THE CARIOGENIC POTENTIAL OF MILK

By

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Submitted to the Faculty of the Graduate School in partial fulfillment of the requirements for the degree of Master of Science in Dentistry, Indiana University-Purdue University at Indianapolis, School of Dentistry, 1971.
TABLE OF CONTENTS
<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Review of Literature</td>
<td>2</td>
</tr>
<tr>
<td>Methods and Materials</td>
<td>30</td>
</tr>
<tr>
<td>Results</td>
<td>34</td>
</tr>
<tr>
<td>Tables and Figures</td>
<td>38</td>
</tr>
<tr>
<td>Discussion</td>
<td>51</td>
</tr>
<tr>
<td>Summary and Conclusions</td>
<td>60</td>
</tr>
<tr>
<td>References</td>
<td>62</td>
</tr>
<tr>
<td>Abstract</td>
<td></td>
</tr>
<tr>
<td>Vita</td>
<td></td>
</tr>
</tbody>
</table>
LIST OF TABLES AND FIGURES
<table>
<thead>
<tr>
<th>Figure No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Photograph of the mouth simulating device</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>Photograph of the mouth simulator</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>Photograph of the plastic glass tray with eight teeth specimens</td>
<td>38</td>
</tr>
<tr>
<td>4</td>
<td>Photograph of the 13 identical mouth simulating devices inside an incubator</td>
<td>38</td>
</tr>
<tr>
<td>5</td>
<td>Graphic representation of the percentage of enamel dissolved by different milk preparations during different time intervals</td>
<td>38A</td>
</tr>
<tr>
<td>6</td>
<td>Photograph of the control group after completion of the experiment and stained with silver nitrate</td>
<td>39</td>
</tr>
<tr>
<td>7</td>
<td>Photograph of the two hours daily exposure to milk formula group after completion of the experiment and stained with silver nitrate</td>
<td>39</td>
</tr>
<tr>
<td>8</td>
<td>Photograph of the two hours daily exposure to bovine milk group after completion of the experiment and stained with silver nitrate</td>
<td>39</td>
</tr>
<tr>
<td>9</td>
<td>Photograph of the two hours daily exposure to bovine milk and honey group after completion of the experiment and stained with silver nitrate</td>
<td>39</td>
</tr>
<tr>
<td>10</td>
<td>Photograph of the two hours daily exposure to human milk group after completion of the experiment and stained with silver nitrate</td>
<td>39</td>
</tr>
<tr>
<td>11</td>
<td>Photograph of the four hours daily exposure to milk formula group after completion of the experiment and stained with silver nitrate</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>Photograph of the four hours daily exposure to bovine milk group after completion of the experiment and stained with silver nitrate</td>
<td>40</td>
</tr>
<tr>
<td>13</td>
<td>Photograph of the four hours daily exposure to bovine milk and honey group after completion of the experiment and stained with silver nitrate</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>Photograph of the four hours daily exposure to human milk group after completion of the experiment and stained with silver nitrate</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>Photograph of the eight hours daily exposure to milk formula group after the completion of the experiment and stained with silver nitrate</td>
<td>41</td>
</tr>
</tbody>
</table>
Table No.  

<table>
<thead>
<tr>
<th>Table No.</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Average composition of bovine milk, milk formula and human milk in percentages</td>
<td>42</td>
</tr>
<tr>
<td>II</td>
<td>Percentage of enamel surface dissolved by different milk preparations during different time intervals</td>
<td>43</td>
</tr>
<tr>
<td>III</td>
<td>Comparison between the area of surface decalcification, expressed as a percentage of the total exposed enamel surface, produced by 2-hour daily exposure to milk formula, bovine milk, bovine milk with honey, and human milk</td>
<td>44</td>
</tr>
<tr>
<td>IV</td>
<td>Comparison between the area of surface decalcification, expressed as a percentage of the total exposed enamel surface, produced by 4-hour daily exposure to milk formula, bovine milk, bovine milk with honey, and human milk</td>
<td>45</td>
</tr>
<tr>
<td>V</td>
<td>Comparison between the area of surface decalcification, expressed as a percentage of the total exposed enamel surface, produced by 8-hour daily exposure to milk formula, bovine milk, milk with honey, and human milk</td>
<td>46</td>
</tr>
<tr>
<td>VI</td>
<td>The decalcifying effect of milk formula on enamel. Comparison between the percentage of the surface area decalcified by 2 hour, 4 hour, and 8 hour daily exposure to milk formula</td>
<td>47</td>
</tr>
<tr>
<td>Table No.</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>VII</td>
<td>The decalcifying effect of bovine milk on enamel. Comparison between the percentage of the surface area decalcified by 2 hour, 4 hour, and 8 hour daily exposure to bovine milk</td>
<td>48</td>
</tr>
<tr>
<td>VIII</td>
<td>The decalcifying effect of milk and honey on enamel. Comparison between the percentage of the surface area decalcified by 2 hour, 4 hour, and 8 hour daily exposure to milk and honey</td>
<td>49</td>
</tr>
<tr>
<td>IX</td>
<td>The decalcifying effect of human milk on enamel. Comparison between the percentage of surface area decalcified by 2 hour, 4 hour, and 8 hour daily exposure to human milk</td>
<td>50</td>
</tr>
</tbody>
</table>
I am deeply grateful to Dr. James Roche, Chairman of Graduate Pedodontics, for his advice, friendship and encouragement, which have made my graduate program the most profitable and pleasant experience that I have ever had.

I owe a great debt of gratitude to Dr. Simon Katz, Associate Professor of Preventive Dentistry, whose constructive criticism, knowledge, and experience guided me throughout every step of my research project.

I want to thank Dean Ralph E. McDonald and Dr. La Forrest Garner, Chairman of the Department of Orthodontics, for their guidance and assistance during my graduate education.

A special thanks to Professor Paul Barton, for his substantial help in editing this work.

I want also to express my profound gratitude to the Kellogg Foundation, which provided me with the support so necessary for this experience.

A word of gratitude is also due to my class-mates, Doctors Alvin Ayers, Douglas Barton, Clifton Dummett, and David Kennedy whose friendship and companionship will be always in my memory.

The help of the staff of the Department of Pedodontics, particularly the Dental Assistants with whom I worked, is greatly appreciated.

I also would like to thank Mrs. Wyonetta Hoffman who kindly accepted the task of typing this thesis and "La Leche League" who furnished the human milk necessary for this study.

I would like to dedicate this thesis to my parents, since it was their sacrifices and love that provided me my whole education.

Finally, I want to thank Carminha, my wife, whose patience, encouragement, and help were mainly responsible for making this work a reality.
INTRODUCTION
"Nursing bottle caries" is a condition 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. The condition, which resembles rampant caries, attacks particularly the four maxillary primary teeth, the maxillary and mandibular first primary molars, and the mandibular primary cuspids. The four lower primary anterior teeth are usually not attacked by the carious process to any extent. This is a means of differentiation from a rampant caries case.

There appears to be little doubt that prolonged use of the nursing bottle may be associated with nursing bottle caries. There is marked disagreement, on the other hand, as to whether milk alone in the nursing bottle could produce the type of lesions described above. For instance, after reviewing the pertinent literature, Finn concluded that insufficient evidence was available to suggest that bovine milk could be cariogenic. On the contrary, there were some indications that milk had no cariogenic potential and could even decrease the enamel solubility in some "in vitro" situations. This protective effect remained even after washing the teeth. However, Michal examined carefully the clinical records of his patients presenting "nursing bottle caries" and concluded that plain bovine milk can cause dental caries.

The purpose of this study was to determine whether different milk preparations, alone or with the addition of fermentable sugars, could produce caries-like enamel dissolution in an "in vitro" situation resembling that occurring in the mouth of a sleeping child. It was hoped that this information would allow the pediatrician and pedodontist to provide adequate guidance and counsel to parents of children prone to use nursing bottles for prolonged periods.
REVIEW OF LITERATURE
Inasmuch as past reports on subjects related to this thesis have comprised different areas of research, this review of the literature will be divided in two sections: (1) In vitro methods for the Study of Dental Caries, (particularly the "artificial mouth") and (2) Milk and Dental Caries. The second section will be subdivided as follows: a) Animal studies, b) Lactose studies, c) In vitro studies, d) Human studies, e) Opinions and Editorials.

In Vitro Methods for the Study of Dental Caries, Particularly the "Artificial Mouth"

The advantages of using in vitro experimentation for the study of dental caries were realized by early research workers, particularly Magitot\(^4\) and W. D. Miller.\(^5-7\) Their procedures were followed by many investigators. Magitot\(^4\) used sound extracted teeth, exposing them to natural oral conditions and observing the development of carious lesions. The procedure was accomplished by mounting sound crowns of extracted teeth on pegs in the canals of remaining roots from which crowns had been removed. A variation of the procedure was to prepare artificial dentures in which natural extracted teeth were mounted. Magitot\(^4\) noted that such teeth in human mouths often showed widespread attack, which he considered as "having every characteristic of ordinary caries."

Magitot's second approach was to place sound extracted teeth in aqueous sugar solutions and to allow them to stand for about two years. To localize the attack, some teeth were covered with wax except for small areas of exposed enamel. To show the effect of fermentative organisms, creosote was added in some experiments, and the solutions were sterilized in others. After two years the enamel and dentin of the teeth exposed to the microorganisms were extensively destroyed.

Magitot\(^4\) stated: "The teeth protected by a coating of sealing wax show at the exposed point an alteration identical to that described above. Since the attack is localized, it produces a cavity having all of the characteristics of caries." He noted also that the teeth in the sterile solutions remained unaltered,
whereas acids had been formed in the non-sterile solutions, which had an effect on the teeth.

W. D. Miller modified Magitot's procedure in ways which many investigators used later. Instead of depending on accidental inoculation of the culture solutions or depending on organisms attached to the teeth, he used human saliva with added sugars, bread, and other foodstuffs. Extracted teeth or tooth sections were incubated in such media for several months. Miller also followed the tooth attack by studying tooth sections. His avowed purpose was "to produce the microscopic appearance of decay of teeth, since it was already proved by Magitot without doubt that by various means macroscopical appearances may be produced which are identical with those found in decay."

Both enamel softening and dentinal destruction were noted. Microorganisms were found to have penetrated the dentinal tubules and in places to have produced "caverns or microscopic holes." Miller evaluated these results as follows: "I affirm that a destruction of the tooth substance may be brought about artificially which the most practical microscopist will not be able to distinguish from real decay as it occurs in the human mouth."

In recent years, more sophisticated attempts have been made to develop in vitro methods for the simulation of oral conditions. The first of these was the MCG apparatus used by Dietz, who mounted tooth sections between microscopic slides. The slides were placed in a chamber exposed to nutrient medium, but in such a way that the tooth section could be continually examined microscopically. The development of carious-like lesions was reported.

Although based on the earlier work, the artificial mouth is an apparatus, with many possible modifications, made to simulate oral conditions more closely than previously and to provide more rigid controls of such important factors as pH, temperature, cleansing factors, and saliva composition.

