CAKE PRODUCTS PREPARED FROM SIMPLE SUGARS AND A SACCHARIDE SIRUP AND THE CARIOGENIC ACTIVITY IN RATS: DIETARY INTAKE AND SALIVA PARAMETERS

Dissertation for the Degree of Ph. D.

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Charlotte May Thompson

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Rachel A Schneeman
Major professor

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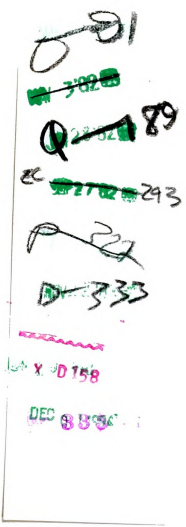

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ABSTRACT

CAKE PRODUCTS PREPARED FROM SIMPLE SUGARS AND A SACCHARIDE SIRUP AND THE CARIOGENIC ACTIVITY IN RATS; DIETARY INTAKE AND SALIVA PARAMETERS

By

Charlotte May Thompson

Dental decay is a major health problem in the U.S. as indicated by the Ten-State Nutrition Survey (1968-1970). Dietary modification becomes a practical approach to control of dental disease through alteration of dietary carbohydrate available as substrate for cariogenic oral microorganisms. Further investigation was considered necessary to evaluate the cariogenic effect of a cake product containing one of the following simple sugars or saccharide sirup: (1) sucrose, superfine quality, (2) fructose, (3) glucose, (4) a high fructose corn sirup, and (5) a mixture of equal parts of crystalline fructose and glucose. The purpose of this study was to develop an acceptable cake product using monosaccharides for sucrose in a cake formula and to test the cariogenic effect of this product in a caries-susceptible animal model.

Butter-type cakes containing fat were prepared by the solution method of mixing and evaluated by both sensory and objective measurements. Eleven taste-panel members scored each of the cakes for grain, crumb color, silkiness, tenderness and flavor. Product

quality was assessed using objective measurements for pH, batter viscosity, moisture loss, moisture content, relative cake volume, resistance to breaking (Allo-Kramer shear press) and color (Hunter Color Difference Meter).

According to objective measurements of product quality, cakes made with monosaccharides were smaller, more easily broken and more moist than the cakes made with sucrose. When compared to other cakes, those which contained fructose were darkest in color as indicated by Hunter Color Difference Meter L or lightness values. As measured by a_L or green-red values, fructose cakes were also the least green. These color differences suggest that increased browning reactions occurred during baking in cakes prepared from fructose. The composite methods used for evaluation of cakes prepared with sucrose, fructose, high fructose corn sirup, and fructose/glucose were sufficiently high so that these products were all considered acceptable whereas cakes made with glucose were considered unacceptable.

Multisurface caries were produced in Osborne-Mendel rats infected with Streptococcus mutans and fed diets containing cakes prepared from one of the simple sugars or saccharide sirup. The baked cakes were dried and used at a level of 70% in rations fed rats. These rations contained on a dry weight basis 32% of one of the simple sugars being tested, 14% fat and 12% protein. Groups of 10 male Osborne-Mendel rats were weaned at 18 days of age and given penicillin in the drinking water (0.5 g/100 ml) for 2 days. The test diet and oral infection with S. mutans was then begun. At

116 days of age, the animals were sacrificed by decapitation, the jaws cleaned of soft tissue, and the molar teeth scored for caries by the method of Keyes (J. Dent. Res. 37, 1088, 1958). The cariogenic effects of sugars incorporated into cakes were greater with sucrose, as compared with glucose, or with fructose, or with high fructose sirup or with a mixture of fructose and glucose.

Techniques for collecting and measuring saliva were investigated to evaluate the feasibility of following nutritional responses in humans associated with changes in dietary intake. Mixed saliva was collected 3 hours after the morning meal. Resting saliva samples (10 min samples) obtained from 7 subjects were analyzed for urea using the Hyland Phenate Hypochlorite method and for glucose using the enzyme glucose oxidase. For subjects who ate 2.3 to 4.9 g protein/100 kcal, the range for urea in saliva was 9.8 to 14.9 mg %. Increasing the protein level from 44 g/day to 115 g/day in one subject resulted in an increase in urea in the saliva from 22.1 to 26.1 mg%. The usual carbohydrate (g/100 kcal) for the 7 subjects in the morning meal was 8.0 to 21.0 g and the glucose level in saliva ranged from 0 to 4.3 mg %. Ingestion of 9 g of carbohydrate (6 g sugar) just prior to the collection of saliva resulted in glucose values of 98.4 mg % as compared to 1.3 mg % salivary glucose when collection was done 1 hour after ingestion of the carbohydrate (10 g caramel), as tested in one subject.

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By

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INTRODUCTION

Currently, dental decay is one of the few health problems that is of importance in both industrial and developing countries. The Ten-State Nutrition Survey (1968-1970) indicated that in areas of the U.S. the incidence of dental decay and the delivery of dental care was a major health problem especially among children. For persons 18 years of age and over, extensive dental decay (10 or more decayed, missing and filled teeth) was found in permanent teeth for 80% of the surveyed population. Values for edentulous persons were not included (Department Health, Education, and Welfare, 1972).

Attention to human dietary habits has been suggested as a means for reducing the incidence of dental caries (Nizel, 1969). This suggestion is based on the finding that certain oral micro-organisms can synthesize extracellular polysaccharides from food debris which, in turn, become an integral part of the dental plaque. Formation of the latter is thought to be the initiating step in dental caries. Cariogenic bacteria enmeshed in the plaque are protected from easy dislodgment; they use the plaque material as substrate for continued cariogenic action. These polysaccharides are formed in vitro from sucrose but not from glucose or other monosaccharides (Newbrun, 1967).

Dietary modification, through alteration of the oral environment, then becomes a practical approach to the control of dental disease (Berman, 1971; Miller, 1973; Hartles, 1970). Studies with human subjects have shown an association between the incidence of dental decay and sucrose consumption. The latter includes not only the amount of sucrose consumed but also the frequency with which it is eaten and the physical consistency of the food containing this carbohydrate (Lilenthal et al., 1953; Gustafsson et al., 1954; Harris, 1963). The effects of a variety of simple sugars on dental caries have been demonstrated recently in hamsters by Campbell and Zinner (1970). They showed that a sucrose containing diet supported a more destructive carious process over a 100 day feeding period than did diets containing fructose, glucose, lactose or a 1:1 mixture of fructose and glucose.

To alter the bacterial flora of the mouth to resemble that of caries-free individuals, Jay and co-workers (1959) recommended a series of low carbohydrate diets. Results from these dietary plans, which restricted carbohydrate to 100 g daily, showed that 70% of the individuals who had been caries-susceptible became caries-inactive. However, the diets were inconsistent with usual eating patterns and therefore inconvenient to maintain. Bibby (1961) has also recommended a dietary approach based on recognition of the need of altering the cariogenicity of foodstuffs.

Before dietary advice for the reduction of dental caries will be accepted, the less cariogenic foods must be well received so that they can be readily incorporated into the individual's

regular diet. Thus, the development of acceptable food substitutes using monosaccharides for sucrose in foods which commonly contain sucrose provides a realistic means for reducing dental caries. The purpose of this study was to develop an acceptable food product made from simple sugars and/or saccharide sirup and to test these in a caries-susceptible animal model.

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PART I

REVIEW OF LITERATURE: DECAY OF ENAMEL IN MULTISURFACE CARIES

CARIOUS PROCESS

Evaluation of the interdependency of diet and caries production is dependent upon the recognition of the multifactorial nature of the carious process. That is, dental decay is primarily an infectious process dependent upon interactions involving microflora, oral dietary substrate, and the resistance of the tooth (host factors) (Orland, 1954; Keyes, 1960). Caries do not develop with only one active factor but control can be effected by altering only one of these conditions (Keyes and Jordan, 1963).

After eruption of the tooth, destructive carious activity begins on an external surface with selective penetration into the interior of the tooth. Formation of a carious lesion in the enamel involves two distinct steps: one of these is demineralization and the other is proteolytic breakdown and destruction of the organic matrix of the tooth structure (Sognnaes, 1955; Darling, 1963). Dietary factors play a role in both the location and extent of these lesions.

Experimental Animal Models

X-ray diffraction studies reveal that the mineral salt in teeth of rats resembles the hydroxyapatite of human enamel (Gilda, 1951). The calcium-phosphorus weight ratio expressed on an ash basis of human enamel is usually in the range of 2.0 to 2.2

(Leicester, 1949). The calcium-phosphorus ratio in the incisor teeth of the rat was calculated to be 1.71 (Matsuda, 1927) and in the enamel to be 2.0 (McClure et al., 1966). In hamsters, Lobene and Burnett (1954) reported the calcium-phosphorus ratio of molars for males and females, respectively, was 1.87 and 1.45.

Patterns of cavitation seen in humans are primarily located in the enamel of crevices (pits and fissures), or on root surfaces, or develop as multisurface cavitation (both sulci and smooth surface). McCollum et al. (1922) reported that the destruction of molar crowns initiated in the occlusal sulci of white rats was similar to that found in man. The developmental pattern of enamel carious lesions from early enamel penetration to deep dentine destruction and cavitation was observed to be analogous to human caries as evidenced by histopathological examination of ground and decalcified sections of rat molars (Agnew et al., 1932). Gross carious lesions in the molar teeth of the Syrian hamster have been described in sulci, occlusal areas and frequently near the cemento-enamel junction and extending around the cervical portion of the teeth (Arnold, 1942).

Smooth-surface caries have also been reported in the albino rat (located on the buccal, lingual and proximal surfaces and also following the gingival line) (Stephan, 1951; McClure, 1952) and on the smooth lingual surfaces of the maxillary second molars of hamsters (Sognnaes, 1948).

A condition of periodontal disease and root surface cavitation (cementum caries) has been observed in the Syrian hamster

and is associated with the gingival accumulation of food debris (King, 1950). König (1965) has observed that early interproximal carious lesions in Sprague-Dawley rats start from the tip of the cusp whereas those lesions in Osborne-Mendel rats originate from the depth of the fissure similar to the pattern of attack seen in human lesions. With respect to distribution, the location of the proximal caries between the two strains of rats was similar.

Carious Lesion in the Enamel

The carious enamel lesion consists of demineralization with loss of calcified external dental tissue, obliteration of dentinal canals, and finally bacterial invasion of the underlying dentine and pulp structure. Experimental caries limited to enamel surfaces permits identification of zones of demineralization while avoiding the complications of proteolytic changes and bacterial invasion with loss of structure. Experimental carious lesions found on bucco-lingual, proximal, and occlusal surfaces correspond in several respects to common types of lesions found in molar teeth of humans (Johansen, 1963; Darling, 1963; Stepehan and Harris, 1955).

The process of carious attack is a selective demineralization of certain preformed structural patterns within the dental hard tissues. Early dental caries are characterized by deep subsurface demineralization (up to 1000 microns). This pattern may extend horizontally involving only the enamel surface area or may penetrate along enamel prisms or rods extending to the dentine-enamel junction (Sognnaes, 1963).

These enamel prisms are composed of apatite crystals in a hydrated organic matrix which is principally protein. In mammalian teeth, the particular apatite present in the enamel is the calcium phosphate salt hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$. In acid media, the hydroxyapatite reacts with lactic acid at a pH of 5.2 to produce tricalcium phosphate, calcium lactate and water. If the acidity goes below pH 5.2, tricalcium phosphate breaks down further to dicalcium phosphate, calcium lactate and water. These calcium phosphate compounds are more soluble than hydroxyapatite and the enamel surface becomes demineralized (Nizel, 1972).

Hydroxyapatite is characterized by a specific crystalline configuration. In enamel structure, developing apatite crystals take a spiraling path to the enamel surface forming the structure of the enamel prisms. The process of mineralization in the enamel involves the displacement of water by minerals and as the enamel matures the crystallites become more densely packed. During early caries development, an increase in sub-microscopic spaces within the enamel structure can be seen as opaque areas representing decalcified lesions (Johansen, 1965).

Within the body of the demineralized lesion, there is a differential decalcification of certain structures. The pattern of attack involves areas between adjacent enamel prisms; regions in which the crystallite orientation is discontinuous. The individual prisms then become more completely involved, often leaving rings of unaffected enamel, which correspond in size to the prisms (Darling, 1963).

A dominant feature of many lesions is demineralization of enamel sections longitudinal to the prisms (the striae of Retzius). These areas are formed due to normal periodic variation in calcification and represent fronts of incremental or normal growth found at an angle to the direction of enamel prisms. Enamel caries spread mainly along those striae of Retzius which are less calcified than neighboring enamel segments (Darling, 1961).

Tooth destruction rapidly occurs once the enamel surface is demineralized. With disintegration of the organic matrix and collapse of the dentine and pulp, loss of tooth surface is seen with evidence of cavity formation (Sognnaes, 1963; Keyes and Jordan, 1963).

CARIES-TEST CHALLENGE FOR MULTISURFACE LESIONS

Experimental multisurface caries (both sulci and smooth surface lesions) develop under specific dietobacterial conditions in vulnerable teeth (Keyes, 1968). Although a number of factors influence the course of the disease, the active carious process involves accumulation of microorganisms and subsequent acid production (Burnett and Scherp, 1962; Gibbons, 1964). A microbial and genetic caries-test challenge is essential in order to evaluate the dietary effect on the caries rate of multisurface enamel caries in erupted teeth.

Bacterial Factors

Bacterial activity is essential to the tooth decay process as shown by Orland et al. (1954) in germ-free studies in the rat. Cariogenic organisms differ with respect to the type of carious lesions they produce (Stephan and Harris, 1955). However, rampant caries are found with organisms that form dental plaque and are also highly acidogenic (Gibbons, 1968).

Cariogenic bacteria were first identified in rodents but have also been isolated from man (Zinner and Jablon, 1968). Certain strains of enterococci and streptococci show specific cariogenic activity (Orland et al., 1955; Fitzgerald et al., 1960). The majority of bacteria known to initiate caries are streptococci

(Gibbons, 1968). Specific anaerobic strains of bacteria of cariogenic importance have been designated as Streptococcus mutans (Clarke, 1924; Edwardsson, 1968; Guggenheim, 1968; and Krasse and Carlson, 1970). These strains of streptococci induce plaque formation and lead to multisurface caries.

Lactobacilli are often present in the oral flora but have not been shown to be associated with highly active carious disease (Fitzgerald, 1963). There are very few lactobacilli in the plaque but as many as 70% of the plaque microflora are streptococci (Bibby, 1938).

Lactobacilli are aciduric and are metabolically active at pH 5.0 (Snyder, 1965) while streptococci have a terminal pH in glucose broth between 4.0 and 4.5 (Zinner and Jablon, 1968). However, Burnett and Scherp (1968) have reported that many streptococci produce 3 to 6 times as much acid as lactobacilli.

Proteolytic properties of microorganisms have not been related to the production of experimental dental caries in animals. Fitzgerald, Jordan and Stanley (1960) produced caries in germ-free rats with a single streptococci strain that was acidogenic but non-proteolytic. Lactobacilli are reported to have little proteolytic activity (Orland, 1959).

Gibbons et al. (1966) have suggested that characteristics of cariogenic microorganisms are the ability to accumulate carbohydrate and to form acid (Gibbons, 1964). In germ-free animals, cariogenic-plaque forming strains of streptococci typically form extracellular polysaccharides of the dextran type whereas non-cariogenic strains

produce large amounts of extracellular levans (Fitzgerald and Jordan, 1968). Cariogenic bacteria typically store intracellular polysaccharide of the glycogen-amylopectin type (Gibbons and Socransky, 1962).

Plaque Formation

Localized sites of demineralization and cavitation have been ascribed to the activity of the adhering plaque of colonies of microorganisms (Black, 1886; Stephan, 1953). When exposed to sucrose solutions, these adhering plaques of dental bacteria have been observed to accumulate polysaccharide material (Manly, 1961). Dextran polymers located outside the bacterial cells within the plaque matrix form insoluble precipitates with proteins (Gibbons and Banghart, 1967) which provide the basis for the adhesive nature of dental plaque and favor initiation of smooth-surface lesions.

Significantly more extracellular dextran is formed from sucrose than from equal amounts of fructose, glucose, maltose, lactose (Gibbons et al., 1966) or fructose and glucose combined (Gibbons and Banghart, 1967). A sucrase enzyme system (Koepsell et al., 1953; Hestrin et al., 1956) on the cell surface of oral bacteria located within the plaque matrix degrades the sucrose entering the plaque and both 1,6 linked dextrans (Gibbons and Banghart, 1967) and levans (Wood, 1964) are formed.

Glycosyl transfer from sucrose is favored due to the high energy yield from hydrolysis (-6600 cal/mole) (Bernfeld, 1963). This extracellular synthesis of dextrans occurs by direct transfer

of the glucose unit to a growing polymer chain (Critchley et al., 1967) with the release of free fructose. The high energy in the bond originally between the 2 anomeric carbons of sucrose, C-1 of glucose and C-2 of fructose, is maintained during the polymer synthesis (Newbrun, 1967).

Extracellular levans are produced in plaque material following incubation with sucrose and Streptococcus salivarius (Wood, 1964); but not with fructose, glucose, or a mixture of fructose and glucose (Manly and Dain, 1961). Only small amounts of levans are accumulated in the plaque; the fructose molecules are readily soluble and can be lost by diffusion and therefore do not readily promote plaque formation (Gibbons and Banghart, 1967). Levans retained in the plaque may be metabolized by the microflora present to yield acid products; however, acid production has been shown to occur only at a very slow rate from these compounds (Manly, 1961; Wood, 1964).

Acid Formation

Acid production by cariogenic bacteria is sufficient to account for the decalcification phase of the carious process. Stephan (1940) showed that a pH (pH 5.0 to 5.5) sufficient to dissolve enamel was reached in plaque on enamel surfaces within 2 minutes after the mouth was rinsed with a 10% sucrose or glucose solution. Stephan (1944) found that plaque from caries-resistant subjects did accumulate less acid than plaque from caries-active subjects.

Acid formation occurs in the oral cavity as a result of microbial fermentation of carybohydrate. Sreebny and co-workers (1950) showed that removal of microorganisms from human saliva by filtration inhibits an increase in titratable acids that is found in whole saliva when these systems are incubated in a glucose solution for 24 hours.

