DENTAL CARIES AND MOUTH MICROORGANISMS IN RATS FED DIETS HIGH IN MALTOSE OR SUCROSE

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ABSTRACT

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The purpose of this study was to compare the cariogenic effects of maltose and sucrose on both caries development and Streptococcus mutans growth.

Forty-eight Osborne-Mendel rats, weaned at 18-20 days of age, were used in this study. The rats were inoculated with Streptococcus mutans and fed a cariogenic diet containing 58 and 27% maltose or sucrose in the form of cake meal or dry powder. Caries incidence and numbers of oral microorganisms were determined in rats fed diets for 6 and 11 weeks. The 6 left molars were scored for buccal/lingual, proximal and sulcal caries (Keyes method, 1958) while the 6 right molars were evaluated for plaque Streptococcus mutans.

Maltose and sucrose diets produced similar caries incidence and Streptococcus mutans growth (range 4.2-7.0 CFU/ml. x 10^5). No significant improvements in caries scores or in decreases of oral bacteria ($p>0.10$) were found when maltose was substituted for sucrose in cariogenic diets.

This book is dedicated to the memory of my father,

James Randolph Ross.

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REVIEW OF LITERATURE

Introduction

Prevalence of Caries in the U.S.

Dental decay is a pervasive problem in this country, affecting more people than cancer, obesity and heart disease (Huis in'T Veld et al., 1979; Miller, 1979). The prevalence of caries in the U.S. population is high especially when compared to other, less developed countries (Newbrun, 1982a). Dental caries incidence is considered a public health problem of particularly affluent, well-fed nations (Miller, 1979; Lauder et al., 1980; Sheiham, 1983). The two internationally recognized indices used by epidemiologists for describing this phenomenon in a population are Decayed, Missing, Filled Teeth (DMFT) and Decayed, Missing, Filled Surface (DMFS) scores (Sardo Infirri et al., 1979).

The prevalence of caries among American children is particularly relevant to this study. The National Institute of Dental Research summarized the findings of a National Caries Prevalence survey for 1979-1980 on a sample representing 45.3 million school children aged 5-17 (Miller et al., 1981). In a permanent dentition of these

youngsters researchers found an average of 4.77 DMFS per child and 2.91 DMFT per child. Age specific analysis of the data showed that by age 12, U.S. children have, on the average, over 4 DMFS, and by age 17 over 11. Percentage distributions of U.S. children according to DMFT status revealed that 37% of the children in the sample were completely caries free. In other words, 63% of the children in this country - almost 30 million youngsters - have dental disease (Miller et al., 1981).

Dietary modifications which lead to decreased sugar, especially sucrose, intake would be an effective means of reducing caries in children (Rytomaa et al., 1980). To the benefit of these youngsters, alternative, non-cariogenic sugars need to be assessed and made available for sweetening foods (Sheiham, 1983). Certain sugars, namely maltose, have been found to reduce plaque formation when compared to sucrose and are therefore presumed less cariogenic (Balekjian et al., 1977; Hamada et al., 1980). To that end, this laboratory sought to examine the effect of maltose and sucrose diets on caries development and oral bacterial populations in laboratory animals.

A Model for Dental Decay

Historical Observations

Already in the 3rd century B.C., Aristotle noted that soft sweet figs adhered to the teeth, putrified and caused

damage (Newbrun, 1982a). But it wasn't until 1889 that researchers recognized microorganisms as important elements in the process of dental decay (Starr et al., 1981). W.D. Miller, an internationally educated scholar, provided the first clear model of dental decay that implicated micro-organisms. The original copy of Miller's seminal treatise, 'The Micro-organisms of the Human Mouth,' has been preserved (Miller, 1973). Miller produced such a thorough and remarkable documentation of his lab work that his ideas would have credibility had they been written yesterday. Miller's idea of germs in relation to dental disease was, however, still general. J.K. Clarke carried Miller's work further by naming a specific pathogen in 1924 (Clarke, 1924). Clarke isolated certain streptococci from human carious lesions, discussed the properties of these particular microbes in his papers, and then named the species group S. mutans (Clarke, 1924). Of equal importance was Clarke's observation that 'colonies of S. mutans adhere closely to the teeth.' With this statement, Clarke provided the first push in a new direction of scientific inquiry which would eventually inspire researchers to look at the deleterious effects of harmful substances adhering to the teeth and to study once again, albeit 30 years after Miller's original studies, oral microbiology.

The absolute connection between caries and S. mutans remained to be established. Not until 1954, 30 years

after Clarke named a specific pathogen, did anyone irrefutably establish the role of Streptococcus mutans as the microbial agent in the formation of carious lesions. This honor was achieved by Frank Orland in an epoch making experiment. Orland used germ-free rats to test the hypothesis that animals, reared from birth, free from living bacteria should not develop caries. While 22 germ-free rats showed complete absence of carious lesions, all but 3 of the 39 rats in the experimental control group, reared conventionally and exposed to uncontrolled germ growth, developed carious lesions (Orland et al., 1954). Corroboration came in one other ingenious study done by Fitzgerald and Keyes in 1960, shortly after Orland's work. This laboratory produced caries by transferring Streptococcal strains from carious rat teeth into the mouths of gnotobiotic rats. The link between S. mutans and caries formation was now firmly established in scientist's minds.

During the ten years that followed 1960, an improvement in available techniques led to an interest in investigating the precise etiology of dental decay. This decade made the bacterial colonies on the surface of the teeth one of the most thoroughly studied microbial communities existing (Bowden et al., 1979). Before the 1960's plaque - now known to be aggregates of flora on the teeth - was relatively ill defined; many assumed plaque to be merely an accumulation of food debris (Bowden et al., 1979).

Although previous studies had shown that plaque was composed mostly of bacteria, their extracellular products and salivary components, few researchers had made more detailed studies. Between 1960 and 1970, scientists developed the techniques necessary for sampling, isolating, and selectively identifying Streptococcal species and new equipment appeared in the form of anaerobic chambers and reduced transport fluid of RTF (Bowden et al., 1979). Along with this introduction of advanced methodology came increased understanding of the characteristics and composition of plaque. By 1970, scientists had accumulated sufficient data on dental plaque to warrant a symposium on its matrix-like structure, its composition, biochemistry, accumulation and pathogenicity (Bowden et al., 1979).

Evidence for a Sugar-Caries Relationship

Several classic studies provided strong support for a relationship between dietary sugar intake and human caries prevalence. The famous Vipeholm study in Sweden during 1949-1954, in which 436 individuals were observed for 5 years, demonstrated a clear relationship between dental caries prevalence and sucrose ingestion (Gustafsson et al., 1954). Sucrose is very likely the most cariogenic sugar in our diet (Dummer et al., 1981). Considerable epidemiological evidence exists in this country linking per capita sucrose intake to dental decay (Newbrun, 1982a). A systematic relation between sugary-food intakes and dental caries or

DMFT scores appears in the sample of 2,514 adolescents participating in the Ten State Nutrition Survey (Garn et al., 1980). Adolescents with high DMFT scores - those in the upper 15th percentile for their sex, race and age - had 10 times the sugar intake of the adolescents with low DMFT values (those in the lower 15th percentile).

Other sugars seem not to be as cariogenic as sucrose (Birkhed et al., 1981). One experiment, which compared the effects of invert sugar (a mixture of 23% glucose and 23% fructose) to that of 56% sucrose in an otherwise similar diet, found in the sucrose fed animals an average of 4.0 times more smooth surface lesions after 8 weeks, $p<0.001$ (Birkhed et al., 1981). The glucose-fructose bond present in sucrose enables Streptococcus mutans to convert sucrose more efficiently than either monosaccharide in unbounded form into cariogenic products (Birkhed et al., 1981). In any event, it is not sucrose alone that is cariogenic. Only in contact with certain oral flora does sucrose produce its deleterious effect. One study of albino rats joined in parabiosis proved this. The blood sugar levels in both groups of rats were equivalent but dental caries flourished only in the one mouth which was fed a sugar solution (Tamura et al., 1980). Sucrose exerts its effect by influencing the colonization and multiplication of specific cariogenic organisms in dental plaque. Streptococcus mutans appeared 3-4 times less frequently in plaque when individuals reduce

dietary sucrose either by choice or by necessity - as in the disease Hereditary Fructose Intolerance (HFI) (Hoover et al., 1980). Frequent intakes of sucrose afford the oral cariogenic bacteria many opportunities to metabolize the sugar, converting it to acids which erode the enamel of teeth. For this reason, many researchers are convinced that how often people eat sugary foods contributes more directly to promotion of decay than how much total sugar they consume (Maiwald, 1981; Firestone et al., 1980; Brown et al., 1980).

