EFFECTS OF CHOCOLATE MILK ON DENTAL CARIES
UNDER MOUTH SIMULATION CONDITIONS

by

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INTRODUCTION
Dental caries continues to be a major public health problem despite significant advances in understanding its complex etiology. It affects over 95 percent of the population and remains the leading oral health problem of children.\(^1\) Dental decay begins early in life, as indicated in a study by Hennon, Stookey and Muhler\(^2\) who reported in 1969 that 8.3 percent of the 18- to 23-month-old children had dental caries and that the proportion increased to 57.2 percent in the children 36- to 39 months old. The dental caries activity increases in childhood and adolescence with dietary exposures to fermentable foodstuffs. Currently, scientific evidence indicates that the fermentable carbohydrate content of foods, especially sucrose, is the most important dental caries-promoting factor in the diet.

Because milk has nutritional properties particularly suited to support growth and development, it is a major food source for newborn infants. During infancy and early childhood, food habits are established that may influence the nutritional status and the general health and welfare of the child. Appropriate dietary recommendations to parents are important for the optimum growth and health of their children.

The effect of chocolate milk on the dental caries process is not clear. Cocoa, with antibacterial and enamel-solubility-reducing properties, may inhibit the formation of dental caries. However, cocoa and sugar as well as other compounds are combined to make a chocolate-flavoring agent; therefore, recommendations for chocolate milk in the diet are controversial because of the potential harmful effect on teeth.
Surveys\textsuperscript{3} for 1972 report that more than 3,419,000 pounds of milk are sold per day as flavored milk in the United States, of which 95 percent is estimated to be chocolate-flavored.\textsuperscript{4} Since chocolate milk contains a significant amount of sucrose (about 5 percent) and some cocoa (about 1 percent),\textsuperscript{5} laboratory testing of the cariogenicity of chocolate milk, both in the form usually sold and in formulations that permit separate evaluations of sugar and cocoa, seemed worthwhile.

The purpose of this investigation was to determine whether under mouth simulation conditions chocolate milk influenced the formation of dental caries as compared to white milk.
REVIEW OF THE LITERATURE
This review will consist of four parts: (1) Definition of Dental Caries - page 3, (2) Role of Bacteria and Cariogenic Streptococci - page 4, (3) Effects of Dietary Factors upon Dental Caries (Sucrose, Milk, Lactose, Cocoa, Chocolate, and Chocolate Milk) - page 12, and (4) In Vitro Dental Caries Research - page 46.

I. Definition of Dental Caries

Dental caries, as recently defined by the World Health Organization, is a "localized, posteruptive, pathological process of external origin involving softening of the hard tooth tissue and proceeding to the formation of a cavity."

Although the exact mode of pathogenesis is not fully known, several theories have been advanced to explain the mechanism for caries initiation and development, including the chemico-parasitic, the proteolytic, the proteolysis-chelation, and others. Most dental scientists and clinicians have accepted as the most plausible the chemico-parasitic theory proposed by Miller in 1890. This "acidogenic theory" maintains that the carious lesion is initiated by acid decalcification of the inorganic component of the enamel, followed by the enzymatic lysis or dissolution of the relatively small amount of enamel protein. Accompanying the acid decalcification, cariogenic bacteria invade the tooth and continue to undermine and destroy the enamel and dentin, resulting in cavitation.
The original theory has been expanded with new knowledge about the metabolic activities of bacteria and biochemical reactions that may occur in dental plaque. More specifically, dental caries results from colonization of vulnerable tooth surfaces by a characteristic group of bacteria. Harbored by many members of a susceptible host species, these bacteria are transmitted from the host to previously uninfected members of the same species. These bacteria ferment dietary carbohydrate primarily to lactic acid which, at susceptible sites, initiates the carious lesion by demineralizing the enamel surface. The dominant group of cariogenic bacteria can metabolize sucrose so that an adhesive extracellular polysaccharide (dextran) is produced from the glucose moiety, and mainly lactic acid is produced from the fructose moiety. Typically, these bacterial plaques store intracellular polysaccharide as amylopectin during periods of environmental abundance; this amylopectin is then utilized with the subsequent formation of lactic acid during periods of carbohydrate deficiency. The development of caries requires critical relationships between surfaces, oral microbiota and dietary carbohydrate. 9,10

II. Role of Bacteria and Cariogenic Streptococci

Miller 6 reported in 1890 that in vitro incubation of teeth in a mixture of saliva and bread resulted in the formation of sufficient acid to produce carious lesions. He demonstrated that this action was caused by living bacterial organisms that fermented carbohydrate. Miller then conducted a series of experiments to examine the role of bacteria in the fermentation of carbohydrates into acids which was thought to be decisive in the initiation of dental caries. Using a mixture of human saliva and
starch, he was able to produce acids only under certain conditions. When a mixture of a small amount of saliva and starch was sterilized, no acid was produced. When starch alone was sterilized and then added to saliva and incubated, acid was produced. If saliva alone was sterilized and added to the starch, however, no acid was produced. This series of experiments demonstrated that a viable entity in saliva was responsible for the formation of acid. As a result of these experiments, Miller proposed that bacteria were the etiological agents of dental caries.6

Dental caries does not attack all dental surfaces indiscriminately, but lesions are confined to localized areas of the teeth. The lesions are most prevalent in pits and fissures which usually have a depth suitable for bacteria to lodge. Equivalent protection for bacterial colonies on smooth surfaces of teeth is provided by a tightly adherent mass of bacterial bodies and debris called plaque. The organisms found in pits and fissures usually are found on smooth surfaces. Stephan11 in 1953 reviewed the historical development of plaque. Knowledge of dental plaque began to develop before the beginning of the twentieth century, with descriptions of the localized, tenaciously adherent masses beneath which acidogenic micro-organisms initiated carious lesions that could be demonstrated in prepared sections. Over 85 years ago, G. V. Black12,13,14 described a special type of "gelatinous microbial plaque" found at the site of carious lesions. He suggested that plaques were formed by the "caries fungus" on dental surfaces as a "gelatine-like" substance in a sucrose containing medium. Black made careful distinction between the tenaciously adherent masses of micro-organisms (microbial plaque) that he thought influenced the initiation of a carious lesion, and the easily
removable superficial accumulations of debris and micro-organisms. These he considered unrelated to the process of caries.

In 1902 Miller\textsuperscript{15} reported that he could find no evidence that bacterial plaque was essential for the initiation of a carious lesion. He argued for the study of the gelatinous substance under which bacteria could produce their acids and asked for direct proof that bacteria on teeth were glue-makers.

Although bacteria had been suspected as causative agents in the process of dental decay, conclusive evidence was not obtained until 1955. Orland et al\textsuperscript{16} showed that rats raised under germfree conditions did not develop molar caries even though they were genetically susceptible and were fed a cariogenic diet. Further investigations by Orland et al\textsuperscript{17} with gnotobiotic rats demonstrated conclusively that bacteria are essential for caries production. Germfree rats were inoculated with known bacterial species and fed a cariogenic diet and all rats developed extensive molar decay.

Keyes\textsuperscript{18} in 1960 showed that in animals, caries is an infectious and transmissible disease. In subsequent experiments, Fitzgerald and Keyes\textsuperscript{19} found that specific streptococci isolated from caries-active hamsters could induce extensive decay in gnotobiotic hamsters.

Streptococci with similar biochemical characteristics have been isolated from human carious lesions by Zinner and co-workers,\textsuperscript{20} Krasse,\textsuperscript{21} and Gibbons et al,\textsuperscript{22} and these streptococci have caused extensive decay when implanted in experimental animals. Streptococci have the ability to colonize on teeth in vivo\textsuperscript{23} as well as on teeth and various other
surfaces in vitro. The formation of a highly polymerized polysaccharide dextran produced by these streptococci when grown in a sucrose-containing medium has also been observed by other investigators. Carlsson in 1967 pointed out that bacteria with the same biochemical characteristics had been described by Clarke in 1924, and proposed that the name used by Clarke, *Streptococcus mutans*, be used. Scherp recently reviewed the dental caries process and concluded that some strains of several other bacterial species have induced coronal caries in teeth of hamsters, gnotobiotic rats and other animals, when implanted in the oral cavity in conjunction with a high sucrose diet. Included were strains of *Streptococcus faecalis, sanguis, salivarius*, *Lactobacillus acidophilus* and *Lactobacillus casei*. On the whole, however, these organisms have induced dental caries with less regularity and to a lesser extent than strains of *S. mutans*, particularly on coronal smooth surfaces.

Research indicates that *S. mutans* produce abundant amounts of extracellular dextrans when grown in the presence of sucrose, but that almost none is produced from other sugars. In a number of tests the formation of plaque, either in vitro or in vivo, is dependent upon the availability of sucrose. Microbial-produced dextrans form insoluble precipitates with salivary proteins and may be adsorbed on hydroxyapatite. Suspensions of glucose-grown (dextran-free) cells of *S. mutans* are agglutinated specifically on addition of high-molecular weight dextrans and such cells attach to dextran-coated teeth. Dextran-producing organisms have been identified in the plaque. Such observations
suggest strongly that the formation of microbial dextrans may be a key factor in the formation of dental plaque. If this observation becomes thoroughly confirmed, excessive intake of sucrose may lead to an unusually severe attack of dental caries.

It has been shown that diet may quantitatively and qualitatively change the oral flora. If the amount of carbohydrate in food is increased or decreased, the variations in the dietary levels of carbohydrate are accompanied by a corresponding variation in the number of lactobacilli. The occurrence of \textit{S. salivarius} in the oral cavity has also been found to vary with changes in the diet. Carlsson found that the numbers of this organism, both in plaque and saliva, increased greatly when a basic diet was supplemented with a frequent intake of sugar totaling about 50 grams per day. Van Houte found that drastic reduction in carbohydrate intake reduced the percentage of polysaccharide-storing micro-organisms in the dental plaque. He demonstrated that the character of the oral flora in both animals and man can be modified to some extent by changes in the diet. These observations support a strong inference that some cariogenic bacteria produce dental plaque and that their formation is mediated principally by the intake of sucrose.

A study by Jay indicated that extreme changes in the level of dietary carbohydrates are necessary to produce marked, prolonged differences in acidogenic organisms of the oral flora. These observations have been made on organisms other than those which form the dextrans that are thought to be a factor in the initiation of smooth surface caries. Jay has shown that sugar must be excluded from the diet, and even the total intake of carbohydrate reduced to 100 grams per day for about two weeks
in order to reduce high counts of lactobacilli to a very low value and keep them there.

The studies of Krasse and co-workers\textsuperscript{38} indicate that certain streptococci can be implanted in the human oral cavity when the intake of sucrose is heavy and continuous. In one experiment, subjects were instructed to chew one lump of sugar (3 grams) every half hour for three days (a total of 75 lumps). Once implanted, the streptococci have been reported to persist for as long as 320 days. These bacteria were isolated originally from human plaque, and were known to induce carious lesions in albino hamsters. This finding becomes important because streptococci can become implanted in an oral cavity which is already heavily populated by a large number of different micro-organisms. With animals, as with human beings, the diet is important for implantation.\textsuperscript{39} When hamsters of a control group were fed the ordinary stock diet containing natural foods such as corn, rice, wheat, alfalfa, yeast, and milk solids, only a few organisms became implanted in the oral cavity. Grinding or powdering the diet made no difference in its effect. The streptococci could be recovered in large numbers from the animals on the test diet. The main ingredients of the test diet were sucrose (56 percent) and a skim milk powder (28 percent) that contained about 50 percent lactose. Another group of hamsters were fed a diet identical with the test diet, except that it contained 56 percent glucose instead of sucrose. Results showed that sucrose was the disaccharide that favored implantation. From the mouths of animals ingesting the glucose, very few streptococci were recovered, very little plaque developed, and several of the animals remained completely free of carious lesions. All of the animals ingesting
sucrose exhibited high caries activity.40 These findings support the hypothesis that sucrose plays an important role in this system both for implantation and for the caries-inducing effect of the streptococci in animals. Man's frequent consumption of sucrose highly favors the implantation of labeled streptococci.

Early attempts to relate the prevalence of cariogenic streptococci in the mouths of human subjects have led to equivocal findings. However, results more recently have provided convincing evidence of a strong relationship between S. mutans and dental caries.

Most populations studied relative to the prevalence of S. mutans have been children and young adults, since they have the highest incidence of dental decay. It is generally agreed that the oral cavities of newborn children are free of S. mutans and remain free until a suitable tooth surface is present upon which colonization can occur. In one study41 it was found that 80 percent of the 2 to 4 year-olds were already carriers of the organism. The percentage of carriers remained fairly constant in a study group of children ranging in age to 16 years of age, and there was no difference between boys and girls in the percentage of isolation of S. mutans. Shklair, Keene and Cullen42 examined a Navy recruit population and found that the prevalence of these organisms was 84 to 93 percent in subjects with active dental caries or a history of decay.

A number of other investigators have also found an association of S. mutans with existing carious lesions.43-49 These organisms, when isolated from lesions, often constitute a very high percentage of the total streptococci found, particularly when compared to organisms isolated
from non-carious sites. For example, in the study of Littleton, Kakehashi and Fitzgerald, the percentage of "caries inducing streptococci" from carious lesions was 41 percent, compared to four percent from non-carious sites. In these studies, there was in general a greater isolation frequency of S. mutans from individuals with greater caries experience.

In the studies of Krasse et al, De Stoppelaar, Van Houte and Dirks, and Woods, there were indications of an increase in caries-inducing streptococci with the development of new smooth surface lesions.

The 1968 study by Krasse and others shows a good correlation between caries experience and the number of cariogenic streptococci found in dental plaque. Persons with high rates of dental caries attack tended to have large numbers of streptococci, while those with low incidence of attack tended to have small numbers of streptococci. Various groups of students, adults, and children were included in this study. A group of 46 school children with an average age of 13 years was re-examined after one year. This examination disclosed that children who developed several lesions on smooth coronal surfaces during this time had a large number of the specific streptococci in their dental plaque. In contrast, most of the children with no new buccal or lingual lesions had a small number of the oral streptococci.

De Stoppelaar, Van Houte and Dirks had similar results. The number of new buccal smooth surface carious lesions was also found to be higher in children who had a greater percentage of S. mutans than in children with smaller proportions of this organism.
In summary, studies in experimental animals as well as in man, have identified certain streptococci, particularly *Streptococcus mutans* as the main causative organism in the initiating of carious lesions.

III. Effects of Dietary Factors Upon Dental Caries

**Sucrose**

An abundance of experimental, epidemiological, and clinical evidence indicates that the carbohydrate component of foods, especially sucrose, is the most important caries-producing factor in the diet.