Extended exposure of teeth to acid within the plaque is due to the continued production by cariogenic microflora of lactic acid from stored carbohydrate. Gibbons and Socransky (1962) showed that streptococci isolated from plaque of humans accumulated polysaccharide when exposed to glucose and formed lactic acid with loss of polysaccharide when the glucose source was removed. It is this prolonged depression of plaque pH during periods of exogenous carbohydrate depletion due to catabolism of intracellular polysaccharide by cariogenic bacteria that contributes to a dietary role in the carious process (Stephan, 1940).

These stored polysaccharides (amylopectin) are generally believed to be polymers of glucose and to function as energy reserves for the cariogenic bacteria (Gibbons and Socransky, 1962). These polymers resemble the starches of plants and the glycogen of mammals and are cleaved by α-1,4-glucan hydrolase (α-amylase) (Gibbons and Kapsimalis, 1963).

Streptococci that are cariogenic are strong amylopectin producers (Fitzgerald and Jordan, 1968). Streptococci do not possess a cytochrome system (do not utilize oxygen) and obtain energy by metabolizing glucose to lactic or other acids (Hartles,

1954). However, Cowman et al. (1974) have reported that aerobic conditions resulted in increased growth of a number of strains of S. mutans due to increased utilization of amino acids.

Microbial-Substrate Factors

Kite et al. (1950) showed that food in the oral cavity is an essential nutrient source for bacteria associated with caries production. Weanling rats fed a caries-producing ration for 16-25 weeks by a stomach tube failed to develop caries. Compared to control littermates fed the same diet ad libitum, the tube-fed rats showed normal growth and molar structure of teeth but no dental decay whereas control rats had an average of 5 molars that were decayed.

B. F. Miller et al. (1940) showed that dental plaque readily produced lactic acid from glucose, maltose and sucrose but less readily from lactose and starch. The role of dietary carbohydrate in the carious process is largely dependent in multisurface caries upon the availability of the carbohydrate substrate to the cariogenic microorganisms lodged in the plaque.

Sucrose has a high energy yield (-6600 cal/mole) from hydrolysis and is capable of producing both intracellular and extracellular polymers which contribute to the carious process in the plaque matrix (Newbrun, 1967; Navia, 1970). When the free glucose molecule is the only carbohydrate substrate available to the cariogenic bacteria, stored amylopectin is formed (Gibbons and Socransky, 1962); but in the absence of a high energy bond the extracellular synthesis of dextran does not occur.

The fact that sucrose is generally consumed in large quantities in human diets and is readily fermented contributes to the cariogenic activity of this sugar. Haldi et al. (1953) observed that the cariogenicity of sucrose fed to rats was greatly reduced by dissolving sugar in water before ingestion. A purified diet free of sucrose was fed to each rat by stomach tube. One group of 13 animals was then fed 9 g of granulated sucrose that was placed in a food cup for oral ingestion; 2 out of 13 rats remained caries free. The second group of 13 rats was provided with measured amounts of 40% sugar solution to equalize the sucrose intake between groups; 7 out of 13 rats remained caries free.

Dietary maltose and lactose do not favor establishment of dextran-synthesizing, plaque-forming streptococci. The glycosidic link in these disaccharides is a hemiacetal between an aldehyde and hydroxyl carbon with a low free energy of hydrolysis; therefore, these compounds do not serve directly as a glycosyl donor in dextran synthesis (Bernfeld, 1963). However, maltose is readily fermented and this carbohydrate may favor acid production in areas where metabolically active microorganisms are already present (areas of plaque or areas of impactation).

Large starch molecules do not readily serve as substrate for the carious process since they do not diffuse into the viscous plaque material (Critchley et al., 1967) and therefore do not come in contact with the surface of the tooth and the metabolically active bacteria. Acid production from starch is also small. Small amounts of maltose may originate from the action of amylase on

the starch in the oral cavity; but it is readily neutralized by the saliva.

Host Factors

Caries occur on vulnerable surfaces of teeth of a susceptible host in the presence of colonies of characteristic groups of bacteria. In the hamster, extensive coronal caries have been shown when animals were infected with streptococci and fed a fine particle, high sucrose caries-test diet (Fitzgerald and Keyes, 1960). Under the same conditions, marked smooth-surface caries are seen in the NIH Osborne-Mendel rat (König et al., 1969). The use of a coarse-particle diet has been associated with the development of carious lesions limited to the occlusal surface (Stephan et al., 1952).

In order to demonstrate that heredity is an important factor in the development of dental caries, Hunt et al. (1944) studied the extent of caries activity with inbred strains of rats. A high prevalence of carious lesions in the dental sulci were found in the caries-susceptible strain fed a coarse ground rice diet for 35 days (Hoppert et al., 1932) while under the same conditions the resistant strain remained free of caries. Substitution of fine-ground rice for the coarse ground particles in the diet delayed the onset of molar sulci caries in these rats while differences between the strains remained consistent (Braunschneider et al., 1948; Hunt et al., 1955). The bacterial flora or salivary secretions in Hunt-Hoppert strains of rats have not been found to differ between caries-resistant and caries-susceptible animals

(Jay, 1948; Rosen et al., 1955; Keller et al., 1954; Sreebny et al., 1956). Therefore, this could not account for the differences in caries susceptibility. Kifer and co-workers (1956) did note that the fissures of caries-resistant rats were narrower than those of the caries-susceptible rats.

An important role in host susceptibility to caries may be dietary-microflora interaction (Larson et al., 1967). A coarse particle diet low in sucrose (diet 585 with 25% cane sugar of Stephan et al., 1952) showed predominantly sulci caries in Hunt-Hoppert caries-susceptible rats (Larson et al., 1968). This occurred regardless of whether or not the animals were infected with cariogenic microflora (exposure to caries active rats).

Using the same experimental conditions, a different pattern of caries development in the Hunt-Hoppert rat was found when a fine particle-high sucrose diet (diet 2000 with 56% confectionary sugar of Keyes and Jordan, 1964) was substituted for the coarse ground-low sucrose ration described above. Uninfected animals again showed predominantly sulci activity with the caries-susceptible rats showing twice as many carious areas. However, infected rats showed the same total number of carious areas in both caries-susceptible and caries-resistant strains of rats; but 60% of the carious areas in the caries-susceptible rats were sulci lesions while 67% of the carious areas in the caries-resistant animals represented smooth-surface lesions. Thus, the site of caries development appears to reflect differences inherent in the host-species while total caries development is dependent upon a response of all tooth surfaces to

an appropriate cariogenic challenge of both microflora and dietary factors.

Differences in caries activity among strains of rats have been attributed to a dietary response involving the pattern and frequency of food intake. Osborne-Mendel rats fed Diet 2000 ad libitum (König et al., 1969) had high caries activity correlated with a long, frequent and slow feeding period (average of 213 minutes eating time and 16 meals/day and 0.65 g/intake/minute). Under the same regimen, NIH Black rats had less caries activity and a feeding period that was shorter, less frequent but with a more rapid food intake (98 minutes eating time and 12 meals/day and 0.77 g intake/minute) as compared to the Osborne-Mendel rats.

SUMMARY

An approach to the study of dental caries prevention or control requires recognition of the multifactorial (bacterial, dietary and host) aspects of the disease. In animal experiments, evaluation of dietary factors that modify the appearance of carious lesions include the composition and pattern of food intake and the residue effect in contact with teeth and the presence of cariogenic bacteria. Fully effective caries control can be achieved under laboratory conditions. This has been shown with tube feeding studies (Kite et al., 1950) and with germ-free studies with animals (Orland et al., 1954). Sucrose, in particular, has been shown to be cariogenic (König, 1968; Pigman, 1970).

The pattern and extent of carious lesions reflect the interaction of diet and microflora in the test animal. Rats fed a coarse cereal diet alone (Hoppert et al., 1932) or in combination with 25% cane sugar develop sulci (occlusal crevice) carious lesions with or without infection of cariogenic microorganisms. Diets high in sucrose (56-66%) in the absence of infection of the animals also primarily resulted in sulci lesions (Larson et al., 1967; 1968).

Carious lesions on the smooth surfaces appear in rats on cariogenic diets containing 56% confectionary sugar (Larson and

Goss, 1967) or 66% granulated sucrose (Larson et al., 1967) only in the presence of cariogenic bacteria. Smooth-surface lesions do not occur in rats fed a diet favoring only sulcal caries even in the presence of bacterial flora that favor multisurface lesions (Larson et al., 1967).

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PART II

USE OF MONOSACCHARIDES AND A SACCHARIDE SIRUP IN BUTTER-TYPE CAKES

INTRODUCTION

Some humans seek sweetness in foods far beyond the need for relieving hunger, even to the detriment of health (Cabanac, 1971). Indeed, the sugar and sirup consumption in the U.S. increased from 25 kg per capita in 1899 to 54 kg in 1924 (Antar et al., 1964) to 57 kg in 1970 (Agricultural Economic Report, 1972). Although these values based on retail market supplies for sugar consumption have stabilized in excess of 50 kg per year for the past 45 years, the total simple sugars as a percent of total carbohydrate intake per day, has increased from 44% to 52% during the same time period; that is, from 1924 to 1970 while there was a concomitant decrease in starch consumed from grain products.

The commercial availability of glucose sirups (Birch et al., 1973) and high fructose corn sirups (Redfern and Hickenbottom, 1972) provide an economical and convenient source of monosaccharides as sweetening agents. Since consumer acceptance reflects sensory and quality characteristics of foods (Rubini, 1974) as well as availability (Yudkin, 1967), changing nutritional intake should be easier if the new food is not excessively changed from the old. Therefore, before advocating dietary modifications in order to decrease the sucrose in the diet, it should be beneficial to first investigate the substitution of monosaccharides for sucrose in foods which contain this ingredient. The purpose of this study

was to investigate, both subjectively and objectively, quality characteristics of butter-type cakes prepared with fructose and/or glucose and to compare these characteristics with those of control cakes made with sucrose.

LITERATURE REVIEW

Overview: Sugars in Cake Products

Functions of Sugar

Structurally, the principal effects of sugar in flour mixtures are to reduce gluten strength, increase tenderness and produce smaller, more uniform cell distribution. These effects on the physical properties of baked products are partially due to the water binding capacity of sugar which in turn delays the hydration of proteins and starch. For this reason if the sugar is too high in relation to the liquid, a sunken center develops in cakes during baking (Kissel and Marshall, 1962). Competition of sugar for water prevents viscosity changes associated with starch gelatinization and leads to failure to develop characteristic cake structure.

All sugars absorb moisture to a certain extent. Moisture is held in a batter or dough product as the solvent primarily for sugar. In plain cake formulas, sugar is found dissolved in the liquid phase (Lowe, 1955). Sugars not well dissolved can produce a sugary crust on cakes. Too much sugar can cause a sticky crust and a gummy texture. In a balanced cake formula, the absorptive ability of sugar contributes to the keeping quality of the product.

The sweetness of cake is dependent upon the concentration and type of sugar used (Dahlberg and Penczek, 1941) and the effect of reactions occurring during product baking. A high sugar to

protein ratio contributes to flavor production by nonenzymatic browning reactions involving mainly reducing sugars with amines, amino acids, peptides and proteins (Hodge, 1966). High heat also causes flavor changes as sugars are decomposed and new compounds are formed (Meyer, 1960).

Color changes also occur in baked products based on sugar content. Browning due to rearrangement of potential carbonyl groups from reducing sugars is accelerated by high temperature, low moisture content and an alkaline media. Reactions of ingredients result in unsaturated polymers of varying composition which contribute color and flavor changes to the final food product. In general, the types of nonenzymatic browning reactions which occur during baking are caramelization of the sugar moiety and the sugar-amine interaction of the Maillard reaction.

Effect of Reducing Sugars

In starch-water-sugar systems, monosaccharides, when substituted for disaccharides at the same concentration by weight, do not delay starch gelatinization of the same extent (Bean and Osman, 1959). Miller and Trimbo (1965) reported that replacement of sucrose with monosaccharides eliminated the surface dip in the center of cakes with a high sugar to water ratio (sugar levels of 140% based on the weight of flour and with water reduced to 80% of normal). However, excessive browning of crust and crumb of the glucose-containing cake was noted.

Optimum flavor and eating quality was found in white and yellow plain cakes to be at pH 7.0 to 7.9 (Stamberg and Bailey,

1939). To minimize excessive browning when sweetening agents containing reducing sugars are substituted for sucrose in butter-type cakes, Miller et al. (1957) maintained the pH of the cake crumb at approximately 6.3 with the use of a leavening acid. However, accelerated loss of CO_2 was also found which resulted in decreased cake volume.

Use of Liquid Sweeteners

Invert sugar sirup, used at a 10% level in baked products, contributes to browning in the crust, fineness of grain, and softness of texture (Anonymous, 1972). Honey, which contains 30% glucose, 30% fructose, and approximately 4% oligosaccharides (Siddiqui, 1970) has been used as an acceptable sweetening agent in cakes for up to 50% of the total sweetening; however, adjustments were made in the added liquid to account for the moisture content of the honey. In addition to water content, the use of honey in baked products makes it necessary to adjust the cake formula to take into account the free-acid content and distinctive flavor (Morgan, 1937).

Corn sirup has been recommended at about 25% replacement level for sucrose (Nesetril, 1967). Corn sirup consists primarily of D-glucose, maltose, and α-1,4-linked oligosaccharides of D-glucose (Grenby and Leer, 1974). Corn sirups have been advocated for use in foods; their main advantage for automated mixing is in the reduction of ingredient and processing costs (Keeney, 1962). Although great strides have been made in production and analysis of

corn sirups, little is known of their actions in foods (Eopechino and Leeder, 1969). Corn and glucose sirups lack the necessary sweetness for total replacement for sucrose in food products (Redfern and Hickenbottom, 1972).

High fructose corn sirups (HFCS) contain more than 10% fructose (Wardrip, 1971) and are produced by enzymatic conversion of cornstarch to a sirup containing both glucose and fructose. ISOMEROSE^{R} 100 Brand High Fructose Corn Syrup (Clinton Corn Processing Co., Clinton, Iowa) is prepared by the enzyme-isomerase and is composed of 42% fructose and 50% glucose on a dry weight basis. In water solutions containing 15% ISOMEROSE^{R} solids, relative sweetness compared to sucrose is 100. The product is available commercially in a 71% solids solution.

Microbiological stability and wholesomeness of the high fructose sirups have been reported to be comparable to invert sucrose sirups. No enzyme activity has been demonstrated in the refined fructose sirup. Furthermore, enzymes derived from strains of Streptomyces (Streptomyces olivochromogenes is used commercially as an enzyme source by CPC International Inc., Argo, Ill.) are nonpathogenic (Kooi and Smith, 1972).

Fructose-containing sirups have ready application in all foods which already utilize liquid sweeteners such as soft drinks or which require low sugar content such as yeast-leavened baked goods. Replacement levels as a total sweetener have been recommended for yellow and chocolate cakes at 40-50% of the sucrose

level and for chewy type cookies at 20-30% of the sucrose level (Redfern and Hickenbottom, 1972).

Properties of Sugars

Solubility

In order of solubility, the sugars in pure form are ranked as follows: fructose, sucrose and glucose. At room temperature, about 4 parts of fructose are soluble in 1 part water and about 1 part glucose is soluble in 1 part water on a total weight basis (American Home Economics Association, 1971). This sugar-to-water ratio is basic to a consideration of substitution of monosaccharides into a cake formula.

The quantity of solids which can be incorporated into a solution is dependent upon the solubility of the solute. Fructose/glucose sirups can be prepared with a high concentration of solids since fructose has a very high solubility and glucose does not readily crystallize. Solubility curves of sucrose-invert sugar mixtures show that the percent of invert sugar increases to a peak solubility and then decreases. The proportion of invert sugar to sucrose gradually decreases from 75% at 40°C to about 60% at 10°C (Davis and Prince, 1955).

The concentration of solute varies with the temperature of a solution. At 40°C, 70.4 g sucrose will solubilize in 100 ml water (Handbook of Chemistry and Physics, 1959). A 78.9% fructose solution is present at 20°C (Jackson et al., 1926) while a 73.1% glucose solution is formed at approximately 55°C (Jackson and

Silsbee,1922). In baked products, the effects of differences in solubility are interdependent upon the hydration capacity of the other ingredients.

The absorptive ability of sugar also prevents the drying of food products. Dittmar (1955) showed that sucrose and glucose absorb less than 1% moisture below 60 to 65% humidity at 25°C whereas fructose and invert sugar hold 20% water in surface solution at 65% humidity. Lowe (1955) reported that products containing fructose do not dry out as rapidly as those made with sucrose.

Sweetness

Relative sweetness of sugars in water varies with the concentration of the solution being tested. At recognition threshold levels (the lowest concentration at which sweetness is tasted under controlled conditions), 19 of 20 subjects detected sweetness in solutions containing sucrose, glucose, fructose and an equal mixture of fructose and glucose at the following % concentrations, respectively: 1.25, 1.75, 0.75 and 1.0% (Willaman, 1925). At 20% levels the relative sweetness of fructose is 119.8 and glucose is 91.7 when compared to a sucrose standard set at 100% (Dahlberg and Penczek, 1941).

Pangborn (1965) has evaluated the intensity of apparent sweetness of aqueous solutions of fructose, glucose, lactose and sucrose in combination with acetic, lactic, tartaric and citric acids. Results showed that citric and acetic acids from among those used had the greatest sweetness depressing effect. Among the sugars,

the apparent sweetness of fructose decreased the most by acid and that of sucrose the least. This report emphasizes the need for further study before direct application can be made from simple aqueous systems to more complex food systems.

Sugar Caramelization

Caramelization refers to the series of reactions that occur when sugars are heated to melting. The sugar decomposes, water is given off, and mixtures of aldehydes and ketones leading to brown colored compounds are formed. This reaction is accelerated in the presence of moisture and alkali.

Products containing fructose and glucose are especially subject to caramelization changes. The melting point of fructose is approximately 104°C while sucrose melts at 186°C. Anhydrous glucose has a melting point at 146°C while that of the hydrate form melts at 86°C (Handbook of Chemistry and Physics, 1973).