Bacteria in the Oral Environment

Indictment and Transmission of *Streptococcus mutans*

In several longitudinal studies, in which researchers followed the distribution of *S. mutans* on tooth surfaces over periods ranging from 18 to 24 months, colonization with elevated *S. mutans* clearly preceded the development of caries (Hamada et al., 1980; Bowden et al., 1979). In another study which compared proportions of *S. mutans* from early carious lesions (white spots) to adjacent caries-free surfaces, significantly more *S. mutans* appeared on decayed surfaces (Hamada et al., 1980; Bowden et al., 1979). Others' conclusions corroborate these in essential details. Among the possible cariogenic microorganisms present in the oral cavity, *Streptococcus mutans* has proven to be the most active (Starr et al., 1981). After

suctioning out 48 human carious fissures, and analyzing the contents for total Streptococcal counts, S. mutans, S. sanguis, S. faecalis, Actinomyces viscous, and lactobacilli Meier's laboratory (1982) reported that S. mutans was the only microorganism common to all carious fissures. Bacteriological analysis shows that carious fissures harbor significantly higher proportions of S. mutans than non-carious fissures (Loesche et al., 1979).

A significant correlation also appeared between S. mutans content per ml of saliva and decayed or filled surfaces when researchers examined 101 teenagers, 13-14 years old (Zickert et al., 1982; Beighton et al., 1982). In another study using 16 monkeys on a high sucrose diet the proportion of S. mutans in first molars was related to a total caries score 6 months later (Beighton et al., 1982). Evidently increasing numbers of S. mutans are associated with increased caries.

Edentulous newborns or aged men and women do not harbor significant quantities of S. mutans (Starr et al., 1981; Beighton et al., 1982). As the number of erupted teeth increases there is a gradual increase in the prevalence of S. mutans (Hamada et al., 1980). It appears that S. mutans is transmitted intrafamilially (Hamada et al., 1980). There is a relationship between numbers of S. mutans in saliva of mothers and colonization in their children (Bowden et al., 1979). Although it is easy for bacteria to colonize

in the mouths of newborns it is difficult to implant new strains of bacteria in mature plaque of humans or animals (Carver, 1982). The transmission of S. mutans between monkeys occurs readily prior to the formation of an established flora (Beighton et al., 1982). The resident S. mutans of mature plaque resists colonization by extraneous strains of bacteria (Beighton et al., 1982).

Physiology of Streptococcus Mutans

The natural habitat for Streptococcus mutans is the entire human mouth - the tongue, gingiva, and teeth - although it prefers to form colonies on one particular site: tooth surfaces (Bowden et al., 1979; Hamada et al., 1980; Chellappah, 1981). Streptococcus mutans is an unusual and complicated organism. For a long time scientists were extremely confused over the classification and nomenclature of the group to which Streptococcus mutans belongs (Starr et al., 1981). The epithet "mutans" was most likely introduced simply to settle taxonomic difficulties (Starr et al., 1981). Some authors believe that no one has satisfactorily described the taxonomic position of S. mutans. The latest edition of Bergey's Manual of Determinative Bacteriology (8th edition, 1974) does not give Streptococcus mutans an independent species position. Its only mention appears in connection with the description of S. salivarius: "Carlsson (1965) has suggested a relation between dental caries and

Streptococcus mutans (Clarke, 1924), an organism quite similar to *S. salivarius*. *S. mutans* has not yet been extensively studied and compared with *S. salivarius*" (Buchanan et al., 1974). Yet in direct contrast, other authors are quick to point out that *S. mutans* is one of the best defined species among the oral streptococci (Hamada et al., 1980; Starr et al., 1981). The International Subcommittee on Nomenclature for Streptococci, for example, has officially designated a neotype strain and reported their consensus: '*Streptococcus mutans* should be conserved as a valid species' (Starr et al., 1981).

S. mutans has a completely unique identity due to a specific set of metabolic processes which no other streptococci possess in their entirety. The standard descriptions of *S. mutans* label it a "gram (+), catalase negative, nonmotile, fermentive cocci" (Hamada et al., 1980). Oral Streptococci are fermentative bacteria because they do not possess cytochrome systems that enable ATP to be generated by oxidative phosphorylation (Roberts et al., 1981). Although *S. mutans* is a facultative anaerobe it will produce larger and more abundant colonies in anaerobic compared to aerobic environments (Skinner et al., 1978). *Streptococcus mutans* growth is inhibited by the hydrogen peroxide which forms when oxygen is present (Skinner et al., 1978). Plaque has been shown to have ten times more anaerobic than aerobic bacteria (Gallagher et al., 1981).

Because plaque is largely anaerobic, though not completely so, the plaque ecosystem is an ideal habitat for S. mutans.

Several properties inherent in S. mutans enable it to survive very well in the oral cavity. In the first place, it produces extracellular polysaccharides of a sticky nature, primarily polyglucans (Starr et al., 1981). The formation of these extracellular polysaccharides (EPS) occurs in two enzymatic steps in which sucrose is practically the sole substrate for the enzymes involved (Hamada et al., 1980). A glucosyl transferase catalyzes the formation of polyglucans with the release of free fructose and a fructosyl transferase catalyzes the formation of levans (Starr et al., 1981). Streptococcus mutans mainly produces the glucans (Starr et al., 1981), which are highly branched at the C-3 and C-6 positions of D-glucopyranose residues (Huis in'T veld et al., 1979). The solubility of these substances decreases as the number of α-1,3-glucopyranosyl linkages increases (Hamada et al., 1980; Huis in'T veld et al., 1979). In addition Streptococcus mutans produces considerably more insoluble than soluble compounds (Starr et al., 1981). These compounds, by the way, are responsible for the ability of S. mutans to initiate plaque formation. The extracellular polysaccharides become the structural skeleton for the plaque matrix. The glucosyl transferase present in Streptococcus mutans acts specifically on sucrose in a manner essential to

production of these compounds.

Plaque contains and concentrates acids which, when formed by S. mutans in the process of fermenting sucrose, destroy tooth enamel (Tilliss et al., 1981). When researchers investigated the in vitro sucrose metabolism of Streptococcus mutans and compared it to the sucrose metabolism of other dominant bacterial cultures in plaque, such as S. sanguis, S. mitis, and lactobacillus casei, they reported that S. mutans formed considerably more lactic acid and 2-3 times more insoluble extracellular glucan than other bacteria (Minah et al., 1977). The amount of sucrose consumed by Streptococcus mutans was greater at each time interval than other bacterial strains. The S. mutans cultures converted half of the sucrose into acids, primarily lactic acid, leaving most of the remainder as polysaccharides (Minah et al., 1977). Noteworthily, acid production by Streptococcus mutans continues even in the absence of an exogenous carbohydrate source (Bowden et al., 1979). The bacterium produces acid as it utilizes stored glycogen-like intracellular polysaccharides to provide its own maintenance energy (Huis in'T Veld et al., 1979). Its ability to form these intracellular polysaccharides (IPS) from sucrose offers S. mutans yet another selective advantage over other oral organisms.

Overall, Streptococcus mutans fits very well into the ecological niche of the oral cavity. It has an ecological advantage during colonization over purely aerobic or anaerobic strains (Starr et al., 1981). Additionally, it tolerates high concentrations of sucrose and can grow under extremely acidic conditions (Svanberg, 1980). In direct contrast, Streptococcus mutans still flourishes without requiring large amounts of sucrose and, as has been mentioned, will even survive relatively short periods without any at all (Starr et al., 1981).

Plaque Formation and Composition

Plaque has been defined in a variety of ways. Hamada's review (1980) refers to it as distinctive patches of microbial origin. Tamura and coworkers (1980) call it the white, gray, or yellow adherent gelatinous material covering the teeth as a result of food (and especially sugar) consumption and neglected oral hygiene. Tamura's paper (1980) describes it as consisting essentially of 2 components: bacterial cells and their extracellular components. Dental plaque contains about 10^{11} organisms per gram, wet weight - a number which matches the density of a bacterial colony on a solid culture medium (Starr et al., 1981). The total number of different strains of facultative and obligate anaerobic colonies isolated so far from plaque samples is 298 (Gallagher et al., 1981). The high

humidity in the oral cavity of humans, ambient temperatures of 35-37^{o}C, and nutrients remaining on the teeth after eating, all provide an excellent environment for the proliferation of oral organisms (Starr et al., 1981).