**Experimental Animals**

Many investigators have confirmed that dental caries experience is considerably more common in experimental animals when sucrose is the major dietary carbohydrate rather than glucose, fructose, maltose, lactose or starch. Exact comparisons between these animal studies are very difficult to make because of differences in strains of animals, consistency and composition of diet, methods of scoring decay, and measures to ensure the presence of cariogenic bacteria. The increase in caries activity has been most pronounced on smooth surfaces of teeth, where development of dental caries seems to depend on *S. mutans* and its adhesion by extracellular dextran produced from sucrose. In the hamster, all dental decay is of this type because of the morphology of the teeth. In contrast, in the deep fissures of the rat molars, food impaction makes adhesion unnecessary and indigenous acidogens, as well as *S. mutans*, can initiate dental caries if provided with various fermentable sugars.

Shafer reported on three groups of hamsters that were fed diets which differed only in the type of carbohydrate present. After 111 days, the results showed that a raw starch diet produced little or no decay,
a 61 percent sucrose-containing diet resulted in a high caries incidence, whereas a 61 percent glucose diet showed an intermediate dental caries score.

Frostell, Keyes and Larson\textsuperscript{32} conducted experiments with hamsters and rats in which the development of all types of lesions (smooth surface, fissure, and proximal) were studied under the influence of caries-producing sugars and sugar substitutes. Fructose, glucose, a mixture of glucose and fructose, maltose, hydrogenated potato starch, and potato starch alone and in combination with sucrose, were exchanged for the 56 percent confectioner's sugar ingredient in a diet known as No. 2000. Hamsters were inoculated with a streptomycin-resistant \textit{S. mutans} which produced an abundance of extracellular polysaccharide. Rats were inoculated by infecting them with feces from rats that had active smooth-surface lesions. The results obtained in this study, as well as those previously discussed by Krasse,\textsuperscript{38} demonstrated that sucrose promoted the implantation of cariogenic streptococci and caries activity. The most active fissure lesions occurred in animals on a diet containing sucrose and mixtures of starch and sucrose. Plaque and smooth-surface lesions were most prevalent in groups that were fed sucrose. The least smooth-surface activity occurred in animals fed maltose, starch and hydrogenated starch.

Grenby and Hutchinson\textsuperscript{51} studied the effects of diets that contained sucrose, glucose or fructose on dental caries in Osborne-Mendel and Wistar rats. These investigators did not find clear differences between glucose and fructose. In most experiments rats that were fed sucrose showed higher caries rates than those fed glucose and fructose, but all three sugars were definitely caries producing.
When Green and Hartles\textsuperscript{52} compared the effects of feeding glucose, fructose, maltose, galactose and lactose on rat fissure caries, they reported no significant differences. In two of three separate experiments, diets that contained sucrose were more cariogenic than similar diets in which sucrose was replaced by glucose. Under the conditions of this investigation, all simple sugars were highly cariogenic, although sucrose seemed slightly more so.

Shaw, Krumins, and Gibbons\textsuperscript{53} also compared the effect of sucrose, lactose, maltose, and glucose on rat caries. Diets that contained sucrose and confectioner's sugar were associated with the highest caries score in the sulci and smooth surfaces. Maltose supported as rapid development of carious lesions as sucrose, but had a lower rate of lesions on the smooth surfaces. Replacement of half of the sucrose with lactose led to a lower incidence of carious lesions in the fissures and smooth surfaces. In these experiments no attempts were made to infect the animals with specific cariogenic micro-organisms, and the diets were then able to select the cariogenic flora on the molar surface.

Stephan\textsuperscript{54} assessed the caries-conducive potential of 52 different human foods or food combinations by offering these substances at will as supplements to the basic diet of rats. The indigenous oral flora of the animals apparently harbored bacteria with the potential to initiate multisurface caries. However, the cavitation appeared to have occurred largely in occlusal fissures. Products containing sucrose were more conducive to caries activity than those containing fructose, glucose and other carbohydrates. When the caries-conducive potential of the basic diet was low, the intake of foods such as popcorn, peanuts, corn
chips, soda crackers, and potato chips was associated with little or no increase in activity. By contrast, the intake of such foods as bread and jam, chewing gum, cookies, candy, bananas, and raisins was associated with an appreciable increase in activity, some of which involved smooth surfaces. These findings support the clinical observation that habitual and excessive between meal intake of sweets favors caries activity.

Several investigators\(^{55,56}\) have shown that merely reducing the frequency of eating a high sucrose diet will significantly reduce caries in rats. In his experiments, Shaw\(^{55}\) used a feeding device that made it possible to make eating intervals resemble human eating patterns of meals and between meal snacks. Doubling the number of feeding periods from 4 to 8 resulted in major increases in carious lesions. Using a similar device, König, Larson and Guggenheim\(^{56}\) found that the difference in caries incidence in two strains of rats was attributable to eating habits, rather than innate caries resistance. When a high frequency eating pattern (36 meals per day) was compared with ad libitum feeding, the average number of fissure lesions increased tenfold.

Other animal feeding experiments have shown that liquid and semi-liquid sweets are less cariogenic than dry types. Haldi et al\(^{57}\) reported on twenty trios of weanling rats that were fed a sugar-containing diet for 90 days in the following manner: (1) complete diet by stomach tube, (2) equal amounts of sucrose in granulated form ingested orally and the remainder of diet by stomach tube, and (3) equal amounts of sucrose in solution and the remainder by stomach tube. No dental caries developed when the entire diet was fed by stomach tube, thus indicating that the carbohydrate must be in contact with the teeth to produce decay. The
average caries score was 7.5 for the granulated sugar group and 1.2 in the sugar in solution group. The rats which were fed sugar in solid form had a highly significant increase in decay over rats in both other groups.

In another study, Gustafsson et al. fed 547 weanling hamsters a purified dry diet containing equivalent carbohydrate portions at the 65 percent level for the following: sucrose, glucose, fructose, maltose, lactose and starch. Sucrose with 20.7 decayed units was the most cariogenic after 150 days, and fructose was nearly as cariogenic. Glucose, lactose, and maltose followed with 11.8, 10.6 and 3.3 decayed units, respectively. Starch produced no dental caries. When sucrose, glucose, fructose and maltose were dissolved in water and added to the diet at 40 - 44 percent concentrations, there was a marked reduction in dental caries, presumably due to decreased retention of food on tooth surfaces.

Lower sucrose levels have not been thoroughly studied for effect on caries incidence. Dental caries in rats can be increased by including sucrose in the diet at levels of 2, 4.37, 8 and 10 percent, according to Zeplin et al., Green and Hartles, Gustafsson et al., and Grenby, respectively.

All these animal experiments may be criticized on the basis that they do not accurately simulate the human situation, as the diets were unbalanced and contained proportions of sugar considerably in excess of that found in human diets. However, these experiments do suggest that sucrose is an important etiological agent in dental caries and that while the mechanism is not necessarily one of simple quantity and exposure time, in certain circumstances a relatively small amount of sucrose may increase
the cariogenic capacity of a diet. Although the results of animal studies cannot be directly applied to man, they have been of great value in providing a better understanding of the biology of dental caries. **Epidemiological Evidence**

Epidemiological studies of isolated populations that have been on low sucrose diets and then changed radically through intake of refined sugars have demonstrated a marked increase in prevalence of dental lesions.

The inhabitants of Tristan da Cunha, a volcanic island about 1,500 miles west of the Cape of Good Hope, have been studied since 1926.\(^{63,64}\) A dental examination of the islanders in 1932 showed that 83.3 percent of them were without carious lesions or missing teeth. In 1952 this percentage had decreased to 22.2 percent. A significant change occurred in 1949 when a canning company was started on the island and furnished employment for many of the islanders. Along with the industry came a greater contact with the outside world. A canteen was opened which provided imported foods, particularly refined carbohydrates that were not previously available. In his report of 1968, Fisher\(^{64}\) stated that the oral health of the islanders had deteriorated from excellent in 1932 to extremely poor 36 years later. In 1932, for ages 6 to 19 years, no carious lesions were found in first permanent molars. In 1966, 80 percent of this age group experienced such lesions. This increase in prevalence has been ascribed to an alteration in diet which accompanied the social changes. Fisher\(^{64}\) stated that Tristan da Cunha is probably the best example of the dental deterioration associated with the consumption of refined foodstuffs by populations that gain an improved standard of living.
Evidence showed a reduction in caries experience during World War II when foodstuffs were restricted. Toverud\textsuperscript{65} reported on a study of 7 to 13 year old Norwegian school children in cities, villages and rural districts during 1940, 1941 to 1948, 1949, 1951, 1952, and 1953. The total number of children each year varied between 4,757 to 6,480. He found decreases in the incidence of lesions for both primary and permanent teeth during the wartime restriction of refined carbohydrates and a gradual rise to prewar levels of incidence after the war when refined carbohydrates became available. Toverud\textsuperscript{65} stated that the magnitude of the decreased rates in occupied countries seemed to be correlated chiefly with the restriction in sugar and sugar products.

A similar finding was reported by Takeuchi\textsuperscript{66} in Japan as a result of rationing during wartime. He found that consumption of sugar per person decreased in 1946 to the low level of 0.2 kilograms from the prewar and postwar levels of about 15.0 kilograms. Prevalence of lesions reached the lowest level in successive years after the lowest years of sugar consumption. The rates for incidence of carious teeth were calculated by observation of cohorts and by calculating the ratio of newly attached teeth to sound teeth. The annual rates for incidence were well related quantitatively and chronologically to the changes in annual consumption of sugar after the eruption of teeth.

Another study that contributed to the understanding of the relationship of fermentable carbohydrates to caries experience was carried out in a home for retarded patients in Vipeholm, Sweden.\textsuperscript{67} Over a period of five years from 1946 to 1951 the investigators varied the diet of 436 subjects by inclusion or exclusion of sticky sweet foods. Seven groups
were defined at the onset of the study. These included: (1) a "control" group which consumed a diet roughly similar to the ordinary Swedish diet; (2) a "sucrose" group which consumed the basic diet plus 300 grams of sugar in beverages at meals; and (3) a "bread" group which consumed the basic diet plus 345 grams of bread containing 50 grams of refined sugar at meals. Four additional between meals groups completed the total of seven, as follows: (4) a "chocolate" group which consumed the basic diet plus 65 grams of commercial milk chocolate containing about 30 grams of sugar; (5) a "caramel" group which consumed the basic diet plus 110 grams of caramels, including 70 grams of different sugars, plus the addition of 200 grams of sugar in solution; (6) a "toffee" group which consumed the basic diet plus eight toffees or 40 grams of sugar plus 250 grams of sugar in solution; and (7) another "toffee" group which consumed the basic diet plus 24 toffees or 120 grams of sugar supplemented with 150 grams of sugar in solution. The findings as summarized by Lundquist, show that the "control," "sucrose" and "bread" groups, eating sugar only at meals, developed uniform increments of carious tooth surfaces per person per year of 0.03, 0.67, and 1.30, respectively. The between-meal "chocolate," "caramel," "8-toffee," and "24-toffee" groups developed 1.17, 2.47, 3.13 and 4.02 carious tooth surfaces per person per year, respectively. The investigators concluded that:

(1) consumption of sugar can increase caries-activity;

(2) the risk of sugar increasing caries-activity is great if the sugar is consumed in a form with a strong tendency to be retained on the surfaces of the teeth;

(3) the risk of sugar increasing caries-activity is greatest if the sugar is consumed between meals and in a form in which the tendency to be retained on the surfaces of the teeth is pronounced with a transiently high concentration of sugar on these surfaces;
(4) the increase in caries activity under uniform experimental conditions varies widely from one person to another; 

(5) the increase in caries activity induced by the consumption of sugar-rich foodstuffs in a manner favoring the development of carious lesions will disappear on withdrawal of such foodstuffs from the diet; and 

(6) carious lesions may continue to appear despite the avoidance of refined sugar, maximum restriction of natural sugars, and total dietary carbohydrates.

In 1956 Weiss and Trithart completed one of the few population studies that examined between-meal habits of eating, and its relationship to caries experience in children. A sample of 783 preschool children was examined, and then parents supplied the between-meal snacking information based on what was eaten for the previous 24 hours. The investigators reported a direct and consistent relationship between caries experience and the frequency of eating items between meals that were high in sugar or high in degree of adhesiveness. The frequency of eating such items between meals induced a corresponding increase in the caries experience. Children who did not eat between meals developed an average of 3.3 def teeth. Those eating one, two, three, and four or more items developed 4.8, 5.7, 8.5 and 9.9 def teeth, respectively. Thus the frequency of eating sweets is a very important caries-determining factor.

Winter, in his review of the relationship of sucrose to cariogenesis, concluded:

(1) in population groups, prevalence of caries experience tends to be associated with the level of sucrose consumed;

(2) sucrose has been found to be more cariogenic than other carbohydrates in experimental animals;

(3) animal and human dietary sucrose results in more extensive formation of plaque than do other dietary carbohydrates;
(4) Cariogenic streptococci produce larger quantities of extra-
cellular polysaccharides (dextrans and levans) than non-
cariogenic organisms do when exposed to sucrose in the sub-
strate; and

(5) A relationship has been established in young children between
consumption of sucrose, the prevalence of carious lesions,
and the ability of the oral microbial flora to produce intra-
cellular polysaccharide.

Clinical Studies

While the Vipeholm study\textsuperscript{67} indicated that liquid sweets are less
retentive and therefore less likely to be cariogenic than solid sweets,
liquid sucrose still has a dental caries-producing potential. Carlsson
and Egelberg\textsuperscript{71} and Carlsson and Sundstrom\textsuperscript{72} have shown that if there is
sticky plaque on the tooth surface and liquid sucrose comes into contact
with it, lactic acid and even dextran will be produced within the plaque.

Stephan\textsuperscript{73} constructed microelectrodes from antimony to measure plaque
pH. He found that when a 10 percent liquid sucrose solution comes into
contact with dental plaque, acid is produced within 20 seconds and may
last up to 20 to 30 minutes before it is buffered by the saliva.

Other studies have shown that plaque pH after rinsing with various
concentrations of sucrose and glucose, was decreased measurably by 0.5
percent rinse solutions, and that 5.0 percent solutions decreased the
pH sufficiently to decalcify tooth mineral.\textsuperscript{74,75} Sugar levels higher than
5 percent further decreased pH and, more significantly, these lower pH
values were maintained for longer periods.\textsuperscript{76}

Von der Fehr, Loe, and Theilade\textsuperscript{77} developed a method for the early
evaluation of the cariogenic potential of different substances. They
claimed that their method allows this determination to be made in a few
days instead of the years required by conventional methods. They reported
detectable signs of enamel alterations in twelve dental students after
23 days of refraining from oral hygiene and being exposed to frequent sucrose ingestions.