Heating solutions of the common sugars (glucose, fructose, maltose and sucrose) yields caramels which are grossly similar (Ramaiah et al., 1957a,b). Greenwood et al. (1961) and Bryce and Greenwood (1963) found that the 300°C pyrolysis volatiles from sucrose, maltose, glucose and starch as determined by gas chromatography, are identical. Hodge (1966) lists carbohydrate caramelization and dehydration products giving rise to bitter flavors as lactic aldehyde, furfuryl alcohol, 5-(hydroxymethyl)-2-furaldehyde and maltol.

Sugar-Amine Interactions of the Maillard Reaction

Reducing sugars also undergo Maillard-type browning. In this case the unsaturated carbonyl compounds responsible for browning are formed mainly as the result of sugar-amine interactions. Condensation between the carbonyl group of a reducing sugar and the free amino group of a protein to form a N-substituted glycosyl amine is an early step in the reaction. This carbonyl-amine condensation is not dependent upon the presence of oxygen (Hodge, 1953).

At moderate temperatures, this first stage of the reaction series is slow, incomplete, reversible to some extent and yields colorless products (Lea, 1950). During later stages of the reaction, rearrangement of the carbonyl-amino compound can occur with splitting off of the labile amine moiety and with dehydration and polymerization of the sugar moiety (Hodge, 1953).

Under mild conditions (50°C, pH 5.5-6.0, low sugar:N ratio), Burton and McWeeny (1964) report that most of the browning occurs due to production of unsaturated carbonyl decomposition products followed by rapid reaction of these compounds with amino groups. The extent of browning is increased with long periods of heating (Burton et al., 1963b) and with increasing temperature (Barnes and Kaufman, 1947).

Color changes during heating.--A key reaction for early color changes is the Amadori rearrangement of the condensation product, an aldosylamine, to form a ketoseamine. This keto

intermediary compound leads to fragmentation, dehydration, and further condensation reactions and provides a basis for the auto-catalytic nature of the system (Hodge, 1953).

Later browning is reported to be dependent upon the degree of unsaturation and the reactivity of the intermediates formed (Burton et al., 1963b). Kato (1960) has suggested that in systems containing aldoses and amines the main cause of browning may be due to reactions between the amine and 2,3-enol compounds. Burton et al. (1963a) reported that saturated aldehydes and saturated and unsaturated α,β-ketones do not readily brown with glycine while the later appearance of highly colored products is accelerated with the use of α,β-unsaturated straight chain aldehydes.

As compared to pH 4, the rate of browning at pH 6 has been shown by Barnes and Kaufman (1947) to double in simple glucose and glycine mixtures. Lea (1950) reported a rise in pH increased the rate of Maillard browning reaction of aldoses and amines approximately linearly between pH 3 and pH 8. However, Cole (1967) found that this expected pH effect on reaction rate was not always seen between pH 6 and 7.

In the presence of alkali, reducing sugars readily form 1,2-enol compounds (Reynolds, 1965). In nearly anhydrous conditions or in the presence of amines in high concentration, browning due to the formation of reductones is favored (Hodge, 1953); while at moisture contents of 20% of greater and with temperatures above 43°C, oxidative browning occurs (Reynolds, 1965).

Within acidic aqueous systems, color changes have been primarily attributed to dehydration of the sugar moiety and production of furfural compounds (Hodge, 1953). Reynolds (1965) reports that organic acids play a catalytic role in promoting decomposition of sugar molecules in mixtures of organic acids, sugars and amino acids. In these systems, the rates of formation of ketoseamine and color changes increase with increasing solids content, and are still further increased in the presence of organic acids.

Flavor changes.--Characteristic flavor changes develop as a result of the Strecker degradation of the α-amino acid to yield the aldehyde with one carbon less. This degradation occurs as a secondary effect of the reaction between aldoses and amines and produces carbon dioxide, carbonyl compounds and free amines. The products are formed as a result of the reaction between amino acids and dicarbonyl compounds. Furthermore, these reactions occur throughout a wide range of pH and temperature conditions (Hodge, 1953; Reynolds, 1965).

More than 80% of the carbon dioxide evolved from the reaction between aldoses and glycine has been reported to originate from the carboxyl group of glycine (Reynolds, 1965). However, Cole (1967) has shown that the limiting factor in the rate of carbon dioxide production is the type of sugar rather than the amino acid or pH.

Barnes and Kaufman (1947) reacted single amino acid-sugar pairs and found that development of flavor in this simple system

was specifically dependent upon the amino acid used. Herz and Shallenberger (1960) reported that, in general, reactions between glucose and 12 different amino acids led to a characteristic bitter reaction product as judged by a 6 member flavor panel. Exceptions were the development of an acid-bitter flavor when histidine was used and a sweet flavor when alanine was used.

Polymer formation.--Final stages of the Maillard browning system are marked by condensation of products formed as a result of the reaction between sugar and amino acids which yield brown polymers and co-polymers known as melanoidins (Hodge, 1953). The presence of brown color, formation of conjugated unsaturated carbonyl compounds, and production of carbon dioxide in sugar-amino acid systems are closely related chemically (Cole, 1967). Rooney et al. (1967) found that the type of sugar used affected the rate and quantity of carbonyl produced while the type of amino acid used mainly affected the kind of aldehyde produced.

Relative Browning Rates

The major mechanism responsible for the chemical alteration of sugars during baking is the Maillard browning reaction since these sugar-amine reactions are autocatalytic and have a lower energy of activation than caramelization reactions (Kinsella, 1971). The rate of early browning associated with the use of carbohydrates increased in the following order: disaccharide, hexose and pentose (Lea, 1950).

Casey (1965) showed that the oxidant effect of fructose was twice as fast as that of glucose. Fructose, when reacted with 5 amino acids under controlled conditions (pH 6.5 for 30 minutes at 110°C), gave higher initial rates of production of low boiling volatiles than did glucose. Burton et al. (1963b) found that when fructose was reacted with glycine the rate of carbonyl development was initially rapid and the rate of increase of browning was approximately twice that seen in glucose-glycine systems.

Others (Lea, 1950; Reynolds, 1965) have noted that while initial rates of reaction between amino acids and fructose are rapid, the extent of later browning may proceed more slowly with fructose than with glucose in buffered systems such as foods. Burton et al. (1963b) also noted that the color density of glucose reacted with glycine did exceed the browning of fructose-glycine systems in later stages of browning.

MATERIALS AND METHODS

Butter-type cakes were prepared using 5 kinds of sweeteners. These sweeteners included (1) sucrose (control cake), (2) fructose, (3) glucose, (4) high fructose corn sirup and (5) fructose and glucose combined. The 4 latter sweeteners were substituted for the sucrose in the cake formula. Ingredients (Table 1) were combined by the solution method of mixing (Stateler, 1950) to permit adaptation of the recipe for use with saccharide sirups available on the market. The cake formula was scaled to produce 3 cakes at each mixing to provide sufficient samples for each evaluation. Each of the 5 varieties of cake was prepared in triplicate.

Preparation

Ingredients

Sugars tested included a representative commercial high fructose corn sirup (HFCS) product[1] as well as crystalline fructose and glucose alone and in a 1:1 ratio (fructose/glucose). Cakes made from sucrose produced a standard-type product and served as a basis against which the other products were evaluated.

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[1] ISOMEROSE^{R} 100 Brand High Fructose Corn Syrup (HFCS) was generously provided by the Clinton Corn Processing Co., Clinton, Iowa. This commercially available product contains 71% solids and is composed of 35.5% glucose, 29.8% fructose and 5.7% other saccharides.

TABLE 1.--Cake formula for one replication.[a]

Ingredients	Amount	Percentage of Flour[b]
Shortening, vegetable	195.0 g	43.3
Sugar,[c] superfine quality	473.0 g	105.0
Vanilla	5.0 ml	1.2
Eggs	225.0 g	50.0
Flour, cake	450.0 g	(b)
Baking powder, SAS-phosphate type	21.0 g	4.6
Salt	7.5 g	1.7
Milk solids, whole	60.0 g	
Water, distilled[c]	446.0 g	112.5[d]

[a]Batter for three 8-inch layers. These were used for sensory and objective measurements.

[b]Weight of ingredients compared to weight of flour taken as equal to 100%.

[c]For cakes prepared with HFCS, 668 g of sirup and 251 g of water were used as compared to sugar (473 g) and water (446 g).

[d]Percentage based on combined weight of milk solids and water.

Substitution of the simple sugars for sucrose in the cake formula was based on an equal weight basis. The cakes were all prepared using the same proportion of ingredients with substitution of the sweeteners based on percent solids for the sirup product. Since the HFCS contained 71% solids and 29% water, each dry sugar was made up as a solution with distilled water and used in that form. This represented 45% of the water required for each cake formula. Although the relative sweetness of sugars varies with their concentration and interaction with other ingredients (Dahlberg and Penczek, 1941), preliminary investigations demonstrated that this was the most fundamental way to modify recipes for the incorporation of the simple sugars in order to produce acceptable butter-type cakes.

All ingredients except eggs were each taken from a common lot and each was weighed to the nearest gram. Sugars as well as cake flour, double-acting baking powder, and salt were weighed prior to baking and stored at room temperature. Just prior to mixing, hydrogenated vegetable shortening, fresh eggs, whole milk powder and distilled water were weighed. Sugar solutions were also prepared at this time. The whole milk powder was then combined with the previously weighed dry ingredients.

Procedure

The sugar solution was placed in a 5-quart bowl and blended with the fat and flour mixture using the whip attachment of a Kitchen Aid mixer, Model K5-A, set at speed 1 (56 rpm) for 2 minutes

and speed 2 (92 rpm) for 2 minutes. After scraping the bowl and beater, the eggs, remaining water and vanilla were added and mixed at speed 2 (92 rpm) for 1 minute and speed 4 (132 rpm) for 1 minute.

For each of the 3 replications, 400 g of batter were placed in each of three 8-inch aluminum layer-cake pans prepared by covering the oiled pan with oiled waxed paper. All cakes comprising a replication were baked simultaneously in a Hotpoint oven, Model HJ225, set and maintained at 177±2°C with a Versatronic controller. For each of the 3 replications of each kind of cake, cakes designated for specific objective measurements and sensory evaluations were baked in a designated spot in the oven according to a preplanned schedule to negate any effects of oven position.

Following baking for 28 minutes, cakes were cooled on wire racks for 15 minutes before removing from the pans and then cooled for 1 hour after removal from the pans. The cakes were then wrapped in plastic food wrap, placed on cardboard trays, labeled, placed in polyethylene bags and frozen at -23°C for later evaluation. This took place at a time more convenient for evaluation and was 2 weeks following baking.

Evaluation

Objective Measurements

The Beckman Zeromatic pH meter was used to determine the pH of all cake batters as well as cake slurries. The latter was prepared from 1 g of baked cake and 60 ml of distilled water at pH 6.8. Using the parameters described by Funk, Conklin and Zabik (1970) and

techniques outlined by Matthews and Dawson (1966), batter viscosity was determined with a Brookfield viscometer. Moisture loss during baking was calculated from the before- and after-baking weight with the latter determined following the first 15 minutes of cooling. Total moisture content was also determined by drying a 2 g cake sample to constant weight in a vacuum oven set at 70°C (Association Official Agricultural Chemists, 1965).

Relative cake volume index based on the height of a center slice of cake, tenderness as determined by the Allo-Kramer shear press, and measurements of crumb color using a Hunter Color Difference Meter have been detailed by Funk et al. (1970). To follow browning changes caused by reducing sugars, color readings were made on the interior surface 3/4-inch from the bottom of the cake.

Sensory Evaluations

Cakes were scored for grain, crumb color, silkiness, tenderness, flavor of crust, and flavor of crumb using a 5-point scale as shown in Table 2. Ratings of 3 and 2 represented intermediate scores for those described in 4 and 1. Instructions explaining the score-card and descriptive terms used were available at each testing session (Appendix A).

The taste panel consisted of 11 departmental faculty and graduate students. No specific training was provided. However, all taste panel members had had previous experience in sensory evaluation of food products.

TABLE 2.--Score-card used to evaluate cake samples.

Attribute	5	4	3	2	1
Grain	Fine, regular, even small round cells with thin cell walls	Slightly small cells evenly distributed or Slightly large cells with slightly irregular distribution			Extremely small, compact cells or Large cells with very irregular shape and distribution
Crumb Color	Creamy yellow throughout	Slight brownish cast or slight irregular color or Slightly pale creamy yellow color			Brownish cast with irregularly colored areas or Too pale, off-white color
Silkiness	Soft, velvety feel or texture	Slightly soggy or Slightly dry			Extremely gummy, wet, soggy or doughy or Extremely harsh, dry and brittle
Tenderness	No resistance to cutting with fork or biting Dissolves easily in the mouth	Slightly fragile or Slightly resistant to cutting or biting			Too tender or Too tough or rubbery
Flavor (crumb)	A delicately sweet, even blended flavor	Slightly tart but acceptable flavor or Moderately perceptible, delicate, sweet flavor			Tart, unacceptable flavor or Imperceptible
Flavor (crust)	A delicately sweet, even blended, caramelized flavor	Slightly tart caramelized flavor or Moderately perceptible caramelized flavor			Sharp, bitter or metallic lingering taste or No taste

The panelists were asked to score each sample independently of the others and to check the appropriate value on the score-card which agreed with their sensory evaluation of the sample. The panelists were encouraged to write comments on the score-card. Distilled water was provided for rinsing the mouth between samples. All panelists were asked to refrain from smoking and consumption of food or liquid for 1/2 hour prior to each tasting session.

Cakes were defrosted for 1 hour at room temperature prior to evaluation. Three to 4 cakes were presented at each tasting session in the same order in which they were baked. The cakes were 8 inches in diameter and were cut into 12 wedge-shaped pieces, one of which was given to each panelist. All cakes were assigned random numbers, consequently no panelist had any prior indication of the sugar present in the cake sample.

Analyses of Data

Means and standard deviations were calculated on all sensory and objective measurements. Scores of all judges for each palatability characteristic were combined and averaged for each cake. Analysis of variance, based on mean values, was computed to determine any differences due to nature of sugar used or differences attributable to replication.

Significance of differences between groups of cakes containing sugars was assessed using Duncan's Multiple Range Test (Duncan, 1955). Correlation coefficients were determined for objective measures of tenderness and moisture.

RESULTS

Objective Measurements

Averages, standard deviations and statistical analyses of objective measurements are presented in Table 3. The pH of batters prepared with HFCS was lower ($P < 0.05$) than batters prepared with glucose, fructose/glucose or sucrose and the pH of fructose-containing batters was lower ($P < 0.05$) than glucose- or fructose/glucose-containing batters. However, after baking, no significant differences were noted in the pH.

Batter viscosity varied with the sugar used and among replication. Lowe (1955) indicated that batters of varying degrees of viscosity can be produced from the same ingredients and the data of this study support her observation.

Moisture losses during baking were not significant. However, moisture determinations of the finished products indicated that cakes prepared with glucose and fructose/glucose contained more moisture ($P < 0.01$) than cakes prepared with other sugars.

From among the five varieties, the cakes prepared with glucose were the smallest ($P < 0.01$) in volume and also required the least force ($P < 0.01$) to shear. Cakes prepared with fructose/glucose required fewer ($P < 0.01$) pounds force per gram to shear than sucrose-containing cakes. In addition, cakes prepared with glucose were more ($P < 0.05$) tender than cakes prepared with

TABLE 3.--Averages, standard deviations and statistical analysis of objective measurements of batter and baked butter-type cakes prepared with monosaccharides and sucrose.

Measurement	Monosaccharides and Sucrose					Significant Differences[a]	
	Sucrose S	Fructose F	Glucose G	HFCS I	Fructose/ Glucose FG	P < 0.01	P < 0.05
pH							
Batter	8.1 ± 0.1	7.9 ± 0.9	8.3 ± 0.9	7.8 ± 0.9	8.2 ± 0.9	I < FG < G F < G	I < S F < FG
Cake	7.0 ± 0.3	7.1 ± 0.4	7.1 ± 0.4	7.0 ± 0.3	7.0 ± 0.4	n.s.	
Viscosity (cps)	263.8 ± 24.2	228.8 ± 6.5	213.8 ± 10.7	196.3 ± 9.8	179.4 ± 54.1	FG,I,G < S FG < F	I < F < S
Baking loss (%)	7.9 ± 1.3	7.2 ± 0.9	7.6 ± 0.2	7.5 ± 0.3	7.7 ± 0.4	n.s.	
Moisture (%)	27.4 ± 1.4	28.0 ± 0.5	31.0 ± 0.4	28.4 ± 0.8	30.9 ± 0.5	S,F,I < FG,G	
Index to volume (cm)	3.7 ± 0.6	3.3 ± 0.6	2.8 ± 0.1	3.3 ± 0.6	3.2 ± 0.6	G < FG < F,I < S	
Shear press[b] (lb force/g)	1.9 ± 0.04	1.7 ± 0.2	1.1 ± 0.07	1.5 ± 0.1	1.4 ± 0.1	G < I,F,S, FG < S	G < FG I < S
Crumb color[c]							
L	84.8 ± 2.9	81.5 ± 0.5	85.1 ± 0.3	82.7 ± 2.9	83.2 ± 0.8	F < I,FG < S,G	
a_L	-1.8 ± 0.9	-0.6 ± 1.4	-1.5 ± 0.7	-1.0 ± 1.1	-1.2 ± 0.8		F < G,S; I < S
b_L	21.0 ± 2.2	23.1 ± 1.9	22.4 ± 1.6	23.2 ± 1.6	22.0 ± 0.5	n.s.	

[a]Values underscored by the same line are not significantly different (Duncan, 1957).

[b]Allo-Kramer shear press.

[c]Hunter color-difference meter values taken 3/4-inch from bottom of cake. For description of measurements, see text.

fructose/glucose; HFCS-containing cakes were more ($P < 0.05$) tender than sucrose-containing cakes. Among the 5 treatments, the glucose-containing cakes were the most tender and also the most moist, suggesting a relationship between solubility and water-binding-capacity. However, for the number of replications it was not possible to establish that there was a linear correlation ($r = -0.36$) between moisture and tenderness.