Dietary sucrose has a direct effect on the amount and characteristic morphology of plaque. With few exceptions, bacterial deposits on media other than sucrose are less solid (Dummer et al., 1981). This change is associated with an increase in sloughing and indicates a reduction in adhesion and cohesion (Dummer et al., 1981). On subjects ingesting a carbohydrate free diet accompanied either by sucrose candies or by xylitol- or sorbitol-sweetened candies during a 4 day test period, scientists found significantly larger amounts of plaque when the surcose candies were selected (Rateitschak-Pluss et al., 1982). Bacterial deposits formed *in vitro* on nichrome steel wire by *S. mutans* produce a greater concentration of extracellular polysaccharides in the media containing sucrose compared to the deposits formed in media containing glucose, fructose and equimolar mixtures of glucose and fructose (Dummer et al., 1981). Apparently sucrose, compared to its constituent monosaccharides, provides optimum conditions for deposit formation and in general supports the formation of a more adherent microbial mass.

Plaque comprises many microbial communities, not just one species of an entire genus. Several bacterial populations

co-exist (Bowden et al., 1979). Although S. mutans has been considered the most cariogenic microorganism among the streptococci, it is only one constituent in dental plaque; other large populations of species include S. salivarius, S. sanguis, and S. mitis (Starr et al., 1981). Numerous microorganisms in the oral cavity still cannot be cultured because their growth requirements are not known; therefore, "it should not be expected that the 'specific pathogen' of a pathological process in the oral cavity of humans in which the participation of bacteria is suspected, can readily be found" (Starr et al., 1981). The correlations between the bacterial composition of plaque and the amount of caries are of a very complex nature (Starr et al., 1981). It seems that all the oral microbiota play a role in the pathological process of dental diseases, although individual organisms or groups can play a prominent role by influencing certain metabolic processes in the progress of the disease (Starr et al., 1981). The effects of varying oral conditions on the composition of bacterial communities, rather than on one species, should be examined as they would better reflect the etiological conditions of an initial lesion. Bacterial communities are associated with initial colonization and early lesioning (Bowden et al., 1979). The potential for caries induction develops when conditions approaching extremes of environment result (Goldner, 1981). We cannot hope to achieve any further degree of caries control unless

we appreciate the way all of these factors work together.

The Pathological Process

Chellappah's investigation (1981) of the relationship between the proportional contents of Streptococcus mutans to total Streptococci in the plaque of carious and non-carious sites showed that simply the absence or presence of S. mutans does not cause caries; caries requires conditions which induce an overgrowth of S. mutans. When S. mutans populations increase to such an extent that they become a proportionally larger part of the total microbial growth, caries are the consequence (Chellappah, 1981). The total cultivable plaque flora of caries-free individuals is now known to have comparatively smaller amounts of S. mutans than the plaque of caries-active individuals (Gallagher et al., 1981). Results of work done by Huis in T'Veld and co-workers (1979) showed that carious surfaces harbor Streptococcus mutans in proportions greater than 5%. Chellappah (1981) demonstrated how salivary levels of S. mutans greater than 10^5 organisms/ml accompanied the caries state. Notably, Streptococcus mutans did not colonize on tooth surfaces when salivary levels fell below 10^4 organisms/ml. Thus a salivary level of Streptococcus mutans greater than 10^5 organisms/ml might be essential for colonization of total surfaces (Chellappah, 1981). In similar studies, Zickert and co-workers (1982) statistically correlated

active caries experience with salivary Streptococcus mutans levels greater than 10^6 organisms/ml, a value one exponent larger than Chellappah's (1981).

The initial events in the colonization of Streptococci on the tooth surface involve salivary factors in a pellicle (Kashket et al., 1979). The pellicle is an amorphous, almost invisible film that covers enamel and is composed primarily of salivary glycoprotein (Huis in'T Veld et al., 1979; Hamada et al., 1980). Cells of Streptococcus mutans can interact with and become aggregated by specific salivary factors (Kashket et al., 1979). Zero to fifteen minutes after teeth have been cleaned, bacteria can be detected on tooth surfaces (Bowden et al., 1979). Increasing evidence suggests that the salivary factors inducing cell aggregation are the same ones which become immobilized on the surface of the hydroxyapatite and mediate cell attachment (Kashket et al., 1979). One possible mechanism proposes that bacterial cells get coated with salivary glycoproteins, which in turn interact with salivary components coating the teeth (Hamada et al., 1980). Kashket's lab (1979) demonstrated that weak bonds formed in the mutans-saliva system. Hamada and Slade (1980) described the interaction between bacteria and pellicle as electrostatic in nature and likened it to a behavior between oppositely charged particles. They asserted furthermore that some H-bonding was part of the interaction. In such a situation the

resulting aggregate resists local cleansing forces and muscle movements, and becomes the foundation upon which the plaque deposit is built.

The volume of plaque continues to increase due to the production of extracellular polysaccharides by Streptococcus mutans. S. mutans produces high molecular weight glucose polymers (glucans) which are divided into two types, water soluble and water insoluble, with the insoluble compounds prevailing (Starr et al., 1981). These polysaccharides have a tendency to clump as clusters of numerous molecules (Starr et al., 1981). The other species in dental plaque, S. mitis, S. salivarius, S. sanguis, also produce extracellular polysaccharides, but these are less important to caries etiology than the glutinous polysaccharides produced by Streptococcus mutans (Starr et al., 1981).

Another group of harmful substances produced by S. mutans are the acidic metabolic end products, particularly lactic acid, that result from carbohydrate hydrolysis (Starr et al., 1981). Acidic end products of carbohydrate metabolism, when present in connection with the formation of glutinous polyglucans, promote the continuous local and destructive action of acids upon dental enamel and dentin (Starr et al., 1981). This eventually leads to initial caries.

The minerals in tooth enamel are dissolved at low plaque pH (Huis in'T Veld et al., 1979). S. mutans' catabolism of simple sugars, particularly sucrose, produces acids which accumulate and cause a large drop in plaque pH. The resting plaque pH monitored in nine subjects who rinsed with a 10% sucrose solution for 1 minute and abstained from oral hygiene for 24 hours had a mean plaque pH of 5.50±0.33 (Jensen et al., 1982). In similar sessions using the same subjects but without the sucrose rinse, the plaque pH was less acidic, the mean was 6.56±0.19.

In summary, the same characteristics of S. mutans which enable it to thrive in the mouth are the ones most detrimental to teeth. It forms extracellular polysaccharides with high viscosity and glue-like character and these, in turn, initiate plaque formation (Balekjian et al., 1979; Huis in'T Veld et al., 1979). Streptococcus mutans will adhere to teeth, produce acid, and survive in the absence of exogenous carbohydrate. Naturally, these characteristics intensify the cariogenic potential of this organism.

Caution is important here, however, because the cariogenic process is neither simple, straightforward, nor easy to follow. For example, the matter of assessing whether a lesion has just commenced is very difficult and, therefore, leaves investigations of the exact conditions surrounding the event suspect (Bowden et al., 1979). Furthermore, no absolute correlate exists between Streptococcus mutans and

the onset of caries; relatively high levels of S. mutans percentages can be detected at surfaces without caries progression (Huis in'T Veld et al., 1979). Besides, the development of a lesion to the point where it can be detected takes almost 2 years (Newbrun, 1982a). The pathological sequence can, of course, be interrupted during these two years by any number of events. The fluctuation in the proportionate level of S. mutans in human plaque during this time cannot be simply attributed to any single cause or agent, such as variations in the level of sucrose in the diet (Beighton et al., 1982). Caries prevention thus requires attention on several fronts.