Clinical evidence specifically implicating sucrose is also provided by subjects with a rare metabolic dysfunction, hereditary fructose intolerance.78-81 These persons are deficient in the liver enzyme aldolase that splits fructose-1-phosphate, an essential step in human fructose metabolism. Since sucrose is broken down to fructose and glucose before it is absorbed or utilized, these persons must abstain from consumption of sugar. They become violently ill if they ingest more than very small amounts of sucrose or fruit containing fructose. Consequently, they tend to avoid sweets of all kinds, and consume starchy foods instead. From every indication, these individuals experience little or no dental caries. In contrast, siblings unaffected by the disorder do not show the same freedom from dental caries.

The intake of sucrose in today's diet far exceeds that of any other sugar. Sucrose is consumed at the rate of about 2.5 pounds per person per week and accounts for up to 37 percent of the total carbohydrate for most people.82

Navia83 reported recently that although the actual consumption of sugar in the United States has not increased in the past 20 years, the manner in which it is consumed has changed. Sucrose consumption from the sugar bowl has decreased, whereas the amount incorporated in manufactured foods has increased at the expense of other nutritionally more important ingredients.84 Therefore, a child selects a sugar-rich food, instead of another food that is a better source of protein and usually has a higher level of vitamins and minerals than the sweeter food.85,86
In a large-scale United States nutritional survey in 1969, the long-term consumption of snack foods containing high levels of sugars was positively correlated with dental caries status. Traditionally, sweet high-sucrose foods have been consumed at the end of the meal, but now they are being increasingly consumed between meals as snacks. This more frequent consumption of sugar-containing foods has stimulated caries development more than ever before.

In summary, research has shown that sucrose is the most common cariogenic carbohydrate. Moreover, the relationship between sugar consumption and dental caries is more than just a quantitative one. As in the Vipeholm study, its cariogenic potential as a component of foods is influenced by a number of other factors: (1) the retentive characteristics of the food (sticky, adhesive foods are more cariogenic than liquid, non-retentive foods), (2) the frequency of ingestion (frequent between meals snacks result in maximum cariogenic potential), and (3) the chemical composition of the food (some foods contain certain substances like fluorides or phosphates which are capable of inhibiting or altering the cariogenic effect of sucrose).

**Milk and Dental Caries**

The local effect of milk, either in powder or liquid form, on dental caries has been the subject of many investigations.

**Animal Experiments with Powdered Milk**

In dry powder form, the effect of heat treatment has been studied extensively. McClure and Folk found that diets containing 35 percent roller-processed skimmed milk powder were more cariogenic than spray-processed skimmed milk powder when fed in the diets of rats. Since higher
temperatures are used in roller-processing than in spray-processing, and since lysine is partially inactivated when dry casein is heated, the authors concluded that the increased cariogenicity of the roller-dried milk probably was related to lysine impairment.

McClure and Folk fed rats diets in which the protein was supplied by three different nonfat milk powders (freeze-dried, spray-dried, and roller-dried), and all three produced smooth-surface caries. The severity of the caries correlated with the degree of heat processing. The freeze-dried milk was associated with the least dental caries and the most severe caries developed in the group fed the roller-dried milk powder.

McClure and Folk later showed that the addition of 1-lysine at levels of 2.5 and .25 percent to two cariogenic diets significantly reduced the caries score in rats.

Similarly, Bavetta and McClure confirmed the cariostatic properties of lysine when they fed a cariogenic diet containing a considerable portion of roller-processed skimmed milk powder to one group of rats, and fed the same diet supplemented with .4 percent lysine to a second group. The lysine-supplement significantly retarded caries development, indicating that under these experimental conditions, lysine exerted a significant cariostatic action.

In an attempt to determine whether this action of lysine was local or systemic, McClure designed an experiment in which lysine was administered in the diet, in the water, by intubation, or by intraperitoneal injection. Significant reduction in rat caries occurred when 1-lysine was administered by diet, water, and intubation. Intraperitoneal injection had a variable and essentially negligible effect.
Other investigators\textsuperscript{93-99} reported similar increases in cariogenicity of various dry milk powders that were proportional to the degree of heat treatment used to process the milk.

In contrast to these investigators, Dreizen, Dreizen and Stone\textsuperscript{100} commented on the work of Mauron, Kinkel and Cremer,\textsuperscript{101} (published in French) and stated that the heating of dry whole milk affects neither the incidence nor the degree of dental caries in the rat, and that there is no significant relationship between the content of utilizable lysine and the cariogenic potency of milk-containing diets.

Dreizen, Dreizen and Stone\textsuperscript{100} came to a similar conclusion, because in their animal-feeding experiment they found that non-fat dry cow's milk fed at a 39 percent level in a cariogenic diet had no appreciable effect on increasing cariogenicity. At the 100 percent level, the non-fat dry cow's milk was noncariogenic for the rat.

Green\textsuperscript{102} suggested that the cariogenicity of skimmed milk powder was related to its stickiness and the frequency with which it was eaten. Natural skimmed milk powder had a more pleasant taste than casein or a casein-rich simulated skimmed milk powder, and therefore was preferentially selected and eaten more frequently. All three diets (natural skimmed milk powder, casein, and casein-rich simulated skimmed milk powder) were found by analysis to have the same protein, carbohydrate and fat content.

Klapper and Volker\textsuperscript{103} studied the effect of powdered whole milk-sorbitol diets on dental caries in desalivated hamsters. Thirty-three hamsters divided into four groups and desalivated at 27 days of age were fed diets consisting of 96 percent powdered whole milk and 4 percent other nutrients, with 0, 20 and 40 percent sorbitol replacing equivalent amounts
of the milk. The fourth group received reconstituted milk made from powdered whole milk. Twenty-nine days later the average caries incidence was: Group 1 (96% powdered whole milk and no sorbitol), 26.0; Group 2 (76% powdered whole milk and 20% sorbitol), 18.8; Group 3 (56% powdered whole milk and 40% sorbitol), 14.0; Group 4 (reconstituted milk and no sorbitol), 0.5. These results suggest that when the diet is in a powdered form some constituent of the whole milk, probably lactose, is primarily responsible for the dental caries observed in desalivated hamsters. Sorbitol seems to have little or no cariogenic effect. When the milk is in fluid form it remains in the oral cavity for so short a time that no cariogenic activity is observed, even in desalivated hamsters, the oral environment of which has proved to be especially prone to the development of dental caries.

Liquid Milk and Animal Experiments

Since milk is fluid and non-retentive in form, most investigators agree that it is unlikely to exert a significant dental caries-promoting effect. Most studies have shown a caries-reducing effect.

Zeplin et al. reported that groups of litter-mate cotton rats were fed for 14 weeks on a natural diet resembling the average human diet with 17 percent sucrose, the proportion consumed by the average person in the United States. This diet was highly cariogenic, and increasing the sucrose to 32 or 47 percent did not appreciably affect the incidence or extent of decay. Reducing the sucrose level of the diet to 0 or 2 percent reduced the occurrence of dental caries by 80 and 60 percent, respectively. When liquid whole milk was given separately as the animals' only source of fluid with the natural 17 percent sucrose diet, the caries scores obtained were the same as those obtained without liquid milk. No protective effect was found.
Schweigert et al. found the cotton rat to be free of carious lesions when fed a diet of while liquid milk. This was in contrast to the extensive dental decay that developed from a diet of dry rations of sucrose and other fermentable carbohydrate. Milk was found to exert a protective action even in the presence of 5 or 10 percent fermentable sugar, although caries scores were not zero as they were on a diet of liquid milk alone. The higher caries score when a dry ration was fed seemed to indicate that the fluidity of the ration might play an important part in this protection.

Anderson et al. reported that milk is protective against dental caries in the cotton rat. Previous findings of zero scores in animals fed only liquid milk were confirmed. Animals receiving milk to which sucrose, glucose or a glucose-maltose mixture had been added at the 5 and 10 percent level exhibited low caries as compared with controls on a cariogenic diet. These sugars produced caries when fed in dry rations. The caries incidence in the animals which received approximately one-third of their caloric intake as liquid milk and the remainder as cariogenic ration was less by 50 percent than in litter-mate controls not receiving milk.

Sperling et al. reported that groups of weanling albino rats composed of 20 males and 40 females per group were fed the following diets ad libitum for their life span: (1) fresh whole pasteurized milk supplemented with traces of Mn, Fe, I, Cu, and cod liver; (2) milk with 10 percent dissolved sucrose; (3) milk and free access to dry sucrose; (4) milk and 10 percent sucrose solution; (5) stock diet plus 10 percent cooked dried whole eggs, and (6) stock diet. Half of the females were bred. In Group 3 (dry sugar) 57 percent to 60 percent of the animals had severe decay in the molar teeth. Of those in Group 4 (sugar solution) 26 to 36 percent had moderate
caries in the molar teeth. The group receiving 10 percent sugar dissolved in the milk (Group 2) had no caries, nor did those on the milk alone. In Group 5, 7 to 10 percent and in Group 6, 12 to 17 percent had slight caries. Milk consumed directly with sucrose seemed to protect the teeth, but caused overweight, failure of reproduction in females, and shortening of lifespan in males.

To compare his results with data obtained on the cotton rat, Shaw\textsuperscript{108} used groups of Sprague-Dawley strain albino rats, feeding them for 14 to 18 weeks with different diets. Feeding either fresh milk or evaporated mineralized milk as the sole nutriment almost completely eliminated decay. Addition of 10 percent sucrose had little effect on decay and improved growth. Substituting mineralized milk for one-third the caloric content of a cariogenic diet did not significantly lower the caries incidence over controls on the cariogenic diet.

Masuda et al\textsuperscript{109} reported that the salivary buffering capacity in rats fed with a milk-containing cariogenic diets was greater than in those fed with a milk-deficient diet. Statistical analysis by t-test showed a significant difference (p<0.01) between the groups regarding buffering capacity. The experimental rats which were on a milk deficient cariogenic diet and had low salivary buffering capacity had a significantly higher incidence and extent of caries than rats on a milk-containing cariogenic diet. Thus it was strongly suggested that the cariostatic action of skimmed milk might be closely related to the buffering capacity of saliva.
Human Milk Consumption and Caries

Milk consumption has been associated with dental caries by several authors. Examinations with mirror and explorers and dietary surveys were made in 1950 by Potgieber et al. on 864 children ages 10 to 16 years. The average DMF rate in 14 to 16 year olds was higher than in a 1944 survey. Diets were found to be moderately good. Lower DMF rates were associated with better diets and the consumption of more milk, fruits and vegetables. There was a marked and consistent drop in DMF rate with the increase in number of cups of milk consumed from an average of 10.65 DMF for those drinking zero to one cups to 5.95 DMF for those drinking 4 to 5 cups per day. In contrast, those drinking over five cups of milk per day showed a slightly higher rate of dental decay with a DMF of 7.12. No definite cause was found.

Milk has been associated with a clinical condition known as "nursing bottle caries." This severe type of dental caries is found in very young children who have developed the habit of requiring a nursing bottle with milk or sugary fluids when lying down to sleep. This condition, which resembles rampant caries, particularly attacks the four primary maxillary incisors, the maxillary and mandibular first primary molars, and the primary mandibular cusps. The four primary mandibular incisors are not affected by the carious process and this serves to differentiate it from a rampant caries case.

Pitts in 1927 found 70 cases of the characteristic pattern of nursing bottle caries. In summary, he suggested that the most important factor in dental caries in young children is the use of a dummy or rag dipped in such substances as sugar, honey and milk.
Many clinicians and investigators have agreed that prolonged use of the nursing bottle can be associated with nursing bottle caries. However, there was marked disagreement in whether milk alone in the nursing bottle could produce these typical lesions. Finn\textsuperscript{112} in 1969, after reviewing the pertinent literature, concluded that there was insufficient evidence to suggest that bovine milk could be cariogenic. On the contrary, there was some indications that milk had no cariogenic potential and could even reduce the enamel solubility in some in vitro conditions. This protective effect remained even after washing.

In contrast, Michal\textsuperscript{113} in 1969 examined carefully the clinical records of his patients presenting with nursing bottle caries and concluded that plain bovine milk can cause dental caries.

It is important to understand that nursing bottle caries represents a rather special set of conditions, in which carbohydrate-containing liquids must remain in contact with enamel surfaces for long periods under relatively stagnant conditions.

Nursing bottle caries can be eliminated by not allowing an infant to fall asleep with a nursing bottle containing milk or sugary fluids in his milk. The infant should be weaned away from the nursing bottle by age 12 months.

\textbf{In Vitro Studies of Milk}

Many in vitro studies using milk suggest that this food is not cariogenic under normal usage patterns. Tooth sections were exposed by Bibby and Soni\textsuperscript{114} to mixtures of saliva and 35 foodstuffs. Polarized light examinations were made for decalcification after 0, 6, 8, and 24 hours, as well as pH and acid formation determinations. There were poor correlations between decalcification and acid formation. Milk products and unrefined cereals produced the most acid but little decalcification.
Weiss and Bibby studied enamel solubility changes upon immersion in different milk preparations. Windows on slabs of bovine enamel were given repeated exposures to decalcifying buffers, and the solubility effects were noted before and after treatment with the milk solutions. Treatment with cow's milk reduced solubility by 20 percent regardless of whether it was raw or pasteurized whole or skimmed milk. Reconstituted whole and reconstituted skimmed milk gave similar reductions. Similar depressions of solubility followed treatment with cream or whey. Butter gave low and inconsistent reductions. No difference was found in the solubility effects of reconstituted dry skimmed milk obtained from 23 locations in the United States. Tests showed that the protective agent in milk reacts rapidly with the enamel and resists washing. That it is a protein in nature was indicated by the finding that full solubility reduction was achieved by treatment with casein solutions and that it was mostly removed by washing with a protein solvent.

Weiss and Bibby stated that whether the solubility-decreasing effects of milk which they have demonstrated have any significance in respect to human or animal caries cannot be decided at this time. However, the findings indicate that, under some circumstances at least, milk can be assumed to exercise a moderating effect on enamel solubility.

In further studies, Weiss and Bibby found that bovine enamel pretreated with casein showed greater reductions in acid solubility than did several other milk protein concentrates. Further tests showed that inclusion of the proteins in the decalcifying buffer produced equal or greater changes in enamel solubility.
Jenkins and Ferguson\textsuperscript{117} carried out three types of experiments: (1) comparing the pH changes during the incubation (without shaking) of 4 ml of wax stimulated saliva with 2 ml of either milk or 4 percent lactose solution; (2) testing the effect of milk on the solubility of tooth substance in buffers (at pH 5.0 or 7.0) or in saliva incubated either with milk or with an equal volume of 4 percent lactose solution; and (3) measuring the changes in the pH of plaque in vivo in subjects who had not cleaned their teeth for three days after placing milk or solutions of 4 percent lactose or 4 percent glucose in the mouth, and leaving them in contact with the teeth for 30 seconds.

