sixteenth and nineteenth weeks after the dose had been increased to 250 mg/kg. On the high level, weight gain was moderately depressed in the males and

slightly depressed in the females while the dose was 100 or 150 mg/kg. Both sexes lost weight when the dose was increased to 200 mg/kg. Weight gain was slightly, but not significantly, decreased in both sexes on the 75 mg/kg level. Food consumption was slightly decreased in the males of the high level while the dose was 100 mg/kg and decreased to a greater extent when the dose was raised. The females on the high level also showed a slight decrease in food consumption when the dose was increased. With the rats losing weight when the dose was increased to 200 mg/kg, the food efficiency values have no meaning. The liver weight/body-weight ratios of both males and females on the 75 mg/kg dose and the liver weight and liver weight/body-weight ratio of the males on the 25 mg/kg dose were increased, but this increase was not accompanied by gross or microscopic alterations in the liver. Other organ weight and organ weight/body-weight ratio changes did not appear to be related to the treatment. No haematological effects or gross or microscopic effects on the tissues of the treated rats, at a dose of 75 mg/kg or less, were seen. The tissues of the high level rats were not examined microscopically (USFDA, 1969).

Comments

Short-term feeding studies with rats show that even the most highly modified starch tested is well utilized.

The metabolic study in rats using radio-labelled material shows that most of the radio-labelled hydroxypropyl containing moiety is excreted in the faeces. No long-term study on this modified starch is available but collateral evidence from the long-term study in rats with hydroxypropyl distarch glycerol, a more high modified starch, indicates that the propylene glycol moiety is causing no adverse effects. The available evidence for the group of modified starches considered suggests that caecal enlargement without associated histopathological changes is of little toxicological significance.

EVALUATION

Temporarily not limited.*

Further work required by 1973

Histological report to complete the two-year rat study on hydroxypropyl distarch glycerol.

Histological report to complete the reproduction study on hydroxypropyl distarch glycerol.

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* Except for good manufacturing practice.

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HYDROXYPROPYL DISTARCH GLYCEROL

Modification is performed by the use of 0.1 per cent. epichlorhydrin and up to 10 per cent. propylene oxide. Cross-linkage would be no greater than expected on modification with 0.1 per cent. epichlorhydrin. Substitution by treatment with 10 per cent. propylene oxide would probably add no more than 15-20 ether linkages per 100 glucopyranose units.

Biological data

Biochemical aspects

The *in vitro* digestibility of a hydroxypropyl distarch glycerol (degree of substitution 0.04) by pancreatin and porcine intestinal mucose was found to be 86 per cent. of that of the native starch (Leegwater, 1971).

Short-term studies

Rat

Groups of 10 male and 10 female were fed for 8 weeks on a diet containing 25 or 50 per cent. modified starch. Although the body-weights of males at the higher level tested were slightly lower than those of the controls, the differences were not statistically significant. The faecal dry matter content was increased at both test levels in both sexes and there was considerable diarrhoea at the 50 per cent. level in both sexes. Slight diarrhoea occurred at the 25 per cent. level in both sexes. Caecal weights were increased in a dose-related manner at all levels in all test groups but no histological abnormalities could be detected in the caeca (de Groot and Spanjers, 1970).

Groups of 25 male and 25 female rats were fed for 13 weeks on a diet containing 1 and 5 per cent. of modified starch or 5 per cent. of native starch. The general appearance and behaviour of test rats was comparable with the controls. One male and one female rat died at the lower test level. Growth, feed consumption, body-weight, haematology at one and three months, urinalysis at one and three months, gross and histopathological findings were comparable in all groups (Knapp, 1967a).

In another experiment, groups of 10 male and 10 female rats were fed for 90 days diets containing 0, 5, 10 or 30 per cent. modified starch. No differences between test and control animals were noted with regard to appearance, behaviour, growth, food consumption, haematology, serum chemistry urinalysis. No definite diarrhoea occurred at any test level. The amounts of dry matter in the faeces did not vary significantly between controls and treated animals. Only at the 30 per cent. level were the caecal weights increased in both sexes. Gross and histopathology were otherwise unremarkable in all groups. Histopathology of the enlarged caeca showed no recognizable abnormality (de Knecht-van Eekelen et al., 1970).

Dog

Three groups of 4 male and 4 female beagle dogs were given in their diet 1 per cent. and 5 per cent. of modified starch or 5 per cent. of native starch for 13 weeks. Haematological studies, blood sugar, blood urea, serum GPT and alkaline phosphatase as well as urinalysis were comparable for all groups. Appearance and behaviour were normal. Body-weight changes were unrelated to the administration of the test substance. Gross and histopathology showed no consistent abnormalities. Organ weights of thyroid, heart, liver, spleen, kidneys, adrenals and testes showed no test-related changes (Knapp, 1967b).

Man

Twelve volunteers consumed 60 g modified starch on each of 4 successive days. No adverse effects were noticed and no abnormalities occurred as regards frequency and amount of faeces or faecal water and lactic acid content (Pieters et al., 1971).

Long-term studies

Groups of 30 male and 30 female rats were fed for 104 weeks modified starch at 0, 5, 10 and 30 per cent. of their diet. No differences were noted between test and control animals with regard to appearance, behaviour, food consumption, haematology, serum chemistry and urinalysis. The mortality of females at the 30 per cent. level was slightly higher. The growth rate was slightly reduced for both sexes at the 30 per cent. level when compared with controls. Caecal weight was increased in a dose-related manner but occurred only in females at the 10 per cent. level. The gross pathology has revealed no undue differences nor was there any obvious evidence of a carcinogenic effect. The histopathology has not yet been completed (Til et al., 1971a).

Reproduction studies

Rat

A three generation study was performed using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10% of the diet consisted of starch modified with 5% phosphorus oxide and 0.1% epichlorhydrin (D.S. of 0.05). No abnormalities were noted regarding health, behaviour, mortality, body-weights, fertility, little size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were increased in both sexes. Similarly kidney and thyroid weights in the females differed in a statistically significant way from the controls. There were no pathological changes on gross examination at autopsy in the F_{3b} generation but the histopathology is still outstanding (Til et al., 1971b).

Groups each of 10 male and 20 female rats were fed the modified starch at a level of 10% in their diet for a period of 3 generations. Rats were mated (P_1, F_1 and F_2) at week 12 and 20 after weaning. Each generation was mated twice and the litter from the second generation used to produce the next generation. No adverse effects were noted on fertility, number of pups per litter, body-weight of young, mortality experience or resorption quotient that could be ascribed to treatment. The average relative cecum weight (filled) of females of the P and F_2 generation was greater than controls, but empty cecum weight did not differ from controls. The relative cecum weight (filled and empty) of males of the F_1 generation did not differ from controls. Growth of the F_{3b} generation maintained on test diet for 3 weeks was normal. Relative organ weights were comparable to control, with the exception of increased thyroid weight and distinctly increased cecal weight (both filled and empty) in male pups, and increased empty cecal weight in female pups. Gross examination of organs at autopsy did not show any compount related changes. Histopathological studies of organs have not been completed (Til et al., 1971).

Special studies

Groups of 10 male and 10 female rats were fed for 7 days on a low-residue diet containing 25 or 50 per cent. modified starch. Thereafter, they were fed an additional 4 per cent. cellulose. Body-weight was slightly reduced at the 50 per cent. test level in

both sexes and faecal dry matter was increased in all groups compared with controls. There was considerable diarrhoea in all test groups which was unaffected by the additional dietary cellulose. Hair loss was pronounced at the 50 per cent. test level in both sexes and was slight at 25 per cent. (de Groot and Spanjers, 1970).

Comments

The feeding studies with rats and dogs show that the modified starch is well utilized. The studies with radio-labelled hydroxypropyl starch provide some information in the rat on the metabolic fate of the propylene glycol released from the starch. Several short-term studies in the rat and dog show no adverse effects even at high dietary levels. The available evidence for the group of modified starches considered suggests that caecal enlargement without associated histopathological changes is of little toxicological significance. The long-term and reproduction studies in the rat indicate no significant adverse effects and may be used for evaluation.

EVALUATION

Temporarily not limited.*

Further work required by 1973

Histological report to complete the long-term rat study.

Histological report to complete the reproduction study.

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* Except for good manufacturing practice.

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DISTARCH PHOSPHATE

Biological data

Native starches are known to contain phosphoric acid esters equivalent to 0.004 per cent. P and some potato starches up to 0.1 per cent. Distarch phosphate is made by the use of sodium trimetaphosphate which cross-links starch chains at an approximate rate of one phosphate link per 620 glucopyranose units. The amounts of phosphate introduced are at the most 0.04 per cent. P (Graefe, 1964).

Cross-linking of starch chains may be produced also by the use of phosphorus oxychloride. Although theoretically likely, there is no relevant chemical evidence to show that modification by phosphorus oxychloride in fact produces distarch phosphate. Although the addition of phosphorus oxychloride to dry material will cause chlorination, the processes involved in starch modification always proceed in the presence of water and/or alkali. Under these circumstances phosphorus oxychloride hydrolyses rapidly with the production of phosphoric and hydrochloric acid and the formation of other products than phosphate cross-linkages is very unlikely (Hudson and Moss, 1962; Grunze, 1959). The maximum number of phosphate bridges could reach one per 100 glucopyranose units.

Biochemical aspects

In vitro digestion of a distarch phosphate using trimetaphosphate by salivary, pancreatic and intestinal amylase was measured by the production rate of reducing sugar. No deleterious effect was shown on enzymic depolymerisation (Rosner, 1960).