Pigman was the first to use this apparatus and he was able to produce 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 are mounted adjacent to each other. Pigman and Sognnaes examined histologically carious lesions produced under this condition and concluded that they closely resembled natural lesions.

Forsianti et al. studied alterations in the behavior of artificially produced lesions during the exposure to ultraviolet (fluorescence) and to polarized light, and concluded that the lesions were very similar to natural carious lesions.

Sidaway et al. studied the conditions necessary within the artificial mouth for the maintenance of a mixed culture of aerobic and anaerobic organisms representative of the oral flora, to induce and maintain bacterial plaque on the enamel surfaces of caries-free experimental teeth, and to produce artificial lesions on sound enamel and dentin. They concluded that the main problems influencing bacterial growth in vitro and consequently the formation of artificial lesions involved incubation temperature, atmospheric conditions, supply of food and, in a continuous culture system, the removal of end products of bacterial metabolism.

Rowles et al. designed an all-glass artificial mouth suitable for in vitro studies on the bacterial plaque and the development of carious lesions. Continuous and intermittent flow feed systems and a siphon for supplying Seitz filtered saliva were developed. These authors also described two new ways to sterilize the teeth specimens, one using antibiotics and the other using Beta-propiolactone (BLP). The results showed that it is possible to produce, by salivary inoculation and the use of an intermittent feed system combined with a continuous salivary flow, a bacterial plaque on a sterile tooth surface. Aerobic and anaerobic organisms from saliva survived together for several months in an induced plaque, and demineralization of enamel occurred beneath such plaque.

Pigman et al. studied in the artificial mouth the ability of single strains of all microorganisms to initiate in vitro caries. The organisms shown to be able to produce lesions were Lactobacillus casei (two strains), Streptococcus salivarius (two strains), Streptococcus faecalis, Micrococcus pyogenes var. aureus, and Clostridium perfringens. One microorganism, 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 teeth. One strain of Lactobacillus casei exhibited intensive decalcifying action; it had only a moderate ability to destroy decalcified dentinal matrix, but it readily destroyed external enamel surfaces. The other extreme action was shown by Clostridium perfringens, which destroyed calcified and decalcified dentinal matrices without any obvious signs of decalcification. Other organisms showed actions intermediate between the extremes. Some organisms destroyed dentin more rapidly than enamel and produced undercutting of enamel. Pigmentation of dentin seemed to be associated with a rate of dentinal decalcification greater than matrix destruction.

The influence of glucose concentration on the type of attack has been studied systematically by Pigman et al. Extracted teeth were inoculated with salivary microorganisms and subjected continuously to fresh media of constant composition except for variable amounts of D-glucose. With a medium containing 0.3% glucose, general destruction of the tooth gradually occurred. After four to five months, most of the enamel and about a third of the dentin had disappeared. Only enamel destruction and decalcification of dentin occurred in a similar experiment when the tooth was exposed to a medium containing 0.5% glucose. Subsequently, however, the decalcified dentin was destroyed extensively when the glucose concentration was decreased to 0.25%.

In similar experiments by Pigman et al., acid-decalcified dentin, sound dentin, and sound enamel were exposed to oral organisms and to solutions having glucose concentrations of 0.0%, 0.125%, 0.25%, and 0.5%. 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 - 0.3% glucose.
Jordan and Keyes developed an automatic artificial mouth which made it possible to control automatically a series of events resembling those occurring in the human mouth. The sequence of events can be programmed as follows: (1) introduction of a preselected solution into the incubation chamber; (2) filling the chamber to a preset level; (3) retention of the solution in the chamber for a time interval which can range from six minutes to six hours; (4) drainage of the chamber at the end of the time intervals and (5) repetition of the above operations on a continuous cycle. If needed, six electromechanical timers can be arranged to operate in sequence, thus a cycle can be programmed to consist of a maximum of six solutions. The different solutions are stored in pyrex glass reservoirs from which they are metered through solenoid valves to the incubation chamber. The chamber is fabricated from a 200-ml large-mouthed, round bottomed flask adapted to provide inlet and outlet openings. In a preliminary experiment the apparatus was programmed to carry out the experimental cycle previously described except that the device cycled continuously.

Human molar teeth were mounted on stainless steel blocks, sterilized in the incubation chamber with ethylene oxide, and inoculated with 10 ml of a broth culture of caries-conductive streptococci on successive days until visible plaque had formed on the teeth.

Streptococci known to be caries-conductive in experimental animals formed heavy bacterial plaques on extracted teeth, artificial teeth, stainless-steel wires, and other objects. This was accomplished by cyclic exposures to a growth medium for three hours, 5% sucrose solution for one hour, and synthetic saliva for three hours, in that order, for a total of two weeks.

Plaques were grown on a glass probe electrode using the above-mentioned methods to obtain an estimate of acidity levels which develop under such deposits. In 5% 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.
In this research of Jordan and Keyes, the individual pure strains of microorganisms were used, and plaque formation was the criterion. This situation is markedly different from that of the artificial mouth, in which an attempt is made to get a representative mixed oral flora.

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 topical treatments of the agent in solution or to a slurry of a dentifrice. The rate of softening the enamel, treated with dentifrice or anticaries agent, is compared with those of untreated controls, and some semiquantitative visual evaluation is used.

Francis and Meckel, using the artificial mouth and their agar-saliva method, reported both sodium and stannous fluorides to be effective after topical treatments, but said that stannous fluoride was superior. A stannous fluoride dentifrice was also found to be effective. The best results in the dentifrice treated group were obtained when the teeth were brushed twice daily with the dentifrice during the experimental period of 148 days.

Pigman and Newbrun used the artificial mouth to evaluate several possible anticaries agents and commercial dentifrices. As topical treatments, stannous fluoride, sodium fluoride, sodium fluorophosphate, calcium fluorophosphate, and sodium N-palmitoyl-sarcosinate were found significantly effective. Tested by exposing the extracted teeth to slurries of commercial dentifrices, two fluoride and two non-fluoride dentifrices were found significantly anticariogenic.

The artificial mouth has been used to produce changes in hardness of the enamel surfaces. Caldwell et al developed a method for measuring the changes in hardness on the enamel surfaces produced in the artificial mouth. Koulourides and Reed have used the artificial mouth to evaluate the efficacy 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. Koulourides et al\textsuperscript{22} recently introduced a variation of this procedure. The nutrient media and a rehardening solution were dripped over the enamel surfaces for periods of one hour each for a total of eight hours. Compared to the control, no significant softening took place.

Koulourides and Volker\textsuperscript{23} and Koulourides and Lastra\textsuperscript{24} went one step closer to actual oral conditions. Their studies were conducted with small slabs of tooth enamel inserted in partial dentures and allowed to remain in the mouth of a given individual for about one week. They used this method to determine the carious activity of a given individual.

Wilson\textsuperscript{25} recently developed some modifications on the original artificial mouth. His apparatus consists of five sources of supply as follows: nutriment, saliva, test product, gas supplement, and bacteria. The fluids are pumped into the incubation chamber by peristaltic pumps. The gas supply is delivered at a constant flow by means of a Rotameter flow regulator. In a preliminary experiment using sucrose as a supplement at a level of 1\% in direct relation to the nutriment, pure inoculums of organisms such as Streptococcus Mutans produced a thick plaque on all surfaces within one week. Biochemical and electron microscopic investigations indicate that plaque produced by this system is similar in many respects to natural plaque.

Bibby\textsuperscript{26} recently mentioned that among several methods for comparing the cariogenicity of food stuffs, the artificial mouth seems to be appropriate.

\textbf{Milk and Dental Caries}

a) Animal studies concerning milk diets:

Numerous reports of animal caries studies involving the use of milk in different forms have been published. McClure and Folk\textsuperscript{27} tested diets containing 35\% of dry skim milk. The milk powders were tested as sold commercially and after heat treatment. Two strains of weanling littermate rats received these diets for 90-91 days. Two roller-process milk powders
were found to be more cariogenic than the low heat spray process powder. The heated powder showed a greater cariogenic effect than the untreated one. Most of the lesions were on the buccal surfaces of lower teeth. These same authors studied the chemical and biologic properties of skim milk powder in relation to their cariogenic effect in rats. Certain residues and extracts of autoclaved skim milk powders were found to be about equally effective in caries production. Dry whey-powders, when present at a level of 25% in rats diets, produced smooth surface caries similar to that produced by skim milk powders. The dry whey powders also showed an increase in their cariogenic effect after a "dry autoclave" treatment. Lossee and Nemes provided 18 male and 18 female Osborne-Mendel weanling rats, for 61 days, McClure-Folk diet 636, which consists of 35% heated skim milk powder, 45% corn starch, 18% cerelose, and 2% liver powder, plus weekly supplements of vitamin A, D, and E concentrate. Lesions developed in 97% of the animals. Occlusal lesions were found in 69.5% of the animals and surface lesions in 91.6% of the animals. All the 12 offspring from an occlusal caries-resistant female (O.-M. strain) developed surface caries, of a pattern and severity comparable to those of the caries-susceptible colony. The authors suggested that there seem to be separate and independent etiologies for occlusal and surface lesions when that diet is used.

McClure and Folk observed that a diet containing a combination of four processed cereal foods ($n^O586$) and a diet containing a roller processed skim milk powder ($n^O635$) for 30, 60, and 90-day periods produced various amounts of caries in Sprague-Dawley and Holtzman strains of rats. The factors causing the variation in the caries incidence seemed to be: (1) number of rats housed together (the more rats, the more caries); (2) freshness of the diet (the more contaminated, staler diet caused increased caries); (3) preweaning feeding (more caries resulted with the early introduction of the cariogenic diet). Both diets produced extensive smooth surface lesions, and more lesions in the
lower molar teeth and buccal surfaces.

In a later study the same authors reported that a diet containing lyophilized skim milk powder produced about one-third as much caries as one containing a spray-process skim milk powder. The greatest amount of caries was produced by a diet containing roller-process skim milk powder. Autoclaving a spray-process whey powder did not change its cariogenicity; autoclaving a roller-process whey powder increased its cariogenicity. Commercial heat treatment seems to be an important factor in increasing the caries-promoting activity of milk powders. The lesions were predominantly on the smooth surfaces or in lower buccal areas. Similar findings were reported by Muhler. He noticed that significantly more occlusal caries occurred when rats were fed a cariogenic corn diet containing a heat-processed whole milk powder than when the milk powder was not heat-processed. If the whole milk powder not subjected to heat was fed by stomach-tube, the caries incidence returned to the level of the group receiving the non-heat-processed milk powder in the diet. Muhler concluded that a heat-labile factor in the milk powder has an anticariogenic effect which acts systemically.