From their results, Jenkins and Ferguson\textsuperscript{117} concluded:

(1) In vitro experiments have been carried out on acid production and the solubility of tooth substance in saliva incubated with either milk or 4 percent lactose solution. Acid production is reduced slightly, and the amount of enamel dissolving is reduced greatly, in the presence of milk.

(2) Studies of the plaque pH in situ after contact with milk show that little change occurs and no evidence was found that clotted milk adheres to the teeth.

(3) Experiments with milk from which various constituents were removed suggest that the calcium and phosphate in milk are largely responsible for the reductions in the amounts of enamel dissolving, but since the effect of milk is still detectable after washing the milk off the teeth, some other constituent must also contribute to the effect.

(4) These experiments suggest that milk is unlikely to have a local effect in promoting caries but could exert a protection against the effects of cariogenic foods such as sugar and biscuits.

(5) The possible effect of milk during tooth formation in producing a tooth with low resistance to caries was discussed and thought to be improbable.
Vianna investigated the capability of human milk, bovine milk, a milk formula and milk supplemented with honey to produce caries-like lesions in an environment which simulated the oral cavity. Enamel slabs of sound human bicuspids were mounted in a mouth simulator, inoculated with human saliva and covered with linen cloths to facilitate bacterial colonization. Each day the teeth of the various study groups were exposed to one of the four milk solutions during a 2, 4, or 8 hour period. After each period, a sterile chemical solution resembling saliva was dropped over the cloth and teeth to simulate the mouth. A control group was not exposed to a milk solution.

In six weeks, all milk solution groups showed signs of decalcification with an intensity proportional to length of the exposure period. Plain bovine milk produced the least decalcification, followed in order by milk formula, human milk and milk and honey. Vianna inferred from these results that milk itself has the potential to produce dental caries if left stagnant over tooth surfaces for a sufficient time. The conditions described for decalcification were intended to simulate those found in young children suffering from nursing bottle caries. Stagnation of carbohydrate-containing fluids, including milk with 3.5 percent lactose, over long periods of time is necessary to produce this artificially induced condition.

Lactose and Dental Caries

The only carbohydrate contained in milk is lactose, and several studies have dealt with its possible cariogenicity.

Green and Hartles used 214 weanling albino rats in five experiments to compare the cariogenic potential of different types of carbohydrate, including lactose. Although sucrose was the most cariogenic, all other
carbohydrates seemed to be highly cariogenic.

Shaw, Krumins, and Gibbons\textsuperscript{53} reported a series of experiments in which different combinations of strains of rats and diets were used to study the caries-producing ability of different sugars. The replacement of lactose for half of the sucrose led to a lower incidence of carious lesions in the sulci and on the smooth surfaces in one experiment but not in a second experiment.

Guggenheim et al\textsuperscript{119} tested the cariogenicity of sucrose, glucose, fructose, lactose, maltose and an uncooked wheat starch in a highly controlled study in which Osborne-Mendel rats had their indigenous flora depressed with antibiotics. The rats were then inoculated with a cariogenic streptococcus (OMZ-61) that is known to induce smooth surface lesions. The eating and drinking patterns were checked and were not found to be altered in the rats that consumed the test diet with 25 percent supplements of the different carbohydrates. As expected, the incidence of dental caries was highest on the sucrose diet. The lactose diet was the next most cariogenic. Caries scores for the sucrose and lactose groups were statistically different from the scores of the other diets in which caries activity was low. These results are relevant solely to the cariogenic streptococci (OMZ-61).

Variations in the plaque pH in six adult men and women were measured by Kurosawa,\textsuperscript{120} by means of an antimony electrode after an oral rinse with solutions of glucose, fructose, maltose, lactose, dextrin, and starch. At the same concentration lower pH values resulted from glucose, fructose, sucrose, and maltose than from lactose, dextrin and starch. The reduction in pH depended on the concentration and viscosity of the solutions and
the duration of the rinse until a certain constant was reached. Mixtures of sucrose or starch 80:20 or 50:50 produced the same drop in pH as sucrose alone.

Steinman and Haley\textsuperscript{121} reported a study in which littermate rats were given the following solutions from the day after birth until weaning at 21 days: 20 percent sucrose, 20 percent lactose, and a 20 percent mixture of glucose and fructose. They also nursed ad libitum. At weaning, all rats in these studies received a cariogenic diet (65 percent sucrose) until 13 weeks of age. Sucrose solutions caused the most caries, and lactose the least. In another study in which 20 percent sucrose solutions were given 1-6, 7-13, and 14-21 days after birth, the highest caries score was found in the group receiving the sucrose at 14-21 days. When 1.85 percent or 5.35 percent of sucrose was added to the milk formula fed from the 14th-21st days to another littermate group, significantly more dental caries resulted at both levels of sucrose. From these results, lactose would be the carbohydrate of choice for infant feeding. The high consumption of sugar by children and adolescents during tooth formation may play an important role in the high caries rates observed currently.

Cocoa

Cocoa is obtained from the cocoa tree (\textit{Theobroma cacao}). The seeds of the cocoa fruit, which are called cocoa beans, are subjected to fermentation causing a considerable transformation of the contents. The cocoa beans are then dried in the sun and shipped to the manufacturer where they are roasted, cracked and freed from shells and germs. The cocoa mass contains about 55 percent fat. Most of this fat is pressed out and the rest, the press cake, is milled and sifted to a fine powder. Cocoa may be made
by one of two methods: the natural or the Dutch alkali process. These are almost the same process, the essential difference being that the Dutch product is treated with alkali at the time of roasting to increase solubility, alter the flavor and darken the color. Often other flavors are added, for example, vanillin. 122, 123

Stralfors 123 interpreted the 1952 investigation by Gustafsson 124 (published in Swedish), and reported that he was able to extract fractions from fermented cocoa beans which reduced the acid production in human saliva of caries-active individuals. Also Stralfors referred to Gustafsson as pointing out that the manufacture of chocolate comprises several procedures in which the cocoa is heated and exposed to air. Therefore, it is doubtful that the finished chocolate product contains any substances which can inhibit acid production. From chocolate it was not possible to extract any fraction with the mentioned effect against saliva.

Other evidence regarding cocoa has been based primarily on work in experimental animals. Stralfors 123 interpreted the study of Rozeik, Cremer and Hannover 125 (published in German) who investigated the effect on rat dental caries of cocoa in different forms including fresh and roasted beans, press cake, water extract, alcohol extract and ash of cocoa bean. They found a decrease in caries by the incorporation into the diet of fresh and roasted beans as well as press cake. The extracts did not produce any significant inhibition, but the ash of beans caused a very marked caries reduction.

Stralfors 123 interpreted the study by Kinkel and Newiger 126 (published in German) on the effect of cocoa mass, cocoa butter and defatted cocoa mass on dental caries in rats. One experiment was performed with diet
given ad libitum and another experiment with paired-feeding technique. In the ad libitum experiment there was caries reduction by cocoa mass but no reduction by cocoa butter or defatted cocoa. In the paired-feeding experiment there was no reduction by any type of cocoa.

Several investigators\textsuperscript{127-131} demonstrated that dental caries is inhibited by different kinds of fat. It would therefore seem likely that fat of cocoa would also inhibit dental caries. However, as Stralfors\textsuperscript{123} has pointed out, Kinkel and Newiger\textsuperscript{126} did not detect any effect of cocoa fat against animal caries.

Stralfors\textsuperscript{123} also interpreted the study by Kinkel and Newiger\textsuperscript{126} (published in German) on the effect of cocoa ash on dental caries in rats. They tested the original cocoa ash, as well as ash which had first been neutralized with phosphoric acid. Both showed inhibition of dental caries. Since the cocoa ash contains considerable phosphate, and since the neutralization with phosphoric acid will increase the phosphate concentration further, this result was expected. Many investigators\textsuperscript{132-135} have reported conclusive evidence for the caries-inhibitive property of phosphates in animals.

Kinkel and Cremer\textsuperscript{136} in 1960 compared the caries-reducing effect of cocoa ash with the effect of reagent grade chemicals comparable to the ash. The intention was to find out whether there might be any cariostatic effect of trace elements in cocoa. The mineral mixture at pH of 6.7 caused the same caries inhibition as the neutralized ash at pH of 7.0, but the non-neutralized ash at pH of 10 had a weaker effect. The authors stated: "It seems unlikely that the caries-reducing effect of cocoa ash is due to the presence of certain trace elements. The caries-reducing action of cocoa as well as of other ashed foodstuffs seems to be mediated by presence of phosphates, chlorides and carbonates."
Stralfors (1966) investigated the effect on hamster caries of whole cocoa powder, defatted cocoa powder and cocoa fat. Whole cocoa powder inhibited caries by 84, 75, 60, and 42 percents, when the cocoa content of the diet was 20, 10, 5, and 2 percent, respectively. Also cocoa powder that was defatted with petrol ether showed a significantly higher caries inhibition than did whole cocoa powder. Furthermore, 15 percent cocoa butter added to the diet had no protective effect but increased caries.

In a similar study, Stralfors showed that after the defatted cocoa powder had been washed six times with water, it still had a fairly substantial anticariogenic effect. This indicates the presence of both water-soluble and nonwater-soluble caries-inhibitory factors.

In a further study of the water-soluble anticaries factor in cocoa, Stralfors used a 1:15 water extract of defatted cocoa powder containing 1.5 percent of solids. This extract, dried onto the potato starch portion of the diet, inhibited hamster caries by 50 percent. The active fraction comprised half the weight of extracted solids, and it adsorbed completely on activated charcoal. About 35 percent of the solids contained about half the anticaries activity and was nondialyzable and ash-free. The high and low molecular fractions appeared almost entirely organic but remained unidentified. By indirect evidence the active fraction appeared to consist of tannins.

In a further study with hamsters, Stralfors showed that several constituents of cocoa: theobromine, xanthine, and tannic acid at the 0.2, 0.2, and 0.01 percent levels, respectively, all significantly reduced dental caries at the indicated concentrations, but did not affect food intake. Higher concentrations of some of them did reduce food consumption.
In addition, two commercial tannins not found in cocoa, but related chemically were tested. Mimosa and quebracho extracts were anticariogenic at 0.05 and 0.2 percent levels, respectively. Stralfors expressed the view that tannins and theobromine, which comprise at least 14 and 3.5 percent, respectively, of fat-free cocoa mass, were the most likely water-soluble factors contributing to the anticariogenicity of cocoa. It appeared unlikely that inorganic factors like phosphate or fluoride were the anticaries factors because the extracts had a low ash content. Also, charcoal adsorption removed the anticaries activity but did not lower the ash value, which further supports the view presented earlier by Stralfors,\textsuperscript{138} that tannins contribute to the caries-inhibitory effect of cocoa.

The possibility that caffeine and other similar compounds in cocoa decreased caries by stimulating salivary flow, became less likely through the fortunate use by Rozeik, Cremer and Hannover,\textsuperscript{125} of albino rats whose salivary glands had been removed.

The cocoa could act as an antibacterial agent since cocoa and chocolate inhibit staphylococci in pastry fillings.\textsuperscript{140} Fuller, Mueller and Swanson\textsuperscript{141} noted that these materials inhibit some bacterial growth in milk and suggested that tannins were the responsible agents. Theobromine is also toxic to some bacteria.\textsuperscript{142} Unheated or autoclaved concentrations of cocoa inhibit the growth of salmonella species.\textsuperscript{143} Both anaerobic and aerobic cultures of saliva were strongly inhibited by 5 and 10 percent concentrations of cocoa in medium.\textsuperscript{144}
On the other hand, Jenkins and Smales\textsuperscript{145} obtained a lowered dissolution of tricalcium phosphate in acetic acid buffer at pH 5 when a water extract of cocoa was added. This result points to the possibility that an enamel solubility change is a factor in the cariostatic action of cocoa.

In summary, there is evidence that cocoa contains one or more substances capable of reducing caries prevalence, at least in experimental animals. In vitro studies show that cocoa possesses antibacterial and enamel-solubility reducing properties. Cocoa appears to be one of the few compounds with true anticariogenic properties, but the exact mechanism of action and convincing, confirmatory human data are still lacking at this time.

**Chocolate**

Chocolate is produced in various ways and many kinds of additions are made to satisfy the varying tastes of consumers. The main components are: cocoa mass, obtained from fermented and roasted cocoa beans; sugar, generally in an amount of 30 to 50 percent; lecithin milk powder and other ingredients.\textsuperscript{122,146}

If no milk powder is added, the product is darker and is called dark or bitter chocolate. As a consequence of omitting the milk powder, the content of fat-free cocoa is higher in dark chocolate than in milk chocolate. By far the larger part of consumption is of milk chocolate.\textsuperscript{122,146}

The manufacturing procedures are very different for cocoa powder and for chocolate. The latter is subjected to the conching procedure, in which the melted chocolate is treated mechanically by rollers moving to and fro over a corrugated granite bed for two to three days. The temperature
is kept at about 80 degrees centigrade for bitter chocolate and about 50 degrees centigrade for milk chocolate. When sugar is heated in the presence of amino acids or proteins, a group of substances called melanoidins are formed. Another change taking place is that the sugar partly undergoes caramelization. Melanoidin and caramel are both very complicated mixtures of chemical compounds. The protracted heating in the conching process will also transform some of the compounds in cocoa into tannin which may tend to increase the caries-inhibitory activity. 146

Stralfors147 investigated the effect of calcium phosphate mixed into chocolate on dental caries in hamsters. The control group received a basal diet plus sweetened chocolate, while the experimental groups received the same diet components but with either 1 or 2 percent calcium phosphate incorporated into the chocolate. The control group exhibited a remarkably low caries incidence, which indicated that there was some anti-caries factor in the chocolate.

In further studies of chocolate in 1967, Stralfors146 performed experiments in hamsters in which 20 percent of the sugar-containing control diet was replaced by sweetened chocolate. There was a reduction of caries by 35 percent for milk chocolate and 73 percent for dark chocolate. In his discussion Stralfors stated that, based upon earlier research, the difference in cocoa content for the two chocolate types is probably the main reason for their differing ability to counteract dental caries.

