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INHIBITION OF DENTAL CARRIES IN FEMALE OSBORNE MENDEL
RATS BY A FOOD GRADE LIPID, LAURICIDIN^R

By

Patricia Lynch

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ABSTRACT

INHIBITION OF DENTAL CARIES IN FEMALE OSBORNE MENDEL RATS BY A FOOD GRADE LIPID, LAURICIDIN^R

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The present study was undertaken to determine the cariostatic potential of Lauricidin^R, a generally recognized as safe (GRAS) antimicrobial lipid.

Eighty four female Osborne-Mendel rats were weaned at 18 days, inoculated with Streptococcus mutans, and fed a cariogenic diet to which 0%, 0.5%, 1% and 2% Lauricidin^R had been added. After six weeks the rats were sacrificed, the jaws were cleaned, and the molar teeth were scored for dental caries by the method of Keyes (J. Dent. Res. 37: 1088, 1958).

Caries scores for rats consuming diets containing 0.5% Lauricidin^R were significantly lower than caries scores for rats consuming diets containing no Lauricidin^R. Caries scores were 35% lower on the sulcal surfaces, 56% lower on the proximal surfaces and 90% lower on the buccal/lingual surfaces of the teeth. Rats consuming diets containing 1% and 2% Lauricidin^R had caries scores on all surfaces which were not significantly different from scores for rats consuming diets containing 0.5% Lauricidin^R.

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INTRODUCTION

Dental caries has been called the most prevalent public health problem in the United States today (Nizel, 1977). Unlike other infectious diseases in which the incidence has decreased over the years, the incidence of dental caries has increased on a national scale (Fitzgerald, 1973). Except in sections of the population that have been exposed to fluoridation of community water supplies, dental caries is a greater problem today than 50 years ago (Prevention and Oral Health, 1973). A "massive attack" against this disease has been urged (Harris, 1970).

Epidemiological studies reveal that almost all developed countries currently have high rates of dental caries. Like those decreases seen in the United States, decreases in other developed countries are seen only where water fluoridation has been applied (Barmes, 1977). Although many developing countries have previously demonstrated low caries rates, the problem of tooth decay is increasing dramatically in such countries (Barmes, 1977). People in urban areas are particularly affected. As these populations take on the ways of modern civilization they also develop higher levels of dental caries (Greene and Suomi,

1977).

Dental caries is not a life threatening disease but it can result in pain, infection, facial disfigurement, chewing impairment, speech impairment, and malnutrition (Nizel, 1977). In 1960-1962, two billion dollars was spent annually to repair damage due to dental caries (Prevention and Oral Health, 1973). More recent figures are not available but obviously have increased due to inflation and increasing population. It has been said that the financial resources and manpower necessary to repair the damage due to dental caries do not presently exist in the United States and are far less available in developing countries (Navia, 1979). The increasing life span of people and the increasing emphasis being placed on the quality of life adds importance to the control of dental caries (Greene and Suomi, 1977).

It has been mentioned that fluoridation has been effective in reducing caries in some areas. The potential effects of water fluoridation are great, however, fluoridation of all community water supplies is not politically possible in this country (Nizel, 1977) and not practical in developing countries (Greene and Suomi, 1977). Even when water fluoridation is present, ingestion of water varies among individuals and among geographic regions (Harris, 1970).

The continuing consumption of sweet, caries-promoting foods despite attempts by the dental profession to motivate

people to change their dietary habits (Morse, 1976) has led to the conclusion that cariostatic food additives are desirable (Harris, 1970). The increasing pattern of frequent snacking in the United States has led to a special need for modification of the caries promoting properties of foods consumed as snacks (Navia, 1979). The addition of cariostatic substances to sugar or flour imported by developing countries has been recommended as a means of preventing decay in such areas (Harris, 1970).

In contrast, other researchers have suggested that the only effective control of dental caries must involve a means of controlling the microbial vectors (Scherp, 1971). Antimicrobial agents could be aimed at eliminating the cause of the disease (Fitzgerald, 1973). An antimicrobial agent which could be used in foods could combine this approach with the suggestions made regarding the development of cariostatic food additives.