Constant et al reported a study in which for 14 weeks weanling littermate cotton rats were fed several different type of diets: (1) basal diet (50% oatmeal and 33% milk) either in dry or fluid form plus 17% natural syrup; (2) basal diet plus 17% cerelose in dry form; (3) basal diet (50% oatmeal and 32% milk) with 18% natural milk or sucrose; (4) basal (50% oatmeal, 32% milk) plus 18% natural syrup and 16% more or 16% less milk, with the oatmeal content correspondingly decreased or increased. There was no significant difference between the cariogenicity of natural and refined sugars. The physical state of the diet was very important. The same sugar levels which in fluid diets produced very low caries scores, were very cariogenic when used in the dry diets. Substitution of dextrin and casein for milk increased caries. Rats on the natural oatmeal-milk diets had little plaque formation whereas those on the purified diets had heavy plaque and darker pigmentation of the lesions.
McClure and Lossee\textsuperscript{34} used strains of different types of rats, originated in two institutions, the National Institutes of Health and the Naval Medical Research Institute. The animals were fed a cariogenic diet containing 35\% of dry autoclaved skim milk powder, 45\% corn starch, 18\% cerelose, and 2\% powdered whole liver. All of the rats developed a significant amount of caries.

Nizel and Harris\textsuperscript{35} reported a study in which 40 weanling hamsters of mixed sex received a modified diet consisting of 63\% corn, 30\% dried whole milk powder, 6\% alfalfa, and 1\% sodium chloride for 15-23 weeks. Another group of 37 received a diet of the same composition for the same period. Corn and milk for the first group's diet were obtained from New England; corn and milk for the second, from Texas. The group fed the New England corn and milk had significantly more caries, about 40\% more carious teeth and 50\% more carious surfaces than the group fed Texas corn and milk.

The same authors reported\textsuperscript{36} a follow-up of that study in which four groups of 35 weanling hamsters were fed modified diets containing 63\% corn, 30\% dried whole-milk, 6\% alfalfa and 1\% NaCl. Group A which was fed New England corn and milk, had statistically more caries than Group B, fed on Texas corn and milk. There was no statistical difference between groups fed Texas corn and New England milk or vice versa. Texas milk contained 2.9 ppm fluorine, New England, 0.3 ppm, but it is not known whether this caused the difference.

McClure\textsuperscript{37} stated that smooth surface caries formed in the teeth of littermate rats fed, for 60 days from weaning, a highly cariogenic, lysine deficient diet, containing autoclaved skim milk powder. Lysine was administered in the diet or water at 1.5\% to some groups, by intubation of 1 ml of a 75 mg solution five days per week to others, and to a last group by intraperitoneal injection of 1 ml of a 100 mg solution, twice daily five days per week. Significant reduction in caries occurred when L. lysine was administered by means of the diet, water or intubation. Injection had a variable but slight effect.
Breveta and McClure reported that when weanling littermate rats were fed diets containing a roller-process skim milk powder, a high incidence of severe caries resulted in spite of supplementation with essential vitamins and amino acids. The highest caries score resulted from a diet containing autoclaved roller-process skim milk powder. The total findings support the theory that the cariogenic factor in heat processed skim milk powders is due to a lysine deficiency and that the quantity of protein in the diet is important.

Patterson attempted to produce smooth surface lesions in the molars of cotton rats by feeding the animals a milk diet known to produce these lesions in white rat molars. The white rat controls developed these lesions but the cotton rats only presented pit and fissure lesions. The effect of autoclaving the skim-milk powder was studied, and this procedure did not alter the cariogenicity of the diet.

Edwards et al. studied four groups of 19 albino rats each (10 male and 9 females) which received four different diets containing skim milk powder, for 13 weeks, after which they were sacrificed using ether anesthesia. The results indicated that the skim milk powder diets produced smooth surface caries in both sexes, but the incidence was higher in the females.

Klapper and Volker studied the effect of powdered whole milk-sorbitol diets on dental caries in desalivated hamsters. Thirty-three hamsters desalivated at 27 days of age, were fed a diet consisting of 96% powdered whole milk, 3% alfalfa and 1% sodium chloride with 0%, 20%, and 40% sorbitol replacing equivalent amounts of the milk. Another group received reconstituted milk made from powdered whole milk. Twenty-nine days later the average caries incidence was: Group 1 (96% powdered whole milk, no sorbitol), 26.0; Group 2 (76% powdered whole milk, 20% sorbitol), 18.8; Group 3 (56% powdered whole milk, 40% sorbitol), 14.0; Group 4 (reconstituted milk, 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 caries
observed in the 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 animals, the oral environment of which has proved to be especially prone to the development of dental caries.

Zeppelin et al. reported that groups of litter-mate cotton rats were fed for 14 weeks on a diet resembling the average human diet with varying amounts of sucrose added and on a synthetic cariogenic diet. Feeding of liquid milk did not reduce caries.

Rathbun and Steinmann studied the effect of cow's milk, soy milk and chocolate milk upon the incidence of caries in the rat. Fifteen-day-old rats were fed chocolate milk, cow's milk, soy milk, and a high-sugar control diet. The rates of the experimental groups were very similar but substantially less than those of the group on the high-sugar diet. In the chocolate milk group the incidence of decay was about three times as great as in the other milks or the cariogenic diet. The other groups, soy milk and cow's milk, under the circumstances of this experiment, developed numbers of lesions similar to those for rats on a high-sugar diet. The statistical analysis of the data gave better than one chance in a thousand that the differences between the chocolate milk and the other foods were not due to chance. The other differences were not significant.

Dreizen and Stone designed a study to permit a more comprehensive evaluation of the relationship between dry cow's milk and rat dental caries. A comparison was done between the dental caries incidence in rats restricted to diets containing 0, 39, and 100% non-fat dry milk. Three randomly selected groups, each containing 30 Sprague-Dawley male albino rats, were given one of the three already mentioned diets. The degree of carious development was noticeably greater in the rats fed the corn meal, wheat flour, sugar and lard mixture enriched with 39% non-fat dry milk (Group 2) than in those given the identical diet without the added milk (Group 1). In contrast, just two lesions were found in the animals given 100% non-fat dry milk. These findings demonstrated that non-fat dry cow's milk is not cariogenic for the rat when
constituting the sole nutrient. The authors were unable, however, to identify any specific anticariogenic factor inherent in this food.

Cohen and Bowen\textsuperscript{45} built up a small colony of monkeys to conduct research on dental diseases. Twenty-two monkeys (macaca irus) were divided as follows: Group 1, 11 monkeys; Group 2, 6 monkeys; and Group 3, 5 monkeys. Each group received the same daily diet except that Groups 2 and 3 were given a flavored milk drink instead of water, four nights a week for periods of two to four years. Group 1 presented two caries-free monkeys and an average of 6.3 carious teeth per monkey. Group 2 had one caries-free and 4.6 carious teeth per monkey. Group 3 presented two caries-free monkeys and 6 carious teeth per monkey. Dental caries in these monkeys seems to resemble that occurring in humans with no significant difference within the groups. In addition, several instances of apparent caries-resistance emerged.

Elliot and Pigman\textsuperscript{46} reported that many common infant formulas have compositions generally similar to those which have been found in numerous investigations to cause extensive dental caries in rats and hamsters. The usual infant formulas consist primarily of a type of commercially processed carbohydrate and of milk in some form with various vitamin supplements. In general agreement with earlier work, these authors showed that diets composed mostly of whole dry milk (30%) and sucrose and an uncooked starch in varying proportions (66%) contributed to caries in hamsters. These results were obtained in a shorter period than usual by placing 17-day old hamsters on the experimental diets for 58 days. During a study to determine the difference between diets containing milk in dry or liquid form, Anderson et al\textsuperscript{47} reported that animals fed dry whole milk and dry skim milk plus butter, had some carious lesions, whereas the animals fed only liquid whole milk, had zero scores. Caries scores were again reduced to nearly zero, however, when the dry rations were reconstituted with water and fed in liquid form. These data indicate that fluidity of the ration is an important factor in producing dental caries in the cotton rat.
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% dissolved sucrose; (3) milk and free access to dry sucrose; (4) milk and 10% sucrose solution; (5) stock diet plus 10% cooked dried whole eggs and (6) stock diet. Half of the females (20) were bred. In Group 3 (dry sugar) 57% to 60% of the animals had severe decay in the molar teeth. Of those in Group 4 (sugar solution) 26-36% had moderate caries in the molars. The group receiving 10% sugar dissolved in the milk (Group 2) had no caries, nor did those on the milk alone. In Group 5, 7% to 10% and in Group 6, 12-17% had slight caries. Milk consumed directly with sucrose seemed to protect the teeth, but caused overweight, failure in reproduction in females, and shortening of lifespan in males.

Law and Bentley reported that three groups of 30 weanling albino littermate rats were fed for 20 weeks as follows: (1) stock diet only; (2) stock diet plus milk equivalent to 22% of the total caloric intake; and (3) stock diet plus chocolate and soft beverage equivalent to 24% of the total caloric intake. There was no difference in caries scores among the three groups. The results suggest that supplements of milk, chocolate, and sweetened beverage had the same effects when the diet was adequate.

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

Shaw and Ensfield studied the effect of a cariogenic diet, either supplemented or unsupplemented by dairy products, on two generations of caries-susceptible rats. The supplements were milk, chocolate milk, or chocolate milk with vanilla ice cream and cheddar cheese. The levels of supplementation
were chosen to fall within likely ranges of human consumption, for the age periods under investigation. The supplementation with dairy products was done in every test by proportional reduction of all ingredients in the cariogenic ration rather than by the replacement of a single dietary component, which in many cases would introduce an immediate bias into the trial. Significant caries reduction was produced by all dairy supplements when fed post-eruptively, but no effects were seen when they were fed pre-eruptively. None of the supplements caused any detectable influence on the development of the teeth which could alter their caries susceptibility. When the supplements were provided continuously through both the developmental and post-eruptive periods, there was no demonstrable effect beyond what could be obtained by post-eruptive supplementation alone.

Masuda et al. reported that the salivary buffering capacity of rats fed with a milk-containing cariogenic diets was greater than that of those fed with a milk-deficient cariogenic diet. Statistical analysis by t-test shows 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, showed 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 skim milk might be closely related to the buffering capacity of saliva.

b. Lactose Studies

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

Guggenhein et al. tested the caries activity on groups of nine Osborne-Mendel rats which were inoculated with cariogenic streptotoccus (OMZ-61) after their indigenous oral flora had been depressed by antibiotics. The OMZ-61 streptococcus is known to induce smooth surface caries. The animals were given low fat test diets containing 25% sucrose, glucose, fructose, lactose, maltose, or uncooked wheat starch. The authors found
that these diets were consumed in amounts not significantly different, and continuous recording of food and water intake revealed similar feeding patterns among the groups. As expected, caries incidence was highest on the sucrose diet. The lactose diet was the next most cariogenic. The caries scores for the sucrose and lactose groups were statistically different from the scores of the other diets in which the caries activity was very low.