Prophylactic Measures

Flouride

Water flouridation produces a major improvement in the dental health of children (Lauder et al., 1980). Numerous experiments have elucidated the various mechanisms involved in the use of flouride to prevent caries. One such mechanism involves its antibacterial action. Sodium flouride (NaF) and stannous flouride (SnF_2), have long been recognized as antimicrobial agents affecting both growth and viability of Streptococcus mutans (Bowden et al., 1979; Ferretti et al., 1982). Minimal amounts of plaque accumulate in the presence of flouride (Streckfuss et al., 1980). Flouride, reportedly reduces the Streptococcus mutans concentrations in plaque.

When Ferretti and coworkers (1982) observed bacterial growth yields *in vitro*, using optical density measurements, more light passed through flouridated samples; in other words, bacterial yields were lower in flouride supplemented media.

While clearly decreasing total microbial growth, flouride affects cell metabolism at several other levels. Flouride interferes with transport and utilization of carbohydrates (Edgar et al., 1981; Roberts et al., 1981; Brown et al., 198; Tananoff et al., 1980). It does this first by inhibiting the activity of enolase, an important enzyme in the glycolytic reaction whereby 2-phosphoglycerate converts to phosphoenolpyruvate or PEP (Bowden et al., 1979; Edgar et al., 1981). The favorable results of diminished PEP production include a less active PEP-phosphotransferase system - the system which translocates sucrose across the bacterial cell wall (Streckfuss et al., 1980; Brown et al., 1981). Any decreased operation of this system coincides with a decrease in both lactic acid and glucan production. Brown and co-workers (1981) demonstrated that sodium flouride gel use in 1% NaF dilutions significantly impairs the *in situ* production of each of the acids D- and L-lactate. Others have established that flouride concentrations of 1 ppm or slightly less will inhibit bacterial acid production (Ferretti et al., 1982).

Finally, flouride helps prevent caries by strengthening tooth enamel (Edgar et al., 1981). Water flouridation results in hydroxyapatite less susceptible to acid demineralization (Bowden et al., 1979). In this mechanism flouride either reduces enamel solubility or favors its mineralization (Edgar et al., 1981).

Saliva

Saliva has the capacity to minimize caries activity in a variety of ways. It has long been established that saliva is a buffering agent for the acidic changes produced by carbohydrate metabolism in Streptococcus mutans (Leach et al., 1981; Bowden et al., 1979; Abelson et al., 1981). In an in vivo study in which changes in human plaque pH were measured after exposure to sucrose, Abelson and coworkers (1981) found that saliva modified plaque pH. Abelson's group reported that the ability in various individuals reflects their caries status. Rosan et al. (1982) showed that the saliva of caries-free and caries-active people differed in one other respect: caries-free people have a greater ability to minimize adherence of bacteria to hydroxyapatite.

Saliva also contains antibacterial factors which can mediate caries resistance (Bowden et al., 1979). A rapid increase in the number of all bacteria occurs in the disorder xerostomia, where saliva flow is severely reduced.

The greater numbers of acidogenic bacteria result in an increase in caries activity (Bowden et al., 1979). The characterization of the salivary factors inherent in caries-free individuals is important in furthering our understanding of caries resistance and susceptibility.

Host Factors

Dental caries is a multifactorial disease, and host factors are one of the principal components in its etiology (Newbrun, 1982a). Gallagher's lab (1981) has shown that certain individuals harbor the oral bacteria which are acidogenic and hence potentially cariogenic, yet still remain caries free. We do not yet know why. Researchers suspect that host factors are genetic in origin. Caries-free people seem to have heritable characteristics which enable them to resist cavity formation by promoting remineralization of tooth surfaces after initial caries attacks; however, these characteristics must at present be considered a generalized ability because no specific mechanism has been provided (Gallagher, 1981). In any case, these systemic factors may explain the large standard deviations and occasional inconsistent values commonly seen in any set of caries score data (Birkhed et al., 1981). More research is needed to understand the role of genetics and how it affects the outcome of dental disease in the host (Birkhed et al., 1981).

Diet

Strong evidence indicates that diet is a major factor in the initiation and continuation of dental caries (Lauder et al., 1980). With an absence of dietary sugar little decay is found (Screebny, 1982b). This is not to say that all carbohydrates are cariogenic and should be strictly limited in the diet. On the contrary, complex carbohydrates should be included in the diet (Krasse, 1982). In a dietary recall study looking for the effects of certain food groups on dental caries prevalence, researchers found a direct and consistent relationship between intake of candy, gum, and caries, but not between caries and buns, breads, and cereals (Newbrun, 1982a). Sugary foods, not starchy foods, cause decay (Newbrun, 1982a). When sixteen monkeys were switched from a regular starch diet to a high sucrose diet, an immediate increase in the proportion of cariogenic bacteria in plaque and an increase in caries resulted (Beighton et al., 1982b). Patients with hereditary fructose intolerance (HFI) consume very few sweets, cakes, and candies but still consume large amounts of complex carbohydrates; not surprisingly, they are commonly referred to as a caries-free group (Newbrun, 1982a).

Sucrose (a disaccharide) was found to be the most cariogenic in rats when compared to monosaccharides, fructose and glucose, at the end of a 13 week feeding trial (Thompson et al., 1976). Although sucrose may be more cariogenic than

monosaccharides such as fructose, glucose and lactose these are still cariogenic and according to Birkhed and co-workers (1981) should be reduced in the diet as an additional preventive measure.

Cumulative evidence suggests that both the amount of sugar consumption and frequency of between-meal sugary snacks are related to caries in humans and animals (Newbrun, 1982a). In a large Canadian study, 7-day dietary recalls were done by dietitians and the population was divided into city, urban and rural communities (Newbrun, 1982a). They found no correlation between the total sugar intake and past caries experience, but a statistically significant correlation between the prevalence of dental caries and the amount of sucrose eaten between meals (Newbrun, 1982a). The frequency of sucrose intake may be more important than the total quantity of sucrose consumed (Lauder et al., 1980). In other words, one might have a relatively high sucrose intake with little increase in carious surfaces per year provided that the additional sucrose is eaten at meal times (Newbrun, 1982a).

In one study Rugg-Gunn and co-workers (1981) investigated the effect of altering the position of a sugary food at mealtime upon the pH of human plaque. The drop in plaque pH caused by the sugary food was reduced if it were eaten with the meal. The favorable effect was negated, however, if the subject paused between the sugary food and the

non-sugary courses.

The level of sugar consumption, namely sucrose, glucose and fructose at which most of the population will not get dental caries is 15 kg/person/year, or 41 gm/day, according to Sheiham's (1983) report on sugars and dental decay. Sheiham did not say how he arrived at this number but the figure does fall in the same range as that derived by Screebny (1982) who used World Health Organization information on caries prevalence and sugar availability in 23 nations. Screebny reports that the ingestion of 50 gm of sugar per day may represent the outer limit of 'safe' or acceptable sugar consumption. The author did not refer to any specific type of sugar.

There is strong evidence that not only specific composition of a diet but its form of ingestion is a major factor in the initiation and continuation of dental caries (Lauder et al., 1980). The physical composition of a food as it comes in contact with the teeth and oral flora is critical to the way in which the flora and teeth interact with it (Kleber et al., 1979). Highly sugared granular foods become trapped in retentive areas of the teeth, and by exposing surfaces to the bacteria for a long time, cause damage to them. Firestone's group (1980) believes that it is the consistency of sucrose consumption rather than simply the sucrose or fermentable carbohydrate content which more strongly determines food cariogenicity. Whitlock and

co-workers (1978) proved that the change in physical composition of a food caused by an increased fat-to-sugar content would decrease the deleterious effects of an otherwise highly sugared, cariogenic product. Dietary reform is essential to the promotion and prevention of dental health and disease (Lauder et al., 1980).

Dental health education aimed at reducing dietary sucrose intake has proven to be an effective means of caries prevention (Rytomaa et al., 1980). A government program of this type in Finland led to improvement in dental health similar to that seen during the war, a time when sucrose rationing officially curtailed the intake of sucrose-containing foods (Rytomaa et al., 1980). Cavitation, once it has occurred, is irreversible. Therefore, serious consideration should be given to increasing the training of dietitians, nutritionists, and dentists as to how dental disease is caused - particularly as it relates to what we eat. Prevention of caries formation is certainly preferable to remediation.