The monoglyceride of lauric acid is an antimicrobial lipid and is generally recognized as safe (GRAS) substance which has been shown to inhibit dental caries in rats fed diets containing 18% skimmed milk and 67% powdered sucrose among other ingredients (Schemmel et al., 1979). The present studies were conducted to extend the conditions under which this substance could inhibit dental caries.

Study I was conducted to determine whether Lauricidin^R, a 90% pure preparation of the monoglyceride of lauric acid,

would inhibit dental caries in rats when included at the 2% level in a diet containing 57% sucrose, 28% skimmed milk, and other ingredients identical to those used in previous studies.

Study II was conducted to compare the effect of a 0.5% and a 1% dosage of Lauricidin ^R with the 2% dosage.

REVIEW OF LITERATURE

The Etiology of Dental Caries

Dental caries has been defined as the localized destruction of tooth tissue (Gibbons, 1975). The etiology of dental caries is not fully understood. However, certain contributing factors are considered essential to the initiation of decay. Fermentable carbohydrates must be in contact with the tooth surface, and specific bacteria must be present in the oral cavity (Nizel, 1977). A third factor, host susceptibility, is equally necessary but much less understood than the other two factors. Variation in the incidence and extent of dental caries among individuals having the same oral bacteria and the same exposure to carbohydrates has been attributed to many possible host factors such as tooth structure, tooth composition, saliva composition and saliva quantity (Shafer et al., 1974).

Dietary Carbohydrates

Carbohydrates must be present in the diet before dental caries will occur (Shaw, 1954). This was proven by experiments in which the carbohydrate portion of a diet was administered to rats by stomach tube (Kite et al., 1950).

Animals given carbohydrates in this manner did not develop caries. Rats allowed to eat the same diet in the normal way developed a considerable number of carious lesions.

There has been much debate and research concerning which type or types of dietary carbohydrates are necessary for the initiation and continuation of dental caries. Although extensive dental caries have been observed in rats fed cornstarch as the only carbohydrate (Shaw, 1972) starch is generally considered the least cariogenic carbohydrate (Nizel, 1969). Sucrose is considered the most cariogenic (Winter, 1968; Newbrun, 1977). Variable results have been obtained with disaccharides other than sucrose, and with monosaccharides. Dental caries have been produced in rats fed glucose, maltose, lactose, or fructose (Green and Hartles, 1969). Other researchers have shown significant reductions in caries when rats were fed diets containing glucose, fructose, or isomerase (Thompson et al., 1976) or maltose (Guggenheim et al., 1966) or lactose (Shaw, 1976) in comparison with controls fed diets containing sucrose. Variability in the results of these animal studies could be due to differences in animal strains, other dietary ingredients, type of microflora, or specific tooth surfaces examined for caries (Makinen, 1972).

Although sucrose is regarded as the most cariogenic sugar the cariogenicity of a diet is not only related to the quantity of sucrose it contains. The frequency of

sucrose consumption and the form in which it is consumed have been proven to be more important than the actual quantity of sucrose consumed. Larson et al. (1962) observed that rats who ate more frequently had a higher caries incidence than rats who ate less frequently. Konig and Muhlemann (1967) observed that rats fed a diet containing powdered sugar developed more caries than rats fed a diet containing granulated sugar. Gustafsson et al. (1954) demonstrated that both of these factors were also important in humans. In this study of institutionalized adults the consumption of sticky sugars resulted in higher caries than the consumption of non-sticky sugars. The consumption of sucrose containing foods between meals resulted in higher caries than consumption of the same amount of sucrose containing foods with meals.

Bacterial Factors

Bacteria are essential for the initiation and continuation of dental caries. Investigators have shown that germ free rats fed highly cariogenic diets do not develop caries (Orland et al., 1954). Several species of bacteria have been found capable of causing dental caries when orally inoculated into gnotobiotic rats (Newbrun, 1977). Transmissibility of cariogenic bacteria from caries active to caries inactive hamsters has been demonstrated (Keyes, 1960) and fulfills one of the requirements that must be met

before a disease can be labeled a bacterial infection (Newbrun, 1977).