In marked contrast to Stralfor's findings, Ishi, Konig, and Muhlemann148 reported that milk chocolate and chocolate wafers produced more fissure and smooth surface caries in Osborne-Mendel rats than did five other between-meal snacks in which orally fermentable and retentive carbohydrates, including sucrose were present.
Milk chocolate was one of the different types of sweets studied in the Vipeholm caries study. One group of patients received 30 grams of sugar daily between meals as 65 grams of milk chocolate. The group that ate chocolate had more caries than the control group, which received no sugar, but less caries than other groups to whom similar amounts of sugar was provided in other forms. This led to the hypothesis that chocolate might contain some kind of caries-inhibiting factor.

In 1975 Grenby compared the deposition of dental plaque in 12 young adults on a chocolate (340-454 grams) and powder (85-113 grams) diet over a 5-day period to a control period when the same 12 subjects ate their normal diets. Each subject had his teeth scaled and polished at the start, but then did not brush during the 5-day period. Dental plaque was then stained by basic fuchsin and a plaque score taken. The normal diet produced a significantly higher plaque score than did the chocolate and skimmed milk powder diet which provided 170-200 grams of sucrose per day and would be expected to have increased the development of plaque.

**Chocolate Milk and Dental Caries**

According to the National Dairy Council, chocolate milk is whole milk flavored with a chocolate syrup or powder. Usually its milk fat content is the same as for whole milk and it contains one percent cocoa or one and one-half percent liquid chocolate plus 5 percent sugar and less than 1 percent stabilizers.

Skimmed, or partially skimmed milk flavored with a chocolate syrup or powder is called chocolate dairy drink. Frequently its milk fat content is about 2.3 percent and its milk solids, (not fat) about 90 percent of the amount in skimmed milk. Otherwise, it contains the same ingredients...
as chocolate milk and is processed in the same manner.\textsuperscript{5}

Research indicates that the addition of normal quantities of good grade chocolate has no appreciable effect upon the availability of either calcium or protein of milk to human beings. Therefore, the nutritional value of milk is not significantly altered by the addition of this flavoring, except in regard to the increased caloric value, chiefly from the added sugar. The sugar and chocolate content brings the caloric value of chocolate dairy drink made of skimmed milk to a slightly higher level than that of plain whole milk but to a lower level than the chocolate milk.\textsuperscript{5}

A commonly used stabilizer for chocolate milk is carrageenan, which is a hydrocolloid unique in its high degree of reactivity with certain proteins. This property is the basis for a number of applications of carrageenan in a variety of foods. The reaction between the milk protein casein and carrageenan is called "milk reactivity,"\textsuperscript{150} and makes it possible to suspend cocoa particles in milk. Carrageenan is added to other foods to improve body or consistency and also for "mouthfeel."

Rathbun, Bond and Steinman\textsuperscript{151} studied the effect of cow's milk, soy milk, chocolate drink and a high sugar (61 percent sucrose) cariogenic control diet on the growth rate and caries incidence of 115 Osborne-Mendel rats. The three experimental groups were given their milk formulas twice daily from large Petri dishes, starting 15 days after parturition. The rats comprising the control group were left with their mothers, with the high sugar diet available as a supplement, and at 21 days they were weaned and placed on the high sugar control diet. The rats were weighed semi-weekly and sacrificed at 45 days.
The growth rate of the experimental rats was similar, but substantially less than that of the high sugar control group. In the chocolate milk group, the incidence of decay was about three times as great as in the other milk or cariogenic diets. The cow's milk and soy milk groups developed numbers of lesions similar to those for rats on the high sugar diet. The statistical analysis gave less than one chance in a thousand that the difference between the chocolate milk and other foods was chance. The other differences were not significant.

Shaw, Ensfield and Wollmann\textsuperscript{152} used a total of 669 Harvard caries-susceptible rats to test the post-eruptive and developmental influence of the supplementation of a cariogenic diet with a variety of dairy products. Supplements consisted of either milk, chocolate drink, chocolate milk, a mixture of milk, vanilla ice cream and cheddar cheese, or a mixture of chocolate milk, vanilla ice cream, and cheddar cheese. The levels of supplementation varied, but were chosen to approximate levels of human consumption.

All supplements of dairy products caused major reduction in the incidence of dental caries when fed on a post-eruptive basis only. No developmental effect was noticed. Chocolate milk or chocolate drink supplements had no significant effect compared to the white milk supplements, although an increased amount of dental caries could have been expected because of the increased sugar content of the chocolate.

Dunning and Hodge\textsuperscript{153} in 1971 reported a study to determine the dental caries incidence in over 300 retarded and institutionalized individuals who received supplements of chocolate-flavored milk products over a two-year period. One pint of chocolate milk per day was given each person to
displace an equivalent amount of whole milk. Four study groups were tested: (1) whole milk control, (2) chocolate milk, (3) cocoa in whole milk with an artificial sweetener, (4) milk sweetened with sugar and colored to resemble chocolate milk. Due to varying degrees of patient disability and cooperation, it was necessary to discard the milk and sugar group.

The final evaluation for pit and fissure caries indicated that the chocolate milk group showed a small, but not significantly higher increment in dental caries over a two-year period than did the whole milk control group. The differences in dental caries experience between the cocoa and milk group and the whole milk control group were not significant. The chocolate milk produced higher caries increments of borderline significance than the cocoa and milk with artificial sweetener.

In summary, after a review of the pertinent literature, the local effect of chocolate milk on dental caries is not clear. Many factors need to be considered.

Chocolate milk may be considered as caries-enhancing because of the relatively high content of fermentable carbohydrate in the form of 5 percent sucrose and 3.5 percent lactose. Although these substances are not in solid, retentive form, frequent between-meal exposures of sucrose and lactose in a milk solution might have a detrimental effect in a caries-conducive mouth.

Chocolate milk may also be considered as caries-protective. There is evidence that cocoa, although present at only 1 percent concentration, contains one or more substances capable of reducing caries, at least in animals. In vitro studies show that cocoa possesses antibacterial and
enamel-solubility-reducing properties. In addition, milk which is high in natural protein, has been shown to also possess enamel-solubility-reducing and acid-buffering properties. In its fluid, non-retentive form and with its high levels of calcium and phosphorus, milk could be considered as a nearly ideal supplement for good dental health.

Dunning and Hodge have reported the only human study comparing the local effect of white and chocolate milk on dental caries. Chocolate milk was found after two years to have produced a small, but not significantly higher, increase in dental caries as compared to while milk. Cocoa and milk with no sugar had a beneficial effect of borderline significance.

Clearly more research needs to be conducted before accurate dietary recommendations can be made concerning chocolate milk as compared to white milk.

IV. In vitro Dental Caries Research

The concept of studying the carious process by using extracted teeth under in vitro conditions was realized and applied early in the development of dental research. Magitot in 1878 conducted experiments in which extracted teeth were placed in an aqueous sugar solution and allowed to stand for about two years. To localize the attack, some of the teeth were covered with wax except for small areas of exposed enamel. To show the effect of fermentative organisms, cresote was added to some specimens and the solution was sterilized in others. After two years the enamel and dentin of teeth exposed to the microorganisms were extensively destroyed.
In 1890 W. D. Miller\textsuperscript{6,155,156} modified Magitot's procedure. Instead of depending upon accidental inoculation of the culture solution or upon organisms attached to the teeth, he used human saliva with added sugar, bread and other food materials. After several months, enamel softening, dentinal destruction and the penetration of the dentinal tubules by microorganisms were observed. The decay which was produced artificially appeared the same under a microscope as decay occurring in a human mouth.

Dietz\textsuperscript{157} in 1943 developed a more sophisticated in vitro method for simulating oral conditions. Tooth sections were mounted between glass slides, placed in chambers and exposed to a nutrient medium in such a way that the teeth could be continuously examined microscopically. Caries-like lesions developed.

In 1952 Pigman et al\textsuperscript{158} increased the value of in vitro studies by the development of the mouth simulator, or "artificial mouth." For this work, extracted human teeth were mounted together and placed in a chamber, and a bacteriological medium or "artificial saliva" was allowed to flow dropwise over the teeth for several months. At certain intervals, the teeth were inoculated with samples of human saliva or bacteria cultures, and rapidly became covered with a mass of microorganisms. These modifications allowed researchers to simulate the oral conditions more closely than before, and to provide more rigid controls of such important factors as pH, temperature, cleansing, and saliva composition.

Pigman et al\textsuperscript{159} using the "artificial mouth" reported localized lesions of both dentin and enamel resembling naturally carious lesions in many respects. The lesions occurred generally in pits, fissures and grooves, in the cervical enamel and interproximally when two teeth were
mounted adjacent to each other. Pigman and Sognnaes examined histologically the carious lesions produced under these conditions and concluded that they very closely resembled natural lesions.

Forsiani et al. used ultraviolet fluorescence and polarized light to study alterations in the behavior of artificially produced lesions and concluded that the lesions were very similar to natural carious lesions.

Sideways et al. studied the conditions within the artificial mouth for the maintenance of a mixed culture of aerobic and anaerobic organisms representative of the oral flora. They then used their system to induce and maintain plaque formation and to produce artificial lesions of sound enamel and dentin. They concluded that the problems influencing bacterial growth in vitro involved temperature, atmospheric conditions, nutrient supply and the removal of end products of bacterial metabolism in a continuous culture system.

Rowles et al. studied bacterial plaque and the development of carious lesions in a specially designed all-glass artificial mouth. The authors developed continuous and intermittent feeding systems, a siphon for supplying Seitz filtered saliva, and two new ways to sterilize teeth by using antibiotics and beta-propiolactone. A bacterial plaque on a sterile tooth surface was produced by salivary inoculated and the use of an intermittent feeding system combined with a continuous salivary flow. Anaerobic and aerobic organisms from saliva survived together for several months in an induced plaque, and demineralization of enamel occurred underneath this plaque, thus indicating its pathogenic similarity to natural plaque.
Pigman et al.\textsuperscript{164} used the artificial mouth to evaluate the ability of single strains of representative oral microorganisms to initiate in vitro caries. The following organisms were able to produce lesions: \textit{Lactobacillus casei} (two strains), \textit{Streptococcus salivarius} (two strains), \textit{Streptococcus faecalis}, \textit{Micrococcus pyogenes vairaureus}, and \textit{Clostridium perfringens}. \textit{Neisseria catarrhalis} was tested and found to be without action. These cariogenic microorganisms exhibited marked differences in their ability to attack the various tissues of the teeth.

Pigman et al.\textsuperscript{165} and Pigman, Hawkins, and Thomas\textsuperscript{166} have also used the artificial mouth to study systematically the influence of glucose concentration on the type of attack. Extracted teeth were inoculated with salivary microorganisms and subjected continuously to fresh media of constant concentration and composition except for the variable concentrations of glucose. The results demonstrated that decalcification and dentinal matrix destruction were inversely related. The rate of decalcification increased with the glucose concentration whereas the rate of destruction of the dentinal matrix decreased. Joint attack upon sound dentin seemed to occur at a maximum in the range of 0.2 to 0.3 percent glucose.

Jordan and Keyes\textsuperscript{167} in 1966 developed a self-operating artificial mouth which made it possible to automatically control a series of events resembling those occurring in the human mouth. Caries-conducive streptococci formed heavy bacterial plaques on extracted and artificial teeth, stainless steel wires and other objects. This happened following cyclic exposure to a growth medium for three hours, 5 percent sucrose for 1 hour, and synthetic saliva for 3 hours in that order, for a total of two weeks.
Streptococci, known to be caries-inactive in animal tests, did not form plaque under these conditions. Sucrose was required for plaque production and could not be replaced by glucose, fructose, galactose, lactose, sorbitol or a mixture of glucose and fructose. In addition, mucin added to the synthetic saliva was found to be essential for plaque formation in this system.

In addition, plaque was grown on a glass probe electrode using the above-mentioned methods in order to obtain an estimate of acidity levels which develop under such deposits. In 5 percent sucrose the pH dropped to a range where enamel would be decalcified (4.5 - 5.0). The low pH values persisted under the plaque for three hours after the electrode had been transferred to synthetic saliva.

Jordan and Keyes\textsuperscript{167} further reported that by using this system and pure strains of microorganisms, extensive plaque formation and incipient carious lesions developed within three weeks. This situation is markedly different from that of the conventional mouth simulator in which an attempt is made to get a representative mixed flora.

Wilson\textsuperscript{168} in 1970 developed an apparatus that attempted to reproduce the oral environment in vitro. The lower of two interconnected chambers is the incubation chamber that houses the specimen (whole extracted teeth, section of enamel or dentin, denture, or filling material). The upper chamber provides nutrients, saliva, supplements such as preventive agents, additives or carbohydrate, bacterial inoculum and gas (95% air, and 5% CO\textsubscript{2}). The fluid drips from the supply chamber to the incubation chamber via a common delivery jet onto the specimens. The fluid supplies are operated by peristaltic valves that may be set to deliver a constant volume.
1 percent supplement sucrose and a pure inoculum of *Streptococcus mutans*, a thick plaque was produced within one week. This plaque was biochemically and electron microscopically similar in many respects to natural plaque.

Anticaries agents and dentifrices have been tested with the artificial mouth and similar in vitro procedures. The usual procedure involves exposing ground enamel surfaces of extracted teeth to a topical treatment of the agent in solution or to a slurry of the dentifrice. The rate of softening of enamel, treated with dentifrice or anticaries agent, is then compared with those of untreated controls, and some semiquantitative visual evaluation is used.

In 1963, Francis and Meckel\textsuperscript{169} used the "artificial mouth" and their agar-saliva method to evaluate dentifrice components. A semiquantitative method of following the enamel changes was developed using fluorescence photography as well as visual examinations. Their results showed both sodium and stannous fluoride to be effective topical treatments, but stannous fluoride was more effective. In addition, a stannous fluoride dentifrice was also found to be effective, especially when the teeth were brushed twice daily during the 148-day experimental period.

Pigman and Newbrun\textsuperscript{170} evaluated several possible anticaries agents and commercial dentifrices. An anticaries index was calculated by measuring the softening rates of teeth treated with different agents. Stannous fluoride, sodium fluoride, sodium fluorophosphate, calcium fluorophosphate and sodium-N-palmitoyl-sarcosinate were found to be significantly effective as topical treatments. In addition, two fluoride and two non-fluoride dentifrices were found to be significantly anticariogenic when tested by exposing the extracted teeth to slurries of the dentifrices.
The artificial mouth has been used to produce changes in hardness of the enamel surfaces. Caldwell et al.\textsuperscript{171} developed a method for measuring the changes in hardness on the enamel surfaces produced in the artificial mouth.