Caloric value and digestibility of a distarch phosphate using trimetaphosphate were tested in groups of 10 rats fed for 7 days on 4 g basal diet with either 0.9 g or 3.6 g starch supplement by observing the gain in body-weight and the organ weights of liver, kidney, heart and spleen after the feeding period. No significant differences were noted between the modified and the unmodified starches (Hixson, 1960). Distarch phosphate using trimetaphosphate was fed to groups of male and female rats on 5 g diets as 1 g or 2 g supplements over 21 days. Weight gains were comparable for modified and unmodified starches tested. Animals appeared normal at autopsy (Whistler and Belfort, 1961).

The in vitro digestibility of trimetaphosphate modified starch by pancreatic amylase was somewhat reduced compared with normal unmodified starch (Kohn and Kay, 1963a). In vivo digestibility was examined in groups of 10 male rats fed for 10 days 5 g basal diet supplemented by 1 g, 2 g or 4 g unmodified or trimetaphosphate modified starch. Weight gains were identical for both types tested at all three levels of supplementation. No unusual behavioural reactions were observed (Kohn and Kay, 1963a).

In vitro digestibility by pancreatin of corn or potato starch modified with 0.05 or 0.1 per cent. phosphorus oxychloride was found to be similar to the unmodified starch. When 0.5 or 1.5 per cent. of phosphorus oxychloride was used, the resulting cross-linkage considerably inhibited digestibility in vitro in a manner related to the concentration of cross-linking agent used. (Janzen, 1969.) Caloric value was determined for starch treated with 0.06 per cent. phosphorus oxychloride in groups of 6 male and 6 female rats receiving 52 per cent. of distarch phosphate for six weeks as sole carbohydrate source in their diet. No differences were noted between modified and unmodified starches (Oser, 1954). The in vitro digestibility by amyloglucosidase of starch modified with 0.035, 0.07 or 0.1 per cent. phosphorus oxychloride varied between 96.4 and 98.3 per cent. (Kruger, 1970).

Short-term studies

Rat

Groups of 10 male and 10 female rats were fed on a diet containing 10 per cent. rising to 35 per cent. of phosphated distarch phosphate (trimetaphosphate) for a total of 60 days. Female rats showed a consistent reduced rate of weight gain throughout the test. Although 4 test and 2 control animals died during the test these incidents were regarded as unrelated to the test substance. All animals behaved normally. Haematological examination and urinalysis were normal and comparable in the various groups. The liver weights of male rats were lower for the test group than for controls and the kidney weights were lower for both sexes but these findings were not associated with any gross or histopathological changes (Kohn et al., 1964).

Groups of 25 male and 25 female rats were fed diets containing 0.2, 1.0 and 5.0 per cent. trimetaphosphate modified or unmodified starch for 90 days. Eleven controls and 3 test animals died from intercurrent disease. There were no obvious gross or histopathological changes attributable to the test substance. Organ weights and haematological examination (days 45 and 90) were normal in both groups. Pooled urinalysis was comparable for all groups (Kohn et al., 1964).

Groups of 10 male and 10 female rats received 0, 5, 15 and 45 per cent. of two types of distarch phosphate (0.085 per cent. esterified and 0.128 per cent. esterified phosphate) in their diet for 90 days. No abnormalities compared with controls were seen as regards general appearance, behaviour, mortality, food consumption, haematology, serum chemistry and urinalysis which could be ascribed to the action of either of the test substances. No diarrhoea or increased caecal weights were observed. Gross and histopathology revealed no abnormalities attributable to the test substances (Til et al., 1970).

Long-term studies

None available.

Comments

Although direct chemical evidence is lacking, it is very likely that phosphorus oxychloride modification will produce distarch phosphates similar to those formed from trimetaphosphate. The extent of phosphate cross-linkage using trimetaphosphate is very small. The metabolic behaviour of the phosphate bridges has not been studied. However, the available short-term studies reveal no adverse changes with either type of modified starch even at high levels in the diet. As these modified starches represent preliminary stages in the manufacture of the more highly modified phosphated distarch phosphates it is appropriate to use the results of the long-term and reproduction studies in rats relating to phosphated distarch phosphate to evaluate all phosphate-modified starches.

EVALUATION

Temporarily not limited.*

Further work required

See phosphated distarch phosphate.

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* Except for good manufacturing practice.

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HYDROXYPROPYL DISTARCH PHOSPHATE

Modification is carried out with 0.1 per cent. phosphorus oxychloride and 8-10 per cent. of propylene oxide. Cross-linkage would be no greater than is experienced on modification with phosphorus oxychloride alone and ether linkages would probably not exceed 20 per 100 anhydroglucose units.

Biological data

Biochemical aspects

Digestibility was tested in groups of 5 rats fed for 7 days a basal diet supplemented by 0, 1 g and 3 g modified or control starch. No difference in weight gain was observed (Prier, 1961).

In a caloric utilization study groups of 10 male rats were fed for 10 days either unmodified starch or starch modified with 3 per cent., 6 per cent. or 8 per cent. propylene oxide as well as 0.0123 per cent. phosphorus oxychloride equivalent to a degree of substitution ranging from 0.085 to 0.23. There was a slight decrease in caloric utilization with increasing degree of modification. Diarrhoea occurred whenever 4 g modified starch was included in the daily ration but also at the 2 g/day level with the more highly modified starches. The relative organ weight of the empty caecum was always increased at the 4 g/day level but also at the 2 g/day level with the 2 highly modified starches. Histological examination of the heart, liver, spleen, kidney and caecum revealed no obvious abnormalities related to treatment. Similar data were obtained in a series of tests using starches modified in the intermediate range of degree of substitution by the use of propylene oxide and phosphorus oxychloride (Porter, 1971).

Short-term studies

Rat

Groups of 10 male rats were fed diets containing 17, 34, 51, or 68 per cent. modified starch for 28 days. Growth and body-weights were reduced compared with controls at the two highest levels tested. The relative organ weights of empty caeca were raised at all levels tested and the extent of the increase was dose-related. No histological abnormalities were seen in heart, liver, spleen, kidney and caecum (Porter, 1971).

Long-term studies

None available.

Comments

Only data from caloric utilization studies are available. Although it might be feasible to use the data from related modified starches to arrive at an evaluation there is a need for at least 1 short-term study at different levels to ensure that no toxicity exists unique to this modified starch.

EVALUATION

Not possible on the evidence available.

Further work required by 1973

Appropriate 90-day studies in 2 species.

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PHOSPHATED DISTARCH PHOSPHATE

Biological data

These modified starches are prepared by the combined use of sodium tripolyphosphate and sodium trimetaphosphate which results in cross-linking and esterification of starch chains. The overall extent of modification is small, the residual phosphate being of the order of 0.4 per cent. P.

Biochemical aspects

The *in vitro* digestibility of this modified starch by pancreatic amylase (Kohn & Kay, 1963) or by pancreatin and porcine intestinal mucosa (Leegwater, 1971) was somewhat reduced compared with unmodified starch.

Short-term studies

Groups of 10 male and 10 female rats received in their diet 0, 25 and 50 per cent. of modified starch (0.3 per cent. P) for 8 weeks. There were no detectable adverse effects on body-weight. Faecal water content appeared to be higher in animals fed the 50 per cent. test level but the results were too variable to allow any definite conclusions. Production of faeces appeared to be unaffected by this modification when compared with controls. No diarrhoea occurred at any test level. Caecal weight was only slightly increased at the 25 per cent. level in male rats but there was no consistent effect on females at any level tested (de Groot & Spanjers, 1970).

Dog

Groups of 3 male and 3 female beagles were given daily for 90 days gelatine capsules containing 50, 250 and 1250 mg modified starch/kg body-weight. No adverse effects were observed as judged by behaviour, body-weight changes, mortality, haematological studies, blood chemistry, urinalysis, liver function tests, organ weights, gross and histopathological findings (Cervenka & Kay, 1963b).

Man

Twelve volunteers consumed on each of 4 successive days 60 g of a phosphated distarch phosphate with 0.35 per cent. introduced P. No adverse effects were noticed. No changes occurred as regards frequency and amount of faeces of faecal water and lactic acid content (Pieters et al., 1971).

Long-term studies

Rat

Groups of 30 male and 30 female rats were fed a modified starch at dietary levels of 0, 5, 10 and 30 per cent. for 104 weeks. No adverse effects were noted on general appearance, behaviour, mortality experience or food intake. Growth rate and food efficiency were similar to controls. Haematology, serum chemistry and urinalysis reveal no consistent changes related to the administration of the test substance. Relative organ weights were comparable with controls except for significantly decreased spleen weight in males and significantly increased spleen and kidney weights in females at the highest levels fed. These changes were not associated with any gross pathological findings. Caecal weights were normal at all test levels. There was no obvious evidence of any carcinogenic effect. The histopathological examination has not yet been completed (de Knecht-van Eekelen et al., 1971).

Reproduction studies

Rat

A three generation study was performed using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations by mating at week 12 and 20 after weaning. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10 per cent. of the diet consisted of starch modified with sodium trimetaphosphate up to 0.04 per cent. P and sodium tripolyphosphate up to a total of 0.35 per cent. P. No adverse effects were noted regarding appearance, behaviour, mortality, body-weights, fertility, litter size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were increased. In the F_{3b} generation spleen weights in females fed this starch differed in a statistically significant way from controls. Gross examination at autopsy did not reveal pathological changes attributable to ingestion of this starch but histopathology is still outstanding (Til et al., 1971).

Special studies

Groups of 10 male and 10 female rats were given 0, 25 or 50 per cent. modified starch in a low residue diet for 7 days. This was followed by 3 further days on a diet containing additional 4 per cent. cellulose. The body-weights of test animals were slightly reduced in both sexes in a dose-related manner but the actual changes were small. No diarrhoea was noted and faecal dry matter was somewhat higher in test animals compared with controls. The

addition of cellulose to the diet had no adverse effect. No histological abnormality of the enlarged caeca was noted (de Groot & Spanjers, 1970).