L values (lightness) for interior color measurments using the Hunter Color Difference Meter 3/4-inch from the bottom of the cake sample showed fructose-containing cakes were darkest ($P < 0.01$). Cakes prepared with HFCS and fructose/glucose were in turn darker ($P < 0.01$) than cakes prepared with sucrose or glucose. Fructose-containing cakes had higher a_L values (least green) than cakes prepared with glucose or sucrose while HFCS-containing cakes were less ($P < 0.01$) green than sucrose-containing cakes. The b_L values (yellowness) showed no significant differences due to sugar used.

Sensory Evaluations

The analysis of variance showed no significant differences among replications for any of the quality characteristics evaluated by the taste panel; however, differences were noted among variables (Table 4). Cakes prepared with glucose scored lower for each of the sensory quality characteristics than did cakes prepared with other sugars. Each of the 4 remaining cakes were judged acceptable by the taste panelists as reflected in average scores of 4 or 5. However, the large standard deviations indicated a range in scores and, no doubt, contributed to the lack of significance.

TABLE 4.--Averages, standard deviations and statistical analysis of sensory evaluations of quality characteristics of butter-type cakes prepared with monosaccharides and sucrose.

Quality Characteristic	Monosaccharides and Sucrose Scores[a]					Significant Differences[b]	
	Sucrose S	Fructose F	Glucose G	HFCS I	Fructose/ Glucose FG	P < 0.01	P < 0.05
Grain	4.4 ± 0.7	4.3 ± 0.8	2.5 ± 0.5	4.4 ± 0.7	4.2 ± 0.9	G < FG,F,S,I	
Crumb color	4.4 ± 0.5	4.5 ± 0.3	4.1 ± 0.7	4.9 ± 0.4	4.7 ± 0.5		G < FG,I
Silkiness	4.6 ± 0.7	4.2 ± 0.4	3.2 ± 0.6	4.2 ± 0.9	4.2 ± 0.6	G < F,FG,I,S	
Tenderness	4.5 ± 0.7	4.7 ± 0.1	3.7 ± 0.5	4.7 ± 0.8	4.5 ± 0.3	G < S,FG,I,F	
Flavor (crumb)	4.3 ± 0.1	4.1 ± 0.1	2.9 ± 0.7	4.3 ± 0.0	4.1 ± 0.4	G < FG,F,S,I	
Flavor (crust)	4.1 ± 0.5	4.1 ± 0.6	2.8 ± 0.5	3.4 ± 0.4	3.7 ± 0.7	G < FG,S,F	G < I

[a]Score of 5 is high.

[b]Values underscored by the same line are not significantly different (Duncan, 1957).

Cakes prepared with glucose scored lower ($P < 0.01$) in grain than did cakes prepared with other sugars. The panelists indicated glucose-containing cakes had small to extremely small, compact cells while scores given to other cakes indicated fine, regular, evenly distributed cells with thin cell walls. However, the large standard deviations indicated a wide variance in the opinions of the panelists.

Cakes prepared with HFCS scored highest in color of crumb but differed significantly ($P < 0.05$) only from cakes prepared with glucose (Table 4). HFCS-containing cakes were creamy yellow throughout although slight browning or a pale color was indicated in some of the scoring. Scores given fructose/glucose-containing cakes indicated a slightly pale creamy yellow throughout as did the scores for cakes prepared with sucrose. For fructose- and glucose-containing cakes, some of the panelists indicated a slight brownish cast to the color while other panelists indicated a slightly pale color for these cakes.

Panelists were asked to evaluate silkiness or the sensation ascribed to the feel of the surface of a cut slice of cake by rubbing lightly with a finger across the freshly cut surface. Glucose-containing cakes scored lowest ($P < 0.01$) in this characteristic. A mean score of 3.2 indicated that panelists found the cake "soggy." Since the objective measure of moisture content (Table 3) was not significantly different between the glucose and fructose/glucose cakes, this probably reflected the compact grain of these cakes. A mean score of 4.2 on the score-card marked "slightly dry" was

chosen by the panelists to describe the texture of cakes prepared with HFCS, fructose and sucrose while scores for fructose/glucose-containing cakes indicated a slightly soggy texture.

Cakes prepared with HFCS, fructose, sucrose and fructose/glucose showed no significant differences in tenderness according to the panelists. These cakes were very slightly resistant to cutting or biting while glucose-containing cakes were more ($P < 0.01$) resistant when tested by the panelists.

Glucose-containing cakes scored lower ($P < 0.01$) in crumb flavor than cakes prepared with other sugars. The standard deviations for crumb flavor were among the lowest for all parameters tested and suggested that there was good agreement among the panelists for this characteristic. The crust flavor of cakes prepared with sucrose and fructose was preferred followed by those prepared with fructose/glucose, HFCS and glucose, in the order given.

Frequency of flavor ratings for interior and crust portions of butter cakes are shown in Table 5. A score of 4 or 5 was considered acceptable while a score of 3 or less was considered unacceptable. Except for the cake made with glucose, the panelists' flavor reactions to the interior of the cakes were very similar. For all of these other cakes, more than 80% of the scores for interior flavor were 4 or 5 indicating an acceptable flavor whereas only a third of the ratings listed the glucose-containing cake in that category. The interior portion of cakes prepared with glucose was scored as unacceptable (rating on score-card of 3 or less) by

TABLE 5.--Frequency of flavor responses to interior portion and crust of butter-type cakes by 11 panelists with 3 replications of each cake.

Score[a]	Cakes Prepared With				
	Sucrose	Fructose	Glucose	HFCS	Fructose/ Glucose
Interior portion					
5	21[b] (63.6%)	13 (39.4%)	3 (9.1%)	16 (48.4%)	12 (36.4%)
4	6 (18.2%)	15 (45.5%)	7 (21.2%)	12 (36.4%)	15 (45.5%)
3	3 (9.1%)	3 (9.1%)	14 (42.4%)	3 (9.1%)	4 (12.1%)
2	2 (6.1%)	2 (6.1%)	5 (15.2%)	2 (6.1%)	1 (3.0%)
1	1 (3.0%)		4 (12.1%)		1 (3.0%)
Crust					
5	14 (42.4%)	8 (24.2%)	1 (3.0%)	8 (24.2%)	5 (15.2%)
4	14 (42.4%)	20 (60.6%)	8 (24.2%)	11 (33.3%)	21 (63.6%)
3	1 (3.0%)	4 (12.1%)	16 (48.4%)	5 (15.2%)	4 (12.1%)
2	1 (3.0%)	1 (3.0%)	3 (15.2%)	6 (18.2%)	1 (3.0%)
1	3 (9.1%)		3 (9.1%)	3 (9.1%)	2 (6.1%)

[a]A score of 5-4 indicated flavor acceptability; 3 or less was rated as unacceptable.

[b]N = 33.

69% of the taste panelists. The flavor was indicated as being tart or sharp, and lingering (score of 2 or less) by 27% of the panel members.

The crusts of cakes containing sucrose, fructose or fructose/glucose as the sweetening agent received favorable or acceptable ratings for flavor in over 75% of the responses. For the sucrose-containing cake, 42% of the panelists chose the score of 5 from the score-card which indicated that the flavor of these cakes was judged as having a delicately sweet, caramelized flavor. However, 42% of the panelists also detected a slightly tart flavor (score of 4) in the crust of sucrose-containing cakes. The crusts of the cakes prepared with HFCS received flavor ratings less favorable (42% of the responses scored 3 or below) than the cakes containing fructose/glucose (21% of the responses scored 3 or below). Glucose-containing cakes scored lowest in crust flavor with 73% of the responses indicating a tart, sharp lingering flavor.

DISCUSSION

Cakes prepared with glucose showed a lack of structure as reflected in a small volume and compact, soggy texture. In addition, glucose-containing cakes scored lower for each of the sensory quality characteristics than did cakes prepared with other sugars (Tables 3 and 4).

Miller and Trimbo (1957) reported that delayed gelatinization of starch granules in white cake formula resulted in a thinner batter and a loss of volume of the baked product. These authors showed that gelatinization of starch was delayed by a high sucrose-low water ratio (140% sugar with 80% water based on the weight of the flour) while monosaccharides and potato starch used in the cake formula resulted in earlier starch gelatinization.

In the present study, a low sugar-water ratio of 1:1 was used which produced an acceptable product when sucrose was the sweetener used. However, when glucose was used at the same concentration by weight for the sucrose in the cake formula, a "dip" appeared in the center of the baked cake with subsequent loss of volume. The explanation for this observation may reside in differences in the solubility of the sugars. Since glucose is the least soluble in water of the sugars used, it would require more water from the batter to solubilize the glucose. Consequently, there would be less water in the glucose-containing cakes for the

hydration of other ingredients important in cake structure and volume. Photomicrographs (Appendix B) taken of suspensions of interior portions of cakes did, indeed, show a greater number of ungelatinized (birefringent) starch granules in the glucose-containing cake as compared to the sucrose-containing cake.

Glucose-containing cakes were judged as resistant to cutting by taste panel members while objective measurements showed that these cake samples required the least amount of force to shear. This apparent disagreement between sensory scores and objective measurements for tenderness for glucose-containing cakes is, no doubt, associated with the lack of structure of these cakes as reflected in the small volume and compact, soggy texture.

In cakes made with fructose, interior color measurements were darkest (low L values) and most red (a_L values nearest to a positive value) (Table 4) which suggest increased browning reactions. These differences in color probably resulted from chemical alterations in the sugars during baking, reportedly due to the Maillard browning reaction (Kinsella, 1971). Rooney, Salem and Johnson (1967) reported that the amount of carbonyl compounds produced and the rate of browning in aldose-amino acid reactions are determined mainly by the sugar type.

Miller, Nordin and Johnson (1957) reported that excessive browning may be minimized when sweetening agents containing reducing sugars are substituted for sucrose in butter-type cakes by maintaining the pH of the cake crumb at approximately 6.3. In the

present study, the pH of the cake crumb for all cakes ranged from 7.0 to 7.1.

Hodge, Mills and Fisher (1972) pointed out that desirable and undesirable flavor compounds can be produced by caramelization of sugars or by other ingredients of cake as well as by aldose-amine reactions. The results of the analyses of flavor scores (Table 5) suggest that more undesirable flavor compounds were present in crusts of cakes produced by monosaccharides than by sucrose.

The results of the cakes prepared with HFCS received flavor ratings for crust less favorable than the cakes containing fructose/glucose. A possible explanation for the high percentage of the unfavorable responses to the crusts of these cakes may be the higher concentration of glucose in the sirup than in the crystalline fructose/glucose mixture. The HFCS had a ratio on a dry weight basis of glucose and glucose polymers to fructose of 48:42 whereas the crystalline monosaccharides were mixed in a 50:50 ratio.

The ability of the panelists to detect undesirable flavors differed significantly ($P < 0.01$). Three of the 11 panelists did not indicate the presence of undesirable flavors in the crusts of glucose-containing cakes while these 3 plus 3 other panelists did not detect these undesirable flavors in the interiors of the cakes. The percentage (30%) of the panel members who were insensitive to a bitter flavor in the crusts of the glucose-containing cakes is similar to the response (28-40% taste insensitive) to the bitter flavor of phenyl-thiocarbamide found among the American population (Amerine et al., 1965).

SUMMARY

This study investigated the use of sucrose, fructose, glucose, high fructose corn sirup (HFCS) and equal weights of fructose and glucose in cakes containing fat and prepared by the solution method of mixing.

Mean pH's of cake batters prepared from various sweeteners were significantly different and ranged from 7.8 to 8.3 and mean batter viscosity ranged from 179.4 to 263.8 cps. However, no significant differences attributable to the type of sugar used in the formula were noted in pH of the baked cakes. All cakes lost 7.2 to 7.9% moisture during baking. Baked cakes prepared with glucose and fructose/glucose contained 31% moisture whereas cakes prepared with other sugars had 27 to 28% moisture. Cakes containing any of the monosaccharides had a measured volume index of 2.8 to 3.3 cm while sucrose cakes measured 3.7 cm. The most tender cakes were those prepared with glucose, fructose/glucose, HFCS, fructose, and sucrose, in that order, as measured objectively with the Allo-Kramer shear press. Cakes prepared with fructose, when compared to other cakes, were darkest in crumb color as indicated by Hunter Color Difference meter L or lightness values of 81.5 compared to 82.7 to 85.1 for all other cakes. The a_L or green-red values also supported these trends and suggest that browning reactions occurring during baking of fructose-containing cakes were greater than for all other cakes.

Subjective scores for grain, crumb color, silkiness and tenderness indicated that equally acceptable cakes could be prepared with sucrose, fructose, fructose/glucose and HFCS. However, cakes prepared with glucose were evaluated as being more compact, soggy and less tender than other cakes.

Cakes prepared with glucose and HFCS scored lowest in crust flavor. Ranked in order of decreasing preference for the interior flavor were cakes prepared with sucrose, HFCS, fructose, fructose/glucose and glucose. The crust flavor of cakes prepared with sucrose followed by those prepared with fructose, fructose/glucose, HFCS and glucose was preferred in the order listed. The undesirable flavors present both in the interior and crust of the glucose-containing cakes influenced the panelists to rate this product as unacceptable. The differences in the ability among the panelists to discern undesirable flavor compounds was highly significant and consistent between replications.

Based on these data, it is concluded that acceptable cakes could be prepared with fructose, fructose/glucose, and HFCS despite the slightly smaller volume of these cakes as compared to cakes prepared with sucrose. However, cakes prepared with glucose were not considered acceptable due to undesirable flavors which consistently influenced the composite product evaluation by taste panel members.

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PART III

CARIOGENICITY OF MONOSACCHARIDES AND A SACCHARIDE SIRUP IN DIETS OF RATS

INTRODUCTION

A number of manipulations, including dietary ones, produce changes in the type and size of carious lesions seen in the rat, or in the human. Laboratory animal experiments allow testing, in a relatively short period of time, of methods for the control of dental decay on carious lesions which appear similar to lesions seen in the human.

Evaluation of the caries potential of foods for humans on the post-eruptive tooth of the experimental animal model requires a caries-test challenge to all tooth surfaces with careful controls for microbial and genetic conditions. With these precautions, the influence of dietary foodstuffs on the course of the cariogenic process can be assessed. The objective of this investigation was to determine the comparative effects of simple sugars (sucrose, fructose and/or glucose) and a manufactured liquid sweetener, HFCS, incorporated into cake products on the incidence of dental caries in Osborne-Mendel rats fed these rations.

LITERATURE REVIEW

The following review includes (1) cariogenicity of dietary foodstuffs tested in an experimental model system using albino rats and S. mutans and (2) the enumeration of factors which alter dietary effects on the carious process.

Testing for cariogenicity of foodstuffs in laboratory animal experiments is advantageous for several reasons. It can lead to a better understanding of the carious process by differentiation of the effects of properties of foods as well as single nutrient content. The appearance or location of lesions may be affected by the level as well as the treatment of nutrients before incorporation into the diet. Also, the physical properties of foods may influence the caries rate by their effect on retention on tooth surfaces.

Cariogenicity of Foodstuffs

The relative cariogenicity of diets containing 67% manufactured biscuit products (5-21% sucrose on a dry-weight basis) was determined by Green and Hartles (1966). Finely ground biscuits were used as a replacement for the 67% sucrose in a cariogenic ration. The biscuit diets contributed up to 14% simple sugar (13% sucrose) and 36% starch as well as 10% fat and 5% protein. Fissure caries were evaluated on the first and second mandibular molars using 14

weanling rats/group which were fed the diets for 31 days. The sucrose diet produced significantly ($P < 0.01$) more sites of decalcified enamel than the biscuit diets. Of the 12 fissure surfaces at risk per rat, a mean of 6 were carious in animals fed the high sucrose diet as compared to 4 sites of decay in biscuit-fed animals. Although the carious process was decreased by substituting biscuits for sugar, the texture of the 2 rations also differed markedly. In addition, fat levels in the diet varied. The original diet contained 5% fat; this was supplemented by fat present in the biscuit to a level of 15% fat present in the biscuit diet.

Stephan (1966) reported results of a clinical study of 80 patients with rampant dental caries. The most frequent dietary finding was a history of frequent or excessive eating of sugar-containing foods, especially between meals. However, no single food or groups of foods were identified as being commonly consumed by most patients.

To further identify the cariogenic properties of foods, 53 of the representative foods commonly eaten by the patients were selected for further study. Eight Osborne-Mendel rats/group were fed basic control diets during two 1-hour feeding periods per day; in the experimental groups one of the test foods was added ad libitum in conjunction with the two 1-hour feeding periods containing the basic diet. Results showed that the most cariogenic foods had a relatively high fermentable sugar content and included foods both natural (fresh and dried fruits) as well as prepared (baked goods and soft drinks).

Caries scores produced in groups of rats fed the experimental basic cariogenic diet (diet 585 with 25% cane sugar of Stephan et al., 1952) 24 hours a day were comparable in cariogenicity to those diets supplemented with foods consumed by humans. These animal experiments lend support to the clinical observations on the effect of food practices on the caries process. In addition, the need for the development of a greater variety of noncariogenic foods was suggested as a positive measure to be explored.

The effects of cooking ingredients of sweet biscuits (39% sucrose) and the subsequent texture effects on cariogenicity were investigated by Grenby and Paterson (1972). Animals were fed, for 8 weeks from weaning, diets containing the same ingredients either in the baked-biscuit or non-processed (control) form. The rations contained 24% sucrose. Among male Osborne-Mendel rats (8/group), more caries were produced from the biscuit diet than from the control diet. The lesion sites were primarily in the molar fissures. The nature of the oral bacterial flora was not specified.

Carious Process and Factors Influencing It

Cariogenic Streptococci in the Rat

Human cariogenic streptococci derived from human teeth are designated on the basis of colonial morphology as strain A (Group AHT), strain B (BHT) and culture H (HHT). Strains cariogenic in the rat resemble the human cariogenic strain BHT; and on plating media (Mitis Salivarius and Trypticase soy agar), colonies exhibit a raised, rough, crumb-like appearance (Zinner and Jablon, 1968).