To compare the cariogenic potential of different types of carbohydrate, Green and Hartless used 214 weanling albino rats in five experiments. Although sucrose was the most cariogenic, all the other carbohydrates seemed to be highly cariogenic. All but one of the lesions found in the study were occlusal ones, and the exception occurred in a rat maintained in the lactose-containing diet.

In studying the cariogenic potential of wheat cereal diets on weanling albino rats fed for 60 days, MacClure noticed that when lactose was added to the whole wheat flour and autoclaved, an increase in the cariogenicity of the diet occurred.

The variations in the plaque pH in six adult men and women were measured by Kurosawa, 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 with lactose or starch 80:20 or 50:50 produced the same drop in pH as sucrose alone.

Shaw, Keremias, and Gibbons 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 one.
Steinman and Haley reported a study in which littermate rats were given the following solutions from the day after birth until weaning at 21 days: 20% sucrose, 20% lactose, 20% mixed glucose plus fructose. They also nursed ad libitum. At weaning, all rats in these studies received a cariogenic diet (65% sucrose) until 13 weeks of age. Sucrose solutions caused the most caries, and lactose the least. In another study in which 20% 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% or 5.35% of sucrose was added to the milk formula fed from the 14th-21st days to another littermate group, significantly more 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.

c. In Vitro Studies

Many in vitro studies using milk suggest that this nutrient is not cariogenic. Tooth sections were exposed by Bibby and Soni to mixtures of saliva and 35 foodstuffs. Polarized light examination 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. They found that treatment with cow's milk reduced solubility by 20% regardless of whether it was raw or pasteurized whole or skim-milk. Reconstituted whole and reconstituted skim 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 skim 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.

Jenkins and Ferguson 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% 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% 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% lactose or 4% glucose in the mouth and leaving them in contact with the teeth for 30 seconds. The results of the incubation experiment showed that while the carbohydrate of milk is used by salivary bacteria for acid production, the pH values reached after four and 24 hours of incubation are somewhat higher than when a 4% lactose solution is used. This difference, although small, (pH 4.58 and 4.69, respectively), was statistically significant (p<0.01).

The experiments on the solubility of enamel and teeth in saliva were consistent in showing that, in spite of the acid production, the amount of calcium phosphate dissolving was much less in the presence of milk. In several experiments the analytical results suggested that no tooth substance whatever had dissolved and occasionally that the concentration of calcium and phosphate in the milk-saliva mixtures fell slightly during the incubation with whole teeth, thus suggesting some tooth recalcification.
Later experiments in the same series by Jenkins and Ferguson (e.g., those on milk from which caseinogen had been removed) showed that the rise in viscosity which occurred after the milk clotted played no important part in the result. The fall of plaque pH in vivo after a milk rinse was compared with that from a 4% lactose rinse (48 sets of observations) and that from a 4% glucose rinse (84 sets of observations). The results showed that the changes produced by milk were not significantly different from those observed during baseline pH determination in 12 experiments. In the remainder it was comparable with 4% lactose in six sets of observations and with 4% glucose in five sets of observations. In general, the effect of lactose was greater than that of milk. In no case were milk curds observed on the teeth or in the interdental spaces.

Another series of experiments by the same authors with milk from which various constituents were removed suggested that the calcium and phosphate in milk are largely responsible for the reductions in the amount of enamel dissolving, but since the effect of milk was still detectable after it had been washed off the teeth, some other constituents must also contribute to the effect. Since milk is often taken with sucrose added to it or with biscuits, Jenkins and Ferguson decided to see whether milk would reduce the potentially cariogenic effect of these other carbohydrates or whether the latter would nullify the protective effect of milk. The experiments in acid production during the incubation of enamel in milk and saliva were repeated but with milk and biscuit or milk and 10% sucrose. The results with 10% sucrose confirmed what had already been shown with 4% lactose, that salivary acid production is lower when milk is substrate than with sugar alone (p<0.01). These results also showed that when sugar and milk are present together, the milk reduces the effect of the sugar on acid production (p<0.01). Experiments with a mixture of biscuits and milk gave similar results on pH change and also showed that less enamel dissolved when incubated with saliva and milk plus biscuit than with saliva and biscuit only.
d. Human Studies

1. Milk Consumption and Caries

Milk consumption has been associated with dental caries by several authors. Examinations with mirror and explorer and dietary surveys were made in 1950 by Potgieber et al. on 864 children, aged 10-16. The average DMF rate in 14 to 16 year olds was higher than in a 1944 survey. Diets were found moderately good. Lower DMF rates were associated with better diets, including more consumption of milk, fruit, and vegetables. However, a higher rate of decay was noted in the heaviest milk drinkers for which no definite cause was found.

In reviewing his records of children under three years of age who were treated for caries in the Dental Department of the Hospital for Sick Children at London, Pitts found that 70 cases of caries in children of three years of age or under were collected during a three-year period. The pattern of caries was a characteristic one, with involvement of the upper incisors followed by the upper and lower molars and seldom the lower incisors. A noticeable factor was the long time that many of these children had suckled. The incidence of caries did not appear to be affected by either breast or artificial feeding. After summing up all his data, he suggested that the evidence warranted his saying that the most important factor in caries in very young children is the use of a dummy dipped in such substances as sugar, honey, milk, or virol.

James, Parfitt, and Faulkner examined 62 children under four years of age who presented decay on their primary incisors. To standardize the history-taking, a questionnaire was printed and completed at the mother's interview. The questionnaires were compiled in an attempt to cover as many as possible of the etiological factors implicated and discussed by previous workers. General and local factors were included, such as illness of the mother during pregnancy, illness of the child in the first year of life,
duration of breast-feeding, sweetened comforters (nature and duration),
sweetened comforter bottles (nature and duration), oral hygiene, and
others. Their control group comprised 187 children under three years
of age, who were given the same questionnaire. By comparing both
groups they concluded that the occurrence of early labial caries of the
primary incisors was strongly associated with local factors involving
the retention of sweet, sticky, and acid substances on the labial enamel
surfaces. The most important vehicles were found to be sweetened com­
forters, either in the form of a dummy dipped in a sugary substance or a
"comforter" bottle containing milk and sugar, fruit juice and sugar, or
sugared water.

Robinson and Naylor, after examining 110 children under five
years of age, found that 59 of them had decayed upper anterior teeth and
51 had sound teeth. By investigating the feeding habits of these children,
they realized that children who were bottle fed were more likely to de­
velop caries, but that the likelihood was reduced if the bottle was given
only as a supplement to breast feeding. It was also suggested that late
weaning from the bottle was more common in children with caries.

2. Milk and "Nursing Bottle Caries"

Milk has been associated with a clinical condition known as "nursing
bottle caries". Investigating the feeding habits of a number of children
afflicted with a pattern of rampant caries, Fass noticed that all were
put to bed, either for the night or for a nap, with a milk bottle from which
they sucked while lying down, to help them fall asleep.

Most parents begin the feeding of the infant on a milk formula and
find that the child falls asleep readily after it is well fed. Then, Fass
continued, a tired mother who realizes that a child goes to sleep more
readily after a feeding, is prone to give her two, three or even four-year
old child a nursing bottle of milk when the child rebels against going to
sleep. It is at this point that a question arises about the possible benefits
of milk, as well as its possible deleterious effects.
For in this way, all of the factors necessary for the carious process to occur are present in child's mouth: (1) milk, with a carbohydrate content from 3.8% to 4.5%, (2) oral microorganisms capable of producing acids, (3) very slow clearance of the oral cavity contents, and (4) decreased salivary flow. Milk usually has a low carbohydrate content, but the greatly reduced rate of swallowing during sleep plus the diminished flow of saliva allows the carbohydrates already in the mouth to remain in contact with the teeth in the presence of acid-forming microorganisms for a much longer time. There is diminished dilution and buffering action from the saliva, and little or no clearance of the fluid from the oral cavity, so that even the small amount of carbohydrate in the milk exerts a marked cariogenic effect. In many instances the nursing bottle remains in the mouth for much of the time that the child spends in bed, and some of the milk from the bottle continues to ooze into the mouth. Fass concluded that "nursing bottle mouth" is a condition similar to rampant caries, and is found in very young children who have developed the habit of requiring the drinking of a nursing bottle with milk when lying down to sleep.

Rosenstein selected 260 children, many of them patients from the Children's Clinic at the School of Dental and Oral Surgery, Columbia University, and the others referred for consultation because of rampant caries. Their ages ranged from one and a half years to about seven years. Thirteen were boys and 129 were girls. Slightly over half of the entire group (140) were classified as having rampant caries with all teeth involved except the lower anteriors. The remaining 120 were children who did not have rampant caries and were thus chosen as a control group.

During the evaluation of the rampant caries cases in this study, it not only became evident that feeding factors are related to the incidence of rampant caries, but also that there are conflicting influences of certain foods and eating habits in terms of systemic and environmental effects.
This means that certain foods like milk and orange juice, which may produce unfavorable reactions from a local oral environmental viewpoint when they are permitted to remain about the teeth for abnormally long periods. Recommendations for the use of such foods for health must include consideration of the method of consumption by the individual.

Kroll and Stone stated that prolonged nocturnal bottle feeding appears to be a prime etiologic factor in a type of rampant caries of infants and young children. The lesions affected most severely the upper primary anterior teeth.

To test their hypothesis of a causal relationship between nocturnal bottle feeding and this type of caries, they sought through statistical analysis to determine the relationship between the occurrence of rampant caries and nocturnal bottle feeding and to analyze only those patients with rampant caries to see whether the pattern of decay might be related to nocturnal nursing bottle feeding. For the first part of their project, 79 consecutive patients from three different dental services were examined. They were all followed up to the age of seven years so that the condition of the anterior teeth could be properly evaluated. All subjective information was taken from the mother. The nocturnal bottle feeding was recorded if it persisted beyond six months of age. Those cases with caries involving most of the teeth except the lower anteriors were labeled typical, while those in which the lower anterior teeth were also involved were labeled atypical.

The second study was undertaken by sending questionnaires to a second group of 130 patients of record who were classified as having had rampant caries. Seventy-nine of these questionnaires were returned and submitted to statistical analysis. The information recorded noted whether the pattern of decay was either typical or atypical for nursing bottle feeding; whether this feeding persisted all night or for less than one hour, and at what age it was discontinued.
The results showed the following: For the first proposition 72% of the entire group did not exhibit rampant caries, 13% showed atypical rampant caries and 15% showed typical rampant decay. Of these 51 patients who were not on prolonged nocturnal milk bottle feeding, 86.3% had no rampant caries, 11.7% showed atypical rampant caries, and 1.96% showed the typical pattern of decay.

Recalculations of Kroll and Stone's data conducted by this writer show that out of 12 children who showed the pattern of decay generally associated with the use of the nursing bottle, 11 have had prolonged nocturnal feeding habits, whereas only one had not. These figures are highly significant in pointing out the relationship of prolonged nocturnal bottle feeding to rampant caries.