Comments

The extent of the modification is small. The metabolic behaviour of the phosphate moieties has not been studied. The available short-term studies in the rat and dog do not reveal any significant adverse effects even at high dietary levels, if one accepts the available evidence for the group of modified starches considered that caecal enlargement without associated histopathological changes is of little toxicological significance. The long-term and reproduction studies in the rat did not reveal any significant adverse effects and can be used for evaluation.

EVALUATION*

Temporarily not limited.**

Further work required by 1973

Histological reports to complete the long-term and reproduction studies in the rat.

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* Includes distarch phosphate prepared using trimetaphosphate of phosphated distarch phosphate or the sum of both. Subject to limits of phosphorus load given in the seventh report of the Joint FAO/WHO Expert Committee on Food Additives, Wld Hlth Org. techn. Rep. Ser., 281, p. 31

** Except for good manufacturing practice.

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Til, H. P., Spanjers, M. Th. & de Groot, A. P. (1971) Report No. 3403 of Centraal Instituut voor Voedingsonderzoek

ACETYLATED DISTARCH PHOSPHATE

Modification is usually performed by the use of up to 0.1 per cent. of phosphorus oxychloride and 5 per cent. acetic anhydride. Vinyl acetate may be used as an alternative acetylating agent. Maximum acetylation amounts usually to 2.5 per cent. acetyl groups.

Biological data

Biochemical Aspects

The digestibilities *in vitro* by pancreatin and porcine mucosal enzymes of acetylated distarch phosphates, modified to 1.6 per cent. and 2.3 per cent. acetyl content, were found to be 93 per cent. and 81 per cent. respectively of that of unmodified starch (Leegwater, 1971).

Short-term studies

Rat

Groups of 10 male and 10 female rats were fed 0, 25 and 50 per cent. of two different modified starches (acetic anhydride and vinylacetate modified) in their diet for 8 weeks. Body-weights of animals on the higher test level were somewhat lower than at the 25 per cent. level and also lower than controls in both sexes. However, the differences were not statistically significant. The faecal water content was variable and could not be related to dietary dosage levels. Production of faecal dry matter was increased in both sexes at the higher level tested and slightly so at the 25 per cent. dietary level. The incidence of diarrhoea was insignificant. A dose-related increase in caecal weight occurred in both sexes. No histological abnormality of the enlarged caeca was noted (de Groot & Spanjers, 1970).

Pig

Groups of 4 male and 4 female pigs were given 0, 35 or 70 per cent. of modified starch in their diet over 14-1/2 weeks. Growth rate and food consumption were satisfactory. Haematology, blood chemistry and urinalysis revealed no treatment-related abnormalities. Ophthalmoscopy showed no abnormalities associated with the test substance. Organ weight, gross and histopathology revealed no abnormalities in test or control groups. Three pigs in the higher test group died suddenly at various intervals during the test without any evidence pointing to the cause of their death.

One pig in the higher test group and another in the 35 per cent. group showed evidence of neurological malfunction. The animal on 70 per cent. test substance died, that on the lower dietary level recovered. No histological evidence of nervous system involvement was noted in these two nor in any other animal (Shillam et al., 1971).

Man

Twelve volunteers consumed on each of 4 successive days, 60 g acetylated distarch phosphates of either 1.6 per cent. or 2.33 per cent. acetyl content. No abnormalities were observed as regards frequency and amount of faeces as well as faecal water and lactic acid content. No other adverse effects were noticed (Pieters et al., 1971).

Long-term studies

Rat

Groups of 30 male and 30 female rats were fed two different modified starches (acetic anhydride and vinylacetate modified) at levels of 0, 5, 10 and 30 per cent. in their diet for 104 weeks. Appearance, behaviour, food consumption and mortality were not adversely affected nor was diarrhoea observed at any level. Growth was slightly depressed at the highest dietary level with both modified starches. Haematology, serum chemistry and urinalysis revealed no effects related to treatment in any group. There was a dose-related increase in the caecal weight in both sexes at the 30 per cent. level but in males only at the 10 per cent. level. All other organ weights showed no treatment-related changes. No gross or microscopic pathological abnormalities were noted which could be related to the test substances or which would point to any carcinogenic effects or to any compound related effects. The caeca appeared remarkably normal (Til et al., 1971a).

Reproduction studies

Rat

A three generation study was performed using 5 males and 10 females for the P generation and 10 males and 20 females of the F_{1b} and F_{2b} to produce successive generations by mating at week 12 and 20 after weaning. The F_{3b} generation was kept for 3 weeks after weaning and then sacrificed for histopathological study. The P, F_{1b} and F_{2b} parents were used for determination of implantation sites. The test material, fed at 10 per cent. of the diet consisted of a starch modified with 5 per cent. acetic anhydride and 0.02 per cent. phosphorus oxychloride (D.S. of 0.093) and another

starch modified with 4.5 per cent. vinylacetate and 1.2 per cent. phosphorus oxychloride (degree of substitution of 0.064, 0.04 per cent. P introduced). No adverse effects were noted in respect of health, behaviour, mortality, growth, fertility, litter size, resorption quotient, weaning weight of pups or mortality of young. Caecal weights were only increased in tests using starch modified with acetic anhydrides. Gross pathology of the F_{3b} generation revealed a significant difference in the average thyroid weights. Gross examination at autopsy did not reveal any pathological changes attributable to the ingestion of the starch but histopathology is still outstanding (Til et al., 1971b).

Special studies

Groups of 10 male and 10 female rats were fed 0, 25 or 50 per cent. of two different modified starches (acetic anhydride and vinylacetate modified) in a low residue diet for 7 days followed by the additional feeding of 4 per cent. cellulose for 3 days. The body-weights of all animals fed 50 per cent. modified starch were somewhat lower than those of the controls. Production of faecal dry matter was increased in the higher groups and less so at the 25 per cent. level. Diarrhoea occurred at the higher level with both modified starches. The addition of 4 per cent. cellulose had no effect on the severity or frequency of the diarrhoea. At the higher level there was also some loss of hair in both sexes (de Groot and Spanjers, 1970).

Comments

The feeding studies with rat and pig show that these modified starches are well utilized. The long-term and reproduction studies in rats indicate no unusual adverse effects apart from caecal enlargement. The suddenness of the death of some of the animals in the pig study indicates an aberrant effect not related to the feeding of modified starches. This is reinforced by the absence of any pointer to a target organ. The available evidence for the group of modified starches considered suggests that this finding, when not associated with any histopathological changes, is of little toxicological significance. It is thus appropriate to use the long-term and reproduction studies for evaluation.

EVALUATION

Temporarily not limited.*

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* Except for good manufacturing practice.

Further work required by 1973

Histological report to complete the reproduction study in the rat.

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DISTARCH GLYCEROL

Treatment with epichlorhydrin under akaline conditions up to 0.3 per cent. tends to produce cross-linking at a rate of one glycerol diether linkage per 220 - glycopyranose units.

Biological data

Biochemical aspects

Much evidence in the literature points to etherified starches being somewhat resistant to enzymic degradation. Probably small fragments containing the ether linkage will remain and will not be absorbed readily. Any material absorbed is likely to be excreted by the kidney. No direct experimental evidence exists for this particular hypothesis (French, 1960). The metabolic fate of methylglucose is cited as collateral evidence. Although methylglucose is absorbed by rats (Sols, 1956) no demethylation is believed to occur (Csáky & Wilson, 1956) and excretion is essentially quantitative (Csáky & Glenn, 1957). The *in vitro* digestibility by amyloglucosidase of distarch glycerol (0.3 per cent. epichlorhydrin) was shown to be 98.3 per cent. of that of unmodified starch (Kruger, 1970). The caloric values of two starches treated at levels of 0.07 per cent. and 0.50 per cent. epichlorhydrin were estimated in groups of 10 weanling male rats by comparing the weight gain obtained after feeding for 28 days a basal diet supplemented with 3 g of each starch against a dose-response curve obtained by feeding daily sucrose supplements of 0, 1.5 g, 3 g, 4.5 g and 6 g equivalent to approximately 0, 6, 12, 18 and 24 calories. Distarch glycerols were only slightly lower in caloric value than unmodified starch, the differences not being significant.

Short-term studies

Rat

Three groups of 10 male and 10 female rats were fed for 90 days unmodified starch or starch treated with 0.07 per cent. or 0.50 per cent. epichlorhydrin at 71 per cent. of their diet. Growth rate, appearance, behaviour and food intake were similar in all 3 groups. Haematological investigations showed no abnormalities related to the feeding of modified starches. Non-protein nitrogen blood levels and blood sugar levels remained normal in all groups. The weights of all major organs were normal and no abnormal gross or histopathological changes related to treatment were seen.

Long-term studies

None available.

Comments

The feeding studies with rats show that the modified starch is well utilized. The available evidence for the group of modified starches considered suggests that caecal enlargement without associated histopathological changes is of little toxicological significance. The short-term rat study shows no adverse effects at the high dietary level used. No long-term study on this modified starch is available and collateral evidence from the long-term and reproduction studies with hydroxypropyl distarch glycerol, a more highly modified starch, indicates that the glycerol linkage is causing no adverse effects.

EVALUATION

Temporarily not limited.*

Further work required by 1973

Histological report to complete the 2-year rat study on hydroxypropyl distarch glycerol.

Histological report to complete the reproduction study on hydroxypropyl distarch glycerol.

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* Except for good manufacturing practice.