Koulourides and Reed\textsuperscript{172} used the artificial mouth to evaluate the efficiency of oral enamel rehardening compositions. Calcium, phosphate, and fluoride ions, at the concentrations known to produce rehardening of softened enamel surfaces, were incorporated in the bacteriological medium. Under these conditions, virtually no softening occurred after operation of the artificial mouth for eight hours. In a variation of this procedure, the nutrient media and a rehardening solution were dripped over enamel surfaces for alternating periods of one hour each for a total of eight hours. Compared to the control, no significant softening took place.

Koulourides and Volker,\textsuperscript{173} and Koulourides and Lastra\textsuperscript{174} went one step closer to actual oral conditions. Enamel slabs were placed in partial dentures and worn in human mouths for about one week. Microhardness was measured before and after exposure. Marked differences were reported between individuals and any one individual may show periods of variable activity at any time.

Bibby,\textsuperscript{175} at a recent workshop examining the role of human foodstuffs in dental caries, mentioned that of a variety of laboratory and intraoral procedures, the "artificial mouth" seems to be appropriate to measure the relative cariogenicity of foodstuffs.
METHODS AND MATERIALS
Test Solutions

A control solution, four milk solutions and a milk solution with toothbrushing were tested over a 20-week experimental period. Included were distilled water, white milk, white milk plus 1.0 percent cocoa powder (equivalent to the amount of cocoa in chocolate milk), white milk plus 5 percent sucrose (equivalent to the amount of sucrose in chocolate milk), commercial chocolate milk, and chocolate milk followed with toothbrushing to remove any residue.

The exact composition of the solutions tested was as follows:

Solution A: (Control) Distilled water, 240 cc.

Solution B: White Milk,\(^a\) 2 percent lowfat, fortified, 240 cc.

Solution C: White Milk\(^a\) with cocoa powder\(^b\)
1. White Milk, 2 percent lowfat, fortified, 240 cc.
2. Cocoa powder, Hershey's, 1 percent (2.4 g.)
3. Calcium carrageenan with mono-and di-glycerides added,\(^c\) stabilizer, .5 percent (1.2 g)

Solution D: White Milk\(^a\) with sucrose\(^d\)
1. White Milk, 2 percent lowfat, fortified, 240 cc.
2. Sugar, granulated pure cane, 5 percent (12 g.).
3. Calcium carrageenan with mono-and di-glycerides added,\(^c\) stabilizer, 0.5 percent (1.2 g).

Solution E: Milk Chocolate Flavored,\(^e\) 2 percent lowfat, fortified, 240 cc.
(Dutch Cocoa processed with alkali)

Solution F: Milk Chocolate Flavored,\(^e\) 2 percent lowfat, fortified, 240 cc.
(Dutch Cocoa processed with alkali)

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\( \text{a. Hillfarm brand, Hillfarm Dairy, Melrose Park, Illinois} \)

\( \text{b. Hershey's brand, Hershey Foods Corporation, Hershey, Pennsylvania} \)

\( \text{c. Stabilizer, provided by Indianapolis District Dairy Council, from a commercial supplier.} \)

\( \text{d. G. W. Pure Sugar brand, The Great Western Sugar Company, Denver, Colorado} \)

\( \text{e. Hillfarm brand, Hillfarm Dairy, Melrose Park, Illinois} \)
Solutions B, E, and F were administered in unaltered form while solutions C and D required additional preparation. A Sartorius balance was used to measure the proper weight of cocoa, sugar and carrageenan. Test solutions C and D were mixed fresh twice daily before use.

Two percent lowfat milk and chocolate-flavored two percent lowfat milk were used since these varieties of the milk products investigated were reported to be the most commonly consumed. 176

Because commercial preparation of chocolate milk involves use of a prepared syrup of cocoa, sugar, and stabilizer to be added directly to white milk, it was necessary to formulate special mixtures to determine what effect each component had by itself. 176

Upon request, the Dairy Council, Incorporated, of Indianapolis, Indiana provided a sample of the stabilizer used in commercial chocolate milk production. The stabilizer consisted of calcium carrageenan with mono-and di-glycerides added.

Typical analyses of the four test solutions for the percentage composition of water, protein, lipid, lactose, sucrose, calcium, phosphorous, sodium and potassium are recorded in Table I. 177,178

Mouth Simulating Device

A mouth-like environment was established by constructing a mouth simulating device based upon Pigman's "artificial mouth." 158 The apparatus is illustrated in Figures 1, 2, 3, and 4 and consists of four parts: (1) Media Supply System, (2) Mouth Simulator, (3) Residue Collecting Device, and (4) Substrate Feeding System.
1. Media Supply System

The media supply system administers a bacterial supporting medium continuously over the teeth and consists of three parts: (A) - the main media reservoir, which is a large 20 liter volume Pyrex glass bottle for the main media supply; (B) - an intermediate reservoir, with connecting rubber tubing to the main media reservoir; and (C) - six plastic tubing connectors. Each connector is fitted with a disposable intravenous-type drop counter to regulate the flow of the media that will bathe the surfaces of the teeth inside the mouth simulator. All six connectors are joined together at a rubber stopper that fits into the intermediate reservoir and forms a closed system.

The entire media system is started flowing by a suction apparatus and continues flowing by gravity feed. All glass and rubber parts are steam sterilized in an autoclave, while the plastic tubing is gas sterilized in ethylene oxide for 24 hours. The entire system is then assembled together using aseptic technique.

2. Mouth Simulator

The mouth simulator consists of six cylindrical plastic chambers mounted on a flat plastic base. Each chamber contains a freely rotating acrylic disc in which tooth specimens are embedded. An electric motor and pulley belt assembly connects all six chambers and rotates all the tooth-discs uniformly at approximately two revolutions per minute inside their respective chambers.

Each chamber is 3 1/2 inches in diameter and 5 inches high and has a feed-in glass entry tube in the top and a discharge hole in the bottom. Each acrylic tooth-disc was rotated continuously so that the embedded
tooth specimens passed directly beneath the feed-in tube.

The mouth simulator and six chambers were specially constructed for this project. One-quarter inch plexiglass plastic sheets and hollow tubing were bonded together with a chloroform plastic solvent. One and one-half inch diameter pulleys were turned down on a lathe from a solid plastic cylinder and then grooved. A small powerful electric motor was mounted on the base, so that a 42 inch O-ring pulley belt would rotate the tooth-discs in each chamber.

3. Residue Collecting Device

Located directly beneath the mouth simulator, the residue collecting device consisted of a large plastic box with one liter glass beakers placed directly beneath each chamber. Each beaker contained a small amount of sterilizer-deodorizer mixture and required daily cleansing. The amount of media in each beaker was monitored daily to equalize flow in the chambers as much as possible.

4. Substrate Feeding System

A test substrate or milk feeding system was designed that consisted of six plastic funnels attached by 1/4 inch rubber tubing to their respective mouth simulator chambers. Hoffman 1/2 inch screw pinch clamps were used to regulate the flow of the respective test solutions. Each funnel was supported with a ring directly above the respective chamber and gravity feed was used to convey the appropriate solution through the respective chamber to a residue collecting glass beaker.
Tooth Selection

A total of 162 sound human permanent molars and bicuspids, extracted from patients by oral surgeons in the Indianapolis area, were collected and stored in 3 percent aqueous formaldehyde. These extracted teeth were then cleaned with a flour of pumice-water mixture and evaluated by two independent examiners in bright light with sharp explorers and were verified as being caries-free teeth. The examiners used clearly defined criteria and were experienced in the detection of dental caries. They had previously demonstrated a high degree of reliability in similar evaluations.

Criteria for Dental Caries

The criteria used in this study for determining pit and fissure carious lesions were established in 1968 by the American Dental Association's Council on Dental Research and Council on Dental Therapeutics:179

1. The area is carious when a sharp explorer "catches" or resists removal after insertion into a pit and fissure with moderate to firm pressure and when this is accompanied by one or more of the following:
   A. A softness at the base of the area.
   B. Opacity adjacent to the pit and fissure as evidence of undermining or demineralization.
   C. Softened enamel, adjacent to the pit and fissure which may be scrapped away with the explorer.
2. The area is carious if there is loss of normal translucency of the enamel, adjacent to a pit, which is in contrast to the surrounding tooth surface. This condition is considered to be reliable evidence of undermining. In some cases, the explorer may not "catch" or penetrate the pit.

Furthermore, both examiners were able to further define and differentiate carious lesions into two further categories: incipient and frank caries.180
Incipient caries consists of beginning carious lesions that satisfy only one of the above criteria. Usually there is a slight "catch" or resistance to removal of a sharp explorer inserted into a pit and fissure with moderate to firm pressure, but there is no accompanying evidence of softening, usually present as a visual component.

Frank caries, on the other hand, meets both requirements that (1) a "catch" or resistance to removal of a sharp explorer inserted in a pit and fissure with moderate to firm pressure be present as well as (2) evidence of a softening of the enamel, usually visible as a discoloration, either a whitening or darkening of the suspected pit and fissure.

After being certified by the two examiners to be caries-free, the teeth were mixed together completely and the molar and bicuspid teeth were separated. Six groups of 27 teeth were computer-selected according to a pre-existing randomization program. Each group consisted of 18 molars and 9 bicuspid teeth. The computer program then randomly assigned each group of teeth to a particular test solution.

Each group of teeth was then mounted in a disc-shaped base of acrylic resin, so that the occlusal surface of each tooth was directed upward and the other surfaces of the crowns were freely exposed (Figure 5). These tooth-discs were then assembled in their respective chambers and sterilized with ethylene oxide for 24 hours.
Bacteria Supporting Medium

Jordan's medium\textsuperscript{181} was used in this study. This medium, which has been used widely in similar studies,\textsuperscript{181,182} has the following formula:

\begin{itemize}
  \item K\textsubscript{2}HPO\textsubscript{4} (anhydrous) 5.0 gms
  \item yeast extract\textsuperscript{a} 5.0 gms
  \item trypicase\textsuperscript{b} 5.0 gms
  \item Jordan's Salt Solution\textsuperscript{*} 0.5 mls
\end{itemize}

q.s. to 900 mls

adjust to pH of 7.2 with 0.1 N HCl

q.s. to 1000 mls.

The formula for Jordan's Salt Solution\textsuperscript{*} is the following:

\begin{itemize}
  \item MgSO\textsubscript{4} \cdot 7 H\textsubscript{2}O 0.800 gms
  \item FeCl\textsubscript{3} \cdot 7 H\textsubscript{2}O 0.040 gms
  \item MnCl\textsubscript{2} \cdot 4 H\textsubscript{2}O 0.019 gms
\end{itemize}

q.s. to 100 mls

Ten liters of Jordan's medium were autoclaved in a 20 liter Pyrex glass bottle for 75 minutes at 250 degrees Fahrenheit. To simulate the glycoprotein found in human saliva\textsuperscript{183} and to facilitate plaque formation,\textsuperscript{167} gastric mucin\textsuperscript{c} was added to the Jordan's medium at a concentration of 120 mg/ml. To prevent alteration in structure, the mucin was sterilized with ethylene oxide for 72 hours under high pressure and added aseptically to the cooled Jordan's medium.

During the operation of the mouth simulator, if the Jordan's medium showed any signs of cloudiness characteristic of contamination, it was discarded. Also the medium supply system was replaced with a sterile system and appropriate new supplies were used.

\textsuperscript{a} Difco, Detroit, Michigan
\textsuperscript{b} BBL, Division of Becton, Dickinson & Co., Cockeysville, Maryland
\textsuperscript{c} Nutritional Biochemical Corporation, Cleveland, Ohio
Daily Inoculation

The occlusal surface of each tooth on each tooth-disc was inoculated once daily with one drop of wax-stimulated human saliva obtained consistently from the investigator under as similar conditions as possible. To ensure the presence of cariogenic bacteria, .2 ml of an 18-hour culture of Streptococcus mutans was added to each 5 ml of saliva to be used for daily inoculation.

Exposure to Test Solutions

Four ounces (120 ml) of each milk solution was allowed to drip continuously onto the occlusal surfaces of the tooth-discs in their respective chambers. In a similar manner, four ounces of distilled water was dripped over the control group. The solutions dripped and remained on the tooth surfaces during a consistently assigned 15-minute period twice a day, once in the morning and once in the evening. Upon completion of the assigned 15-minute period, Jordan's medium was allowed to flow drop by drop at a rate of 8 to 12 ml per hour or 2 to 3 drops per minute over the teeth. To ensure uniform flow over the teeth, the tooth-discs were rotated continuously during the feeding periods as well as during exposure to the Jordan's medium.

Random Placement

To ensure that no chamber occupied a more favorable position in the mouth simulator during feeding or medium exposure, each chamber was rotated daily to a different position. A randomization program ensured that each chamber spent an equal amount of time in each position.
A standardized brushing technique was used for the chocolate milk plus brushing group. A standard synthetic multi-tufted medium bristled toothbrush and distilled water were used for the gross residue removal procedure. Groups of five adjacent teeth received 10 strokes on each of the occlusal, buccal and lingual surfaces by wielding the brush in a back and forth movement between interproximal areas. The brushing procedure was performed after each exposure to chocolate milk.

Following the completion of these procedures, the mouth simulator was maintained in an incubator at 37 degrees Centigrade for 12 hours, at which time the process was repeated except that once each week all six chambers were cleaned and sterilized with ethylene oxide and each acrylic tooth-disc was brushed according to the previously described procedure and stored in moist paper towels until the start of a new cycle. The weekly sterilizing and brushing procedure was performed to reduce the accumulation of altered milk and oral flora and thus minimize the possibility of undesirable surface decalcification. A separate but identical type of toothbrush as previously described was used for each group.

The study was continued for a 20-week period sufficient to produce some carious alterations in several of the groups without producing extensive surface decalcification. Upon completion of the study, the teeth were re-evaluated individually by the same two examiners using sharp explorers and the same criteria to check for pit and fissure caries.

The final examination was conducted under blind and random conditions so that: (1) neither examiner knew the results of the other examiner and (2) the examiners could not identify the individual teeth with their respective test groups because the specimens were coded. The teeth were
evaluated for either the presence or absence of dental caries on each occlusal, buccal and lingual surface with a maximum allowed of one lesion per surface (as in the Decayed, Missing and Filled Surfaces Index). For Examiner I, the data of each test group were compared with data of the remaining test groups and the differences were analyzed by means of repeated "t" tests. A similar but separate analysis was done for the data from Examiner II. A computer program was used to this effect and the Level of Significant (P) was determined for each "t" value.
RESULTS
The results of two groups, white milk plus cocoa and white milk plus sugar, were invalidated because an improper proportion of carrageenan additive was used. The higher level of carrageenan resulted in a greatly increased viscosity for these two test solutions and complicated the interpretation of the results for these two groups. Figures 6 and 7 show the increased milk residue for these two groups after one week's exposure. The data from these two groups have been dropped from the final analysis.