Strain BHT induces coronal caries in the rat (Jordan and Keyes, 1966) and is a culture similar to rat streptococcus FA-1 (Fitzgerald et al., 1960). This rat strain of streptococcus had been isolated from swabbings of the teeth of a Sprague-Dawley rat receiving a cariogenic coarse cereal, 25% cane sugar diet (diet 585) (Fitzgerald and Keyes, 1965).

This organism was micro-aerophilic and optimum growth occurred at 37°C in 95% nitrogen and 5% carbon dioxide. The strain produced acid but not gas in 24-48 hours from media containing glucose, fructose and sucrose while starch was not hydrolyzed. The final pH attained with growth in the presence of fermentable carbohydrates was approximately 4.3. This organism did not have proteolytic activity (Fitzgerald et al., 1960).

Streptococcus mutans strain K1-R has been used for production of smooth surface and sulcal lesions in the rat (Larje and Larson, 1970). This strain had been isolated from molar scrapings from a hamster that had been infected with human plaque material (Jordan and Keyes, 1966). The organism grew best in an atmosphere of 95% nitrogen and 5% carbon dioxide at 37°C and formed long tangled chains in thioglycollate broth media. These strains also produced acid but not gas from fructose, glucose, and sucrose. The final pH in glucose- or sucrose-containing media was 4.1 to 4.3 after 48 hours. Starch was not hydrolyzed. This strain of streptococcus showed no proteolytic activity as judged by the inability to liquify gelatin or peptonize milk (Fitzgerald and Keyes, 1960).

Use of Penicillin in Caries Research

Antibiotics have been used in caries research in animals (relative gnotobiosis) to limit specific bacterial activity and to permit control of the carious process. Acid-producing microflora conducive to multisurface caries in the rat can be suppressed with antibiotics with primarily a gram-positive spectrum of activity such as penicillin (Fitzgerald, 1955). Fitzgerald (1972) has demonstrated the *in vitro* inhibition of growth of *Streptococcus mutans* by penicillin. The anti-microbial mode of action of penicillin is related to the inhibition of bacterial cell-wall synthesis (Gibbons, 1968).

Nutrient Factors

Foods may alter susceptibility to dental caries by means of individual nutrient effects as well as by dietary influences due to physical or chemical properties. Assessing the role of dietary foodstuffs requires caries-promoting diets that fulfill nutritional requirements for growth and maintenance of the experimental animals as well as allowing for the growth of cariogenic flora responsible for the carious lesion.

Using sucrose at a level of 56% and 28% of the diet, Frostell *et al.* (1967) studied multisurface caries under known caries-test conditions in Osborne-Mendel rats using cariogenic microflora (from caries-active rats). The low sucrose diet (28%) had a less carious effect on both fissure and smooth surfaces than the 56% sucrose diet. In addition, monosaccharides and starch fed

at a level of 56% of the diet had a less active cariogenic effect than the equivalent sucrose-containing diets.

Green and Hartles (1970) have suggested that a level of sucrose at approximately 24% in the diet of rats might produce a maximum cariogenic effect. Although increasing the level of sucrose in the diet increases tooth decay, significant differences were not found between the total number of lesions produced by diets containing 70% sucrose as compared to 35% sucrose plus 35% starch diets. At levels of 17.5% sucrose/52.5% starch and with lower quantities of sucrose, the cariogenic effect of the diet was decreased.

Proteins have a cariostatic effect upon development of smooth-surface lesions in the newly erupted teeth in animals (reviewed by Nizel, 1970). Bavetta and McClure (1957) reported a reduction in incidence from 70% to 20% carious rats if the animals were fed a diet with 24% casein rather than 13% (diets also contained 18% glucose plus 18% lactose). No pronounced differences in the rate of growth (daily gain of Sprague-Dawley rats of 2.6 and 2.8 g, respectively) were noted as the protein content was increased. Under similar conditions (McClure and Folk, 1953), the use of 35% commercial non-fat dry milk solids (13% protein) that had been spray processed (low heat treatment with a high dispersibility of solids in water) resulted in a caries incidence of 13% with an average daily weight gain of 2.11 g. The relative insolubility of casein in oral fluids has been suggested as promoting formation of dental plaque and development of smooth-surface caries

due to the stickiness and retentive qualities (Shaw, 1966). Cariostatic properties have also been reported (reviewed by Nizel, 1970) with supplementation of the amino acids L-lysine, L-arginine, or L-histidine in otherwise cariogenic diets that contained minimum protein (10%) and low sugar content (18%).

Dietary fats in animal studies have been reported to reduce caries incidence (reviewed by Volker, 1956). McClure et al. (1956) reported that a 13% substitution of Crisco for starch in diets of rats reduced smooth-surface caries significantly. The diet containing 45% cornstarch and 18% glucose was fed to weanling Sprague-Dawley rats for 90 days. Weight gain for rats fed the control diet was 0.6 g/day and weight gain for rats fed the experimental diet was 0.4 g/day. Green and Hartles (1966a) reported that the addition of 5% groundnut oil (using a 67% sucrose diet) significantly reduced total caries in the albino rat. Weight gain from weaning (22-28 days) for the experimental period of 20 days was 54 g for the control diet and 59 g for the experimental diet.

MATERIALS AND METHODS

Experimental Design

Fifty male Osborne-Mendel rats were randomly distributed at 20 days of age among 5 diet treatments so that littermates were not all in the same diet group. The rats were bred in the laboratory of the Department of Food Science and Human Nutrition and were offspring of NIH stock. The original NIH colony of Osborne-Mendel rats was Caesarian derived and then infected with 4 organisms. These were rodent strains of 2 lactobacilli, an enterococcus and a bacteroid (Fitzgerald and Larson, 1971). Presumably, the animals in the Department of FSHN carried these same organisms since the colony is maintained under conditions which minimize entry of extraneous bacteria.

Five experimental groups of 10 rats each were fed diets (Table 6) which contained one of various dried crumbled cakes. These cake rations consisted of 70% by weight of dried cake crumbs (Thompson et al., 1974) made from 1 of the following 5 sugars: (1) sucrose, superfine quality (S), (2) fructose (F), (3) glucose (G), (4) a high fructose corn sirup (HFCS)[1] and (5) a mixture of equal parts of crystalline fructose and glucose (FG). Hereafter, these rations will be referred to as S, F, G, HFCS and FG,

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[1] ISOMEROSE^{R} 100 (HFCS) was generously provided by Clinton Corn Processing Co., Clinton, Iowa.

TABLE 6.--Composition of cake rations.

Ingredients	Percent by Weight
Cake[a] crumbs, dried and sifted[b]	70.0
Skim milk powder[c]	15.0
Sugar, same as in cake[a]	5.0
Yeast powder[d]	4.0
Dehydrated alfalfa meal[e]	3.0
NaCl	2.0
Liver powder[f]	1.0

[a]Cakes were made using one of the following: superfine sucrose, fructose, glucose, 1:1 ratio of fructose and glucose and ISOMEROSE^{R} 100 (HFCS).

[b]Cake formula with 34-37% moisture removed. During baking, 7-8% of the water was lost; the cake was then grated into small pieces and placed in a 7°C oven to dry the sample to the percent moisture loss as indicated above. The dried cake was then sifted through an 1/8-inch screen prior to mixing with other ingredients in ration.

[c]Instant non-fat dry milk purchased from Carnation Co., Los Angeles, Calif.

[d]U.S.P. Brewers yeast purchased from E. R. Squibb & Sons, Inc., New York, N.Y.

[e]Alfalfa meal from Consolidated Milk, Inc., Blissfield, Mich. Composed of crude protein, 17%; crude fat, 1.5%; and crude fiber, 27%.

[f]Liver powder from Nutritional Biochemicals, Cleveland, Ohio.

respectively. The baked cake was grated into small pieces, weighed and then placed in a 7°C oven to dry the sample until 34-37% of the moisture had been removed. The dried cake was then put through an 1/8-inch screen before combining with other ingredients in the ration.

The protein level in the dried cake [4.5% protein derived from eggs (1.6%), milk (0.9%) and flour (2.0%)] was supplemented by skim milk powder in the diet so that the ration fed to the rats contained 12.9% protein (Table 7). The ration contained 14.2% fat; the primary fat source was from the partially hydrogenated vegetable shortening (Crisco) used in the cake. Total carbohydrate in the ration was present at the level of 66.6% with 32.5% of this total present as the simple sugar that was under study (Table 7).

Additional sugar (Table 6) was added to the cake ration at a 5% level (the same type of sugar as present in the cake) to increase the sugar level from 27.5% found in the dried cake to 32.5% present in the diet. This was done to assure sufficient substrate to permit evaluation of caries differences between monosaccharide-containing diets. Sodium chloride was added to the cake ration to promote a longer, more frequent and slower feeding period in order to enhance dietary substrate effects (König, 1964).

The S ration contained 4.42 kilocalories per gram of diet (Table 7) while the monosaccharide-containing rations provided 4.35 kilocalories per gram. The protein:kilocalorie ratio in the diets, as calculated from the mg of dietary protein consumed per kilocalorie, was 29.2 for the ration containing cake made with sucrose

TABLE 7.--Percentage of carbohydrate, protein and fat in the partially dried cake rations.

Component	Percentage
Protein[a]	12.9
Fat[b]	14.2
Carbohydrate (32.5% sugar)[c]	66.6
Kilocalories/g[d]	4.35-4.42

[a]Dried cake (70 g) supplied 1.96 g protein from cake flour, 0.9 g protein from whole milk solids, 1.68 g protein from egg; skim milk powder (15 g) supplied 5.3 g protein; dried yeast (4.0 g) supplied 1.9 g protein; alfalfa meal (3.0 g) supplied 0.5 g protein; and liver powder (1.0 g) supplied 0.7 g protein. (Agriculture Research Service, Handbook No. 8, 1963; National Academy of Sciences, 1972.)

[b]Dried cake (70 g) supplied 11.3 g fat from Crisco, 0.21 g fat from cake flour, 0.9 g fat from whole milk solids, 1.5 g fat from eggs; dried yeast (4.0 g) supplied 0.04 g fat; and liver powder (1.0 g) supplied 0.16 g fat. (Values obtained from Agriculture Research Service, Handbook No. 8, 1963; National Academy of Sciences, 1972.)

[c]Dried cake (70 g) supplied 27.5 g carbohydrate from sugar, 20.8 g carbohydrate from cake flour, 1.3 g carbohydrate from whole milk solids, 0.1 g carbohydrate from egg, 0.36 g carbohydrate from baking powder; added sugar (5 g) supplied 5 g carbohydrate; skim milk powder (15 g) supplied 7.7 g carbohydrate; dried yeast (4.0 g) supplied 1.6 g carbohydrate; alfalfa meal (3.0 g) supplied (2.1 g) carbohydrate; and liver powder (1.0 g) supplied 0.14 g carbohydrate. (Values obtained from Agriculture Research Service, Handbook No. 8, 1963; National Academy of Sciences, 1972.)

[d]Values used for calculating kcal were 9, 4 and 4 for 1 g of protein, complex carbohydrate and fat, respectively. The energy value of monosaccharide is calculated as 3.68 kcal/g and sucrose is calculated as 3.87 kcal/g (Merrill and Watt, 1955).

and 29.6 for the rations-containing cake made with the monosaccharides.

The rats were housed by littermate-pairs in metal screen suspended cages. The environment was controlled with 12 hours of light of each 24 with a temperature maintained at 23±1°C. Diets and distilled water were available ad libitum for a period of 15 weeks.

Every week from age 20-111 days, rats were weighed and food and water intakes recorded. Food in porcelain cups was weighed and placed in each cage for each pair of rats. At the end of the week, the record of weekly food intake was determined by subtracting the weight of the cup and remaining contents from the original weight of the filled food cup. Values for food consumption were adjusted for spillage by adding the spilled ration collected on a paper towel under each cage to the cup weight at the end of the week. Fluid intake was determined by weighing cylindrical glass water bottles equipped with ball-tipped drinking spouts when filled and subtracting from this weight the weight of bottle and spout at the end of a week. Minimum leakage was measured in 2 sample bottles over a period of a week. Leakage per day consisted of 0.2% (1.4 g/100 g water/7 days).

At 116 days of age, the rats were sacrificed by decapitation. Food cups were removed at 7:45 A.M. Sacrificing occurred between 8:30 and 10:00 A.M.; animals were sacrificed on a random schedule following the same order used in assignment to diets.

Infection of Rats

Rat litters were weaned at 18 days of age and were fed a grain diet (Schemmel et al., 1972) and distilled water ad libitum for 2 days. The distilled water contained sodium penicillin[2] (0.5 g/100 ml) and was prepared fresh daily. Rats were weaned at an early age to permit maximum cariogenic challenge to tooth surfaces. Penicillin was used to reduce the normal oral flora in the rat.

On the 20th day of life, each animal was weighed and those littermates weighing between 35-40 g were housed by pairs and the experimental ration assigned. Fresh drinking water was provided which consisted of distilled water without the added penicillin. This day without antibiotic was scheduled to permit loss of the penicillin from the gastrointestinal tract before infection of the animals was begun.

Infection was carried out using a pure culture of Streptococcus mutans 6715-15[3] on animals of age 21 through 25 days. A drop (0.1 ml) of the 30-hour working culture (Appendix C) was placed in the mouth of each rat using a 1 ml syringe without needle on the 21st, 23rd and 25th day of age. Throughout this time period of days 21-25, drinking water was prepared fresh daily by adding the pure culture of S. mutans to distilled water (1/100 ml). The

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[2]Penicillin-G (Benzylpenicillin) sodium salt, 25 million units, was obtained from Sigma Chemical Company, St. Louis, Missouri.

[3]Cariogenic streptococci cultures and instructions for maintenance were generously provided by Dr. Rachel H. Larson; Chief, Preventive Methods Development Section; Caries Prevention & Research Branch; NIDH.

remainder of the experimental period, the animals were provided with one of the experimental cake rations and distilled water ad libitum (26-116 days of age).

The animals were exposed to the total cariogenic challenge (diet plus infection) for 95 days (day 21-116). Preliminary experiments showed that 95 days was sufficient to permit accurate identification of decalcified areas within each cake ration. Accurate evaluation of differences in caries activity is based on the number of individual lesions and the extent of each carious lesion. Within this time period, each experimental group of animals showed lesions large enough to be detected yet no group had decayed areas so extensive as to prevent identification of individual lesions.

Scoring Carious Lesions[4]

Preparation of Jaws

Animals were sacrificed by decapitation. The fur and skin were removed with the use of surgical scissors and then the heads were autoclaved at 121-123°C for 5 minutes to facilitate removal of soft tissue. Following these procedures, the skulls were then further cleaned by placing them in a 2% solution of ammonium hydroxide for 30 minutes. The jaws were then rinsed, dried and stored until scored.

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[4]Dr. Rachel H. Larson, NIDH, generously provided the procedures and training for dental scoring and verified the scores used in the present study.

Values for Linear Area of Decay

Three molars in each jaw quadrant were scored for caries by the method of Keyes (1958) to identify the location and the quantitative size of the lesions. Lesions evaluated were located at 1 of the following 4 sites on the tooth: buccal, lingual, proximal and sulci.

The incidence and extent of smooth-surface caries in unstained jaws were determined by examining moist jaws using a Bausch and Lomb dissecting microscope under low power magnification (20 X) while the teeth were rapidly dried under a stream of compressed air. The decalcified enamel dehydrates more rapidly than the sound enamel and appears as chalky white areas which can then be identified. The method of assigning values on the buccal or lingual surface involved judging by eye the extent of decay of enamel using an established (Keyes, 1958) linear scale of enamel units based on the size of the molar (the maximum number of buccal plus lingual linear units in the lower jaw quadrant is equal to 28). At proximal sites, each surface is assigned a linear value of 1 unit (the maximum number of linear units assigned to proximal lesions for the 3 molars per jaw quadrant is equal to 4).

Sulci lesions were evaluated after dyeing the jaws in a saturated solution of Kernechtrot B salt[5] for 17 hours. The wet jaws were then held with a small straight hemostat beneath a slow

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[5]Kernechtrot nuclear fast red dye distributed by Roboz Surgical Instrument Co., 810 18th Street, N.W., Washington, D.C. 20006.

drip of water under magnification of 20 X. The molars of each jaw quadrant were sliced[6] into equal halves at an angle parallel to the bucco-lingual surfaces. The method of assigning values for decalcified enamel on these exposed sulcal surfaces was based on the number of linear surfaces showing dye penetration. Maximum values for total linear sulci area in the lower jaw quadrant is equal to 14.

Analyses of Data

Means and standard deviations were determined for the groups of 10 rats on each of the 5 cake diets in the study. Extent of enamel caries for smooth surfaces (buccal plus lingual plus proximal) and for sulcal surfaces were evaluated for each rat; the summation of these linear values then represented the total linear area of enamel decay for each rat. Analysis of variance was performed by the 3600 Controlled Data Corporation (CDC) computer, Michigan State University.

Significance of differences between mean values for caries was assessed by Dunnett's test (Dunnett, 1955) using sucrose as a control group. The Student's paired t-test (Sokal and Rohlf, 1969) was used for analyses of mean values for growth, intake and wet tissue weights. Correlation coefficients were determined between each caries location (smooth surfaces, sulci surfaces and total surfaces) and for cumulative 13-week food intake, fluid intake and weight gain for rats fed each of the cake rations.

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[6] A steel disc saw, 0.004 inch thickness and 0.75 inch in diameter was mounted on a mandrel held in a straight handpiece of a standard dental engine.

RESULTS

Body Weights

Animals fed each of the 5 cake-containing rations increased in weight as they grew older (Figure 1). Mean live body weights at 111 days of age and body weight gains from 20 to 111 days of age are presented in Table 8. Rats fed the S ration gained a mean weight of 343±34 g and attained a final mean weight of 379±36 g while rats fed diets which included fructose (FG, HFCS, and F alone) gained a mean range of 305±18 to 316±39 g and attained a final mean weight for the 3 groups of 345±30 g, but differences were not significant.

Diet and Fluid Consumption

Rats fed the S cake diet consumed a mean of 109±9 g of diet per week over the 13-week feeding period from age 20 to 111 days (Table 8). Glucose and FG fed animals ate a mean of 114±9 and 111±5 g of diet per week, respectively, while rats fed F and HCS ate a mean of 105±14 and 103±9 g diet per week, respectively. There were no significant differences in mean food intake among the different dietary treatments.