ACETYLATED DISTARCH GLYCEROL

Modification is carried out with epichlorhydrin to a maximum of 0.3 per cent, and acetic anhydride to a maximum of 8 per cent. The amount of acetyl groups introduced does not exceed 2.5 per cent. Further treatment, such as bleaching is often carried out.

Biological data

Biochemical aspects

The *in vitro* digestibility of acetylated distarch glycerol (0.3 per epichlorhydrin, 1.2 or 2.5 per cent. acetyl content) varies with the acetyl content but is not affected by cross-linkage. At 1.2 per cent. acetylation some 82 per cent. of the modified starch are digestible, at 2.5 per cent. only 68.5 per cent. are hydrolysed enzymatically (Kruger, 1970). The caloric value was determined by reference to a dose-response curve established in groups of 10 male rats given a basic diet with graded supplements of 0, 0.75 g, 1.5 g, 3.0 g and 4.5 g sucrose equivalent to 0, 3, 6, 12 and 18 calories per day. Starches modified by the use of 0.1 per cent. epichlorhydrin + 5.5 per cent. acetic anhydride and 0.3 per cent. epichlorhydrin + 5.5 per cent. acetic anhydride were tested against native starch at levels of supplementation of 1.5 g and 3.0 g per day for 28 days. Weight gain on modified starch supplement was slightly reduced compared with that on unmodified starch. All rats remained normally active and healthy. The caloric estimates were similar for both levels of epichlorhydrin treatment (Oser, 1961).

Short-term studies

Rat

Groups of 15 male and 15 female rats were fed for 90 days on diets containing 50 per cent. either native or modified starch (0.3 per cent. epichlorhydrin + 10.5 per cent. acetic anhydride). The growth rate of male rats was significantly lower compared with controls on unmodified starch. The full and empty caecal weights of both male and female rats in the test groups were significantly greater than in controls. Haematology, blood chemistry, urinalysis, organ weights and gross as well as histological examination were normal (Oser, 1964).

Comments

The feeding studies with rats show that the modified starch is well utilized. The available evidence for the group of modified starches considered suggests that caecal enlargement without associated histopathological changes is of little toxicological significance. The short-term rat study shows no other significant effects related to treatment, the observed growth depression being the obvious result of the only high dietary level used. No long-term study is available but the collateral evidence from starch acetate and distarch glycerol indicates that neither of the modifications singly applied cause any adverse effects.

EVALUATION

Temporarily not limited.*

Further work required by 1973

Histological reports to complete the 2-year and reproduction rat studies on starch acetate.

Histological reports to complete the 2-year and reproduction rat studies on hydroxypropyl distarch glycerol.

REFERENCES

Kruger, L. (1970) Unpublished reports Nos 405 and 406 submitted by National Starch & Chemical Co.

Oser, M. (1961) Unpublished report of Food and Drug Research Laboratories Inc. No. 81774, submitted by the National Starch and Chemical Co.

Oser, B. L. (1964) Unpublished report of Food and Drug Research Laboratories Inc. No. 85554, submitted by the National Starch and Chemical Co.

* Except for good manufacturing practice.

ACETYLATED DISTARCH ADIPATE

Modification is carried out by the use of 8 per cent. acetic anhydride and a maximum of 0.12 per cent. adipic acid, the latter acting as cross-linking agent. The maximum number of acetyl groups introduced is 2.5 per cent. The number of adipic cross-links does not exceed more than 1 in about 1000 glucopyranose units, or not more than 0.09 per cent. adipyl groups introduced in the starch.

Biological data

Biological aspects

In vitro studies with pancreatin have shown that only the acetate ester bond splits easily while the adipic acid ester linkages are not attacked. No free adipic acid could be demonstrated in the hydrolysate (Morgareidge, 1959a). In vitro digestion with amyloglucosidase of acetylated distarch adipate showed a digestibility of 98.3 per cent. (Kruger, 1970). The metabolic fate of adipate-modified starches was investigated in male rats in vivo using adipic acid labelled at C1 and C6 with C^{14}. The rate of appearance of $14CO_2$ was compared between starch modified with 1,6 - C^{14} adipic acid. Starch adipate is absorbed and/or metabolized more slowly as evidenced by the delayed appearance of $14CO_2$ in the respired air. 99.3 per cent. of the C14 activity of the free adipic acid was recovered in the respired air; 5.8 per cent. appeared in the urine while none was detected in the faeces, in the gastro-intestinal tract or in the carcass. 70.5 per cent. of the C14 activity of the esterified adipic acid appeared in the respired air and 7.2 per cent. in the urine; 24.5 per cent. was found in the faeces. No activity appeared in the carcass (Morgareidge, 1959b). The caloric equivalent of the modified starch was determined in groups of 10 male rats fed for 28 days on a basal diet containing either 1.5 or 3.0 g of starch supplements. The modified starch had been treated with 0.2 per cent. adipic anhydride and 5.5 per cent. acetic anhydride. Native starch was used as control. Caloric values were determined from a dose/response curve obtained by the use of 0, 0.75 g, 1.5 g, 3.0 g and 4.5 g of sucrose supplements equivalent to 0, 3, 6, 12 and 18 calories per day. There was no difference in caloric value as between the modified and unmodified starches (Oser, 1961).

Short-term studies

Rat

A 90-day feeding study was carried out in groups of 15 male and 10 female rats at a dietary level of 50 per cent. modified or

unmodified starch. The growth rate of males was significantly lower in the test group and the full and empty caecal weights of both sexes were significantly greater in rats fed the treated starch. All rats survived the test period and no differences were observed between the groups in respect of liver and kidney weights, haematology, blood chemistry, urinalysis, gross and histopathology. The modified starch used had been treated with 0.12 per cent. adipic acid and 10.5 per cent. acetic anhydride resulting in 3.1 per cent. of acetyl groups being present (Oser, 1964).

Long-term studies

None available.

Comments

A study with C^{14}-labelled material showed that the reacted adipic acid entered the metabolic pool more slowly but followed the normal pathways for adipic acid. No retention of label was found in the carcass. Short-term feeding studies with rats at a high dietary level showed no significant pathological changes. The available evidence for the group of modified starches considered suggests that caecal enlargement without associated histopathological changes is of little toxicological significance. The observed decrease in growth rate at the dietary level used appears to be related to the use of an extremely high level. No long-term studies are available but the collateral evidence from starch acetate and acetylated distarch phosphate indicates that acetylation causes no adverse effects.

EVALUATION

Temporarily not limited.*

Further tests required by 1973

Histological report to complete the long-term and reproduction studies in the rat on starch acetate.

Histological report to complete the reproduction study in the rat on acetylated distarch phosphate.

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* Except for good manufacturing practice.

REFERENCES

Kruger, L. (1970) Unpublished reports Nos 405 & 406 submitted by National Starch and Chemical Corporation

Morgareidge, K. (1959a) Unpublished report No. 78522 of Food and Drugs Research Laboratories Inc., submitted by National Starch and Chemical Corporation

Morgareidge, K. (1959b) Unpublished report No. 79408 of Food and Drugs Research Laboratories Inc., submitted by National Starch and Chemical Corporation

Oser, M. (1961) Report No. 81776 of Food and Drugs Research Laboratories Inc., submitted by National Starch and Chemical Corporation

Oser, B. L. (1964) Report No. 85555 of Food and Drugs Research Laboratories Inc., submitted by National Starch and Chemical Corporation

WHO '72
Bib No. 1681

World Health Organization; W.H.O. Food Additives Series 2:45-50. 1972.

CHEMICALLY TREATED STARCHES *

DEFINITION

Functional Use in Foods — Thickening agents

Structural Formula and Molecular Weight

Chemically treated starch is a macromolecule composed of anhydro glucose units in linear and branched form and modified as indicated below under "Chemical Description". Its molecular weight varies greatly depending upon botanical origin and is generally lower than that of the native starch from which it is obtained.

DESCRIPTION

Appearance

Most chemically treated starches are white or off-white, tasteless and odourless powders. According to the drying method these powders can consist of whole granules having the appearance of the original native starch, of aggregates consisting of a number of granules (pearl starch, starch-grits) or, if pregelatinized, of flakes, amorphous powder or coarse particles.

Chemical Description

Chemically treated starches are food starches which have one or more of their original characteristics altered by chemical treatment in accordance with good manufacturing practice with one or more of the agents listed in Table I. In the case of starches treated with heat in the presence of acid or with alkali, (1,2, and 3 in Table I) the alteration is a minor fragmentation of the chains.

* This supercedes the specifications given in FAO Nutrition Meetings Report Series No. 46B; WHO/Food Add./70.37;1970 pp. 19-36.

When the starch is bleached, (4 in Table I) the change is in the colour only. Oxidation (5 in Table I) involves the production of carboxyl groups.

The treatments indicated under 6 and 7 of Table I result in partial substitution in the 6, 3 or 2-position of the anhydro glucose unit unless the 6-position is occupied for branching.

In case of crossbonding, where a polyfunctional substituting agent as mentioned under 6 in Table I connects two chains, the structure can be represented by: Starch-O-R-O-Starch, where R = crossbonding group and Starch refers to the linear and/or branched structure.

CHARACTERISTICS

Identification

A. Solubility Water (cold) : Insoluble (if not pregelatinized)

Water (hot) : form typical colloidal with viscous properties

Ethanol : insoluble

B. Chemically treated starches which have not been pregelatinized retain their granular structure and can be identified as starches by microscopic observation. Shape, size and sometimes striations are characteristics of the botanical origin. In polarized light under crossed nicols the typical polarization cross will be observed.