Research Postulates

Purpose

Sucrose substitutes that interfere with plaque formation are desirable in a food (Balekjian et al., 1977). Many other laboratories have examined the usefulness of sucrose substitutes by testing them for plaque and/or caries reduction (Firestone et al., 1980; Grenby et al., 1982; Kleber et al.,

1979; Ooshima et al., 1983; Thaniyavarn et al., 1981). Certain sugars such as maltose and fructose have been found to significantly reduce the yield of insoluble glucan and because of this are considered less cariogenic (Hamada et al., 1980). When S. mutans is grown with a variety of different carbon sources, different balanced growth rates are achieved. S. mutans grown on maltose supplemented media has a slow doubling time (Cuffini et al., 1982). When Osborne-Mendel rats were fed dry powdered diets (Keyes diet) containing 56% w/w crystalline maltose, pulverized sucrose, lactose and combinations of the above sugars, the rats eating the maltose diet had significantly fewer S. mutans on their teeth ($p<0.01$) (Schemmel et al., 1982). It has been shown that maltose promoted less plaque formation on glass rods by S. mutans when compared to sucrose or lactose supplemented media (Balekjian et al., 1970). The adherence of S. mutans to glass surfaces is also inhibited by maltose (Hamada et al., 1980). This might be explained by experiments carried out in vitro in the lab of Skinner et al. (1982b) which showed that it was the production of the adhesive, sticky extracellular polysaccharides by S. mutans which were nearly halved when cultures were supplemented with maltose syrup as opposed to sucrose. In a cross-over, double blind study involving human subjects this time, the same lab (Skinner et al., 1982b) reported differences in plaque samples of subjects eating

maltose-sweetened diets compared to those sweetened with sucrose. The extracellular polysaccharide content of plaque in the maltose-fed group was lower than in the sucrose fed group ($p=0.052$) (Skinner et al., 1982b). Certainly maltose cannot replace sucrose as a substrate for glucosyl transferase, because the hydrolysis of maltose is thought not to yield the specific energy for the synthesis of glucose polymers (Newbrun et al., 1969). Apparently maltose interferes with the enzymes acceptor specificity and chain initiation (Skinner et al., 1982a).

There has been extensive research done to investigate the effect of sucrose on dental caries. However, the work with maltose is limited. If maltose were proven to be a less cariogenic sugar than sucrose, it would be useful as a sucrose substitute in certain hard candy. The purpose of this investigation was to test the hypothesis that maltose supports less S. mutans growth in the oral cavity than does sucrose and that it, concomitantly, produces less caries in rats.

MATERIALS AND METHODS

Diets

Diet Composition

Two dry powdered diets and two dry cake meal diets were used in this experiment. The composition of the nutritionally defined dry powdered diet is given in Table 1, and the cake meal diet, in Table 2. The dry powdered diet contained 31% more sugar and 11% less fat in it compared to the cake diet. Mineral mix added to the diets was similar to that used by Cerklewski in his caries test diet (Cerklewski, 1981). The mineral mix was prepared using the compounds listed in Table 3. The adequacy of this mix was determined using the information given in the standard text: Nutrient Requirements of Laboratory Animals, p. 64 (Rogers et al., 1978). A comparison between the mineral mix used and actual laboratory rat requirements is presented in schematic form, Table 4. The mineral mix was nutritionally borderline; that is, it contained 88-90% of the requirement for calcium, copper, manganese, and phosphorus and 82% of the iron requirement. Rats fed dry powdered diets which contain high levels of calcium and phosphorus do not get caries (Navia, 1977).

Table 1. Modified caries test diets.

Ingredients	Percent by weight
Maltose[a] or sucrose sugar[b]	58
Cornstarch[c]	10
Lactalbumin[d]	20
Cellulose powder[e]	5
Vitamin mix[f]	1
Mineral mix[g]	3
Corn oil[h]	3

[a]U.S. Biochemical Corporation, Cleveland, Ohio.

[b]Amstar Corporation, New York, New York.

[c]Best Foods, Englewood Cliffs, New Jersey.

[d]Teklad Test Diets, Madison, Wisconsin.

[e]Teklad Test Diets, Madison, Wisconsin.

[f]Teklad Test Diets, Madison, Wisconsin.

[g]Composition, Table 3, pg. 33.

[h]Best Foods, Englewood Cliffs, New Jersey.

Table 2. Modified caries test diet with cake crumbs.

Ingredients	Percent by weight
Maltose[a] or sucrose cake[b]	68
Lactalbumin[c]	20
Cellulose powder[d]	5
Vitamin mix[e]	1
Mineral mix[f]	3
Corn oil[g]	3

[a,b]Cake composition, Table 6, pg. 38.

[c]Teklad Test Diets, Madison, Wisconsin.

[d]Teklad Test Diets, Madison, Wisconsin.

[e]Teklad Test Diets, Madison, Wisconsin.

[f]Composition, Table 3, pg. 33.

[g]Best Foods, Englewood Cliffs, New Jersey.

Table 3. Composition of mineral mix used in experimental diets.

Mineral	gms./kg. mineral mix
Calcium phosphate $CaHPO_4$	568.1800
Sodium chloride NaCl	103.8600
Potassium sulfate K_2SO_4	54.5400
Potassium carbonate (anhydrous) K_2CO_3	58.9100
Potassium citrate $K_3C_6H_5O_7 \cdot H_2O$	149.3000
Manganese chloride $MnCl_2 \cdot 4H_2O$	6.0046
Ferric citrate $FeC_6H_5O_7 \cdot 5H_2O$	6.3337
Manganese carbonate $MgCO_3$	51.2800
Zinc subcarbonate $ZnCO_3$	.9615
Cupric chloride $CuCl_2 \cdot 2H_2O$	.4472
Potassium iodate KIO_3	.0112
Sodium selenate $Na_2SeO_4 \cdot 10H_2O$	.0078
Chromium potassium sulfate $CrK(SO_4)_2 \cdot 12H_2O$	.1601

Note: The mineral mix was pulverized thoroughly for 40 minutes with mortar and pestle.

Table 4. Mineral mix composition relative to mineral requirements for rats.

Mineral	Mineral requirements (gms)* kg diet	Mineral present (gms) kg diet	Percent of requirement
Calcium	5.6	5.022	90
Chlorine	0.6	1.96	327
Copper	0.0056	0.005	89
Iodine	0.00017	0.0002	118
Iron	0.0389	0.0317	82
Magnesium	0.4	0.44	110
Manganese	0.0556	0.05	90
Phosphorus	4.4	3.89	88
Potassium	2.0	3.45	173
Selenium	0.00004	0.00005	125
Sodium	0.6	1.23	205
Zinc	0.0133	0.015	113

*Values tabulated using information given on pg. 64, Nutrient Requirements of Laboratory Animals, Third Revised Edition, 1978; National Academy of Sciences, Washington, D.C.

Cakes

Cakes used in the two diets which required them (Table 2) were prepared using a standard batter type recipe (Table 5) (Thompson et al., 1976). The sugars used in both the cake and dry diets were sifted first in order to equalize particle size and provide for similarity in texture. A Fisher Scientific 100 mesh sieve was used for sifting. The sugar, hydrogenated shortening, flour, baking powder, salt, dried whole milk and 223 gms of distilled water were then placed together in a 5 quart bowl. The ingredients were blended in a Kitchen Aid mixer, Model K5-A, set at speed 2 (92 rpm) for 2 minutes. After scraping the bowl and beater, the dried eggs were sifted and added along with the remaining water and vanilla. The batter was mixed at speed 2 (92 rpm) for 1 minute and speed 4 (132 rpm) for 1 minute. Three aluminum 8 inch pans were oiled with corn oil and covered on the bottom with waxed paper which had also been oiled. Each pan was filled with 600 gms of batter and baked for 28 minutes at 177±2°C in a Hotpoint Deck Oven, model HJ225. The cake was weighed before and after baking.

Following baking, cakes were cooled for 15 minutes before being removed from the pans and cooled for an additional hour after being removed and placed on wire racks. The cakes were grated into small pieces by hand and placed in drying ovens (61±2°C) until a constant weight was reached. This usually required 60 hours of drying. A

Table 5. Cake formula for one replication.

Ingredient	Amount
Shortening, vegetable[a]	195.0 gms
Maltose[b] or sucrose sugar[c]	473.0 gms
Vanilla[d]	5.0 gms
Egg solids[e]	45.0 gms
Flour[f]	450.0 gms
Baking powder[g]	21.0 gms
Salt[h]	7.5 gms
Milk, whole dried[i]	60.0 gms
Water, distilled[j]	626.0 gms
Total	1882.5 gms

[a]Crisco[R], Procter and Gamble, Cincinnati, Ohio.