The mean intake of distilled water per week by rats fed either G, S, FG, F or HFCS cake rations was 265±58, 228±28, 220±11, 206±37 and 205±23 g, respectively (Table 8). The fluid intake of rats fed F and HFCS cake diets was significantly ($P < 0.05$) lower than the intake of rats fed G diets.

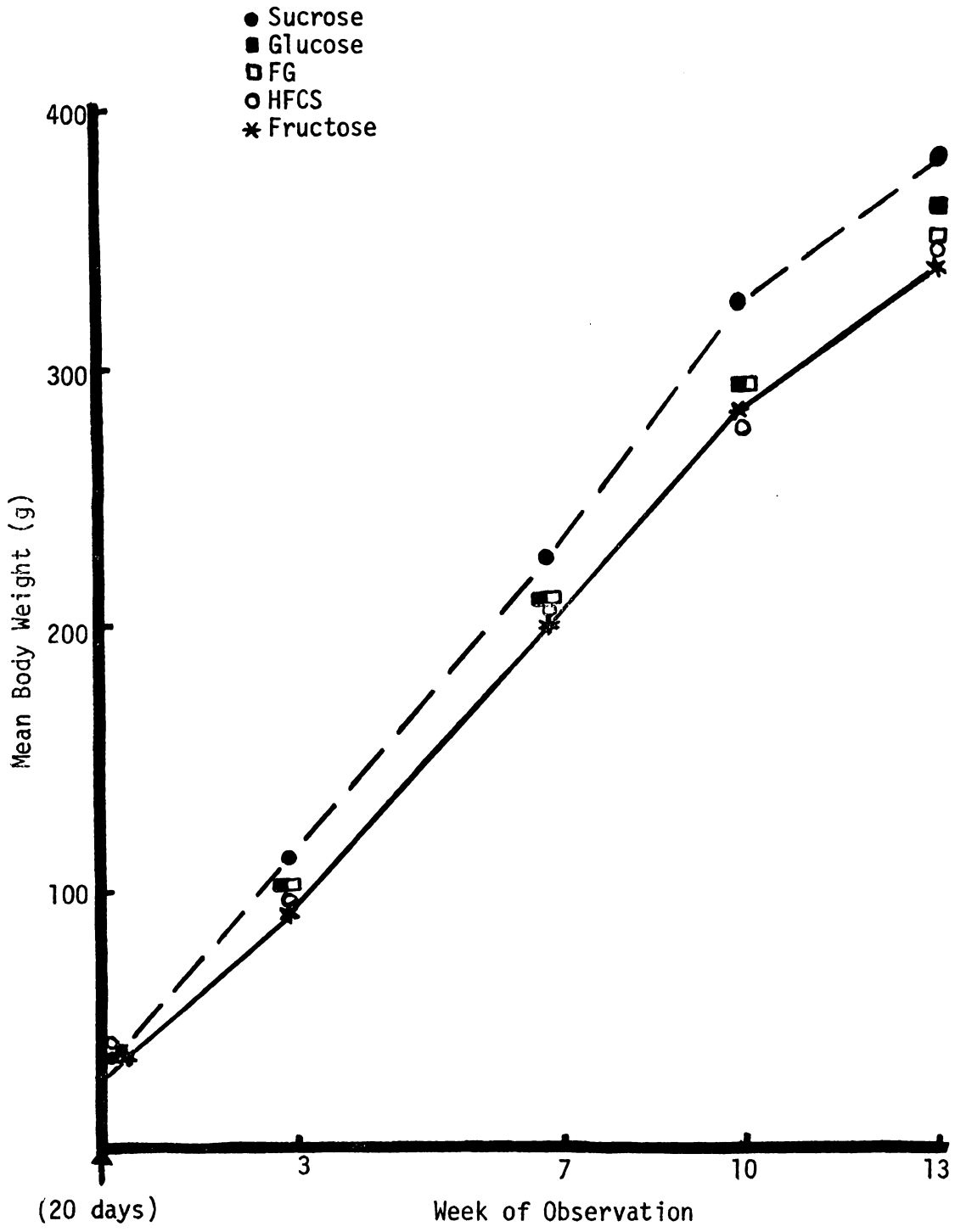

Figure 1.--Mean growth rates of male Osborne-Mendel rats fed diets containing 70% dried cake made from 1 of 5 sweeteners for 13 weeks.

TABLE 8.--Means, standard deviations and statistical analyses in growth of Osborne-Mendel rats fed either a sucrose- or monosaccharide-containing cake diet for 13 weeks.[a]

Parameter[b]	Cake Rations					Significant Differences
	Sucrose S	Glucose G	Fructose F	HFCS I	Fructose/ Glucose FG	
Body weight (g)	379 ± 36	363 ± 39	345 ± 45	346 ± 30	344 ± 20	n.s.
Gain/rat (g)	343 ± 34	323 ± 40	316 ± 39	306 ± 29	305 ± 18	n.s.
Food intake (kcal/week)	482 ± 38	495 ± 41	456 ± 61	446 ± 39	483 ± 21	n.s.
Food intake (g/week)	109 ± 9	114 ± 9	105 ± 14	103 ± 9	111 ± 5	n.s.
Fluid intake (g/week)	228 ± 28	265 ± 58	206 ± 37	205 ± 23	220 ± 11	F,I < G[c]

[a]There were 10 rats/group.

[b]Mean values/rat/111 days of age (13 weeks on diet).

[c]There is a statistically significant ($P < 0.05$) difference between fluid intake means for F and I as each is compared to the mean for fluid intake for G (Student's two-sided paired t-test) (Sokal and Rohlf, 1969).

Dental Caries Incidence

Means, standard deviations and statistical analyses of dental caries incidence are presented in Table 9. From among all treatments, rats fed the S-containing cake ration showed the greatest mean cariogenic effect in terms of the number of carious teeth per animal and the greatest loss of enamel surfaces. The mean number of carious teeth involved was 7.3±4.2. That is, on the average, in any one rat, 7 out of the 12 teeth scored were decayed. However, there was a wide range with one rat free of decay while 5 animals had 8 or more teeth showing some enamel decay; this wide range is reflected in the standard deviation of 4.2. Mean total enamel decay per animal was 20.0±14.8 linear units. A linear unit represents extent of decay out of a maximum arbitrary measure of total surface area of 172 linear units (Keyes, 1958). On enamel smooth surfaces alone, S fed rats showed 10.6±8.9 mean linear units of decalcified enamel from a possible area of 124 linear units. An approximate equal area of decay (9.4±7.9 linear units) was located in the sulci.

Rats fed G and FG cake rations had a mean of 4.5±2.3 and 4.1±2.3 carious teeth per animal, respectively. One hundred percent of the rats fed the G cake ration showed some carious enamel. For these rats, the mean total carious linear area was 8.8±5.4. This decay was approximately equally divided between smooth-surface areas (4.5±4.6 linear units) and sulci areas (4.3±5.4 linear units). Also, 100% of the rats fed FG rations showed 1 or more of the 12 molar teeth decayed. The mean linear area of total enamel involved

TABLE 9.--Means, standard deviations and statistical analyses for dental caries incidence of male Osborne-Mendel rats fed either a sucrose- or monosaccharide-containing cake diet for 13 weeks.[a]

Dental Caries Incidence	Cake Rations					Significant Differences[d]
	Sucrose S	Glucose G	Fructose F	HFCS I	Fructose/ Glucose FG	
Carious teeth (no./rat)	7.3 ± 4.2	4.5 ± 2.3	4.0 ± 3.3	3.9 ± 3.8	4.1 ± 2.3	n.s.
Carious rats (%)	90	100	80	70	100	
Smooth surface[b]	10.6 ± 8.9	4.5 ± 4.6	4.2 ± 3.9	2.5 ± 3.5	3.4 ± 3.9	G, F < S[d,e] I, FG < S[d,f]
Sulci surface[c]	9.4 ± 7.9	4.3 ± 5.4	2.1 ± 2.1	5.1 ± 5.9	4.6 ± 4.9	n.s.
Total surfaces[c]	20.0 ± 14.8	8.8 ± 5.4	6.3 ± 5.7	7.6 ± 8.5	8.0 ± 6.5	G,FG,I < S[d,e] F < S[d,f]

[a]Ten rats/group; 12 molars scored/rat.

[b]Represents lesions expressed as linear areas of enamel surfaces located on buccal + lingual + proximal sites.

[c]Extent of lesions expressed as linear units of enamel on designated surfaces.

[d]Statistical significance from control (sucrose) calculated using Dunnett's test $(\bar{y}_1 - \bar{y}_c) / \sqrt{MS_E \left[\frac{1}{r_1} + \frac{1}{r_c}\right]} \pm t_D \alpha, t, \gamma$ (Dunnett, 1955).

[e]$P < 0.05$

[f]$P < 0.01$

with these FG rats was 8.0±6.5 with an approximate equal division between smooth-surface areas (3.4±3.0) and sulci areas (4.6±4.9).

Eighty and 70% of rats fed the F and HFCS cake rations, respectively, showed evidence of enamel tooth decay. The mean number of carious teeth per animal fed F and HFCS rations, respectively, was 4.0±3.3 and 3.9±3.8. Smooth-surface lesions in rats fed F and HFCS were 4.2±3.9 and 2.5±3.5, respectively; whereas sulci lesions involved 2.1±2.1 linear areas in the F fed rats as compared to 5.1±5.9 linear area in the HFCS fed rats.

Significant differences were noted among treatments in the extent of carious linear areas located on smooth surfaces. Rats fed the S diet had significantly more smooth-surface caries than those fed G and F rations ($P < 0.05$) and FG and HFCS ($P < 0.01$). No differences were noted among variables for carious sulci areas. However, total enamel decay in rats fed the S diet was significantly ($P < 0.05$) higher than total enamel decay in rats fed G, FG and HFCS rations and significantly ($P < 0.01$) greater than total enamel decay in rats fed F diets.

Correlation coefficients (r) were determined (Table 10) between enamel decay at smooth, sulci and total surfaces and food intake, fluid intake and weight gain for rats fed each of the 5 cake diets. For rats fed the G diet, an inverse correlation ($r = -0.64$) was found between total enamel caries and g fluid intake. This association was significant ($P < 0.05$). For rats fed the F cake ration, significant linear relationships were found between enamel decay involving each of the 3 locations on the tooth

TABLE 10.--Correlation coefficients (r) and significance between caries and intakes and weight gain at 13 weeks in male Osborne-Mendel rats fed dried cake diets prepared with 5 different sweeteners.

Caries Location	Food Intake (kcal)	Fluid Intake (g)	Weight Gain (g)
10.1.--Sucrose Cake Ration			
Smooth surface	-0.05	-0.14	-0.18
Sulci surface	0.17	0.01	-0.05
Total surfaces	0.07	-0.08	-0.13
10.2.--Glucose Cake Ration			
Smooth surface	-0.57	-0.09	-0.19
Sulci surface	-0.08	-0.56	-0.44
Total surfaces	-0.40	-0.64[b]	-0.61
10.3.--Fructose Cake Ration			
Smooth surface	0.05	0.69[a]	-0.20
Sulci surface	0.24	0.67[a]	-0.09
Total surfaces	0.13	0.73[a]	-0.17
10.4.--HFCS Cake Ration			
Smooth surface	0.45	0.42	0.90[b]
Sulci surface	0.62	0.60	0.60
Total surfaces	0.62	0.59	0.79[b]
10.5.--Fructose/Glucose Cake Ration			
Smooth surface	0.06	0.13	-0.18
Sulci surface	-0.05	0.14	0.05
Total Surfaces	0.00	0.18	-0.07

[a]Significant relationship between the 2 variables at $P < 0.05$.
[b]Significant relationship between the 2 variables at $P < 0.01$.

(smooth, sulci and total surfaces) and fluid intake. Rats fed the HFCS cake ration showed a highly significant ($P < 0.01$) linear association between smooth-surface caries and weight gain ($r = 0.90$) and total enamel caries and weight gain ($r = 0.79$).

Wet Tissue Weights

Means, standard deviations and statistical analyses of relative weights of wet tissues at sacrifice are shown in Table 11. In increasing order, relative weights of livers from rats fed S, FG, HFCS and F cake rations were, respectively, 3.09±0.2, 3.13±0.4, 3.26±0.2, 3.44±0.4 and 3.74±0.6. Relative weights of liver from rats fed F cake diets were significantly heavier than weights of liver from rats fed FG, G and S cake rations.

Relative weights of kidneys of rats fed F cake rations were significantly larger than kidneys of rats fed all other cake rations. The kidney weights in mg/100 g body weight in decreasing order were for F, HFCS, FG, S and G cake diets and were, respectively, 672±86, 585±48, 579±70, 531±35 and 517±66.

Left inguinal fat depots were heaviest (1.56±0.2 g/100 g body weight) in relative weight in rats fed HFCS cake diets and were lightest (1.27±0.3 g/100 g body weight) in rats fed FG diets. Differences between these mean values for these 2 groups of rats were significant at the level of $P < 0.05$. The experimental error due to dissection techniques of inguinal tissue in male Osborne-Mendel rats weighing 300 g approximates 2% of tissue weight (Schemmel et al., 1970).

TABLE 11.--Means, standard deviations and statistical analyses of wet tissue weights of male Osborne-Mendel rats fed either a sucrose- or monosaccharide-containing diet.

Wet Tissue	Cake Rations					Significant Differences[a]
	Sucrose S	Glucose G	Fructose F	HFCS I	Fructose/ Glucose FG	
Liver[b]	3.09 ± 0.2	3.26 ± 0.2	3.74 ± 0.6	3.44 ± 0.4	3.13 ± 0.4	FG < F[c] G,S < F[d]
Kidneys (2)[e]	531 ± 35	517 ± 66	672 ± 86	585 ± 48	579 ± 70	S,G < F[d] I < F[c] FG < F[f]
Inguinal fat depot[b]	1.52 ± 0.3	1.52 ± 0.3	1.43 ± 0.2	1.56 ± 0.2	1.27 ± 0.3	FG < I[f]
Cecum plus contents[b]	0.95 ± 0.2[g]	1.08 ± 0.4	1.25 ± 0.1	1.05 ± 0.3	1.11 ± 0.2[g]	S < F[d]

[a]Statistical significance determined using Student's two-sided paired t-test (Sokal and Rohlf, 1969).

[b]g/100 g body weight at sacrifice

[c]$P < 0.01$

[d]$P < 0.001$

[e]mg/100 g body weight at sacrifice.

[f]$P < 0.05$

[g]Eight rats/group; remainder of values based on 10 rats/group.

Wet weight of the cecum plus content in g/100 g body weight were smallest from rats fed S (0.95±0.2) as compared to the monosaccharide diets (relative weights for animals fed HFCS, G, FG and F were, respectively, 1.05±0.3, 1.08±0.4, 1.11±0.2 and 1.25±0.1). The mean weights for cecum plus contents were significantly different between rats fed S as compared to rats fed F cake diets.

DISCUSSION

In the present study, the S fed rats weighed 379 g with a daily weight gain from the start of the experimental diet (20 days) to 111 days of 3.8 g. For the rats fed monosaccharides, the weight gain/rat/day ranged from 3.4 g for the F, FG and HFCS diets and was 3.6 g for the rats fed the G ration.

In diets containing highly metabolizable energy (low fiber diet) and containing a digestible protein of high biological value, the protein requirement to support growth of the rat can be expressed on a dry weight basis as a protein level of 12% (whole egg protein) to 13% (casein) and an average protein/kilocalorie ratio of 29 mg protein/kcal of diet (Warner and Breuer, 1972). In the cake rations used in the present study, the fiber content was approximately 0.9% and was derived primarily from the alfalfa. Casein of milk and whole egg protein supplied 61% of the protein in the ration and the mg protein/kcal ratio averaged 29.4.

Full-fed rats have been reported to consume water and food, respectively, at about a 1.9:1 ratio with interdependence between food and water intake (Adolph, 1947). Conversion of the intake values (Table 8) as a ratio of g water/g food for the diet groups S, G, F, FG and HFCS were, respectively, 2.1, 2.4, 2.0, 2.0 and 2.0. The higher ratio for rats fed G rations reflects the significant increase in fluid intake, proportionately more than the

increase in food intake, found in this group. Increased water absorption in the small intestine is associated with active glucose transport by a sodium-carrier transport system located on the membrane of the intestinal cell (Herman, 1974). Decreased carious lesions have been reported by Harris and Stephan (1953) with the consumption of an increased ratio of fluid to diet by rats.

As seen in Table 10.3, rats fed the F ration which drank more fluid had more caries regardless of type; however, total fluid intake for this group was significantly less than total fluid intake ingested by the G fed rats (Table 8). Manly and Dain (1961) have shown that fructose introduced into salivary sediment does lead to polysaccharide formation; these compounds are water soluble (Manly, 1961) and can be rapidly hydrolyzed to acid by oral microflora (Wood, 1964; Gibbons and Banghart, 1967).

Rats fed HFCS diets showed a linear correlation between weight gain and smooth-surface caries (Table 10.4). A look at the individual rats within this group did reveal that the 3 rats that gained the most (mean weight gain of 342 g compared to the remaining 7 rats with a mean weight gain of 261 g) also had the greatest smooth-surface decay (a range of 4-10 linear units compared to a range of 0-2 linear units for the remaining 7 rats in the group). Susceptibility of individual rats within the HFCS group did not appear to be a factor in caries development. Rats showing smooth-surface decay involved 2 litters; however, rats from these same 2 litters fed different diets did not show an increased smooth-surface activity within their respective diet groups.

Stephan and Harris (1955) reported that Osborne-Mendel rats showed no correlation of weight gain and susceptibility to caries. However, with strains of slower growing rats (Sprague-Dawley), the greatest amount of smooth-surfaces caries were found in rats which gained the least weight. In the present study, rats fed HFCS consumed the least food (446±39 kcal/week) and the least fluid (205±23 g/week) among all the dietary treatments (Table 8). Energy utilization for rats fed S, F, HFCS, G and FG for the 13-week experimental feeding period was, respectively, 18.27, 18.76, 18.95, 19.92 and 20.59 kcal/g weight gain.