For the second proposition the results showed the following: The 79 children with rampant caries were classified according to the pattern of decay. Sixty-three were classified as typical. The age to which nocturnal bottle feeding persisted ranged from 0 to 48 months. Twenty-eight patients were permitted to suckle throughout the night whereas 51 partook for less than an hour or not at all. There was a statistical difference between the typical and atypical group regarding nursing bottle habits. The period of time that the bottle was used at night was then related to the pattern of decay. A 10 X 10 frequency count showed that there were no atypical cases who had an all-night bottle. Comparing this to the counts of the typical cases, a relationship between the night bottle and the pattern of decay can be assumed.

Michal² reviewed his office records, called parents of some old patients, and interviewed all parents of new patients as to the exact ingredients placed in the nursing bottle. Some children were given fruit juices and other commercial formula, but most were given formula until 6-9 months of age and then transferred to plain milk. With no statistical analysis, but from his own observations and those of other pedodontists, he concluded that plain milk can cause dental caries. According to Michal² "bottle mouth" caries is a common
phenomenon in infants and young children who have had prolonged bottle feeding. Almost all children with this type of dental caries have taken milk, formula or fruit juices from bottles when going to sleep past twelve months of age.

**e. Opinions and Editorials**

The possible cariogenic potential of milk has also been the subject of editorials and review type articles.

Brass\(^{69}\) stated that basically milk contains protein 3%, fat 4%, lactose 3.5%, water 88.5%, salts 1%. The pH of fresh cow's milk is 6.4. When milk sours, the proteins coagulate, causing a caseous precipitate and pH falls to about 5.5. Furthermore, proteins in solution tend to spread over surfaces. Both of these factors contribute to the formation of a sticky plaque on the surface of the teeth after the ingestion of milk. There is present in the saliva a small amount of lactic acid. Brass noted that the presence of plaque on the teeth is conducive to acid production and dental decay and that the pH of plaque is lower than that of saliva and is not effectively buffered by it. He concluded that milk is likely to form an adherent plaque because of its physical and chemical characteristics, and since plaques are related to dental decay, milk must be connected with dental decay.

In response to a request by the Secretary of the Canadian Dental Association, the Research Committee of that Association\(^{70}\) reviewed a number of studies dealing with the effect of milk consumption on dental caries. The conclusion was that these studies do not support the concept that drinking milk increases the incidence of dental caries. The diets of caries-immune children have been reported to contain from three to six glasses of milk per day.

Reinarz\(^ {71}\) expressed the opinion that milk is an excellent prophylactic agent for dental caries. In the enzymatic degradation of milk protein, soluble fractions are produced which have high calcium content. This permits mineral components of milk to be absorbed more easily than
the ordinary inorganic calcium salts. In a non-controlled type of study, comprising a group of farm children which was compared with a group of village children, the farm children had fewer cavities despite their inferior oral hygiene. This result was attributed to the fact that they have more milk and dairy products in their diet.

Mendel suggested that several studies in the United States have shown that a comparable caries pattern can be found when a similar type of carbohydrate ingestion occurs. He states that dental caries on the labial surfaces of maxillary primary incisors is usually associated in the U.S. with nocturnal bottle feeding. A pattern of dental caries has been described as "nursing bottle caries", and it is most prevalent if the milk is supplemented with sugar, or the formula has a high sugar content, or fruit juices are used. Finn reviewed the literature concerning milk and dental caries, and said that there is in vitro evidence as well as clinical evidence that milk reduces dental caries when its total carbohydrate content does not exceed roughly 10%. Furthermore, he states that there is need for statistically valid studies in infants to show the relative caries potentiality of milk with and without added sugars.

From the preceding review it is obvious that there is no consensus concerning the cariogenic potential of milk. Some investigators have found that milk in powdered form increases the incidence of caries in experimental animals. Several factors appear to play a role in its cariogenicity, such as the type of procedure used in preparation of the milk powder, whether a liquid or solid form of milk is used, and whether the milk is autoclaved or not. Other researchers claim that milk exerts a protective action against dental caries. This effect might be due to the formation of protective films (formed at the expense of milk proteins), to the contribution of milk to the buffering capacity of saliva, or to a decrease in enamel solubility in acids. Perhaps the discrepancy between these groups of workers is due to the fact that they tested milk under different conditions. Other factors should also be considered.
For example, if there is a protective effect, how long would it last? Would the protective film coating produced by milk proteins resist bacterial metabolism for several hours? Would the decrease in enamel solubility be of such magnitude as to prevent enamel dissolution at pH's obtained after prolonged fermentation of milk carbohydrates in the mouth?

A third group of research workers, as well as several pedodontists, has described a special condition which is found in children who are given nursing bottles, generally containing milk, for several hours in an attempt to induce them to sleep. This condition is characterized by severe caries in the maxillary primary anterior teeth since they are among the earliest teeth to erupt, and are the most readily exposed to the liquid environment during the nursing function.

The other affected teeth are the maxillary and mandibular primary molars on the occlusal surface. The mandibular primary anterior teeth are generally unaffected. This particular condition has been given the name of "nursing bottle caries". Since fermentable carbohydrates like sugar or honey have often been added to the milk contained in the bottle, a controversy has arisen as to whether milk alone, without added carbohydrates, has the potential of producing "nursing bottle caries". Much of this controversy is based on the lack of agreement, noted above, on the cariogenic potential of milk.

Many authors, based on the protective effect of milk reported by several workers, believe that milk alone, in a nursing bottle, will not produce caries. Others claim that even without added carbohydrates, milk provided in the conditions described for "nursing bottle" patients is able to produce the lesions typical of this condition. This study was designed to investigate whether or not different milk preparations, with and without added fermentable carbohydrates, have the potential of producing caries-like lesions during in vitro systems which resemble both
the conditions typical of nocturnal bottle feeding continuously for several hours and those existing in the oral cavity (temperature, bacterial flora, plaque).
METHODS AND MATERIALS
Four milk solutions were tested during a six-week period: milk formula, bovine milk, bovine milk plus honey, and human milk. The composition of these four preparations is given in Table I. The mouth-like environment was established by constructing a mouth-simulating device based on Pigman's artificial mouth (Figure 1). The apparatus consists of a feeding system, a mouth simulator, and a residue-collecting device. The feeding system is a standard 1000 ml bottle, commonly used in intravenous solutions, fitted with a disposable-type drop counter regulating the media flow that will bathe the surfaces of the teeth inside the "mouth simulator". The mouth simulator is represented by a plastic cylinder containing the tooth specimens (Figure 2).

Sound bicuspid teeth extracted for orthodontic purposes from adolescents in the Indianapolis, Indiana, area were stored in 3% aqueous formaldehyde solution. The extracted teeth were all cleaned with a zirconium silicate-water prophylactic paste and divided mesio-distally through the occlusal fissure. Subsequently, the root was removed with a disc, resulting in a separate buccal and lingual crown surface. Eight bisected crowns were mounted on a circular plastic glass tray using dental modeling compound and red sticky wax (Figure 3). The mounted enamel surfaces were evaluated by two independent examiners with explorers to verify the complete absence of cavities or decalcifications, and were then assembled in the mouth simulator to be sterilized.

This study required a special medium which chemically and physically simulated human saliva. A synthetic saliva was formulated on the basis of the work of Afonsky, Jenkins, Caldwell and McCann, which could support bacterial growth with an extremely low carbohydrate content and could be sterilized without modification of the organic components.

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* Similac, Ross Laboratories, Columbus, Ohio.
** Cutter Laboratories, Berkeley, California
The following is the formula for the synthetic saliva:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaHPO₄</td>
<td>1.02 g</td>
</tr>
<tr>
<td>KCNS</td>
<td>1.10 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>3.25 g</td>
</tr>
<tr>
<td>KCL</td>
<td>4.20 g</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.70 g</td>
</tr>
<tr>
<td>Na₂CO₃</td>
<td>2.80 g</td>
</tr>
<tr>
<td>Urea 8</td>
<td>1.00 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Mucin</td>
<td>17.5 g</td>
</tr>
<tr>
<td>H₂O q.s.</td>
<td>5 liters</td>
</tr>
</tbody>
</table>

Six hundred milliliters of the medium was siphoned into sterilized bottles which had been autoclaved for 30 minutes at 250°F. To prevent altering the structure of the mucin in the synthetic saliva, the bottles containing the medium were sterilized with ethylene oxide gas for eight hours. The sterilization procedure was evaluated by transferring samples of the bottled media to blood agar and thioglycollate media and incubating them for 24 hours at 37°C to determine the presence or absence of aerobic and anaerobic bacteria. Thirteen identical mouth simulating devices, each containing eight enamel surfaces, were assembled and sterilized by ethylene oxide gas for eight hours. Subsequently, the mouth simulators were placed in an incubator and the temperature was controlled thermostatically at 37°C (Figure 4).

The enamel surfaces in the mouth simulators were inoculated every 10 days with 10 ml of stimulated human saliva.

To facilitate plaque formation, a linen cloth was positioned directly over the enamel surfaces to capture and disperse the human saliva, synthetic saliva, and study solutions.
Ten milliliters of each milk solution was then poured onto the linen cloth in each of 12 mouth simulators. The solutions remained on the enamel surfaces of the study groups during a consistently assigned 2, 4, or 8 hour period. Upon completion of the appropriate time periods, the synthetic saliva was allowed to flow drop by drop over the linen cloth at a rate of 8 to 12 ml per hour. No milk was poured over the control group, so that the teeth in this group were under a continuous flow with the synthetic medium, except during the inoculation periods.

Each group was photographed after the first, third, and sixth week periods. The sixth week Kodachrome slides for the 13 groups were projected onto paper and the image of each tooth and decalcified area was traced.

The outline of each tooth was cut from the tracing and weighed to provide a numerical value for the total surface area exposed to the test solutions, human saliva and artificial saliva. The decalcified area of each tooth was also traced, cut out and weighed to provide a numerical value for the total decalcified area of that particular tooth. The area of decalcification of each tooth was compared on a percentage basis with the total exposed surface, and the oral percentage of decalcification was computed for each group in terms of means and standard errors. The mean percent of enamel decalcification for each study group was compared to that of the control group and a statistical analysis of the data was performed by a t-test.

The decalcifications produced by each individual milk solution at the three time periods were compared with one another, and a statistical analysis of the data was performed by a t-test.

The decalcifications produced by each individual milk solution at each time period were also compared with the decalcifications produced by the other three milk solutions at the same period, and a statistical analysis of the data was performed by a t-test.
At the completion of the experiment, to confirm the visual grading of this study, all teeth were rinsed thoroughly with water to remove any debris deposited by the medium or milk solutions. They were then soaked in 1% silver nitrate solution for 10 minutes, rinsed thoroughly in running water for 10 minutes to remove excess silver nitrate, and then immersed in 0.5% hydroquinone solution for 5 minutes. The teeth were next rinsed in running water for 5 minutes and fixed with 2% sodium thiosulphate for 5 minutes before a final rinse. All areas of decalcification were delineated by the black silver stain. This method of silver staining for incipient lesions and decalcification of enamel is essentially the method devised by Von Kossa in 1901.
RESULTS
The results of this study are given in Tables II through IX and Figure 5. The percentages of enamel dissolved by the four milk preparations at the end of six weeks and for the different time intervals were compared with those for the control group (Table II and Figure 5). Group 1, in which the eight enamel surfaces were allowed to stay in contact with the milk formula for two hours a day, had a mean percentage of decalcified enamel of 11.28%. This is 153% more decalcification than in the control group. This difference is not statistically significant.