C. Add a few drops of 0.1 N KI solution to an aqueous suspension of the product. These starches stain with iodine in the same way as native starches. The colour can range from dark blue to red.

D. Boil 2.5 g of the treated starch with 100 ml of hydrochloric acid solution (3 % w/w) under reflux for 3 hours. Glucose can be identified in the hydrolysate by any usual method.

E. The appropriate tests to differentiate between the various treatments of the starches appear in the section entitled "Analytical Methods for Chemicall Treated Starches" appearing immediately below this specification.

Specifications

The article of commerce can by specified by the parameter specific for the particular type of modification indicated in Column 3 of Table 1, and further as to loss in weight on drying, to pH and to contents of ash, chloride, protein and fat.

LIMITS OF IMPURITIES

Arsenic:	not more than 3 mg/kg
Lead:	not more than 5 mg/kg
Heavy metals:	not more than 40 mg/kg
Sulphur dioxide:	not more than 80 mg/kg

TABLE I

Column 1	Column 2	Column 3	Column 4
Treatment (may be combined)	Maximum amount of substance reasonably required to accomplish the intended physical or technical effect	Limitation and maximum residual traces in the finished product	Commercial Name
1. Roasted starch with addition of acid	0.15% acid, calculated as hydrochloric acid anhydrous (100%) and based on dry starch	Final pH 2.5 to 7.0	White or yellow dextrin, dextrinised starch
2. Acid treatment in slurry	7% hydrochloric acid, 2.0% sulphuric acid or 7% phosphoric acid	Final pH 4.8 to 7.0	Acid-thinned Starches
3. Alkaline treatment with	Sodium or potassium hydroxide not to exceed 1.0%	Final pH 5.0 to 7.5	Alkali-treated Starches
4. Bleached starch	Sodium hypochlorite, sodium chlorite, hydrogen peroxide, potassium permanganate, peracetic acid, ammonium persulphate, sulphur dioxide in amounts not more than that sufficient to bleach the material	No residues, no carboxyl groups, except 50 mg/kg Mn when permanganate used	Bleached Starches

TABLE I

Column 1	Column 2	Column 3	Column 4
Treatment (may be combined)	Maximum amount of substance reasonably required to accomplish the intended physical or technical effect	Limitation and maximum residual traces in the finished product	Commercial Name
5. Oxidized by treatment with	Chlorine, as sodium hypochlorite, not to exceed 5.5% of chlorine, based on dry starch	0.5% sodium chloride; Carboxyl groups not more than 1% corresponding to 1 carboxyl group per 25 glucose units	Oxidized Starches
6. Esterified by treatment with	Acetic anhydride, not to exceed 10% ; Vinyl acetate, not to exceed 7.5% ; Phosphorus oxychloride, not to exceed 0.1% ;	Acetyl groups, not to exceed 2.5 % ;	Starch Acetate; Acetylated Distarch phosphate ($POCl_3$); Acetylated Distarch glycerol; Acetylated Distarch Adipate.
	Sodium trimetaphosphate	residue phosphate in starch treated with sodium trimetaphosphate not to exceed 0.04% calculated as phosphorus	
	Adipic anhydride, not to exceed 0.12%		Acetylated Distarch Adipate
	Sodium tripolyphosphate and/or sodium trimetaphosphate and/or orthophosphoric acid and/or sodium or potassium salts thereof	Residual phosphate, not to exceed 0.4%, calculated as as phosphorus	Mono-Starch Phosphate; Distarch Phosphate (sodium trimetaphosphate) Phosphated Distarch Phosphate

TABLE I

Treatment (may be combined)	Maximum amount of substance reasonably required to accomplish the intended physical or technical effect	Limitation and maximum residual traces in the finished product	Commercial Name
7. Etherified by treatment with	Propylene oxide, not to exceed 10%	Propylene oxide, 5 mg/kg, Propylene chlorohydrin, 5 mg/kg.	Hydroxypropyl Starch; Hydroxypropyl Distarch Phosphate* ($POCl_3$ or sodium trimetaphosphate)
	Succinic anhydride, not to exceed 4%		Starch sodium succinate
	Phosphorus oxychloride, not to exceed 0.1%	Residual phosphate not to exceed 0.04% calculated as phosphorus	Distarch phosphate (phosphorus oxychloride)
	Epichlorohydrin, not to exceed 0.3%	(Epichlorohydrin, 5 mg/kg (Glycerol monochlorohydrin, (5 mg/kg (Glycerol dichlorohydrin (5 mg/kg	Distarch glycerol; Acetylated Distarch glycerol; Hydroxypropyl Distarch glycerol

Identification tests for the various treatments are given in the Annex

* Tentative

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UTILIZATION OF SWEET POTATO STARCH BY RATS AND ITS EFFECT ON THE DIGESTION OF DIETARY PROTEIN

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It is known that the degree of protein utilization is influenced by the nature of carbohydrate in the diet. Monson et al. ('50) observed that chicks on a dextrin diet grew markedly faster than those on a sucrose diet and that the dextrin diet passed through the digestive tract of chicks more slowly than a sucrose or lactose diet. Monson et al. ('54) also have reported that chicks receiving dextrin grew more rapidly than those receiving sucrose when the level of casein was 18% or less. However, when the casein content of the sucrose diets was increased above 18%, growth approached that of chicks receiving 18% casein with dextrin. These authors suggested that dextrin exerts this effect largely by permitting better utilization of dietary protein. Several Wisconsin workers have reported the superiority of dextrin to sucrose as a source of carbohydrate in the diet of rats (Krehl et al., '46; Henderson et al., '47; Hankes et al., '48; Lyman and Elvehjem, '51), and the increase of xanthine oxidase activity in the liver of rats fed dextrin in the place of sucrose in the diets (Kring and Williams, '51; Harper et al., '53). Harper and Katayama ('53) reported that rats fed 9% of casein grew faster when cornstarch replaced sucrose as the dietary carbohydrate. Harper et al. ('53) reported further studies along this line and they suggested that the utilization

of dietary protein is improved when the sucrose in low-protein diets is replaced by dextrin. Marshall and Wommack ('54) have shown that rats fed 0.1% of essential amino acids with sucrose had more negative nitrogen balances than rats fed dextrin. Recently, Dreisbach and Nasset ('54) have compared the absorption rates of glucose arising from corn starch, banana meal and glucose and have reported that absorption of glucose arising from cornstarch is the slowest.

On the other hand, it has been shown that certain starches, mainly those derived from tubers, are poorly utilized by animals. Booher et al. ('51) have reported that cereal starches are assimilated nearly perfectly and those from arrowroot, white potato and sago palm showed low degrees of digestibility. They suggested that digestion-resistant properties of the latter reside in the outermost layer of granules. Sweet potato starch occupied an intermediate position in their results. Jelinek et al. ('52) have reported the inadequacy of unmodified potato starch as a source of dietary carbohydrate for rats and the improvement of its utilization by several treatments. Harper et al. ('52) have shown that raw and autoclaved potato starch in the diet induced a relatively large excretion of endogenous nitrogen and amino acids.

It is the purpose of this paper to report a comparison of the digestibility of sweet potato starch with that of corn starch and potato starch, and to study the effect of these starches upon the digestion of dietary protein. Also some further data on the effect of steaming and purification of sweet potato starch upon digestion are presented.

EXPERIMENTAL

Design of experiment. The experiment was composed of three series. Series 1 consisted of a comparison of the digestibility of three kinds of starch, i.e. corn, sweet potato and potato starch. Eight male rats of Wistar strain, weighing 300 to 400 gm. were housed in individual cages with raised screen floors. The feeding plan is shown in the first 5 columns of table 2. Each diet was fed, with the free supply of water,

for 10 days and the feces were collected on a screen set under the cage for the latter half of each period. Fifteen grams of diet were given to each rat daily and all of the food was consumed. Raw diet was given mixed with water, steamed diet was mashed and steamed for 15 minutes. Test 7 was done in May 1954 and was designed to check the seasonal change in digestibility, the rats being fed the same diet as that in test 1 which was finished in January 1954.

The effect of purification of sweet potato starch was studied in series 2, using 6 rats of 230 to 310 gm body weight. This series consisted of a comparison of the digestibility of raw cornstarch and three kinds of sweet potato starch, i.e. raw, purified and autoclaved, in the same manner as in series 1, except that the daily food intake was 18 gm. It is supposed that steaming of the diet decomposed some of vitamins and protein. To avoid this, sweet potato starch was autoclaved in the dry state at 110°C. for 24 hours, powdered after cooling and mixed with the other nutrients. The diets in series 2 were given mixed with water.

In series 3, metabolic fecal nitrogen was determined by the method advocated by Mitchell ('24). The same rats as those of series 2 were fed two kinds of low protein diet, one of which contained raw cornstarch as the carbohydrate source, and the other, raw sweet potato starch. Five per cent of whole egg powder was given as the protein source in both diets. Eighteen grams of diet were given daily mixed with water.

Starch. Starches used were the first grade commercial starches, the chemical composition of which is shown in table 1. In series 2, sweet potato starch was suspended and settled 4 times in distilled water. The upper and lower layers were discarded and the starch was washed twice with alcohol and once with ether, suspended and settled 12 times with 0.2% sodium hydroxide and washed with distilled water till supernatant became neutral.