Liver weight (absolute and relative) was reported by Winnie et al. (1973) as increased in rats fed semipurified rations high in sucrose or fructose compared to rats fed grain, or semipurified rations high in either glucose or cornstarch. These same authors found rats fed semipurified diets high in sucrose had heavier fat depots than rats fed semipurified diets high in glucose or fructose. In the present study, the rats fed F cake rations showed the heaviest relative liver weights (Table 11) while the absolute left inguinal fat depots were the heaviest in rats fed S diets.

Chalvardjian and Stephans (1970) noted that the volume of cecum in dextrin fed rats was 2.5 times larger than those of rats fed sucrose; this was attributed to a change in intestinal microflora. Others (Pronczuk et al., 1973; Tanka et al., 1975) have suggested that Maillard reaction products which pass into the colon are degraded by cecal bacteria. In the present study, weight of

the cecum plus contents (Table 11) was greatest in the rats fed F cake rations. Color changes noted in the fructose-containing cakes also suggested the greatest Maillard browning in these products.

SUGGESTIONS FOR FURTHER STUDY

The present study establishes the feasibility of using food sweeteners which are acceptable as sucrose substitutes in food products such as cakes. Furthermore, feeding trials with rats indicated these products reduce the incidence of dental caries in at least one species of animals. However, investigations into the nutritional consequences of metabolism of the different sweeteners in these cake products warrants continued study. Sodium chloride was included in all the cake diets fed to Osborne-Mendel rats in order to enhance the cariogenic effects by favoring more frequent feeding and elongation of feeding time. Nutritional interpretation of growth data, food and fluid intakes and growth of selected tissues of rats consuming cake rations composed of different sweeteners must necessarily be considered as being comparative within the limits of this study. Grenby and Leer (1974) studied the effects in Osborne-Mendel rats (11 males and 11 females per group) of the administration of drinking water containing 20% sucrose as compared to a 20% solution of spray-dried glucose syrup (dry weight basis contained 19.3% dextrose, 14.3% maltose and 66.4% higher saccharides). While mean daily sucrose intake was less than that of glucose, the rats consuming the sucrose regimen gained more weight and accumulated more body fat and liver fat. Food intake of powdered cereal diets for the groups drinking sucrose and glucose

solutions were, respectively, 12.7 g/rat/day and 13.0 g/rat/day over an 8-week experimental period.

The fact that the cakes prepared from fructose were darker in color than those cakes prepared from the 4 other sweeteners suggested differences in the extent of sugar-amine interactions of the Maillard reaction. To assess any possible color changes caused by reducing sugars, color measurements were made in preliminary experiments on the top crust, on the interior surface 3/4-inch from the bottom and after a 1/8-inch portion had been cut from the bottom of a wedge of cake. Significant differences were found on samples taken 3/4-inch from the bottom of the cake sample and were used to follow color differences between the different sugar variables. Preliminary values taken 1/8-inch from the bottom of the cakes did indicate decreased lightness values for the cakes containing reducing sugars. Top crusts of the cakes prepared with fructose were brownest as indicated by Hunter Color Difference Meter readings although not significantly browner than cakes prepared with sucrose. The overall nutritive effect of these changes due to heat treatment of the protein-carbohydrate components in these food products needs to be further evaluated. Additional study might include the effect of the formation of Maillard reaction products on the digestibility and utilization of the protein in feeding experiments in rats.

SUMMARY

This study investigated the comparative effects of simple sugars and a saccharide sirup in cake rations fed to caries-susceptible Osborne-Mendel rats. There were no significant differences in body weight (mean range of 344±20 in rats fed FG to 379±30 g for rats fed S), in weight gain (mean range of 305±18 in rats fed FG to 343±34 g for rats fed S), and in food intake (mean range of 446±39 for rats fed HFCS to 495±11 kcal/week for rats fed G) among the different dietary treatments (Table 8). The fluid intake of rats fed F and HFCS were significantly lower than the intake of rats fed G diets. The range of values for fluid intake was 205±23 g for rats fed HFCS diets to 265±58 for rats fed G diets.

Dental caries (Table 9) in rats fed S cake rations showed the greatest enamel decay with 7.3±4.2 mean number of carious teeth/rat, 10.6±8.9 mean linear units of enamel decay on smooth surfaces, and 9.4±7.9 mean linear units of enamel decay on sulci surfaces. Rats fed monosaccharide-containing diets showed a mean number of carious teeth/rat ranging from 4.5±2.3 for rats fed the G ration to 3.9±2.8 for rats fed HFCS diets. Enamel decay on smooth surfaces for rats fed monosaccharide cake rations ranged from 4.5±4.6 mean linear units for rats fed G to 2.5±3.5 mean linear units for rats fed HFCS diets. Enamel decay on sulci surfaces in rats fed

monosaccharide diets ranged from 5.1±3.5 mean linear units (rats fed HFCS diets) to 2.1±2.1 mean linear units (rats fed F diets).

Rats fed F diets had the heaviest relative wet weight (Table 11) for liver (3.74±0.6 g/100 g body weight), kidneys (672±86 mg/100 g body weight) and cecum plus contents (1.25±0.1 g/100 g body weight). Left inguinal fat depot relative weights were heavier (1.56±0.2 g/100 g body weight) in rats fed HFCS diets than in rats fed FG diets (1.27±0.3 g/100 g body weight).

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PART IV

DIETARY INTAKE AND SALIVA PARAMETERS

INTRODUCTION

Saliva analysis has been proposed as a simplified research tool for studying nutritional response in humans (Kaniuka, 1971). Updegraff and Lewis (1924) reported that combined urea and ammonia nitrogen levels in saliva reflect approximately 76% of corresponding blood values and for nonprotein nitrogen the saliva values represent 87% of blood values. Holmes (1964) found a pattern of reduced salivary flow in uremic patients which persisted through the first month of the post-transplant period and then gradually increased to a normal range found in control subjects.

Dietary effects on the oral environment can be both of a local or a systemic nature. Nikiforuk et al. (1956) found a significantly higher value for blood urea for 40 caries-free children when compared to 94 control children. A significant correlation was also noted between blood urea nitrogen and salivary urea and ammonia nitrogen. The data were interpreted as a reflection of different dietary habits of the two groups with respect to protein and/or carbohydrate intake.

The objectives of this investigation are to describe changes in salivary parameters associated with various dietary intake of humans. Possible implications for the dietary control of the carious process will be made based on an examination of dental caries experience, salivary flow, salivary composition (urea and glucose), and usual nutrient intake.

LITERATURE REVIEW

A number of manipulations, including dietary ones, produce changes in salivary secretion and composition. The effects of diet on saliva must then be separated from the effects of such non-dietary variables as source of saliva; flow rate; nature, duration and length of stimulation; time of day; and health of the subject (Dawes, 1970).

Saliva Secretion

Mixed Saliva

Saliva is a mixed secretion from the parotid, submaxillary, sublingual and mucous glands. The submaxillary and sublingual glands are called mixed glands because they contain both a mucous type and a serous type of secretory cell. In man, the serous cell predominates in the submaxillary gland while the mucous type is more frequent in the sublingual gland. Saliva from mucous cells is viscous and contains amylolytic enzymes and a high percentage of mucin.

The parotid, the largest of the salivary glands, is composed of serous cells and secretes saliva that is usually colorless, transparent, and of low viscosity. The parotid secretion also contains amylolytic enzymes and small amounts of mucin (Gore, 1938; Kerr, 1961; Eastoe, 1961).

The rate of secretion of saliva from submaxillary glands has been shown to respond readily to mechanical stimulation of the oral mucosa while parotid secretion rate shows a delayed response (Kerr, 1961). Schneyer (1956) collected separately the outflow from the three major glands with suction and found with 43 subjects that 69% of resting saliva volume was derived from the submaxillary glands, 26% was contributed by the parotid glands and 5% derived from sublingual glands while mucosal glands made no significant contribution to total volume.

Resting Flow

Rate.--The resting flow of saliva varies from 0.4 to 1.8 ml per minute (Eastoe, 1961) and is characteristic for individuals over time. Kerr (1961) found that resting rates of secretion of the individual salivary glands fluctuate around a mean value which is reasonably stabilized over a 10-minute period.

Becks and Wainwright (1939) compared resting and stimulated saliva flow in 40 individuals and found that stimulation tends to minimize the differences in rates of flow between different persons. Also, the resting flow was more consistent on different days than was individual response to stimulation. In addition, long collection periods led to greater fluctuation in rate of secretion than that obtained by sampling over a shorter period of time on different days. Thus, mean resting saliva flow has been suggested as being more characteristic of individuals than output of stimulated saliva and thus preferable for investigating differences between individuals with different caries experience.

These same authors (Becks and Wainwright, 1943) observed average rates of flow of resting saliva collected from 661 individuals who were healthy (with the exception of the presence of dental caries). No differences due to sex or age after 10 years of age were reported in resting flow rate among these subjects. In general, slower rates of salivary flow were observed from 5 to 9 years of age (54 individuals).

Collection.--Procedures used to collect secretion of whole mixed saliva with minimal stimulation include tilting the subject's head forward and allowing the saliva to run to the front of the mouth to prevent its being swallowed (Wainwright, 1934). Kerr (1961) compared methods of emptying the mouth by draining, suction or spitting and concluded that spitting at a constant rate was the most efficient and effective method of clearing the mouth of saliva collected.

Flow Rate and Salivary Composition

The composition of mixed saliva can be changed by altering flow rate which leads to changes in relative proportions of the secretions from the individual glands. Time of day has a decided influence on salivary flow rate and composition. Dawes (1970) reported a gustatory effect on saliva samples collected within an hour of consumption of a meal. This author suggested that dietary studies on saliva secretions be made 3 or 4 hours after a previous meal or gustatory stimulation.

Unstimulated salivary flow rate is affected by degree of hydration of the body tissues to a great extent (Dawes, 1970). Holmes (1964) has shown that conditions that lead to dehydration cause a decrease in salivary flow rate. Hyperhydration of tissues has also been shown to increase the flow rate of unstimulated saliva (Shannon and Chauncey, 1967) but not the flow rate of stimulated saliva (deWardener and Herxheimer, 1957).

Properties of Saliva

pH

Pure parotid saliva is more acid than mixed saliva with a pH range from 5.5 to 6.0 (Hardwich, 1949). Resting saliva ranges from pH 5.6 to 7.6 with a mean of approximately pH 6.7 while stimulation produces a more alkaline secretion with a range of pH 7.2 to 7.6 (Eastoe, 1961). Upon standing, pH will rise in saliva if CO_2 is allowed to escape. This change can be avoided by testing unstimulated samples within a few minutes of collection or by collecting samples under oil (Starr, 1922).

Near the normal pH of the mouth, saliva has a pronounced buffer capacity due to the presence of bicarbonate and phosphate ions. The more important buffer ion, quantitatively, in saliva near pH 7.0 is bicarbonate (Leung, 1951).

The buffer capacity power of the saliva has been found to be higher in the afternoon than in the morning (Krashow, 1932). Also, the ability of saliva to neutralize acid is increased with increased flow rate and with stimulation of the salivary glands since these actions increase the bicarbonate content of saliva (Dawes, 1970).

Urea

The combined urea and ammonia nitrogen value of saliva in normal persons is reported to range from 6 to 13 mg per 100 ml (Hench and Aldrich, 1922). Urea is thought to be the main substrate for base formation by dental plaque (Dawes, 1970).

The parotid gland secretes a greater part of the urea present in saliva. Increasing the flow rate of saliva slightly above the unstimulated rate or stimulation with acid, decreases the urea concentration in the parotid saliva (Barnett and Bramkamp, 1929).

Negligible amounts of ammonia are found in parotid secretions (Schmitz, 1922). However, upon standing, ammonia nitrogen in mixed saliva increases while the urea nitrogen decreases. Ammonia nitrogen is derived in the saliva from urea by the action of bacterial enzyme systems (Hench and Aldrich, 1922).

Specific Gravity

The specific gravity of saliva is reported to be 1.002 to 1.020. Mixed saliva contains a variety of nonsalivary components such as gingival crevice fluid, leukocytes and epithelial cells, oral bacteria, and occasionally dental plaque and food debris (Eastoe, 1961). Suspended matter may interfere with analytical determinations whereas centrifuged saliva may alter values for some constituents and has been shown to increase pH values (Starr, 1922).

Glucose

Values obtained for glucose in unstimulated saliva are low, approximately 11-30 mg per 100 ml (Jenkins, 1954); and the quantity of sugar secreted by the salivary glands is negligible in amount (Volker, 1955). With the ingestion of carbohydrate and sugar containing foods, peak values exceeding 300 mg % are reached within 5 minutes and then fall to the original fasting value by 20 minutes (Volker and Pinkerton, 1947).

Values for glucose levels in saliva have been calculated based on analysis for reducing sugars. Volker (1955) measured the *in vivo* saliva sucrose to glucose conversion and found that within 2 minutes saliva-sucrose mixtures increased values calculated as glucose by 4 to 10 times over the control levels of resting salivary glucose.

Dietary Factors

pH

Ingestion of acids and carbohydrate containing beverages in man has been shown to cause a decrease of short duration in the pH of the saliva and plaque (Haggard and Greenberg, 1951). However, plaque obtained after a glucose rinse showed considerable difference with lower pH values over a longer period of time. The difference between the carbohydrate rinse and drink was thought to be due to the increased contact of the glucose rinse with teeth as compared to the time needed for drinking the carbohydrate containing beverage (Ludwig and Bibby, 1957).

Ludwig and Bibby (1957) also compared acid formation following the eating of carbohydrate foods and found pronounced pH changes. Within 10 minutes, the pH of the plaque had fallen to between 5.0 and 5.5 in the 5 subjects and remained low for the remainder of the half-hour test period. This rapid change in pH in the mouth following ingestion of foods is thought to reflect the different types of fermentation of different food materials.

Dietary effects can also be produced by systemic body changes which alter the composition of salivary secretions. Thus, the ability of saliva to neutralize acid has been shown to be decreased by a high cereal diet (Forbes and Gurley, 1932). Wills and Forbes (1939) have also shown the acid neutralizing power of saliva to be decreased by a high (67%) carbohydrate diet and increased with consumption of a high (40%) protein diet. However, the addition of 5% vegetables to either the high carbohydrate or high protein diet resulted in an increase in the acid neutralizing power of the saliva. An increase in both the amount of alkalinity of the saliva has been reported by Pickerill (1924) with the consumption of an acid diet (fruits, vegetables and meat in an acid sauce).

Rate of Flow

The effect of materials in the mouth may reflect a response to chewing. Wills and Forbes (1939) compared the acid neutralizing power of resting and stimulated saliva. Although the acid neutralizing power was increased with stimulation, there was no apparent relationship between the resting and stimulated conditions and the

effects were not repeatable over time and were inconsistent between individuals. The authors concluded, in agreement with McClelland (1922), that the effect due to chewing was in general proportional to the resistance offered by the material within the mouth.

Gore (1938) used a saliva ejector system with suction to collect resting saliva output of parotid glands and mandibular (submaxillary and sublingual) glands from one subject. He reported that wax stimulated saliva greatly increased the amount of saliva and especially the secretion from the parotid glands when compared to resting saliva output.

Carbohydrates

Fermentable carbohydrates in the mouth are derived solely from ingested foodstuffs (Volker, 1955). The relative caries-producing capacity of foods has been investigated (Bibby et al., 1951; Bibby, 1955). Differences between foods containing sugars have been attributed to effects on salivary and plaque acid production, concentration and persistence of reducing sugars in the saliva, and quantity of food adhering to the teeth after eating.

Gore (1938) noted the effect of the carbohydrate level of the diet on salivary flow rate. A marked increase in the amount of mandibular saliva was found when a high carbohydrate diet was compared to secretions obtained on a low carbohydrate diet.

METHODS

Ten sets of mixed saliva samples were collected over a 30-minute period from 7 female subjects. Samples of saliva were collected 3 hours after the morning meal in order to obtain resting saliva values for subjects eating various diets. Seven samples (one from each subject) obtained while subjects consumed their regular diets served as controls. Saliva was also collected 3 hours after the morning meal for 3 of the same subjects when fed experimental diets. One subject consumed a high protein diet (115 g protein compared to a usual intake of 44 g of protein). A second subject consumed a high sugar diet with the ingestion of 11 caramels/day; one consumed 1 hour before saliva collection and then one just prior to saliva collection. A sample Dietary-Dental Data Sheet completed by each subject is shown in Appendix D.

Instructions used for saliva collection and saliva measurements are given in Appendix E. Saliva samples were collected at the end of 5, 10, 20 and 30 min. Urea, glucose, pH and resting flow were determined on the 10 min saliva samples. Beck and Wainwright (1939) have suggested that resting saliva flow and resting saliva composition obtained in the first 10 min of unstimulated collection is more characteristic of any one individual over time than is output of stimulated saliva. Total saliva volume and specific gravity are calculated based on values using 30 min total saliva

samples. Salivary urea nitrogen was determined colorimetrically (Appendix F) by the Hyland Phenate Hypochlorite method (modified Berthelot reaction).[1] Glucose levels in saliva were tested (method in Appendix G) using the enzyme glucose oxidase and the chromogenic O_2 acceptor, O-dianisidine, present in the "Glucostat" commercially prepared reagent kit.[2]

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[1]Hyland Division Travenol Laboratory, Costa Mesa, Calif. 92626 (Chaney and Marbach, 1962).

[2]Worthington Biochemical Company, Freehold, N.Y.

RESULTS AND DISCUSSION

Urea

The g protein intake/100 kcal (Table 12) in the morning meal of 7 persons consuming a control diet (their normal intake) ranged from 2.3 (Subject 4) to 4.9 g (Subject 7). The range for urea mg % determined in the 10-minute saliva sample was 9.8 (Subject 4) to 22.1 mg% (Subject 2-A). The range for normal persons for combined urea and nitrogen values in whole resting saliva is reported to be from 6-13 mg % (Hench and Aldrich, 1922).

Even though Subject 7 consumed the highest relative protein intake from among the control subjects the salivary urea value was similar (10.9 mg %) to the others. However, the resting flow rate was also the highest among the subjects consuming the control diets, 4.6 ml/10 min. Thus, dilution of the urea may be a factor here since urea diffuses passively from the blood supply into saliva (Caldwell, 1968) and increasing the flow rate decreases the urea concentration (Barnett and Bramkamp, 1929).