[b]U.S. Biochemical Corporation, Cleveland, Ohio.

[c]Generously contributed by Amstar Corporation, New York, New York.

[d]McCormick and Co., Inc., Baltimore, Maryland.

[e]Hentex 10, generously provided by Henningson Foods, Inc., White Plains, New York.

[f]Pillsbury Company, Minneapolis, Minnesota.

[g]SAS phosphate type, Calumet, General Foods Co., White Plains, New York.

[h]Hardy Salt Co., St. Louis, Missouri.

[i]Wisconsin Dairies, Madison, Wisconsin.

[j]Michigan State University, Food Science and Human Nutrition Department, East Lansing, Michigan.

batter weight, baked weight and a drying weight of 24, 48, and 60 hours were recorded. Sixty hours was generally sufficient drying time. The 48 hour and 60 hour drying weight were within 3 grams or 3 percent by weight of each other. These cake crumbs had lost 34-37% of their weight as moisture after this time and were the actual product used in two of the four diets. However, to insure a uniform texture between the diets the cakes were ground into fine uniform particles using a Comminuting Machine or otherwise called Fitz Hammer Mill (W.J. Fitzpatrick Co., Chicago, IL) with U.S. standard sieve size 125. The mesh opening of this sieve was .125 inches in diameter. The final product was refrigerated in metal containers until a complete cake diet was prepared. Calculated dry matter and protein for the cakes are given in Table 6.

Diet Preparation

For all 4 diets, batches were made in 5 kilogram amounts. The lactalbumin and oil used in both diets (Tables 1 and 2) were added to a bowl in which the vitamin mix and mineral mix had first been stirred together. These four ingredients were mixed with a wooden spatula to a crumbly consistency. This mixture was then combined with the cellulose powder and the maltose or sucrose sugar or cake and blended for 30 minutes at speed 2 in a 5 kilogram capacity Hobart mixer; model A 200.

Table 6. Cake formula, calculated dry matter and protein content.

Ingredient	Amount (gms)	Dry Matter (%)	Dry Matter (gms)	Protein (%)	Protein (gms)
Shortening[a]	195.0	100	195	0	0
Sugar[b]	483.0	100	473	0	0
Vanilla[c]	5.0 mls	0	0	0	0
Egg solids[d]	45.0	100	45	10	4.5
Flour[e]	450.0	88	396	7.5 (wet)	33.9
Baking powder[f]	21.0	100	21	0	0
Salt[g]	7.5	100	7.5	0	0
Dried whole milk[h]	60.0	100	60	28	7
Distilled water[i]	626.0	0	0	0	0
Totals			1197.5		54.3
Protein (gms/100 gms dry matter)					4.53

[a]Hydrogenated, Crisco[R], Procter and Gamble, Cincinnati, Ohio.

[b]Maltose-U.S. Biochemical Corp., Cleveland, Ohio.
Sucrose- Amstar Corporation, Englewood Cliffs, New Jersey.

[c]McCormick and Co., Inc., Baltimore, Maryland.

[d]Hentex 10, Henningson Foods, Inc., White Plains, New York.

[e]Pillsbury Company, Minneapolis, Minnesota.

[f]SAS phosphate type, Calumet, General Foods Co., White Plains, New York.

[g]Hardy Salt Co., St. Louis, Missouri.

[h]Wisconsin Dairies, Madison, Wisconsin.

[i]Michigan State University, Food Science and Human Nutrition Department, East Lansing, Michigan.

The two caries test diets using the maltose or sucrose alone were prepared in the same fashion as the two diets using cake crumbs made from the 2 sugars. All diets were stored in metal containers and kept in a refrigerator at $29\pm2^{o}F$.

pH of Diet Slurries

Diet slurries were prepared in a beaker by adding 1 gram of diet to 60 mls of distilled water. A magnetic mixer (Cenco Brand) was used to stir the contents of the beaker. The pH values for all diets were taken using Beckman's Expandomatic.

Animals

Forty-eight Osborne Mendel rats were used in this study. The rats had been bred in the animal laboratory of the Department of Food Science and Human Nutrition (FSHN), Michigan State University, and were offspring of National Institutes of Health Stock (Bethesda, MD, U.S.A.). Twenty-four rats were fed the cake meal diet and twenty four rats were fed the dry powdered diet. The groups were further divided such that 12 rats consumed a sucrose diet and 12 a maltose diet. The rats were kept in a room which alternated with twelve hours of light and dark and was maintained at an ambient temperature of $23\pm1^{o}C$ for the entire period of the experiment. Diet and distilled water

were available ad libitum.

In order to control for post-natal, pre-weaning feeding conditions all pups were raised in litters of eight rats each. Rats were weaned at 18-20 days of age and three or four pups of one sex and fed the same diet were housed in suspended stainless steel cages (10 inches x 17 inches x 7 inches) for 3 weeks. This promoted cross infection with inoculum bacteria, Streptococcus mutans. Rats were identified using ear clippings on one, neither or both ears. Body weights of the experimental groups were as similar as possible at the initiation of the experiment. It should be noted that after this initial 3 week period rats were housed individually in suspended cages (8 inches x 9.5 inches x 7 inches) during the 3 or 8 additional weeks of the experiment. A total of 24 rats, divided into 6 rats/diet group, were fed in 2 separate trials of the experiment.

The original culture of S. mutans used in Trial 1 was obtained from the National Institute of Dental Research through the generous contribution of Rachel Larson, Ph.D. The 24 rats used in Trial 2 were inoculated with newly arrived culture 6715-15 obtained from American Type Culture Collection, Rockville, MD. The inoculum bacteria was removed from a 48 hour working culture standardized to 25% transmittance at 500 nm. The spectrophotometer used to standardize the culture was a G.K. Turner Associates

model 330. At weaning, a syringe was used to inoculate half of the rats with 3 drops of pure culture Streptococcus mutans 6715-15. The rats were also provided with drinking water which contained Streptococcus mutans in solution. This solution was prepared by mixing 5% sucrose and 1% standardized culture of Streptococcus mutans in double distilled water. The rats drank this for 3 days post weaning until the water bottles were replaced and filled with plain double distilled water.

Rats were weighed and food intakes were tabulated on a weekly basis. Food spillage was adjusted for by subtracting the spilled ration which had been collected on a paper towel under each cage from the food intake measured for that week. Food cups, food savers and water bottles were changed every 7 days.

The rats were exposed to the total cariogenic challenge, diet plus infection, for 6 weeks (60-62 days of age) or 11 weeks (95-97 days of age). Rats were then anaesthesized with carbon dioxide and decapitated. The head was sectioned in the sagittal plane with surgical shears and the molar teeth from the mandibular and maxillary jaws of the anatomical right side were prepared for evaluation of microorganisms. Identical teeth on the anatomical left side were prepared for and subsequently scored for dental caries.

Caries Scoring

Jaw Preparation

Jaws were coded to remove bias and provide for blind scoring. The left sides of the heads were autoclaved in glass jars at 121-123^{o}C for 15 minutes at 15 lbs. pressure in a Castle Thermatic 60 autoclave. Soft tissue was removed from the jaws using tweezers. Following this, the skulls were further cleaned by soaking them in a 2% solution of ammonium hydroxide for 1 hour. Dissecting equipment was again used to scrape flesh from the jaws. This process was repeated if necessary. Finally, the jaws were rinsed with double distilled water, air dried and stored until scoring.

Teeth Scoring

The three rat molars from the upper and lower left jaw quadrants were scored for buccal, lingual, sulcal and proximal caries using the method of Keyes (Keyes, 1958). This scoring procedure permits immediate comparisons of caries patterns. The selected method identifies the location of the lesion and quantifies the intensity of attack.