Diet. The percentage composition of the diets fed in series 1 was as follows: starch 65, butter fat 10, casein 10, fish meal 5, brewers' yeast 4, cellulose (flour of filter paper)

3, McCollum's salt mixture no. 185 3. In series 2 and 3, whole egg powder was used as protein source instead of the mixture of casein, fish meal and yeast in series 1. The percentage composition of the diets fed in series 2 was as follows: starch 68, whole egg powder 23, butter fat 2, cellulose 3, salt mixture 3, and vitamin mixture 1. The low protein diet used in series 3 contained 77% starch, 5% whole egg powder, 10% butter fat, 3% cellulose, 4% salt mixture, and 1% vitamin mixture. One gram of vitamin mixture contained 2,500 I.U. of vitamin A, 200 I.U. of vitamin D, 1.0 mg of thiamine, 1.5 mg of riboflavin, 10 mg of niacin, 1.0 mg of pyridoxine, 0.5 mg of folic acid, 0.5 mg calcium pantothenate,

TABLE 1

Chemical composition of starch

STARCH	MOISTURE	NITROGEN FREE EXTRACT	TOTAL OTHERS DETERMINED
	%	%	%
CS [1]	14.15	85.61	0.24
SPS	16.61	83.01	0.38
SPS (purified)	16.88	82.99	0.13
PS	18.46	81.24	0.30

[1] CS = cornstarch; SPS = sweet potato starch; PS = potato starch.

1.0 μg of vitamin B_{12}, 1.0 mg of vitamin E, 0.2 mg of vitamin K, and 37.5 mg of ascorbic acid. Ingredients other than starch showed no starch-reaction with iodine.

Analytical methods. The feces of each rat were collected for 5 days and were air-dried and powdered. Their starch and nitrogen contents were analyzed by the following method: the sample was hydrolyzed with 0.6 N hydrochloric acid, neutralized with 0.5 N sodium hydroxide and centrifuged. The reducibility of supernatant solution was determined iodometrically with Somogyi's phosphate reagent ('45). In series 1, starch content was determined at the same time by the method of Pucher et al. ('48). Starch was extracted with perchloric acid, precipitated as starch-iodine complex, liberated by alcoholic sodium hydroxide and de-

TABLE 2

The digestibility of three kinds of starch

SERIES	TEST NO.	DIET: Source of carbohydrate	DIET: Content: Protein	DIET: Content: Starch	WEIGHT[1] OF FECES	NITROGEN: Daily intake	NITROGEN: Daily output	NITROGEN: Apparent[2] digestibility coefficient	STARCH: Daily intake	STARCH: Apparent[2] digestibility coefficient
			%	%	gm	mg	mg	%	gm	%
1	1	Raw CS[3]	15.8	50.9	1.25	378	43.2	88.6 ± 0.69	7.6	99.3 ± 0.04
	2	Raw SPS	15.6	50.1	1.76	374	75.7	79.8 ± 1.37	7.5	99.8 ± 1.21
	6	Raw PS	14.7	48.9	5.65	354	93.6	73.7 ± 1.62	7.3	57.2 ± 6.11
	3	Steamed CS	15.8	50.9	1.37	378	52.2	86.3 ± 1.35	7.6	98.9 ± 0.16
	4	Steamed SPS	15.6	50.1	1.28	374	48.0	87.2 ± 0.77	7.5	99.2 ± 0.09
	5	Steamed PS	14.7	48.9	1.34	354	48.8	86.2 ± 1.12	7.3	99.0 ± 0.06
	7	Raw CS	15.8	50.9	1.27	378	43.1	88.6 ± 0.76	7.6	99.4 ± 0.05
2	8	Raw SPS	9.8	56.2	1.85	281	73.0	74.1 ± 2.28	10.1	97.8 ± 0.89
	9	Autoclaved SPS	9.3	55.8	1.73	269	69.1	74.3 ± 2.00	10.0	98.5 ± 0.17
	10	Purified SPS	11.4	53.5	1.56	329	61.3	81.3 ± 2.69	9.6	98.6 ± 0.41
	11	Raw CS	11.2	56.6	1.26	324	41.0	87.4 ± 0.52	10.2	99.4 ± 0.04
3	12	Low protein CS	2.9		1.26	83	24.8			
	13	Low protein SPS	2.6		2.58	74	54.2			

[1] Mean value of daily excrement per rat.

[2] Mean value ± standard deviation.

[3] CS = cornstarch; SPS = sweet potato starch; PS = potato starch.

termined by the method above described. Nitrogen content of the diet was determined by the Kjeldahl method and that in feces was determined by the micro-Kjeldahl method of A.O.A.C. ('50).

RESULTS

The results of this experiment are summarized in table 2. The digestibility of raw sweet potato starch was significantly lower than that of cornstarch, though the difference was less than 3%. Sixty per cent of raw potato starch was digested. Both raw sweet potato and raw potato starch decreased the

TABLE 3

Variance analysis of series 1

FACTOR	DEGREES OF FREEDOM	F-RATIO		
		Wt. of feces	Digestibility coeff.	
			Starch	Nitrogen
I[1]	7	1.6	1.8	0.6
M	2	536[2]	621[2]	243[2]
T	1	743[2]	834[2]	208[2]
I × M	14	0.7	1.8	2.4
I × T	7	0.8	1.5	3.2
M × T	2	528[2]	624[2]	221[2]
I × M × T	14	1	1	1

[1] I: individual difference, M: material, T: treatment.
[2] Significant at 1% level.

apparent digestibility of dietary protein. These effects of raw starches upon the digestibility of dietary starch and protein were not detected when the diets were steamed. Autoclaving of sweet potato starch in the dry state, however, showed little influence.

After the transformation of digestibility coefficient to arcsine and weight of feces to cubic root, the data of series 1 were analyzed statistically following the three-way layout method (Wilks, '44). The effects of material, heat treatment and individual difference and their interactions were studied. F-ratios of this variance analysis are given in table 3. The results of analysis show that factors of treatment, material

and interaction between material and treatment are significant at the 1% level. This means that the digestibility of dietary starch and that of protein vary according to the kind and the state of starch in the diet and that the effect of steaming varies according to the kind of starch. The treatment was most effective upon the potato starch diet and had little effect on the cornstarch diet.

The difference in digestibility between test 1 and test 7 was too small to be statistically significant. Since this result means that the seasonal difference in digestibility within the period of this experiment is negligible, the difference due to the order and the time of test may be left out of consideration.

Tables 4 and 5 show diagrams of the differences between the digestibility coefficients of the diets. Differences between raw starch diets were always significant but those between steamed diets were too small to be significant. The apparent digestibility of protein when fed with steamed sweet potato starch and potato starch was much improved. As shown in table 5, the digestibility of both starch and protein was inferior when sweet potato starch replaced corn starch. Nutrients in purified sweet potato starch diets, however, were digested better than those in raw sweet potato starch diet. Purified sweet potato starch seemed to take an intermediate place between raw cornstarch and raw sweet potato starch in nutritional value. There was no significant difference between autoclaved sweet potato starch and raw sweet potato starch. It was observed that raw sweet potato starch made very soft feces. Soon after the sweet potato starch diet was given, some rats had diarrhea for several days. Especially, when low protein sweet potato starch diet was fed, every rat suffered from severe diarrhea throughout the test period. That the weight of feces from rats on this diet was twice that of rats fed the low-protein cornstarch diet shows this fact clearly. The diarrhea disappeared as soon as diet was changed to the cornstarch diet. Raw potato starch made

TABLE 4

Diagram of difference between digestibility coefficients

(Results of series 1)

DIET		RAW CS	RAW SPS	RAW PS	STEAMED CS	STEAMED SPS	STEAMED PS
		%	%	%	%	%	%
Raw	CS [1]		8.8 [2]	14.9 [2]	2.3 [2]		
Raw	SPS	2.5 [2]		6.1 [2]		7.4 [2]	
Raw	PS	42.1 [2]	39.6 [2]				12.5 [2]
Steamed	CS	0.4				0.9	0.1
Steamed	SPS		2.4 [2]	0.3			1.0
Steamed	PC			41.8 [2]	0.1	0.2	
		CS	SPS	PS	CS	SPS	PS
		RAW			STEAMED		

Protein Starch

[1] CS = cornstarch; SPS = sweet potato starch; PS = potato starch.
[2] Significant at the 1% level.

TABLE 5

Diagram of difference between digestibility coefficients

(Results of series 2)

		CS RAW			SPS RAW	SPS AUTOCLAVED	SPS PURIFIED
		%	%		%	%	%
Raw	CS [1]				13.3 [2]	13.1 [2]	6.1 [2]
Raw	SPS	1.6 [2]				0.2	7.2 [2]
Autoclaved	SPS	0.9 [2]	0.7				7.0 [2]
Purified	SPS	0.8 [2]	0.8 [2]	0.1			
		RAW	RAW	AUTOCLAVED	PURIFIED		
		CS	SPS				

Protein Starch

[1] CS = cornstarch; SPS = sweet potato starch; PS = potato starch.
[2] Significant at the 1% level.

feces very large, white and sometimes caused diarrhea. This may be due to the excretion of much undigested starch.

Dietary and fecal starch contents determined by the direct hydrolysis method and by the method of Pucher et al. ('48) are compared in table 6. Rather small differences between the figures obtained by both methods are observed. Especially the agreement of dietary starch content is satisfactory. Very few reducing compounds other than starch are contained in the diet used.

TABLE 6

Comparison of starch intake, output and digestibility coefficient determined by direct hydrolysis (1) and by the method of Pucher et al. (2)

DIET		CONTENT IN THE DIET		DAILY INTAKE		DAILY OUTPUT		DIGESTIBILITY COEFF.	
		(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
		%	%	gm	gm	mg	mg	%	%
CS[1]	raw	50.9	51.1	7.6	7.7	53	2	99.3	99.97
	steamed	50.9	51.1	7.6	7.7	83	15	98.9	99.8
SPS	raw	50.1	51.2	7.5	7.7	242	85	96.8	98.9
	steamed	50.1	51.2	7.5	7.7	61	7	99.2	99.9
PS	raw	48.9	48.6	7.3	7.3	3144	2875	57.2	60.7
	steamed	48.9	48.6	7.3	7.3	71	9	99.0	99.9

[1] CS = cornstarch; SPS = sweet potato starch; PS = potato starch.