The mean percentage of enamel dissolved by two hours of daily contact with bovine milk was 15.56%, or 249% more than in the control. Again, this difference is not statistically significant.

Two-hour daily contact with milk and honey caused 91.56% enamel surface decalcification, or 1957% more than in the control group. This difference is statistically significant (p<0.001).

The group with a two-hour daily exposure to human milk had 57.67% of its enamel surface decalcified, or 1600% more than the control group. This difference is statistically significant (p<0.001).

Four-hour daily exposure to milk formula produced 96.8% of decalcification, or 2075% more than the control. This is statistically significant (p<0.001).

The mean percentage of enamel dissolved by four hours daily contact with bovine milk was 54.39%. This group had 1122% more decalcification than the control, and this is statistically significant (p<0.001).

Four-hour daily exposure to milk and honey resulted in 98.16% of decalcification or 2105% more decalcification than the controls. This is statistically significant (p<0.001).
Four and eight hours of daily exposure to human milk, and eight hours of daily exposure to milk and honey produced 100% decalcification. This means 2147% greater decalcification than the control group and is statistically significant (p<0.001).

Eight-hour daily exposure to milk formula produced 98.8% of decalcified enamel surface, or 2119% more than the control group. This is statistically significant (p<0.001).

Eight-hour daily exposure to bovine milk followed a similar pattern with values of 89.78% and 1917%. The difference was significant (p<0.001). A comparison between the decalcifications produced by two-hour daily exposure to the four milk solutions (Table III) showed that the most cariogenic was the bovine milk plus honey, with 91.56% decalcification. The least cariogenic was milk formula with an 11.28% decalcified area. Bovine milk produced a 15.56% area of decalcification and human milk, a 75.67% decalcified area. The analysis of these data, using a t-test, shows no statistically significant difference between the bovine milk and the milk formula groups. When the milk and honey group was compared with the human milk group, a statistically significant difference at the 0.02 level of confidence was found. There was a statistically significant difference at the 0.001 level of confidence when the bovine milk group or milk formula group was compared with the milk plus honey group and the human milk group.

The comparison between the decalcifications produced by four-hour daily exposure to each of the four milk solutions (Table IV) showed that the most cariogenic was human milk, followed by bovine milk and honey, milk formula, and bovine milk. The statistical analysis of the data showed no statistically significant difference between the decalcification produced by milk formula, bovine milk plus honey, and human milk. Bovine milk produced significantly less decalcification than milk formula (p<0.01). Bovine milk also produced significantly (p<0.001) less decalcification than
the comparison of the decalcifications produced by the four milk solutions at an eight-hour daily contact (Table V) showed that the most deleterious were bovine milk plus honey and human milk, both producing 100% of decalcification. Milk formula produced 98% and bovine milk about 90% surface area decalcification.

The differences between bovine milk and all other groups are significant (p<0.01). No statistical significance was found in any of the other comparisons. The decalcifying effects produced by the milk formula on enamel at the three time variables were compared and a statistical analysis performed (Table VI). The longer the daily exposure, the greater the area of decalcification proved to be. The differences were significant in all but one case, that is, when the four-hour group was compared with the eight-hour group. The same procedure was performed for the bovine milk solutions and the same trend was found. Table VII shows these results. It is to be noted, however, that the decalcification was much more pronounced at the four- and eight-hour exposure in the milk formula than in the bovine milk group.

The tooth surfaces exposed to milk and honey (Table VIII) showed a very high rate of decalcification at all the exposure times. The differences between the two-hour group are significant when compared to either four-hour or eight-hour group. The other differences are not significant.

The last comparison performed (Table IX) was between the decalcifying effect of human milk on the enamel surfaces at the three time variables. Again, the longer daily exposures resulted in greater areas of decalcification. The statistical analysis of this data showed the difference to be significant (p<0.01) between the two-hour daily exposure and the four and eight hours daily exposure. On the other hand, no statistically significant difference was found between the four and eight-hour daily exposures groups. As in
the milk and honey group, there was a high rate of decalcification.

The staining with silver nitrate of all groups corroborates the data reported above (Figures 6 to 18).
Figure 1. Photograph of the mouth simulating device.
A. Feeding system. B. Flow regulating device.
C. Mouth simulator. D. Residue-collecting device.

Figure 2. Photograph of the mouth simulator.

Figure 3. Photograph of the plastic glass tray with eight teeth specimens.

Figure 4. Photograph of the 13 identical mouth simulating devices inside an incubator.
Figure 5. Graphic representation of the percentage of enamel dissolved by different milk preparations during different time intervals.
PERCENTAGE OF ENAMEL SURFACE DISSOLVED BY DIFFERENT MILK PREPARATIONS DURING DIFFERENT TIME INTERVALS

- Control
- Bovine Milk
- Milk Formula*
- Human Milk
- Bovine Milk + Honey

* Similac - Ross Laboratories, Columbus, Ohio

Figure - 5
Figure 6. Photograph of the control group after completion of the experiment and stained with silver nitrate.

Figure 7. Photograph of the two hours daily exposure to milk formula group after completion of the experiment and stained with silver nitrate.

Figure 8. Photograph of the two hours daily exposure to bovine milk group after completion of the experiment and stained with silver nitrate.

Figure 9. Photograph of the two hours daily exposure to bovine milk and honey group after completion of the experiment and stained with silver nitrate.

Figure 10. Photograph of the two hours daily exposure to human milk group after completion of the experiment and stained with silver nitrate.
Figure 11. Photograph of the four hours daily exposure to milk formula group after completion of the experiment and stained with silver nitrate.

Figure 12. Photograph of the four hours daily exposure to bovine milk group after completion of the experiment and stained with silver nitrate.

Figure 13. Photograph of the four hours daily exposure to bovine milk and honey group after completion of the experiment and stained with silver nitrate.

Figure 14. Photograph of the four hours daily exposure to human milk group after completion of the experiment and stained with silver nitrate.
Figure 15. Photograph of the eight hours daily exposure to milk formula group after completion of the experiment and stained with silver nitrate.

Figure 16. Photograph of the eight hours daily exposure to bovine milk group after completion of the experiment and stained with silver nitrate.

Figure 17. Photograph of the eight hours daily exposure to bovine milk and honey group after completion of the experiment and stained with silver nitrate.

Figure 18. Photograph of the eight hours daily exposure to human milk group after completion of the experiment and stained with silver nitrate.
Table I
Average Composition of Bovine Milk, Milk Formula** and Human Milk in Percent. 78, 79

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Bovine*</th>
<th>Formula**</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>87</td>
<td>87.2</td>
<td>87.5</td>
</tr>
<tr>
<td>Protein</td>
<td>3.5</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>Lipid</td>
<td>3.5</td>
<td>3.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.5</td>
<td>7.0</td>
<td>7.2</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>0.09</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.1</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>Ash</td>
<td>0.75</td>
<td>0.4</td>
<td>0.2</td>
</tr>
</tbody>
</table>

* 5 ml of honey were added to 45 ml of bovine milk for the milk plus honey group.

** Similac, Ross Laboratories, Columbus, Ohio.
### Table II

Percentage of Enamel Surface Dissolved by Different Milk Preparations During Different Time Intervals

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean % of Dissolved Surface</th>
<th>Standard Error</th>
<th>% Difference with the Control</th>
<th>Statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>4.45</td>
<td>2.25</td>
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<td>----</td>
</tr>
<tr>
<td>2 hr. F</td>
<td>8</td>
<td>11.28</td>
<td>4.06</td>
<td>153.52</td>
<td>1.47 N.S.</td>
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<tr>
<td>2 hr. M</td>
<td>8</td>
<td>15.56</td>
<td>7.41</td>
<td>249.51</td>
<td>1.43 N.S.</td>
</tr>
<tr>
<td>2 hr. MH</td>
<td>8</td>
<td>91.56</td>
<td>2.95</td>
<td>1957.06</td>
<td>23.49 .001</td>
</tr>
<tr>
<td>2 hr. HM</td>
<td>8</td>
<td>75.67</td>
<td>5.03</td>
<td>1599.92</td>
<td>12.92 .001</td>
</tr>
<tr>
<td>4 hr. F</td>
<td>8</td>
<td>96.8</td>
<td>2.71</td>
<td>2074.64</td>
<td>26.22 .001</td>
</tr>
<tr>
<td>4 hr. M</td>
<td>8</td>
<td>54.39</td>
<td>10.54</td>
<td>1121.85</td>
<td>4.63 .001</td>
</tr>
<tr>
<td>4 hr. MH</td>
<td>8</td>
<td>98.16</td>
<td>.9</td>
<td>2105.31</td>
<td>38.70 .001</td>
</tr>
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<td>4 hr. HM</td>
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<td>100.00</td>
<td>0.0</td>
<td>2146.56</td>
<td>42.51 .001</td>
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<tr>
<td>8 hr. F</td>
<td>8</td>
<td>98.76</td>
<td>.63</td>
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<td>40.40 .001</td>
</tr>
<tr>
<td>8 hr. M</td>
<td>8</td>
<td>89.78</td>
<td>3.46</td>
<td>1916.93</td>
<td>20.68 .001</td>
</tr>
<tr>
<td>8 hr. MH</td>
<td>8</td>
<td>100.00</td>
<td>0.00</td>
<td>2146.56</td>
<td>42.51 .001</td>
</tr>
<tr>
<td>8 hr. HM</td>
<td>8</td>
<td>100.00</td>
<td>0.00</td>
<td>2146.56</td>
<td>42.51 .001</td>
</tr>
</tbody>
</table>
Table III
Comparison Between the Area of Surface Decalcification, Expressed as Percent of the Total Exposed Enamel Surface, Produced by 2 Hour Daily Exposure to Milk Formula, Bovine Milk, Bovine Milk With Honey, and Human Milk

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percent Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>8</td>
<td>11.28</td>
<td>4.06</td>
</tr>
<tr>
<td>B. Milk</td>
<td>8</td>
<td>15.56</td>
<td>7.41</td>
</tr>
<tr>
<td>B Milk + Honey</td>
<td>8</td>
<td>91.56</td>
<td>2.95</td>
</tr>
<tr>
<td>H. Milk</td>
<td>8</td>
<td>75.67</td>
<td>5.03</td>
</tr>
</tbody>
</table>