Using this technique, four surfaces of each tooth were identified: the buccal surface is the side of the tooth closest to the cheek, the lingual surface is closest to the tongue; the sulcal surface is the top surface and is associated with chewing of food; and, lastly, the proximal surface, which includes the surfaces facing the front or

back of the mouth. The proximal surface on a middle molar interfaces proximal surfaces of the teeth directly adjacent to it. The rat has three molars per jaw and each molar surface is divided into a certain number of linear units depending upon molar structure and size. These units are standardized boundaries which separate the various teeth into fractions or theoretical slices. The caries scoresheet includes drawings of each rat tooth surface and one can easily visualize these standard sub-units. Each sub-unit is evaluated and scored according to size and depth of lesion. Four different depths of a lesion have been described. Each tooth surface has a score recorded for each of 4 different depths. The number of sub-units of that tooth surface which are decayed to the depth in question are recorded in a box labelled as that depth. For example, if the buccal (cheek) side of a tooth had only the enamel decayed and the tooth was one of the smaller molars which have only 4 sub-units on the buccal surface and furthermore all four sub-units had enamel decay then the box labelled "E" for enamel under a picture of that tooth surface should contain a 4. The three other boxes (depths) would contain zero. The three other depths are slight dentinal (D_s), moderate dentinal (D_m) and extensive dentinal (D_x).

The teeth are examined under 20 power magnification and the differences in depth can be distinguished. When water

is allowed to evaporate off a tooth surface under a stream of compressed air any decalcified enamel dehydrates more rapidly than sound enamel and appears as chalky white. If the surface is chalky but unbroken the caries involvement is said to be E. If the surface is slightly loose and flaky the involvement is D_s. If a probe can be inserted into the lesion the involvement is D_m. If a probe can be extended all the way into the pump chamber the involvement is D_x. The total caries score for a given tooth surface is the sum of all the linear units involved at any depth, i.e., the entries in the four boxes labelled E, D_s, D_m, and D_x are summed together. Using this method it is possible to compare total caries per surface area within one rat. It is also possible to compare enamel caries or slight dentinal caries separately. Buccal and lingual surface caries are not usually compared separately but are added instead and referred to collectively as the smooth surface caries. Proximal caries are also typically included in a smooth surface caries score. The sulcal and proximal lesions could not be identified until after staining.

The teeth were first scored for buccal/lingual caries. Following this they were placed in a Murexide staining solution for 24 hours. Murexide[1] or ammonium purpurate (60 gms) was dissolved in 75 mls of distilled water and

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[1] Murexide purchased from Sigma Chemical Company, St. Louis, MO.

175 mls absolute alcohol. This was filtered through a Buchner funnel using a vacuum and filter paper circles 9.0 cm in diameter. After 24 hours the staining solution was discarded and the teeth were allowed to air dry for several days. The buccal and lingual scores were then verified under 20 power magnification.

In order to fully examine the sulcal surfaces of the teeth each jaw quadrant was sectioned mesiodistally. A standard dental engine was mounted with a steel disc saw (0.04 in thick, 0.75 in diameter) and was accelerated to full speed during the slicing process. Before slicing, the teeth were coated with nail polish to minimize shattering. While being sliced the teeth were held with a small hemostat beneath a slow drip of water. The sliced teeth were air dried for 24 hours and then examined for sulcal and proximal caries.

The depth of the sulcal and proximal lesions was determined by the extent of stain penetration. When the tooth is cut the border between the enamel and dentin can be seen. If the pink stain has not penetrated this border the sulcal or proximal lesion is enamel (E) only. If the stain is 1/4 of the way into the dentin then the lesion is slight dentinal (D_s). If the stain has colored 1/4 to 3/4 of the dentin then the lesion is moderate dentinal (D_m). A final score for a sulcal lesion is assigned on the basis of half having the greatest involvement. If the dentin is

completely stained then the lesion is extensive dentinal (D_x).

Proximal surfaces were also scored by measuring stain penetration, however, if no proximal lesions were visible a probe was used to separate the molars and expose the entire proximal surface. Uneven slicing could cause some of the proximal caries to be overlooked and would require that the teeth be separated from each other.

Microbial Evaluation

The molar teeth from the anatomical right were evaluated for microorganisms. Teeth were aseptically removed from the jaws and transferred to 5 mls of reduced transport fluid (RTF). All RTF samples were serially diluted by 10 fold (10^{-1}, 10^{-2}, 10^{-3}). These were plated on two different media. Medium one: modified media 10 agar (mm 10) is a non-selective all purpose medium which provides for the growth of all microorganisms (Schemmel et al., 1982). Medium two: Mitis Salivarius with Streptomycin supports the growth of one specific organism, Streptococcus mutans. One complete set of plates were incubated aerobically and one set anaerobically. Therefore, total colony forming units (CFU) and Streptococcus mutans colony forming units were counted and recorded in the results.

Statistical Evaluation

The data for *Streptococcus mutans*, total bacterial count, sulcal caries, smooth surface caries and total caries was transformed to the log scale. The hypothesis was statistically tested on a log scale because the original data, when plotted, was not distributed as a normal, bell-shaped curve. The data collected for weekly food intake and total weight gain was left unaltered.

Means and standard errors were calculated for each group using the following data as recorded for each individual rat: a smooth caries score which included buccal, lingual and proximal caries, a sulcal caries score, a total caries score, a total weight gain during the experiment, an average weekly food intake, a total bacterial count and a *Streptococcus mutans* bacterial count.

Student's paired t-test (Navia, 1977) was used to compare caries formation and bacterial growth between rats consuming the diets containing one sugar or the other. The same one way t-test was performed to compare weight gain and food intake between these groups. Statistical analysis was performed using the CDC 6500 computer, Michigan State University.

RESULTS

Food Intake

There were no significant differences ($p<0.10$) in average weekly food intakes among rats fed the sucrose or maltose diets either in the dry powdered or cake meal form, Table 7. Weekly food intake values were those collected while rats were caged individually, that is, weeks 3 through 6 or 3 through 11 of the experiment. Male rats fed the dry powdered diet containing either sucrose or maltose for 6 weeks had an average weekly food intake of 125 and 137 g, respectively, females, 94 and 88 g, respectively, Table 7. Male rats fed the same dry powdered diet with maltose or sucrose for 11 weeks both had an average weekly food intake of 131 g and females had an average of 107 and 101 g, respectively (Table 7). Male rats fed the cake meal diet for 6 weeks consumed an average of 120 g/week of the sucrose formulated cake diet and 140 g/week of the maltose formulated diet (Table 7). Female rats fed the cake meal diet for 6 weeks consumed an average of 86 g/week of the sucrose cake meal and 84 g/week of the maltose cake meal. Rats fed the same cake meal diet during an 11 week period consumed an average of 119 g/week of sucrose (males) and

Table 7. Average weekly food intake - males and females.

Food intake (g)	Treatment[a]	
	6 weeks	11 weeks
Diet	Males	
Dry powdered sucrose	125[b]	131
Dry powdered maltose	137	131
Cake meal sucrose	120	119
Cake meal maltose	140	109
	Females	
Dry powdered sucrose	94	107
Dry powdered maltose	88	101
Cake meal sucrose	86	97
Cake meal maltose	84	94

[a]Feeding trials began at weaning or 18-20 days of age.

[b]Food intake values collected while rats were caged separately.

109 g/week of the maltose diet (males), Table 7. For females fed the diet for 11 weeks the values were 97 and 94 g for sucrose and maltose cake meal, respectively.

Body Weights

All animals in each of the treatment groups increased in weight over the 6 and 11 week experimental periods. Mean cumulative body weight gain data for these rats is presented in Table 8. Male rats which were fed sucrose and maltose dry powdered diets for 6 weeks (from age 18 days) gained a mean weight of 221 g and 232 g, respectively (Table 8). Males fed the sucrose and maltose dry powdered diet for 11 weeks (from age 18 days) gained a mean weight of 337 g and 312 g, respectively (Table 8). Male rats which were fed the sucrose and maltose cake meal diets for 6 weeks gained a mean weight of 270 and 246 g, respectively (Table 8). Males fed the sucrose and maltose cake meal diets for 11 weeks gained a mean weight of 357 and 331 g (Table 8). Differences between mean weight gains in males or females for maltose or sucrose comparisons were not significant at the 90% or 95% confidence level.

Dental Caries Incidence

Means, standard errors and statistical analysis of dental caries severity in the 6 molar teeth from the left mandible and maxilla of all 48 rats are presented in Tables 9 and 10. In all treatment groups there were no

Table 8. Cumulative weight gain data - males and females.