The digestibility of whole egg with raw cornstarch, calculated from the data of tests 11 and 12, is 95%, which agrees with the results of Mitchel and Bert ('54). As the rats had diarrhea, it may not be valid to assume the fecal nitrogen of test 13 as the endogenous value for rats fed the raw sweet potato starch diet. Therefore, true digestibility of whole egg with raw sweet potato starch is not known.

DISCUSSION

Coefficients of digestibility of starches shown in table 2 agree with those of Booher et al. ('51), whose coefficients were calculated from the content of nitrogen-free extract, not on direct measurements. It can be said that the direct hydrolytic

procedure is satisfactory for the analysis of starch contained in the diet and feces under the conditions of this experiment. In series 1, starch was also analyzed by the extraction method of Pucher et al. ('48). The result is compared with that obtained by the direct hydrolysis method in table 6. It seems reasonable to suppose that there exist in the feces fractions of starch which are not absorbed and which are so small that they do not precipitate with iodine and can not be determined by the method of Pucher et al. Therefore, the digestibility coefficient of starch based upon the data obtained by this method does not represent exactly the degree of utilization of dietary starch. The direct hydrolytic procedure always involves the risk that the figures obtained thereby may be higher than the true values on account of the possible existence of reducible materials other than sugars in the feces. Comparing those merits, the direct hydrolysis method was considered to be more satisfactory than for this specific determination and was used in series 2.

Cornstarch supported better utilization of dietary protein whether the protein was supplied as whole egg or a mixture of casein, fish meal and yeast. Both raw sweet potato starch and potato starch suppressed the utilization of dietary protein and they also caused diarrhea to a certain extent. However, the cause for this seems to be different for each starch. Forty-three per cent of dietary raw potato starch, 92% of which was in the form of starch large enough to precipitate with iodine, was excreted little attacked by digestive enzymes, in agreement with many previous workers' observations (Booher et al., '51; Jelinek et al., '52; Sakurai et al., '51). This may be explained by the digestion-resistant properties of the uppermost layer of the starch granule as Booher et al. ('51) suggested. The undigested starch granule, which will excite the intestinal canal, and which will swiftly pass through the digestive tract as Booher et al. ('51) observed, may be the main cause of diarrhea and of low utilization of protein in the potato starch diet. The digestion-resistance of potato starch disappeared on steaming. In the case of sweet potato

starch, on the contrary, fecal excretion of starch, determined by the method of Pucher et al. ('48), was very slight and this means that a very small quantity of sweet potato starch was excreted in the form of glucose chains long enough to precipitate with iodine. It is probable that some component of the sweet potato which is carried along with the starch caused diarrhea and that this in turn caused the decreased utilization of dietary protein, rather than that this is caused by the small quantity of digestion-resistant sweet potato starch residue. The finding that the purification process was effective in increasing the protein utilization and that the low-protein sweet potato starch diet (test 13) caused severe diarrhea without exception, adds support to the suggestion of the existence of some component which caused diarrhea. This component may combine with starch firmly, may be heat labile and rather water insoluble matter, and can be excluded by washing with alcohol, ether and sodium hydroxide solution.

SUMMARY

The digestibility of sweet potato starch and the degree of its effect upon the digestion of dietary protein were compared with those of cornstarch and potato starch, by feeding adult white rats diets containing one of these starches as the sole source of carbohydrate. The coefficients of digestibility of corn, sweet potato and potato starch were found to be 99, 97 and 57%, respectively. The differences are statistically significant. When the diets were steamed, all starches were utilized nearly perfectly and no significant differences were observed.

Raw sweet potato starch caused diarrhea and markedly decreased the apparent digestibility of dietary protein. This effect was decreased when sweet potato starch was purified, and disappeared when it was steamed. It is suggested that some component of the sweet potato which caused diarrhea is carried along with the starch, and that it would appear to be heat labile and can be removed by washing with certain solvents.

Raw potato starch was little utilized and it also lowered the apparent digestibility of the dietary protein. It is suggested that the main reason for this is the heat-labile digestion-resistant property of the potato starch granule.

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THE ROLE OF DIETARY CARBOHYDRATES AND INFECTION ON LIVER LIPID AND COLLAGEN

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ONE of the features of the geographical pathology of the liver in man is the high incidence of hepatic cirrhosis found in Africa. Many explanations for this have been suggested, including nutritional imbalance and hepatic infection. (Patek, 1963). As the nutritional imbalance is frequently a relative excess of carbohydrate and the infection is often schistosomiasis, it was decided to investigate the role of starch and sucrose on hepatic lipid and collagen formation in 2 groups of mice, one of which was infected with *Schistosoma mansoni* and another which was uninfected.

MATERIALS AND METHODS

Male albino mice weighing 15 g. were placed on a diet containing calcium caseinate as the protein and either maize starch or sucrose as the carbohydrate. The diet, which was given *ad libitum*, contained 12 per cent calcium caseinate, 81 per cent carbohydrate, 5 per cent salt mixture, 2 per cent dried yeast and added vitamins A and D. Other mice of the same weight were put on a normal diet. Each group of mice was weighed weekly. The experimental design was such that after 6 weeks each group of 6 mice was either transferred to a normal diet, or transferred to one of the high carbohydrate diets or maintained on the same diet. At the same time half of the groups of mice were infected, to approximately the same extent, with *S. mansoni*. The arrangement of the groups is shown in the Table. The mice were killed in comparable groups 17-24 weeks after the beginning of the diet. Animals which died before 17 weeks were rejected. Statistical comparison was based on Student's *t*-test, and only *P* values of less than 0·025 were considered significant. The wet liver was weighed and a portion taken for section. The material taken for section was fixed in 10 per cent formol-saline, and the sections were stained by Ehrlich's haematoxylin and eosin method and Gomori's silver impregnation method for reticulin (1937). Quantitative estimation of collagen and reticulin can at best represent only an approximate and relative measure between the controls and the experimental animals. The amount of collagen and reticulin present in each of four sections taken from one liver was graded numerically and mean value assessed. The estimation was carried out by one observer with no knowledge of the type of diet used in the particular group from which the sections were obtained.

The remainder of the liver was dried in a vacuum desiccator and the lipid extracted in a Soxhlet apparatus using 40°-60° petroleum ether. The lipid was methylated and the proportions of palmitic, palmitoleic, stearic, oleic and linoleic acids were found using gas chromatography. The collagen in the dry, fat-free liver was estimated by the method of Neuman and Logan (1950).

RESULTS

Weight.—The mean weights of animals on the experimental diets are shown in Fig. 1. The mice after infection tended to increase in weight less rapidly than the non-infected. The animals on the sucrose diet did not show the weight gains of the starch or control-fed animals.

Liver collagen and reticulin.—The sucrose diet resulted in a more extensive collagen reaction, mainly in the portal tract, than did the starch diet. An interesting finding in the livers of the animals fed on the sucrose diet initially and then changed to the control diet after 6 weeks, was that the early fibrotic changes did not appear to be reversed during the subsequent period on the normal

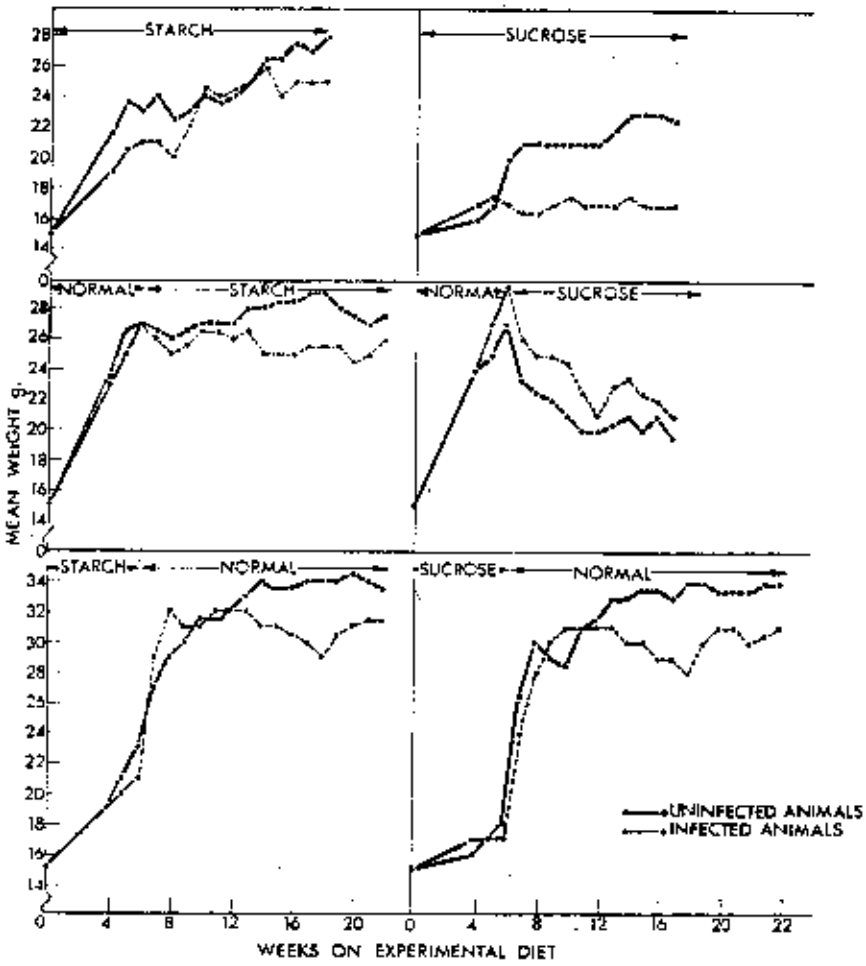

FIG. 1.—The mean weights for each group of mice on the high carbohydrate diets.