Statistical Analysis (t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>37.86</td>
<td>.5</td>
<td>N.S.</td>
<td>711.39</td>
<td>15.98</td>
<td>.001</td>
<td>570.51</td>
<td>9.95</td>
<td>.001</td>
</tr>
<tr>
<td>M</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>488.56</td>
<td>9.53</td>
<td>.001</td>
<td>386.37</td>
<td>6.71</td>
<td>.001</td>
</tr>
<tr>
<td>MH</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>17.36</td>
<td>2.72</td>
<td>.02</td>
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Table IV
Comparison Between the Area of Surface Decalcification, Expressed as Percent of the Total Exposed Enamel Surface, Produced by 4 Hour Daily Exposure to Milk Formula, Bovine Milk, Bovine Milk with Honey, and Human Milk

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percent Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>8</td>
<td>96.8</td>
<td>2.71</td>
</tr>
<tr>
<td>B. Milk</td>
<td>8</td>
<td>54.39</td>
<td>10.54</td>
</tr>
<tr>
<td>B Milk + Honey</td>
<td>8</td>
<td>98.16</td>
<td>0.9</td>
</tr>
<tr>
<td>H. Milk</td>
<td>8</td>
<td>100.00</td>
<td>0.0</td>
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</table>

Statistical Analysis (t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Diff.</th>
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<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>F</td>
<td>-43.81</td>
<td>3.89</td>
<td>&lt;.01</td>
<td>1.41</td>
<td>0.48</td>
<td>N.S.</td>
<td>3.31</td>
<td>1.18</td>
<td>N.S.</td>
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<tr>
<td>M</td>
<td>-----</td>
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<td>---</td>
<td>80.49</td>
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<td>&lt;.001</td>
<td>83.87</td>
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<td>---</td>
<td>-----</td>
<td></td>
<td></td>
<td>1.87</td>
<td>2.04</td>
<td>N.S.</td>
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</table>
Table V
Comparison Between the Area of Surface Decalcification, Expressed as Percent of the Total Exposed Enamel Surface, Produced by 8 Hour Daily Exposure to Milk Formula, Bovine Milk, Bovine Milk with Honey, and Human Milk.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percent Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>8</td>
<td>98.76</td>
<td>.63</td>
</tr>
<tr>
<td>B. Milk</td>
<td>8</td>
<td>89.78</td>
<td>3.46</td>
</tr>
<tr>
<td>B Milk + Honey</td>
<td>8</td>
<td>100.00</td>
<td>0.0</td>
</tr>
<tr>
<td>H. Milk</td>
<td>8</td>
<td>100.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Statistical Analysis (t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td></td>
<td></td>
<td>MH</td>
<td></td>
<td></td>
<td>HM</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
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<td>2.55</td>
<td>.01</td>
<td>1.26</td>
<td>1.97</td>
<td>N.S.</td>
<td>1.26</td>
<td>1.97</td>
<td>N.S.</td>
</tr>
<tr>
<td>M</td>
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<td>---</td>
<td>11.38</td>
<td>2.95</td>
<td>.01</td>
<td>11.38</td>
<td>2.95</td>
<td>.01</td>
</tr>
<tr>
<td>MH</td>
<td>----</td>
<td>----</td>
<td>---</td>
<td>----</td>
<td>----</td>
<td>---</td>
<td>0.0</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

Statistical Analysis (t test)
Table VI

The Decalcifying Effect of Milk Formula on Enamel. Comparison Between the Percentage of the Surface Area Decalcified by 2-Hour, 4-Hour, or 8-Hour Daily Exposure to Milk Formula

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percent Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h Formula</td>
<td>8</td>
<td>11.28</td>
<td>4.06</td>
</tr>
<tr>
<td>4h Formula</td>
<td>8</td>
<td>96.8</td>
<td>2.71</td>
</tr>
<tr>
<td>8h Formula</td>
<td>8</td>
<td>98.76</td>
<td>.63</td>
</tr>
</tbody>
</table>

Statistical Analysis (t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>4h</th>
<th>8h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Diff.</td>
<td>T</td>
</tr>
<tr>
<td>2h</td>
<td>758.16</td>
<td>17.51</td>
</tr>
<tr>
<td>4h</td>
<td>------</td>
<td>-----</td>
</tr>
</tbody>
</table>
Table VII

The Decalcifying Effect of Bovine Milk on Enamel. Comparison Between the Percentage of the Surface Area Decalcified by 2-Hour, 4-Hour, or 8-Hour Daily Exposure to Bovine Milk.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percent Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h Milk</td>
<td>8</td>
<td>15.56</td>
<td>7.41</td>
</tr>
<tr>
<td>4h Milk</td>
<td>8</td>
<td>54.39</td>
<td>10.54</td>
</tr>
<tr>
<td>8h Milk</td>
<td>8</td>
<td>89.78</td>
<td>3.46</td>
</tr>
</tbody>
</table>

Statistical Analysis (t-test)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h</td>
<td>249.55</td>
<td>3.01</td>
<td>.01</td>
<td>476.99</td>
<td>9.07</td>
<td>.001</td>
</tr>
<tr>
<td>4h</td>
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<td>----</td>
<td>---</td>
<td>65.07</td>
<td>3.19</td>
<td>.01</td>
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</tbody>
</table>


Table VIII

The Decalcifying Effect of Milk and Honey on Enamel. Comparison Between the Percentage of the Surface Area Decalcified by 2-Hour, 4-Hour, or 8-Hour Daily Exposure to Milk and Honey

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percentage Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h Milk-Honey</td>
<td>8</td>
<td>91.56</td>
<td>2.95</td>
</tr>
<tr>
<td>4h Milk-Honey</td>
<td>8</td>
<td>98.16</td>
<td>.9</td>
</tr>
<tr>
<td>8h Milk-Honey</td>
<td>8</td>
<td>100.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Statistical Analysis (t test)

<table>
<thead>
<tr>
<th>Group</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h</td>
<td>7.21</td>
<td>2.14</td>
<td>.05</td>
<td>9.21</td>
<td>2.86</td>
<td>.01</td>
</tr>
<tr>
<td>4h</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>1.87</td>
<td>2.04</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Table IX
The Decalcifying Effect of Human Milk on Enamel. Comparison Between the Percentage of Surface Area Decalcification by 2-Hour, 4-Hour, or 8-Hour Daily Exposure to Human Milk

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean Percentage Decalcified Area</th>
<th>S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h Human Milk</td>
<td>8</td>
<td>75.67</td>
<td>5.03</td>
</tr>
<tr>
<td>4h Human Milk</td>
<td>8</td>
<td>100.00</td>
<td>0.0</td>
</tr>
<tr>
<td>8h Human Milk</td>
<td>8</td>
<td>100.00</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Statistical Analysis (t test)

<table>
<thead>
<tr>
<th>Groups</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
<th>% Diff.</th>
<th>T</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2h</td>
<td>32.16</td>
<td>4.38</td>
<td>.001</td>
<td>32.16</td>
<td>4.83</td>
<td>.001</td>
</tr>
<tr>
<td>4h</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>0.0</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>
DISCUSSION
In a study of this nature, care should be exercised to differentiate between nutritional and local effects of milk. It is universally recognized that milk is a highly nutritional food, which provides calcium and phosphorus in nearly ideal proportions and is thus of great value to build good bones and teeth. The situation is not so clear as far as local effects of milk are concerned, i.e., reactions produced or induced by milk on teeth with which it comes in contact during its ingestion. This thesis covers only the second type of milk effects.

As stated in the review of the literature, there are different opinions relative to the potential of milk to produce or induce caries formation: some authors claim that milk is cariogenic, others that it protects the teeth against caries.

A cursory look at the chemical composition of milk (Table I) discloses immediately the potential cariogenic component of this food: lactose. Like many other fermentable sugars, lactose is metabolized by plaque bacteria to form acids. According to some authors, it forms as much acid as the other fermentable sugars but dissolves less enamel. Others claim that there is no difference in either parameter; still others say that lactose forms less acid than other sugars. In Jenkins' experiments, 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 have little practical significance.

The results of this study suggest that lactose in milk has cariogenic potential. Table I shows that the concentration of lactose in human milk is about 1.6 times greater than in bovine milk. At the same time, much more enamel was dissolved in the human milk group than in the bovine milk group, in any of the time periods studied. Of course, the difference could also be due to another factor, such as the presence of a hitherto unknown protective principle which exists in bovine milk but may be absent
(or present to a lesser degree) in human milk. Consistent with this way of thinking, the data for the two-hour period demonstrate that milk formula, prepared from bovine milk but having a lactose concentration similar to that of human milk, produced a rather small amount of de-calcification, which was not significantly different from that produced by bovine milk and was less than that produced by human milk. This suggests that a protective effect may inhibit the effect produced by lactose in the milk formula and in the bovine milk group. At the four hour period the situation has changed and milk formula is approximately as damaging as human milk, with a percentage of decalcified surface area of nearly 100%. At this same time period, the proportion of decalcified area for bovine milk, was only 54%. This would suggest that the protective principle present in bovine milk and thus also present in milk formula has markedly decreased or even disappeared, after four hours of stagnation in the artificial mouth. At this point the fact that the formula produced much more decalcification than the bovine milk could be explained on the basis of the higher lactose content in the former (7.0%) as compared to the latter (45%).

Other differences between bovine milk, formula and human milk are the phosphorus, calcium, and ash content, higher in bovine milk than in the other two study solutions. The total amount of minerals in all three may have some bearing on their buffering capacity and will be discussed later.

The addition of fermentable carbohydrate (honey) to bovine milk increased dramatically its potential to produce decalcification, particularly at the two-hour treatment period. This is in complete agreement with the literature, and confirms the dangers of adding fermentable sugars to foods.
A second factor that has been mentioned in the literature as a cause of cariogenicity in milk is the formation of surface films, which may enhance plaque formation. The same films, according to other authors, may afford a protective effect against dental caries. The formation of protein films on hydroxyapatite surfaces is well documented. Whether these films promote plaque or protect against caries is at present a moot question. Our results would suggest that, if these films are protective, their effectiveness is markedly reduced as a function of time. Again, this may be due to bacterial metabolism, the films being hydrolyzed as their stay in the artificial mouth environment increases. It may also be added that the lesions observed in our specimens were of the so-called "white spot" type, i.e., subsurface decalcifications. This type of lesion is produced in vitro only when a polymeric coating is formed on the surface of the teeth. In other words, there is a film which protects the surface enamel from being attacked by the hydrogen ions but is permeable to these ions, so that they may proceed through permeable structures of enamel (interprismatic substance, Striae of Retzius, etc.) and produce the subsurface lesion. In this regard, the film may be protective for the surface of the enamel and thus decrease so-called enamel solubility (which is usually tested chemically, measuring the amount of surface enamel dissolved in acid buffers in a given time) but may have little, if any, protective effect on subsurface enamel.

The fact that milk plus honey, which due to the addition of sugar should generate initially many more hydrogen ions than the other forms of milk used in the study, produced a very high amount of dissolution at the two-hour period suggests that, if the milk films are truly protective, they are also easily permeated by the hydrogen ions. Furthermore, the fact that human milk was found to be markedly active in decalcifying enamel, even at the 2-hour daily exposure time, suggests that human milk either produces fewer films
than bovine milk, or films which are less protective.