Weight gain (g)	Treatment	
	6 weeks	11 weeks
Diet	Males	
Dry powdered sucrose	221	337
Dry powdered maltose	232	312
Cake meal sucrose	270	357
Cake meal maltose	246	331
	Females	
Dry powdered sucrose	125	229
Dry powdered maltose	123	200
Cake meal sucrose	146	216
Cake meal maltose	139	222

Table 9. Incidence of caries in rats consuming dry powdered diets-comparisons among means.

Tooth surfaces (N=6)	Treatment		Significant difference[c]
	Sucrose	Maltose	
	6 weeks		
Buccal/lingual/ proximal tooth surface (log carious units)	0.9±0.1[b]	0.7±0.2	n.s.
Sulcal tooth surface (log carious units)	1.1±0.2	0.9±0.1	n.s.
Total[a]	1.3±0.1	0.8±0.1	n.s.
	11 weeks		
Buccal/lingual/ proximal tooth surface (log carious units)	1.1±0.1	0.8±0.2	n.s.
Sulcal tooth surface (log carious units)	1.4±0.1	1.3±0.1	n.s.
Total	1.6±0.1	1.5±0.1	n.s.

[a]Values represent an accumulated score which takes into account the width and depth of the lesions on 6 teeth.

[b]Mean±SEM (log values).

[c]Student's t-test, separate variance estimate, $p \geq 0.10$.

Table 10. Incidence of caries in rats consuming cake meal diets-comparisons among means.

Tooth surfaces (N=6)	Treatment		Significant difference[c]
	Sucrose	Maltose	
	6 weeks		
Buccal/lingual/ proximal tooth surface (log carious units)	0.7±0.1	0.6±0.1	n.s.
Sulcal tooth surface (log carious units)	0.8±0.2	1.1±0.7	n.s.
Total[a]	1.1±0.1	1.2±0.1	n.s.
	11 weeks		
Buccal/lingual/ proximal tooth surface (log carious units)	0.4±0.2	0.7±0.1	n.s.
Sulcal tooth surface (log carious units)	1.0±0.2	1.1±0.1	n.s.
Total	1.2±0.1	1.3±0.1	n.s.

[a]Values represent an accumulated score which takes into account the width and depth of the lesions on 6 teeth.

[b]Mean±SEM (log values).

[c]Student's t-test, separate variance estimate, $p \geq 0.10$.

significant differences in mean carious units using log values of buccal/lingual/proximal surfaces, sulcal surfaces and total caries scores when maltose treatment was compared to sucrose treatment. The differences between the log values of these figures did not reach the critical values to be significant at the 10% level ($p \geq 0.10$). A separate variance estimate was more conservative than a pooled variance estimate in testing for significant differences. The original data as mean±SD (Table 11) is presented for reference, although statistical tests were performed using only the log values.

Microbial Analysis

Means, standard errors and statistical analysis of microorganisms in the six molar teeth from the right mandible and maxilla of all rats are presented in Tables 12 and 13. Only the data from the aerobically grown set of plates was reported since no difference was found between anaerobically or aerobically grown plates. For technical reasons, only half the microbial counts were included in comparisons. The statistical analysis showed that when sucrose fed rats were compared to their maltose fed counterparts there were no significant differences in total bacterial counts (log CFU/mlx10^5) or *Streptococcus mutans* species counts (log CFU/mlx10^5). The differences between these means did not reach the critical value to be significant at the 10% level ($p \geq 0.10$).

Table 11. Incidence of dental caries in rats fed diets containing maltose or sucrose.

Diet N=6	Carious units		
	Buccal/lingual/ proximal	Sulcal	Total
		6 weeks	
Sucrose - dry powdered	9.7±3.4[a]	17.2±11.5	26.0±12.8
Maltose - dry powdered	8.5±6.3	9.2±3.8	17.7±7.3
Sucrose - cake meal	5.3±2.5	9.2±6.9	14.5±6.5
Maltose - cake meal	4.3±2.7	14.8±8.6	19.2±8.7
		11 weeks	
Sucrose - dry powdered	14.8±6.9	30.0±16.1	44.8±20.7
Maltose - dry powdered	8.8±6.2	22.3±5.7	31.2±9.8
Sucrose - cake meal	3.7±3.1	14.3±7.9	18.0±5.4
Maltose - cake meal	5.3±2.6	14.8±9.3	20.2±9.5

[a]Mean±S.D.

Table 12. Total bacterial counts and *Streptococcus mutans'* species counts in rats consuming dry powdered diets (N=3).

	Treatment		Significant difference[b]
	Sucrose	Maltose	
	6 weeks		
Total bacteria (CFU/ml x 10^5)	7.0±0.3	6.7±0.1	n.s.
Streptococcus mutans (CFU/ml x 10^5)	5.9±0.2	6.0±0.4	n.s.
	11 weeks		
Total bacteria (CFU/ml x 10^5)	5.9±0.2	5.4±0.3	n.s.
Streptococcus mutans (CFU/ml x 10^5)	5.2±0.4	4.4±0.4	n.s.

[a] Mean±SEM (log values).

[b] Student's t-test, separate variance estimate, $p \geq 0.10$.

Table 13. Total bacterial counts and *Streptococcus mutans'* species counts in rats consuming cake meal diets (N=3).

	Treatment		Significant difference[d]
	Sucrose	Maltose	
	6 weeks		
Total bacteria (CFU/mlx10^5)	6.6±0.4	6.2±0.3	n.s.
Streptococcus mutans (CFU/mlx10^5)	5.4±0.2	5.6±0.1	n.s.
	11 weeks		
Total bacteria (CFU/mlx10^5)	5.7±0.5	5.2±0.1	n.s.
Streptococcus mutans (CFU/mlx10^5)	4.2±0.2	4.5±0.2	n.s.

[a]Mean±SEM (log values).

[b]Student's t-test, separate variance estimate, $p \geq 0.10$.

Diet Slurry pH Measurements

Very small differences in diet slurry pH values were found (Table 14). The pH values for cake sucrose and cake maltose diets were 6.0 and 5.9, respectively. The pH values for the dry powdered sucrose and dry powdered maltose diets were 5.9 and 5.6, respectively.

Table 14. Diet slurry pH values.

Diet	pH
Dry powdered sucrose	5.9
Dry powdered maltose	5.6
Cake meal sucrose	6.0
Cake meal maltose	5.9

DISCUSSION

The present work shows that rats have no significant improvement in caries incidence when fed diets with maltose substituted for sucrose. This result occurred when two different diets were used. Maltose has been reported to be a less suitable carbon source than sucrose *in vitro* (Balekjian et al., 1977) and *in vivo* (Schemmel et al., 1982) for sustaining *Streptococcus mutans* growth. Compared to sucrose, maltose also decreases extracellular glucan production (Skinner et al., 1982b). However, apparently the decrease was not sufficient to effect the outcome of caries incidence. That these changes do not effect caries incidence is in agreement with the findings of Skinner et al., 1982a, and Schemmel et al., 1982. In Skinner's study rats were fed a 67% maltose or sucrose diet for 4 weeks.

Bacterial fermentation of maltose most likely produces acid in quantities sufficient to decay teeth. All strains of streptococci readily ferment sucrose and maltose as well as their monosaccharidic components - glucose and fructose (Starr et al., 1981). Skinner's group (1982b) demonstrated that the acid production from the metabolism of these two

sugars is almost identical. Analysis of plaque pH showed similar values - 6.37 and 6.32 - when maltose-rich and sucrose-rich diets respectively were fed to human subjects. Although it did not prove possible in this experiment to evaluate the pH of plaque in our animals, the pH of the 4 diets were checked and found similar - range 5.6-6.0. This information, combined with the results showing no significant difference in oral Streptococcus mutans counts between rats fed maltose and sucrose circumstantiates the similar caries incidence scores.

It is important to note, at this point, that we were in fact able to get caries, at all, using the nutritionally defined diet that was formulated in this lab. This was an achievement because, while the rats did get caries they remained otherwise healthy and continued to gain an average of 20-25 grams per week. The most commonly used cariogenic diet - NIDR Diet 2000 - does not provide for optimum rat growth. Among other things NIDR Diet 2000 contains brewer's yeast, alfalfa meal, liver powder and dried skim milk as nutrient sources and is not a nutritionally defined diet.

CONCLUSIONS

Sucrose may be slightly more cariogenic than maltose but the present work shows that maltose is not significantly different in the amount of caries produced and should not be considered as a good sucrose substitute.

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