EXPLANATION OF PLATE.

FIG. 2.—Reticulin in the liver of an animal on control diet (×45).
FIG. 3.—The collagen reaction around the ova of *S. mansoni* (×240).
FIG. 4.—The reticulin fibres in an infected liver (×45).

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diet. Indeed the changes in the collagen were similar to those found in the animals which had been on the sucrose diet for more than 12 weeks. There was no difference, histologically, in the reticulin response in the liver between the animals on either of the 2 types of carbohydrate diet and the control diet (Fig. 2).

Infection with *S. mansoni* produced an intense localised collagen reaction around the ova, which were found principally in the portal tract (Fig. 3), as well as a very marked reticulin response. The formation of collagen was essentially a local tissue response around the ova resulting in the development of spherical fibrous nodules. The reticulin fibres, however, tended to radiate from the lesions into the lobule, and indeed there was considerable reduplication and thickening of the reticulin fibres (Fig. 4). The group of mice transferred from the control diet to the starch diet after infection, had more collagen and reticulin, as judged histologically, than the comparable group on the sucrose diet. The intensity of fibrosis was less marked in the infected mice on either of the carbohydrate diets than in the infected control group. Mice transferred from either of the carbohydrate diets to a control diet immediately after infection had more extensive fibrosis than those remaining on a high carbohydrate diet throughout the experiment. Indeed, the intensity of fibrosis in the former group compared closely with infected mice on the control diet.

Liver collagen was expressed as the total amount of collagen in the liver, and the mean value for each group and the standard error are shown in the Table. The non-infected animals consuming sucrose had significantly more collagen than both the controls and the comparable starch-fed group. It is difficult to understand how the animals fed on sucrose for only 6 weeks seemed to have most liver collagen, except perhaps that the time on the diet was in the post-weaning period with its more active metabolism.

In the infected group the collagen, in the mice on a control diet was, as expected, higher than in the non-infected control group. The control-fed animals transferred to the starch diet after 6 weeks had significantly more collagen than the comparable mice on the sucrose diet. Furthermore, the mice on the starch and sucrose diets throughout the experiment had less collagen than the control fed group. This unexpected finding could be due to a co-existing nutritional deficiency for the schistosoma; whereas in mice on a control diet or transferred to a control diet at the time of infection such a deficiency for the parasite would not occur.

Liver lipid.—The mean values of the amount of liver lipid for each group are shown in the Table. In every experimental group there was a significant increase compared with control. The presence of infection did not seem to be reflected in the amount of liver lipid. The mice on sucrose throughout the dietary period had a significantly greater amount of lipid than those eating starch. A similar finding has been observed in experimental rabbits (Macdonald, 1962).

The mean values for the proportion of each fatty acid in each group are also shown in the Table. In the non-infected group the striking alteration brought about in the fatty acid pattern by the high carbohydrate; low fat diet is the reduction in the concentration of palmitic acid and the increase in oleic acid. In the groups fed for 6 weeks on the high carbohydrate diet and then for 16 weeks on the normal diet the concentration of the palmitic and oleic acids had still not reached the normal values. There were no marked differences in the fatty acid pattern of the liver between any of the starch and sucrose-fed groups.

TABLE.- *The Experimental Plan and Biochemical Findings in the Livers I = infected with S. mansoni at the 6th week of the experiment*

Diet: First 6 weeks	Diet: Remainder of time	n	Liver collagen (mg.) $\bar{X}$	Liver collagen (mg.) S.E.	Liver lipid (mg.) $\bar{X}$	Liver lipid (mg.) S.E.	Palmitic acid (per cent) $\bar{X}$	Palmitic acid (per cent) S.E.	Palmitoleic acid (per cent) $\bar{X}$	Palmitoleic acid (per cent) S.E.	Stearic acid (per cent) $\bar{X}$	Stearic acid (per cent) S.E.	Oleic acid (per cent) $\bar{X}$	Oleic acid (per cent) S.E.	Linoleic acid (per cent) $\bar{X}$	Linoleic acid (per cent) S.E.
Normal	Normal	5	5·1	1·23	49	18·3	60·3	5·83	4·1	0·97	4·4	0·08	18·2	0·86	7·0	1·67
Starch	Starch	8	7·0	0·48	97	15·6	12·6	1·08	5·5	0·66	1·0	0·44	73·0	1·12	7·0	0·88
Sucrose	Sucrose	5	16·1	0·32	253	35·4	18·6	1·66	4·1	0·42	2·9	1·10	70·4	2·23	4·0	0·53
Normal	Starch	6	7·8	0·87	181	9·8	12·0	0·93	3·3	0·30	1·1	0·17	79·2	2·00	3·5	0·85
Normal	Sucrose	6	16·5	1·00	169	10·5	13·1	1·33	5·0	0·71	2·6	0·57	75·8	2·56	3·5	0·37
Starch	Normal	6	9·9	0·67	102	19·3	46·5	2·93	3·7	0·47	4·7	0·95	40·1	2·86	5·0	0·53
Sucrose	Normal	6	18·7	0·82	79	9·4	52·9	3·56	4·6	0·47	5·1	0·62	31·7	2·32	5·7	1·31
Normal I	Normal	4	22·4	2·21	92	13·3	45·8	2·23	2·2	0·02	2·7	0·48	40·3	2·54	9·0	2·34
Starch I	Starch	5	15·6	1·90	79	18·0	23·7	1·72	3·0	0·09	3·3	1·33	65·0	1·16	3·2	0·54
Sucrose I	Sucrose	4	14·6	0·84	135	10·3	20·5	1·03	4·8	0·42	1·7	0·36	71·2	0·91	1·8	0·24
Normal I	Starch	5	24·9	1·02	171	13·0	20·3	1·36	4·0	0·89	1·6	0·40	72·4	2·48	1·7	0·32
Normal I	Sucrose	6	14·1	0·79	179	32·4	14·9	1·73	3·2	0·58	1·1	0·21	78·6	2·80	2·2	0·44
Starch I	Normal	6	25·3	1·48	99	24·0	57·1	2·28	3·8	0·86	3·0	0·57	31·1	0·94	5·0	1·50
Sucrose I	Normal	5	22·7	1·17	84	12·0	41·0	1·05	5·6	0·26	8·5	0·30	34·8	0·54	0·8	0·48

In the infected groups, the mice on the normal diet showed a reduction in palmitic and an increase in oleic acid concentrations compared with the non-infected normals. The fatty acid pattern in the infected mice showed the same trend as in the non-infected animals with the exception of linoleic acid which was significantly lower in the infected groups, except those which were on the high carbohydrate diet for only 6 weeks. The non-infected carbohydrate-fed groups tended to have a lower concentration of linoleic acid than the normally fed, but in those groups the differences were not significant.

DISCUSSION

Two questions were asked at the outset of this investigation: firstly, does a high proportion of carbohydrate in the diet aggravate hepatic fibrosis whether liver infection with a fibrinogenic agent is present or not, and secondly does dietary starch differ from dietary sucrose in this respect? Under the present experimental circumstances, excess of dietary carbohydrate increases hepatic fibrosis where no fibrinogenic agent such as schistosomiasis is present. One possible explanation for the failure of the high carbohydrate diet together with the fibrinogenic agent to produce the greatest amounts of collagen and reticulin may lie in the fact that the schistosomal infection itself was adversely affected by the high carbohydrate diet. Some support for this view is obtained from the infected groups which were not on a normal diet at any time during the experiment, since these groups had relatively low amounts of collagen and reticulin. Regarding the problem of the difference between dietary starch and sucrose on the extent of fibrosis it is evident, in the non-infected group, that in each case the sucrose-fed animals had more collagen than the comparable starch-fed groups. This difference did not exist in the infected groups.

While studying the collagen response of the liver the opportunity was also taken to answer the original two questions as far as the liver lipid was concerned in terms of both amount and fatty acid composition. The proportion of protein in the diet of these mice was not low, as the mice on the starch diet gained weight as rapidly as those on the control diet, and therefore any lipid changes were due directly or indirectly to the high carbohydrate content of the diet. It was found that a high proportion of carbohydrate in the diet increased the amount of liver lipid in both the non-infected and infected animals confirming earlier experiments (Macdonald, 1962). Infection alone, however, caused an increase in the amount of liver fat. The only difference in the amount of liver fat observed between the mice fed on starch and sucrose was seen in the non-infected groups which were on the high carbohydrate diet throughout the experiment, where the animals fed on sucrose had about twice as much fat in the liver. The changes in the fatty acid pattern were very consistent and consisted of a decrease in the proportion of palmitic acid and an increase in the proportion of oleic acid in all the animals on a high carbohydrate diet, with no marked differences between the starch and sucrose diet.

SUMMARY

Mice were given diets containing 81 per cent carbohydrate as starch or sucrose and the hepatic lipid and collagen responses to these diets were determined histologically and biochemically in the presence and absence of schistosomiasis.

The sucrose diet resulted in a greater amount of hepatic collagen and lipid than did the starch diet. In infected livers this difference was not present.

The amount of collagen in the liver infected with schistosomiasis was less in mice on the high carbohydrate diet than on the normal diet. It is suggested that this may be due to malnutrition affecting the parasite also.

The high carbohydrate diets resulted in a fall in the palmitic acid and rise in the oleic acid proportions in the liver lipid.

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