The results from four valid test groups are shown in Tables II, III, IV, V and VI and indicate the presence of several trends, as follows:

**Chocolate Milk with Brushing Group:**

Both examiners found that this group showed the lowest overall caries activity of all the groups tested. The differences were significant ($P = .05$ or less) in a majority (9 out of 12) of the comparisons for both the incipient and total caries category. Concerning these incipient and total lesions, a very significant ($P = .001$ or less) preventive effect was noted by both examiners when this group was compared with the chocolate milk group. No significant differences were found when the same comparisons were made for frank lesions.

**Control Group and White Milk Group:**

Both examiners found that the caries activity for these two groups was approximately equal, with no significant differences. Overall, both groups were intermediate in caries activity between the low caries rate of the chocolate milk with brushing group and the high caries rate of the
chocolate milk group. Relative to incipient and total lesions, both the control and the white milk groups experienced caries activity that always was numerically higher and, in a majority (5 out of 8) of the comparisons, was also significantly higher (P = .05 or less) than that of the chocolate milk with brushing group. No similar effect was noted in the frank lesion category.

In the great majority (7 out of 8) of the comparisons, both for incipient and for total lesions, the control and white milk groups showed a significantly smaller (P = .05 or less) caries experience than the chocolate milk group. Both examiners agreed that for incipient caries, the chocolate milk demonstrated a significantly greater (P = .05 or less) caries experience than the white milk or the control group.

**Chocolate Milk Group:**

Both examiners agreed that this group showed the highest caries activity of all groups tested. Concerning the incipient and total number of lesions, chocolate milk was found by both examiners to have a very significant (P = .001 or less) caries-inducing effect in all (4 out of 4) of the comparisons performed with the chocolate milk with brushing group. No significant differences were found when the frank lesion category was compared.

Also in the great majority (7 out of 8) of the comparisons of the chocolate milk group with the control and white milk groups for the incipient and total lesion categories, both examiners found that the chocolate milk group was significantly (P = .05 or less) more cariogenic than the control or white milk groups.
FIGURES AND TABLES
Figure 1. Mouth simulating device
A. Medium supply system consisting of
   a - 1 Main medium reservoir
   a - 2 Intermediate media reservoir
   a - 3 Plastic tube connectors
B. Mouth simulator
C. Residue collecting device
Mouth simulator and residue collecting device are located in an incubator.

Figure 2. Mouth simulator, top view, showing six plastic chambers, electric motor and pulley belt system.
Figure 3. Mouth simulator, lateral view, showing medium supply plastic tube connectors (a-3) entering mouth simulator chambers (B) with residue collecting device (C) located in incubator.

Figure 4. Mouth simulator (B) and substrate feeding system (D).
Figure 5. Six tooth-discs for test groups A-F.

Figure 6. Tooth-disc for white milk plus cocoa group after one week in mouth simulating device, showing excessive accumulation of milk residue.
Figure 7. Tooth-disc for white milk plus sugar group after one week in mouth simulating device, showing excessive accumulation of milk residue.
TABLE I

Major components of milk products in percent

<table>
<thead>
<tr>
<th>Component</th>
<th>Distilled Water Control</th>
<th>2% White Milk</th>
<th>2% Chocolate Milk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>100</td>
<td>87.0*</td>
<td>80.0*</td>
</tr>
<tr>
<td>Protein</td>
<td>0.0</td>
<td>3.5*</td>
<td>3.6*</td>
</tr>
<tr>
<td>Fat</td>
<td>0.0</td>
<td>2.0*</td>
<td>2.0*</td>
</tr>
<tr>
<td>Lactose</td>
<td>0.0</td>
<td>5.6*</td>
<td>5.6*</td>
</tr>
<tr>
<td>Sucrose</td>
<td>0.0</td>
<td>0.0*</td>
<td>4.0*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5.0**</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.0</td>
<td>0.14**</td>
<td>0.11**</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.0</td>
<td>0.11**</td>
<td>0.09**</td>
</tr>
<tr>
<td>Sodium</td>
<td>0.0</td>
<td>0.06**</td>
<td>0.04**</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.0</td>
<td>0.18**</td>
<td>0.15**</td>
</tr>
</tbody>
</table>

*Reference 177

**Reference 178
TABLE II

Dental caries experience after 20 weeks for different mean number of carious surfaces per tooth and standard of the mean, According to Two Examiners

<table>
<thead>
<tr>
<th>Group</th>
<th>Examiner 1</th>
<th></th>
<th>Examiner 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Incipient Lesions</td>
<td>Frank Lesions</td>
<td>Total Lesions</td>
<td>Incipient Lesions</td>
</tr>
<tr>
<td>Control</td>
<td>2 = .07 ± .05</td>
<td>0 = .00 ± .00</td>
<td>2 = .07 ± .05</td>
<td>13 = .48 ± .10</td>
</tr>
<tr>
<td>Wh Mk</td>
<td>2 = .07 ± .05</td>
<td>3 = .11 ± .06</td>
<td>5 = .18 ± .08</td>
<td>14 = .52 ± .10</td>
</tr>
<tr>
<td>*Wh Mk + Co</td>
<td>*11 = .41 ± .10</td>
<td>*0 = .00 ± .00</td>
<td>*11 = .41 ± .10</td>
<td>*23 = .85 ± .07</td>
</tr>
<tr>
<td>*Wh Mk + Su</td>
<td>*11 = .41 ± .10</td>
<td>*3 = .11 ± .06</td>
<td>*14 = .52 ± .10</td>
<td>*18 = .67 ± .09</td>
</tr>
<tr>
<td>Ch Mk</td>
<td>9 = .33 ± .09</td>
<td>0 = .00 ± .00</td>
<td>9 = .33 ± .09</td>
<td>22 = .81 ± .08</td>
</tr>
<tr>
<td>Ch Mk + Br</td>
<td>0 = .00 ± .00</td>
<td>0 = .00 ± .00</td>
<td>0 = .00 ± .00</td>
<td>5 = .18 ± .08</td>
</tr>
</tbody>
</table>

Key to Groups:
- Control - Distilled Water
- Wh Mk - White Milk
- *Wh Mk + Co - White Milk plus Cocoa
- *Wh Mk + Su - White Milk plus Sugar
- Ch Mk - Chocolate Milk
- Ch Mk + Br - Chocolate Milk plus Brushing Group

*invalidated results, dropped from further analysis
TABLE III

GRAPH COMPARING DENTAL CARIES EXPERIENCE IN TEST GROUPS ACCORDING TO FRANK, INCIPIENT AND TOTAL (INCIPIENT + FRANK) CARIES AS FOUND BY TWO EXAMINERS

--- EXAMINER I ---- EXAMINER II

- FRANK - INCIPIENT - TOTAL
- FRANK - INCIPIENT - TOTAL
- FRANK - INCIPIENT - TOTAL
- FRANK - INCIPIENT - TOTAL

WHITE MILK GROUP
CHOCOLATE MILK GROUP
CHOCOLATE MILK + BRUSHING GROUP

MEAN NUMBER OF CARIOUS SURFACES PER TOOTH
TABLE IV
Statistical Analysis (Repeated t Tests) for Incipient Lesions

Level of Significance (P) for the comparisons between the different groups. If $P \geq .05$, the notation N.S. will be used to express lack of significance.

<table>
<thead>
<tr>
<th></th>
<th>Examiner 1</th>
<th>Examiner 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wh Mk (0.07)</td>
<td>Ch Mk (0.33)</td>
</tr>
<tr>
<td>Control (0.07)*</td>
<td>N.S.</td>
<td>$&lt;0.020$</td>
</tr>
<tr>
<td>Wh Mk (0.07)</td>
<td>-----</td>
<td>$&lt;0.020$</td>
</tr>
<tr>
<td>Ch Mk (0.33)</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

*The number in parentheses represents the mean number of lesions per group.

*The number in parentheses represents the mean number of lesions per group.
TABLE V

Statistical Analysis (Repeated t Tests) for Frank Lesions

Level of Significance (P) for the comparisons between the different groups. If \( P = \geq 0.05 \), the notation N.S. will be used to express lack of significance.

<table>
<thead>
<tr>
<th></th>
<th>Examiner 1</th>
<th></th>
<th>Examiner 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wh Mk (.11)</td>
<td>Ch Mk (.00)</td>
<td>Wh Mk (.07)</td>
<td>Ch Mk (.07)</td>
</tr>
<tr>
<td>Control (.00)*</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Wh Mk (.11)</td>
<td>-----</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ch Mk (.00)</td>
<td>-----</td>
<td>-----</td>
<td>N.S.</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

*The number in parentheses represents the mean number of lesions per group.
**TABLE VI**

Statistical Analysis (Repeated t Tests)
for Total Lesions (Incipient and Frank Caries)

Level of Significance (P) for the comparisons between the different groups. If $P \geq .05$, the notation N.S. will be used to express lack of significance.

<table>
<thead>
<tr>
<th></th>
<th>Examiner 1</th>
<th>Examiner 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wh Mk (.18)</td>
<td>Ch Mk (.33)</td>
</tr>
<tr>
<td>Control</td>
<td>N.S.</td>
<td>$&lt; .020$</td>
</tr>
<tr>
<td>Wh Mk (.18)</td>
<td>-----</td>
<td>N.S.</td>
</tr>
<tr>
<td>Ch Mk (.33)</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

*The number in parentheses represents the mean number of lesions per group.

---

The number in parentheses represents the mean number of lesions per group.
DISCUSSION
The primary objective of this study was to evaluate the local, post-eruptive effect on dental caries of various milk formulations. This effect is to be differentiated from the systemic effect, for which milk is universally recognized as an important food for infancy and childhood. Milk is of great nutritional value based on its content of minerals, vitamins and proteins. However, the situation is not clear for the localized effects of milk on teeth which it contacts during ingestion. Even less is known about chocolate milk with its various additives and their effects on dental caries. This thesis relates only to the local effect of milk on teeth.

A cursory examination of the chemical composition of milk and chocolate milk (Table I) immediately discloses two potentially cariogenic components: lactose and sucrose.

Lactose was present in study Groups B, C, D, E, and F at the 5.6 percent level. Like many other fermentable sugars, lactose is metabolized by plaque to form acids. According to some researchers, it forms as much acid as other fermentable sugars, but dissolves less enamel. Others claim that there is no difference in either effect; still others say that lactose forms less acid than other sugars. In Jenkins' experiment, for instance, the fermentation by salivary bacteria of lactose produced a pH slightly higher than the fermentation of glucose. However, the differences were so small (0.12 of a pH unit on the average) that they are of little practical significance.
Sucrose, in addition to the lactose, was present in study Groups D, E, and F at the 4 to 5 percent level. As reported earlier, clinical and laboratory studies have shown that plaque pH is reduced by sucrose rinses at the 0.5 percent level. At the 5.0 percent level the pH reduction is sufficient to decalcify tooth mineral. In combination with the lactose already present, Groups D, E and F would seem to have a greatly increased caries potential due to the amount of available fermentable carbohydrate.

Milk for study Groups B, E and F was used in unaltered form, while Groups C and D required additional preparation. Upon addition of 1 percent cocoa to milk it was found that the cocoa powder was not soluble in milk unless a stabilizer was used. The local dairy council,* through a commercial supplier,** provided a commonly used stabilizer in the form of calcium carrageenan. According to one source,\(^5\) chocolate milk contains "less than one percent stabilizer." Several different milk formulations were tried and finally carrageenan at the 0.5 percent level was used. The cocoa dissolved completely but there was a readily observable increase in viscosity. This was thought to be due to different methods of mixing and handling compared to the commercial preparation.

At the conclusion of the experiment, in an effort to learn more about the properties of carrageenan, it was found that in chocolate milk production only a very small amount of carrageenan (0.025 percent) is used to create a thixotrophic system and thereby form a weak gel, which only slightly increases the viscosity of the milk. If a higher level (0.15 percent) is used, strong gels are formed.\(^{150}\) Thixotrophism is defined as the property

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*Dairy Council, Inc., Indianapolis, Indiana  
**Robert A. Johnston Co., Milwaukee, Wisconsin
exhibited by certain gels, which become fluid when shaken and then become solid again. Since the milk formulations of Groups C and D were used directly after mixing, no gel formation was seen, but the viscosity was increased.

Figures 6 and 7 (photographs of Tooth-Discs C and D) show the increased residue of milk after one week exposure. This is thought to be one of the reasons for the increase in dental caries over what could have been expected according to the percentage composition of these two solutions.

Interestingly, carrageenan has been found to increase the rate of erosion in teeth in certain animal experiments when included in the diet at a low level.

The Appendix shows the dental caries data from the two evaluators after the teeth underwent 20 weeks of mouth simulation conditions and exposure to the pre-mentioned test and control solutions. Each of the 162 teeth was evaluated individually under blind conditions, in random order using the dental caries criteria mentioned earlier. Three teeth were reported fractured during the evaluation and all three were noted to relatively smaller-sized bicuspids.

Table II shows the average number of carious surfaces per tooth in the different study groups, along with the standard error of the mean as calculated from Appendix.

A rather large variability in the diagnosis of dental caries is evident in the data from Table II. Table III compares the dental caries experience found by Examiner I and II for the different groups. Examiner II found a higher caries rate than Examiner I in a great majority (10 out of 12) of the comparisons, and the rates were generally higher by about the
same amount, showing a degree of consistency. However, more important than the actual numerical differences in caries experience is the similarity in trends found by both evaluators for all six groups, which indicates that both examiners were looking at the same process at different levels of acuity.

Using the data from Table III, a statistical analysis by the more conservative Neuman Keul's method showed results of only borderline significance. A more liberal analysis, using the Repeated t-Test, showed a slightly higher degree of significance between the groups. The results of the Repeated t-Test analysis are reported in Tables V, VI and VII, and help further delineate certain trends.

Three distinct levels of caries activity are present. The highest caries activity was found in the chocolate milk, white milk and cocoa and white milk with sugar groups. Unfortunately, the latter two groups contained an undesirable factor that limits their usefulness for comparative purposes in this experiment. The level of carrageenan used was much greater (about 33 times) than is usually found in commercial chocolate milk. This higher level of carrageenan resulted in a greatly increased viscosity and complicated the interpretation of the results for these two groups. Thus, the data from these two groups were dropped from the final analysis.