Another protective factor attributed by different authors to milk is its buffering capacity. Among the potential buffers in milk, protein and phosphate are considerably more concentrated in bovine than in human milk. This may explain the differences in the respective decalcifying potential observed in this study. However, milk formula also has much less protein and phosphate than bovine milk, and yet the decalcifying potentials of these two preparations were similar at the two-hour daily exposure group. This would suggest that the protective principle apparently present in bovine milk is not related to its buffering capacity, or at least not to its phosphorus (and mineral) content.

At any rate, the data indicate that even the buffering capacity of bovine milk may be overcome as a function of time (compare two-hour with four-hour data).

Throughout this discussion, repeated mention has been made of the influence of time of exposure relative to the decalcifying potential of milk. Table II makes it clear that time increases markedly the damaging local effects of all types of milk used in this study. Another factor that must be considered jointly with exposure time is stagnation. Time plays a major role in the less actively decalcifying milk preparations, such as bovine milk and milk formula, which at the two-hour exposure period did produce decalcifications not significantly different from those in the control group. From a clinical viewpoint, this suggests that the local deleterious effects of these preparations at rather short exposure times are minimal. As time increases, in the stagnant condition in which the system is kept, the intensity of decalcification increases markedly.

Lindquist produced evidence that carbohydrates which are slowly cleared from the oral cavity favor caries initiation. The longer the combination of fermentable carbohydrates and acid-producing microorganisms were allowed to remain in the oral cavity, the greater was the caries activity.
Schneyer and Levin have demonstrated that the rate of salivary flow and secretion is important in caries etiology. The less the flow of saliva, the greater was the attack of caries.

In the clinical condition known as "nursing bottle caries", all of the maxillary primary anterior teeth, the maxillary and mandibular primary first molars, and the mandibular primary cuspid teeth develop decay ranging from severe in the maxillary anterior teeth to milk in the mandibular cuspid teeth. Contrary to what would be expected in a case of rampant caries, the mandibular primary incisor teeth are either unaffected or are very slightly carious. The older the child, the more severe the lesions seem to be. The maxillary primary incisors are most extensively involved, with deep carious lesions apparent on the labial and lingual surfaces of the teeth. The mesial and distal surfaces may or may not be carious, but if they are, the carious process is continuous with the carious labial and lingual surfaces. If the outer layer of decayed tissue is removed with an excavator, softened tooth structure is revealed and leaves very little of the tooth crown may remain. The first primary molars are the next most seriously affected teeth, revealing deep occlusal caries, less deep buccal surface damage, and milder damage on the lingual surface. The primary cuspid teeth seem least affected, with lesions found on the labial and lingual surfaces. The second primary molars, if present, are relatively unaffected but, if caries is present, the occlusal surfaces may be seriously involved.

A typical case history of nursing bottle caries may reveal that neither parent has ever presented a high caries rate, that no prenatal disturbances were present, that the child is not or was not a fussy eater, although he may be a reluctant eater now, and that the child always drank plenty of milk. The older the child, the longer the presence of the nursing bottle habit, and the earlier the eruption of the primary dentition, the greater the possibility of
damage to the teeth. Further investigation into the methods of feeding
the child will reveal a factor which is common to all of the affected
children. All children are placed in bed, either for a nap or for the
night, with a "nursing bottle" of milk from which they drink, while
lying down, to help them to fall asleep. Thus, at this point we find
in the mouth the following ideal conditions for caries to occur: (1) milk
with a minimum sugar content of 4.5%; (2) oral microorganisms capable
of producing acids; (3) very slow clearance of the oral cavity contents;
and (4) decreased salivary secretion and decreased salivary flow.

These conditions can readily be seen in an analysis of the process
involved in the child's use of the nursing bottle. The child lies down,
holding the nursing bottle in the mouth. The nipple rests against the
palate while the tongue, in combination with the cheeks, forces the con­
tents of the nipple into the oral cavity. In the course of this action the
tongue extends almost out of the mouth, in contact with the lips, at the
same time covering the mandibular primary central and lateral incisors.
At first the force of sucking on the nipple is great, the salivary secretion
and flow are increased, and swallowing goes on, but as the child grows
drowsier and finally falls asleep the rate of swallowing decreases, the
salivary secretion and flow diminish, and the milk in the mouth bathes
the oral environment in a stagnant puddle. The tongue remains in contact
with the lips, extended, and covering the lower anterior teeth, thus pre­
venting the milk from puddling around these teeth. Although milk usually
has a low carbohydrate content, the greatly reduced rate of swallowing during
sleep, plus the diminished flow of saliva, allows the carbohydrate to remain
in contact with the teeth in the presence of acid-forming microorganisms for
a greatly increased period of time (stagnation). There is diminished dilution
and buffering action from the saliva, and little or no clearance of the fluid
from the oral cavity. In many instances, the nursing bottle remains in the
mouth most of the time that the child is asleep, and milk from the bottle
continues to ooze into the mouth.
The test system used in the study provided an environment with the following features: (a) temperature and humidity comparable to those existing in the mouth; (b) contamination by oral bacteria; (c) exposure of the teeth to milk under typical conditions of stagnation for rather long periods; and, (d) a situation in which the flow of "saliva" was interrupted during the periods of milk contact, and reinitiated after these were finished. In other words, the environment closely resembled the mouths of a "nursing bottle" child described above.

The fact that lesions observed in the study were subsuperficial supports the view that the system indeed resembled the real mouth. Under these conditions, the results clearly show that milk alone, with no added carbohydrate, has the potential to decalcify human teeth.

The addition of honey markedly enhances that potential. In regard to ranking the four study solutions used in the study, bovine milk is the least damaging, followed by milk formula, human milk, and milk and honey.

Some of the possible reasons for these differences have already been discussed; there may be several others which are as yet unknown. The very high decalcification potential of human milk came as a surprise, and deserves further study.

Two points warrant further discussion: the effect of time and the addition of honey. Only milk and honey was extremely decalcifying at the two-hour exposure time. The amount of decalcification for the bovine milk and milk formula at that interval was not significantly different than for the controls. Human milk was only moderately active at this time exposure. Thus, from a clinical viewpoint, it seems that infant feeding should be restricted to the time needed for nutrition, and that in no case should fermentable carbohydrates be added to the milk preparation.
The results of this study seriously incriminate the habit of providing the children with nursing bottles of milk, even without added carbohydrates, to induce their sleep. On the other hand, there is no indication from this study that milk, without the addition of fermentable carbohydrate, has any significant cariogenic potential if maintained in contact with the teeth for a short time.

The in vitro system used for this study deserves some comments and perhaps some criticism as well.

In general terms, the mouth simulator performed as expected, and the fact that subsurface decalcifications were produced is an indication of its resemblance to the real oral situation. The system proved to be uncomplicated to operate, maintain and standardize. Contrary to what the literature review, it was possible to obtain complete sterilization of artificial saliva by ethylene oxide gas.

The use of rate flow valves similar to those used for intervenous feeding permitted a rather easy control of the solution flow. The following are potential deficiencies which should be considered in future studies. First, the inoculation of the tooth surfaces in the mouth simulator should probably be done more often, perhaps every day or two, so that these flora will keep their resemblance to the child's oral flora, particularly several hours after the milk has been administered.

In this study it is possible that the bacterial flora, upon successive re-introductions of milk (between periods of incubation) was different from the flora initially inoculated.

In the second place, more frequent changes of the "linen plaque" could also have given the in vitro system a closer resemblance to the oral cavity of infants.

* Cutter Laboratories, Berkeley, California.
Third, it might be convenient to add a second feeding system, so that saliva could be added, at an extremely reduced rate, during the time that the milk is in the mouth. Also it might be well to design a somewhat more sophisticated device which could allow the introduction of other foods intercalated with both saliva and milk, so as not to favor excessively the selection of a given type of bacteria (lactic acid ones) and at the same time to make the in vitro conditions conform more closely to the in vivo situation.
SUMMARY AND CONCLUSIONS
Reports differ concerning the cariogenic potential of milk. Some authors indicate that plain milk can cause dental caries, while others believe that the disease results from adding carbohydrates to the milk. Still others suggest that milk has a protective effect and may contribute to caries prevention.

The present study investigated the capability of human milk, plain bovine milk, a milk formula, and milk with honey to produce caries-like lesions in an environment which simulated the oral cavity. The mouth-like conditions were established by constructing a mouth-simulating device based on Pigman's artificial mouth (Figure 1). The apparatus consists of a feeding system, a mouth simulator, and a residue collecting device. The feeding system is a standard 1000 ml bottle, commonly used in intravenous solutions, designed in the same way as for intravenous injection with the drop counter regulating the media flow that will bathe the surfaces of the teeth inside the "mouth simulator," a plastic cylinder containing the tooth specimens.

Four groups of eight sound bicuspids each, which had been extracted for orthodontic reasons were mounted in a mouth simulator. The teeth and complete apparatus were sterilized with ethylene oxide. The teeth were then inoculated with human saliva and covered with linen cloth to facilitate bacterial colonization. Each day the various groups of study teeth were exposed to one of the four milk solutions during a 2, 4, or 8 hour period. After each period, a sterile chemical solution simulating human saliva was dropped (8-12 ml/hr) over the cloth to provide a mouth-like environment. A control group was not exposed to a milk preparation.

After six weeks, all milk solution groups showed unequivocal signs of decalcification, with an intensity proportional to the period of exposure to the study solutions. Plain bovine milk produced the least decalcification, followed in order by milk formula, human milk, and milk and honey.
On the basis of the available results, it is possible to speculate concerning the causes of the carious effect of milk and the differences obtained with various milk preparations.

Concerning the first point, the results strongly suggest that lactose plays a role in the decalcifying potential of milk. This is borne out by the differences found between human and bovine milk effects. In addition, it is speculated that milk, particularly bovine milk, contains a "protective" ingredient that reduces its cariogenic potential. The investigation of such a protective ingredient is beyond the scope of this thesis.

Two factors played an important role in determining the decalcifying effects of milk: time of contact and stagnation. The results support the contention that milk, particularly bovine milk, is probably not conducive to caries if its contact with the teeth is kept within reasonable limits. On the other hand, the results clearly demonstrate that milk itself, without extra carbohydrates, can produce dental caries if left stagnant long enough over the tooth surfaces. If fermentable carbohydrate is added, the deleterious effects are notoriously increased, even in a rather short exposure time. The conditions of time and stagnation found to be necessary for milk to produce marked enamel decalcification are similar to those found in the "nursing bottle caries" patients. It can be concluded that the habit of prolonged use of the bottle, even with milk alone, should be systematically discouraged. This conclusion is of marked importance for both pedodontists and pediatricians.
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The Cariogenic Potential of Milk
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VITAE
Vitae

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