Saliva values were compared in Subject 2 while consuming a control diet (2-A) average 1200 kcal and 44 g of protein per day for 4 days as well as when followed by a high protein diet (2-B) average 1500 kcal and 115 g of protein per day for 7 days. Urea values determined on the final day of each dietary period in this subject were, respectively, 22.1 and 26.1 mg %. It is noted

TABLE 12.--Dietary intake in the morning meal, selected salivary parameters, and dental information in 7 subjects consuming either their regular food intake or experimental meals high in protein or carbohydrate.

Subject	Morning Dietary Intake				Selected Salivary Parameters						Dental Information	
	Kcal	Carb[a]	Pro[a]	Fat[a]	Urea (mg%)	Glucose (mg%)	pH	Resting Flow (ml/10 min)	Total Flow (ml/30 min)	Specific[b] Gravity	Filled Teeth (%)	Dental Visit
1	124	20.1	3.7	0.5	10.7	0	7.1	4.4	16.6	0.977	18	past year
2[c]-A	220	20.4	2.7	1.1	22.1	8.2	6.4	2.2	5.6	0.941	21	past month
2[c]-B	171	8.9	9.5	2.5	26.1	0	6.5	2.0	5.9	0.881		
3	184	8.0	4.5	6.6	14.6	0	7.2	2.2	4.4	1.030	25	past year
4	174	19.3	2.3	1.4	9.8	0	7.5	3.8	14.2	1.019	29	past week
5[d]-A	263	17.5	3.8	1.9	14.9	2.6	6.4	2.0	4.3	1.106	37	past 1/2 year
5[d]-B	585	8.6	6.2	5.3	9.3	98.4	6.8	6.5	18.0	1.006		
5[d]-C	485	11.8	3.9	4.6	12.1	1.3	6.1	2.5	7.0	1.028		
6	186	21.0	4.0	0.9	9.9	0	6.6	0.9	6.5	1.052	46	past week
7	243	10.8	4.9	7.5	10.9	4.3	7.1	4.6	12.6	0.855	50	past week

[a]g nutrient/100 kcal intake.

[b]Calculation on 30 minute saliva sample.

[c]2-A usual dietary intake for subject; 2-B dietary intake of 115 g protein and 1500 kcal/day.

[d]5-A usual dietary intake for subject; 5-B saliva tested immediately after ingestion of caramel candy (10 g); 5-C saliva tested 1 hour after ingesion of caramel candy.

(Table 12) that Subject 2 had proportionately higher salivary urea values in relation to protein consumed than the other subjects consuming control diets. This subject reported that she was allergic to milk but, nevertheless, the ratio of animal protein to plant protein in her control diet was 2:1. Bolourchi et al. (1968) have reported that normal adult subjects show a reduction of blood urea values by one-half when consuming a diet providing approximately 90% of their protein from wheat as compared to a mixed diet which was isonitrogenous to the wheat diet. In addition, this subject was taking thyroxine medication. This hormone alters energy metabolism by increasing glucose production from body stores and stimulating growth hormone which promotes retention of nitrogen (increased protein stores and decreased excretion of urinary nitrogen) (Lowrey, 1973).

Glucose

The g carbohydrate intake/100 kcal in the morning meal during the control period when subjects consumed their regular diets ranged from 8.0 to 21.0 g (Table 12). The range for salivary glucose for the control period was from 0 to 8.2 mg %. The normal range for glucose for resting saliva has been reported to be 11-30 mg % (Jenkins, 1954).

Subject 5 increased the carbohydrate content of the control diet (5-A) by the inclusion of caramel candy snacks during the experimental diet period (5-B,C). The normal glucose level for the subject (5-A) was 2.6 mg % glucose. Ingestion of the caramel just

prior to saliva testing (5-B) increased salivary glucose values to 98.4 mg %, increased pH at 10 min to 6.8 (compared to 6.4 when fed the control diet), increased the resting saliva flow by approximately 3 fold to 6.5 ml, and increased the total 30-minute saliva flow by approximately 4 fold to 18 ml. Volker (1955) noted that fermentable carbohydrates in the mouth are derived solely from ingested carbohydrates. Eastoe (1961) reported that increased flow rate of saliva produces a more alkaline secretion; this is due to an increased bicarbonate content (Dawes, 1970).

Values seen for Subject 5 when saliva was tested one hour after ingestion of caramel (5-C) indicated a glucose value of 1.3 mg %; this drop in glucose from the control value of 2.6% may reflect a dilution factor due to the increased saliva flow of 2.5 ml at 10 min as compared to 2.0 ml flow at 10 min in the control period. A drop in pH is seen in this experimental period (pH 6.1 in 5-C) as compared to the control period (pH 6.4 in 5-A). Haggard and Greenberg (1951) have reported a decrease of short duration in the pH of the saliva following ingestion of carbohydrate-containing substances.

Salivary Parameters and Dental Data

Salivary flow and composition are important host factors in the carious process. Subject 6 had 46% of 28 teeth that had received dental repair (fillings, 1 crown, 1 false tooth). It is noted (Table 12) that the usual morning dietary intake was high in carbohydrate, 21 g. The urea value of 9.9 mg % is lower than the average

of 11.4 mg % recorded for subjects fed control diets. The salivary pH value of 6.6 for Subject 6 is lower than the average of pH 6.9 for the subjects on their usual dietary intakes. In addition, this subject had the lowest resting saliva flow/10 min; that is, 0.9 ml. Each of these factors plays a role in the carious process; Nikiforuk et al. (1956) noted a higher salivary urea in caries-free children. Hardwich (1949) reported the mean of resting saliva was pH 6.7 as compared to a mean pH of 7.4 for saliva that had been stimulated with an increased flow rate.

Values for this subject and the response of saliva urea values to increased protein intake (Subject 2) and the response of saliva glucose and pH levels to carbohydrate intake (Subject 5) suggest that dietary manipulations can play a role in the carious process through changes in salivary secretion and composition, especially within caries-susceptible individuals or age groups.

SUMMARY

This study investigated changes in salivary parameters in 7 subjects associated with consumption of their normal intake (control diet) or with the consumption of a high protein (Subject 2-B) or a high carbohydrate intake (Subject 5-B,C) (Table 12). The g protein intake/100 kcal in the morning meal of the 7 subjects consuming their normal intake ranged from 2.3 to 4.9 g. The range for urea mg % determined on the 10 min saliva sample for 6 subjects (Subject 2 excluded due to the possible effect of thyroxine medication on energy metabolism) was 9.8 to 14.9 mg %.

The g carbohydrate intake/100 kcal in the morning meal during the control period when subjects consumed their regular diets ranged from 8.0 to 21.0 g (range of 7 subjects). Salivary glucose for subjects consuming the control diets ranged from 0 to 4.3 mg % (Subject 2-A excluded).

For one subject, urea mg % increased from 22.1 to 26.1 in response to an approximate 3-fold increase in protein intake. In another subject, a local oral effect was seen in an increase in glucose values to 98.4 from 2.6 mg% in response to ingestion of carbohydrate just prior to saliva testing as compared to saliva testing 3 hours after carbohydrate consumption.

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APPENDICES

APPENDIX A

GENERAL SENSORY INSTRUCTIONS

APPENDIX A

GENERAL SENSORY INSTRUCTIONS

1. Please refrain from smoking, eating or drinking one-half hour prior to evaluation.

2. Please do not give any facial or vocal reactions as you evaluate your samples.

3. The samples are coded with random numbers and are presented in a randomized fashion. Please start with the sample in the upper left-hand corner of the tray and proceed toward the right. The lower row should be evaluated in the same order.

4. Each sample is to be evaluated on a separate score sheet using a 5-point scale, a score of 5 being the highest given. Six attributes are to be judged. Descriptive terms for the scores of 5 indicate those desirable in a high quality cake. For each attribute, descriptive terms given for the scores of 4 and 1 indicate the two different directions that deviations from the high quality attributes could take. Therefore, please indicate the direction and extent of any deviation.

5. Place a check in the block which most nearly fits the evaluation of each quality characteristic of the sample. Score each sample independently of others.

   BE SURE THE PLATE CODE MATCHES THE SCORE-CARD.

6. You may rinse your mouth between sample evaluations with the water provided.

* *

GRAIN. Evaluate the grain of the cut surface by visual observation. Large holes or tunnels are not considered as a part of grain.

CRUMB COLOR. Evaluate the color of the cut surface by visual observation. If necessary, hold the sample so that any shadows are eliminated from the surface.

SILKINESS. Evaluate the sensation ascribed to the feel of the surface of a cut slice of cake. Rub the fingers lightly across the freshly cut surface.

TENDERNESS. Evaluate tenderness by cutting or biting the cake sample and by the rate and ease of dissolving in the mouth.

FLAVOR. Taste the inside of the cake and the crust separately. Rinse your mouth with the water provided between the evaluations.

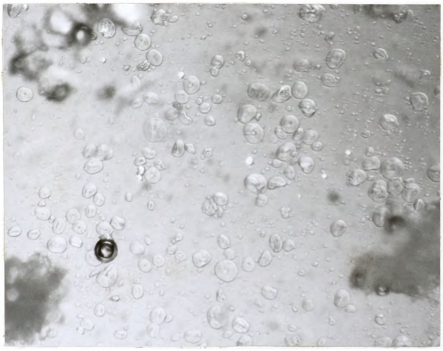
APPENDIX B

PHOTOMICROGRAPHS

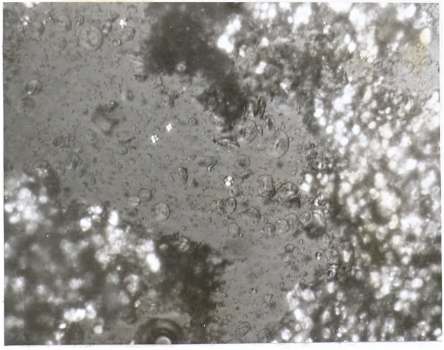
APPENDIX B

PHOTOMICROGRAPHS

Suspensions of interior portions of sucrose-containing cakes (A) and glucose-containing cakes (B) under polarized light at 10 X magnification. The lack of starch gelatinization (greater number of granules retaining birefringence) in the cake containing glucose suggests that the quantity of water available to the starch was limited during baking due to greater binding of water required to solubilize glucose.



A



B

APPENDIX B

APPENDIX C

CARIOGENIC WORKING CULTURES

APPENDIX C

CARIOGENIC WORKING CULTURES

Stock cultures of Streptococcus mutans 6715-15[a] were received from the National Institutes of Dental Health maintained in N.I.H. fluid Thioglycollate Medium plus 20% horse meat infusion plus calcium carbonate. One tube of culture was frozen at -23°C for immediate use while the remaining 4 tubes were lyophilized and then frozen.

Prior to use, 12 ml screw-capped tubes of sterile fluid thioglycollate media (2.95 g/100 ml) were innoculated from the stock culture. These subcultures were incubated at 35-37°C for 30 hours. Turbidity of these subcultures were determined using the Bausch and Lomb Spectronic 20 photocolorimeter set at 686 millimicron wave length (Benson, 1972). Dilutions were made using sterile fluid thioglycollate media until a transmittance of 50 was reached. These working cultures were prepared prior to use for the infection of animals and were maintained in the refrigerator during this week time period. Unused working cultures were destroyed by autoclaving.

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[a]Streptococcus mutans strain 6715 was derived from strain K1-R (laboratory culture and parent strain) (R. J. Gibbons and R. J. Fitzgerald, J. Bacteriol. 98, 345, 1969).

APPENDIX D

DIETARY-DENTAL DATA SHEET

APPENDIX D

DIETARY-DENTAL DATA SHEET

GENERAL HEALTH STATUS Date:
Age, height, weight:

Are you afflicted with colds or hay fever?

Are you on medications?

DIETARY INFORMATION
Are you on a special diet? What kind? How long?

Do you use vitamin or health food supplements? Describe.

Describe appetite, food dislikes, food allergies.

Do you have access to supplementary or seasonal sources of food (garden, family resources, rely on convenience foods)?

DENTAL CARIES EXPERIENCE
Number of teeth present in mouth (designate wisdom teeth, false tooth, etc.).

Number of teeth that contain fillings?

Date for last visit to dentist.

Comments on dental history: (fluoride treatments, hereditary factors)

BREAKFAST MEAL PRIOR TO SALIVA TEST

Substance Consumed	Amount (g or other measure)

APPENDIX E

SALIVA COLLECTION AND MEASUREMENTS

APPENDIX E

SALIVA COLLECTION AND MEASUREMENTS

GENERAL--A 30 minute saliva sample is collected 3 to 4 hours after the morning meal. No toothpaste or mouthwash is used following the meal; however, the usual morning tooth brushing routine (minus toothpaste) is followed. During the interval between breakfast and saliva collection, no smoking, food or drink (other than water) should be used. No water should be taken 15 min prior to sample collection.

Collection equipment includes: four 15 ml pharmaceutical graduate cylinders, minute timer, 4 funnels with 50 mm stems and 4 one-oz sample bottles with caps. Four timed samples are collected at one time; one at the end of 5, 10, 20 and 30 minutes.

Record weight of flasks to nearest 0.1 g. Locate chair and bench space and setup funnel and flasks and be prepared to remain sitting quietly without talking for the 30-min saliva collecting period. As you collect each sample, cover with parafilm until you are ready for saliva testing.

COLLECTION--Begin by tilting the head slightly forward to allow saliva to start to collect in the mouth. Note the time. An effort should be made during this time to minimize movements of the mouth or tongue which may change the rate of saliva flow.

At the end of 5 min, spit the saliva into the funnel draining into the graduated flask marked as the 5 min sample. This sample is usually quite watery and will quickly drain into the flask. Note an additional 5 min time period, repeat this collecting procedure and spit this second sample into the flask marked as the 10 min sample (time indicates min from initial collecting point). For the third sample, again note the time for an additional 10 min interval and collect into the flask marked as the 20 min sample. And then collect the final sample into the 30 min flask to mark the end of the total 30 min collection period.

Measurements should be made on each of the 4 samples collected each day at each of the time periods. These values can later be used individually to show changes over time or they can be averaged to represent a single 30-min pooled sample.

MEASUREMENTS--For each of the 4 samples, determine the pH of the sample using the Beckman glass electrode pH meter. To prevent loss of CO_2, samples are tested within minutes after collection or

are covered with parafilm. The volume of saliva is recorded to the nearest 0.1 ml. The weight of each saliva sample is determined to the nearest 0.1 g. The empty flask is first weighed and then the weight of the flask plus sample is determined and the difference calculated. Store each sample in labeled sample bottles for later determinations of urea and glucose.

Calculate resting flow secretion rate as ml/min using the combined collection volume covering the first 10 min collection period (5 min sample volume plus 10 min sample volume). Determine specific gravity of the total 30 min saliva sample by calculating the weight of the total sample divided by the volume of the total sample.

APPENDIX F

SALIVARY UREA NITROGEN DETERMINATION

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SALIVARY UREA NITROGEN DETERMINATION

1. Preparation of reagents-Hyland UN-TEST[a]

   a) Reconstituted urease
   b) Solution of phenol with sodium nitroprusside catalyst
   c) Solution of sodium hypochlorite with sodium hydroxide
   d) Nitrogen standard (15 mg/100 ml)

2. Incubate at 50°C for 5 min in test tube (150 x 16 mm) the following: 0.2 ml urease, 0.02 ml undiluted saliva (or nitrogen standard or distilled water for blank).

3. Add the following: 1.0 ml phenol color reagent and 1.0 ml alkali (NaOH)-hypochlorite reagent.

4. Incubate 6 min at 50°C.

5. Add 8 ml distilled water.

6. Read absorbance (O.D.) on Beckman DB spectrophotometer at 630 nm using blank to zero photometer.

7. The absorbance of the unknown is proportional to the ammonia nitrogen concentration. Calculate:

$$\frac{\text{O.D. of unknown sample}}{\text{O.D. of standard}} \times 15 = \text{urea nitrogen concentration in sample (mg/100 ml)}$$

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[a]Hyland Division Travenol Laboratory, Costa Mesa, Calif. 92626.

APPENDIX G

ENZYMATIC DETERMINATION OF GLUCOSE

APPENDIX G

ENZYMATIC DETERMINATION OF GLUCOSE

1. Preparation of reagents-Glucostat X 4,[a] semi-micro method

   a) 1000 mg % glucose stock solution in 0.25% benzoic acid
   b) 4N hydrochloric acid
   c) Deproteinizing reagents: Make up 300 ml each of 1.8% $Ba(OH)_2 \cdot 8H_2O$ crystals and 2.0% $ZnSO_4 \cdot 7H_2O$. These solutions should neutralize each other. To test, dilute 10.0 ml zinc sulfate with 50 ml water and add 2 drops of phenolphthalein. Titrate with barium hydroxide to a faint pink; 10 ml 0.05 ml should be required.
   d) Reconstituted glucostat reagent (chromogen and glucose oxidase)
   e) Glucose standards made up daily:

mg %	20	50	100	150
glucose stock, ml	0.2	0.5	1.0	1.5
distilled water, ml	9.8	9.5	9.0	8.5

2. Deproteinize samples: Add to test tubes (7.5 x 1 mm) the following--1.9 ml distilled water, 0.1 ml undiluted 10 min saliva sample (or glucose standard or distilled water for blank), 1.0 ml barium hydroxide and 1.0 ml zinc sulfate. Centrifuge[b] to precipitate protein.

3. Assay for color change: Transfer 2 ml deproteinized filtrate to test tube (150 x 16 mm). At 15 sec intervals, add 2.0 ml glucostate reagent; let stand 10 min at room temperature. At 15 sec intervals, add 1 drop 4N HCl to stabilize color; let stand 5 min at room temperature.

4. Read absorbance on Beckman DB spectrophotometer at 420 nm using reagent blank to zero photometer. Calculate mg % of unknown:

$$\frac{\text{mg \% of standard}}{\text{absorbance of standard}} \times \text{absorbance of unknown}$$

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[a] Worthington Biochemical Company, Freehold, N.Y.

[b] International Clinical Centrifuge, Model CL; International Equipment Company; Needham, Mass.

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