**Chocolate Milk Group**

As far as the incipient and total lesions are concerned, chocolate milk was found by both examiners to have a very significant (P = .001 or less) caries-inducing effect in all four of the comparisons performed with the chocolate milk with brushing group. No differences were observed when frank carious lesions were compared.
Also, in the great majority (7 out of 8) of the comparisons within the incipient and total lesion category, both examiners found that the chocolate milk group was significantly \( P = .05 \) or less) more cariogenic than the control or white milk groups.

A more specific comparison of white milk groups with chocolate milk groups showed that in the frank lesion category there was no significant difference. In the total lesion category, Examiner II found that the chocolate milk was significantly \( P = .02 \) or less) more cariogenic than white milk, while Examiner I found that although chocolate milk had a higher caries activity, it was not significantly higher.

In the incipient lesion category, both examiners found that chocolate milk exhibited a significantly greater \( P = .02 \) or less and \( P = .05 \) or less, respectively) cariogenic effect than the white milk. From these results, it is evident that no protective effect against pit and fissure caries was exerted by the chocolate milk group under the conditions tested. In contrast, according to the two evaluators, chocolate milk had a dental caries-promoting effect, at least in the incipient caries category. In addition, there was no significant difference in the frank lesion category between the two milk formulations. It may be speculated that the time was short for the difference in incipient lesions to become apparent in the frank lesions category. Had the study continued longer, more frank lesions might have been found in the chocolate milk group than in the white milk group. In addition, it seems logical that if a protective effect had been present, it would have been more effective at the incipient stage than the frank caries stage. In fact, the opposite effect seems to be true, with a higher caries rate occurring in the incipient stage category for chocolate milk.
It is not known whether the increased cariogenicity of chocolate milk that was observed was due to the presence of sucrose, or to the presence of other additives such as carrageenan. Possibly, both factors interact to produce a caries-enhancing effect.

**Control Group and White Milk Group**

The control group and the white milk group showed caries activity intermediate between the high caries rate of the chocolate milk group and the low caries rate of the chocolate milk with brushing group. Both evaluators agreed that the caries activity for these two groups was approximately equal, with no significant differences. Thus, under the conditions tested, white milk had neither a caries-protective effect nor a caries-enhancing effect in comparison with the control group of distilled water.

On the one hand, an increase in dental caries might be expected due to the presence of carbohydrate in the form of lactose at the 5.6 percent level. Vianna showed in his study\(^{118}\) that white milk has a cariogenic potential, but apparently only under nursing bottle or relatively stagnant long-term conditions.

On the other hand, a decrease in dental caries might be predicted based on certain animal and in vitro tests. These tests showed that milk possessed certain protein and mineral factors that should reduce enamel dissolution.

It is interesting that even the distilled water control group developed dental caries. This would seem to indicate that the bacteriological supporting medium as used in this experiment has a certain caries-producing potential itself. Upon analysis of the ingredients of Jordan's medium, it is noted that only three sources of carbohydrate are present: 1) yeast extract, the water-soluble portion of autolyzed fresh yeast; 2) tryp ticase,
prepared from the pancreatic digestion of the protein casein; and 3) gastric mucin, a mucopolysaccharide or glycoprotein derived from intestinal lining. All three consist primarily of protein found to be essential for bacterial growth. It is possible that the mixed oral flora present have enzymes capable of forming low levels of carbohydrate from the above protein sources.

This very low level of possible carbohydrate, however, coupled with the formation of dental caries in the distilled water control group, raises some thought-provoking questions concerning the etiology of dental caries and the acidogenic theory of dental caries.

The proteolysis-chelation theory by Schatz and Martin is founded upon non-acid mechanisms of enamel destruction. In brief, it can be summarized as follows:

(1) Any organic matrix in the enamel (for example: interprismatic substance, interprismatic sheath, lines of Retzius, keratin) may be attacked by oral microorganisms.

(2) The resulting degradation products liberate substances capable of complexing calcium and thereby destroying enamel apatite.

(3) Complexing agents are those compounds or ions that can snatch or chelate metals such as calcium away from their parent molecule by a chemical process known as coordination, which is a complex sharing of electrons between atoms. This mechanism is to be differentiated from the typical acid degradation reaction which results in a more simple electrostatic bond between the atoms.

(4) Complexing agents may also arise from the dental plaque and saliva.

(5) The above process can occur in acid, neutral or alkaline media.

It is obvious, therefore, that the enamel structures under this theory are destroyed by chelation of metal-binding processes while under the acidogenic theory, they are broken down by direct acid degradation. Unfortunately, little is known of the chelation mechanism and there is little
conclusive or supportive evidence for the theory.

**Chocolate Milk with Brushing Group**

The lowest overall caries activity was found in the chocolate milk with brushing group, which when compared to the chocolate milk group for incipient and total caries, exhibited a very significant ($P = .001$ or less) caries-preventive effect in all four comparisons. In addition, the chocolate milk with brushing group revealed a significant ($P = .05$ or less) caries-protective effect in a majority (5 out of 8) of the comparisons with the control and white milk groups in the incipient and total lesion categories.

The importance of mechanical disruption and removal of plaque and food debris from enamel surfaces for the prevention of dental caries becomes obvious. Even under the influence of cariogenic foodstuffs and bacteria, if good oral hygiene is performed, dental caries can be prevented to a large extent. Further use of other preventive measures, including dietary control and application of topical fluorides would be expected to reduce the incidence of dental caries even further.

The only other study showing the specific non-supplemented effect of chocolate milk on dental caries as compared to white milk, was conducted in rats by Rathbun, Bond and Steinman. The trend for high caries rate found for chocolate milk coincides in both studies, although in the rat study, the chocolate milk exhibited a much greater relative cariogenicity when compared to white milk. Also, in the rat study the white milk showed no difference in caries rate than in the 62 per cent sucrose highly cariogenic control diet. In the mouth simulating device, however, white milk showed neither a caries enhancing nor a caries protective effect.
Two other studies, reporting on the effect of supplementation of the diet with chocolate milk, have shown a tendency for the diet to decrease the relative cariogenicity of chocolate milk when compared to white milk. Shaw, Ensfield and Wollmann\textsuperscript{152} found in 669 rats that all supplements of dairy products caused major reduction in dental caries. No significant difference in effect was found between chocolate milk and white milk supplements.

In Dunning and Hodge's study\textsuperscript{153} on over 300 retarded patients who received dairy supplements over a two-year period, there was no significant difference between white milk and chocolate milk supplements on pit and fissure caries. However, the chocolate milk supplements did show increased caries increments of borderline significance when compared to cocoa and milk with artificial sweetner supplements.

It is not clear exactly how closely the mouth simulating device duplicates the actual oral milieu. Other investigators\textsuperscript{165,166,184} have observed that dental decay proceeds at a faster rate in the artificial mouth than would normally be expected in the human mouth. This might be due to the semi-stagnant condition in the mouth simulator. No self-cleansing natural features such as the lips, tongue or buccal mucosa are present. In addition, a medium is used to support bacterial life rather than actual saliva with its accompanying enzymes and antibodies. To what extent this faster rate of decay modified the end result is not known.

It should also be noted that there is a definite variation in caries response of the teeth collectively within the individual mouths, even when subjected to identical treatments.\textsuperscript{186} This variation is probably due to a number of factors, including biologic variation of the teeth, different
environmental backgrounds of the teeth, differences in flow rates of the medium onto the teeth, contamination problems, and so forth. In view of this situation, data obtained using only one "mouth" per group, as was done in this study, should be interpreted with some reservation. Even so, this author feels that the mouth simulator remains a valid laboratory procedure in dental caries research. Many factors such as pH, temperature, cleansing and saliva composition are well controlled and allow for much closer comparison of the cariogenicity of foodstuffs than would otherwise be possible.

In general, the operation of the mouth simulator proceeded well and accomplished its intended goal. Using the randomization program to rotate the positions of the tooth discs, it is felt that all groups were treated in a similar and equal fashion.

Problems of a minor nature did occur, such as contamination of the medium. Several times the gravity feed medium system was disrupted, but only for short periods. Any irregularities were believed to have occurred randomly and to have averaged out overall.

Use of a peristaltic pump for the medium feed system would assure more uniform media flow and prevent any possible contamination of medium due to back flow.

In one respect, this experiment created an artificial factor that normally would not have occurred. The diet of the mouth simulator consisted of only one food or resulted in exposure to only one milk formulation and the bacteria support medium. This would not normally occur in human circumstances where the effect would be modified and the end result would be an interaction of many dietary factors rather than just one.
However, it is this limitation that allows for a better comparison of the relative cariogenicity of the milk formulations than could be made otherwise. Only the ingredients of the test solution are given exposure on the teeth, and a more exact comparison can therefore be made.
SUMMARY AND CONCLUSIONS
Dietary recommendations vary concerning the role of chocolate milk in the dental caries process. Some investigators have shown that cocoa exhibits antibacterial and enamel-solubility-reducing properties and thus may inhibit the formation of dental caries. Other investigators point out that five percent sucrose is added to cocoa to make chocolate milk, and that this sweetener, in addition to the fermentable lactose already present in milk, could exert considerable cariogenic potential. Laboratory testing of the cocoa, sugar and milk ingredients together, and in separate formulations, was undertaken in the present study.

The relative cariogenicity of five milk formulations and a control was investigated. Included in the groups were: 1) distilled water control, 2) white milk, 3) white milk plus cocoa, 4) white milk plus sugar, 5) chocolate milk, and 6) chocolate milk plus brushing.

Mouth-like conditions were established by constructing a mouth simulating device based on Pigman's artificial mouth with certain modifications. The apparatus consisted of a medium supply system, a mouth simulator, a residue collecting device and a test substrate feeding system. The medium supply system consisted of an intermediate reservoir and six plastic tube connectors with intravenous type drop counters to regulate the medium flow bathing the surfaces of the teeth inside the mouth simulator.

A total of 162 teeth, which had been extracted for reasons other than dental caries, were mounted in the mouth simulator in six groups of 27 teeth each. Two independent evaluators had certified the teeth to be caries-free and a computer program was used to ensure complete randomization of the teeth into groups.
After the mouth simulator and teeth were sterilized by ethylene oxide, the teeth were inoculated with a mixture of a culture of *Streptococcus mutans* and saliva to facilitate bacterial colonization. Each group was exposed to one of the milk formulations for 15 minutes twice daily. After each period, a sterile bacterial medium was dripped (8-12 ml/hr) over the teeth in the mouth-like environment. A control group was similarly treated, except that it was exposed only to distilled water and the bacterial medium.

After 20 weeks, the teeth were separated, coded and re-evaluated for pit and fissure dental caries by the same two examiners. A statistical analysis of the results indicated the presence of three levels of relative cariogenicity: the chocolate milk group had the highest caries rate, the control group and the white milk group were intermediate, and the chocolate milk with brushing group showed a marked reduction in dental caries. The results of two groups, the white milk plus cocoa and the white milk plus sugar groups, were invalidated due to the use of an improper proportion of carrageenan.

In summary, for pit and fissure dental caries under the conditions tested in the mouth simulating device, chocolate milk exhibited a significant cariogenic potential relative to white milk, especially in the early incipient caries stage. In addition, white milk was found to exert neither a caries-protective nor a caries-enhancing effect when compared to distilled water. Moreover, the chocolate milk with brushing group exhibited a very significant decrease in dental caries compared to the chocolate milk group. These results stress the importance in the prevention of dental caries of limiting the tooth's exposure to sucrose-
containing foods, and after the exposure, the importance of brushing and flossing.

It may be concluded from this study that in an individual with high dental caries susceptibility, it would seem unwise to recommend frequent ingestion of chocolate milk, unless proper and immediate oral hygiene follows the ingestion.
APPENDIX
### APPENDIX

Dental caries data from two examiners after twenty weeks in mouth simulating device.

<table>
<thead>
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\[a = 2 \quad b = 0 \quad a + b = 2\]

\[
\begin{align*}
\text{Code:} & \quad E-1 = \text{Examiner 1} \\
& \quad E-2 = \text{Examiner 2} \\
& \quad a = \text{incipient caries} \\
& \quad b = \text{frank caries} \\
& \quad S = \text{sound tooth} \\
& \quad 1 = \text{occlusal surface} \\
& \quad 2 = \text{buccal surface} \\
& \quad 4 = \text{lingual surface} \\
& \quad * = \text{tooth fractured upon final evaluation} \\
& \quad a + b = \text{total caries} \\
& \quad A = \text{Control Group} \\
& \quad B = \text{White Milk Group} \\
& \quad C = \text{White Milk + Cocoa Group} \\
& \quad D = \text{White Milk + Sugar Group} \\
& \quad E = \text{Chocolate Milk Group} \\
& \quad F = \text{Chocolate Milk + Brushing Group}
\end{align*}
\]
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Professional Societies

American Dental Association
Indiana State Dental Association
American Society of Dentistry for Children
American Academy of Pedodontics
Indiana University Pedodontic Alumni Association
Abstract
Dietary recommendations concerning chocolate milk remain controversial since the effect of chocolate milk on the dental caries process is not clear. Cocoa with antibacterial and enamel-solubility-reducing properties may inhibit the formation of dental caries. Since chocolate milk contains a significant amount of sucrose (about 5 percent) and some cocoa (1 percent), laboratory testing of the cariogenicity of chocolate milk seems valuable.

The present study investigated whether or not under mouth simulation conditions chocolate milk influenced the formation of dental caries compared to white milk.

A control solution, four milk solutions and a milk solution with toothbrushing were tested over a 20-week experimental period. A mouth-like environment was established by constructing a mouth simulating device. One-hundred-and-sixty-two-teeth were mounted in the mouth simulator in six groups of 27 teeth each. Two independent evaluators had certified the teeth to be caries-free and a computer program was used to ensure complete randomization of the teeth in groups.

After initial sterilization by ethylene oxide, the teeth were inoculated with a mixture of a culture of Streptococcus mutans and saliva. Each group was exposed to one of the milk formulations for a 15 minute period twice daily. After each period, a sterile bacterial medium was dripped (8 to 12 mls/hr) over the teeth in the mouth-like environment.
After 20 weeks the teeth were separated, coded, and re-evaluated for pit and fissure caries by the same two evaluators. A statistical analysis by Repeated t Tests indicated the presence of three levels of relative cariogenicity: the chocolate milk group had the highest caries rate, the control group and the white milk group were intermediate and the chocolate milk with brushing group showed a marked reduction in dental caries. The results of two other groups were invalidated.

In summary, for pit and fissure dental caries under the conditions tested in the mouth simulating device, chocolate milk exhibited a significant cariogenic potential relative to white milk, especially in the early incipient caries stage.

It may be concluded from this study that in an individual with high dental caries susceptibility, it would seem unwise to recommend frequent ingestion of chocolate milk, unless proper and immediate oral hygiene follows the ingestion.