The oral cavity of man and various other animals contains numerous bacteria which may be causative agents of dental caries when fermentable carbohydrates and susceptible tooth surfaces are present. At this time all known cariogenic strains of bacteria are gram positive and with the exception of a few filamentous organisms all are either streptococci or lactobacilli (Fitzgerald, 1972). Cariogenic species that have been used to produce pit and fissure caries in gnotobiotic rats include the following: Streptococcus mutans, Streptococcus salivarius, Streptococcus sanguis, Lactobacillus acidophilus, Lactobacillus casei, Actinomyces viscosus, Actinomyces naeslundii, and Actinomyces israelii (Newbrun, 1977). All of these organisms with the exception of the Lactobacilli and A. israelii have also been shown to produce root caries. Streptococcus mutans is one of the only species capable of causing decay on smooth enamel surfaces in test animals.

Most of the same organisms used to produce dental caries in gnotobiotic rats have been found on human tooth surfaces. Lactobacilli are found both in human saliva and on human tooth surfaces. Since they constitute only a small fraction of plaque flora and since their numbers tend to increase only after decay has already begun they are thought to be secondary invaders which contribute to the

progression of decay (Gibbons, 1975). Streptococcus mutans has been isolated from almost all human enamel carious lesions examined (Littleton et al., 1970) and has been reported in a significant proportion of cemental lesions also (Sumney and Jordan, 1974). S. sanguis and S. mitis have been found on the teeth of humans, however, their proportions have been found to correlate inversely with caries activity (de Stoppelaar et al., 1969). A. viscosus and A. naeslundii have been found in human plaque and dental calculus in significant numbers (Collins et al., 1973) and have been isolated from advanced human dentinal lesions (Loesche and Syed, 1973).

Dental Plaque

Dental plaque is a soft bacterial deposit which forms on the teeth (Löe, 1969). Plaque is 70% microorganisms and 30% other material, mostly polysaccharide (Schroeder and De Boever, 1970). Plaque can be removed but begins to reform immediately after cleaning. Initially it is a thin layer near the gingiva on the smooth surfaces of the teeth. In the absence of oral hygiene it gradually spreads over all surfaces of the teeth (Prevention and Oral Health, 1973). Various organisms have been isolated from dental plaque at different stages of development. Regardless of the age of the plaque streptococci are the predominant organisms (Newbrun, 1977). Specific polysaccharides, both

glucans and levans, have been isolated from dental plaque (Guggenheim, 1970). The glucans are partially insoluble and may serve as the structural component of the plaque matrix. The levans and some of the glucans are soluble and may function as energy reserves for the bacteria (Newbrun, 1977). Enzymes responsible for the synthesis of both glucans and levans have been isolated and purified from S. sanguis and S. mutans (Carlsson, 1970; Carlsson et al., 1969). These enzymes are highly specific for sucrose and will not utilize fructose, glucose, maltose or lactose (Newbrun, 1977). The enzymes are able to conserve the high energy link between glucose and fructose that is found in sucrose and use this high energy to transfer glucose or fructose units directly to the growing polysaccharides. The high energy of hydrolysis of sucrose makes it much more chemically reactive than other disaccharides (Nizel, 1969). The polysaccharides in plaque enable the bacteria to stick to the teeth and therefore remain in contact with the tooth enamel. Fermentation of dietary carbohydrates while in this location results in the release of acid close to the tooth surface and the subsequent initiation of a carious lesion (Nizel, 1977). Mutants of S. mutans that cannot form polysaccharides do not cause dental caries (Tanzer et al., 1974).

Production of Acid

A drop in plaque pH from 6.5 to 5 following a glucose or sucrose rinse was first demonstrated with the use of fine antimony electrodes embedded in plaque (Stephan, 1940). Modern methods have confirmed the formation of acid by microorganisms in plaque. A variety of acids have been found in plaque and in carious lesions (Bibby, 1965). Lactic acid is the strongest of the acids produced and is therefore the one most implicated in enamel demineralization. If the pH drops below 5.2 in the environment of the tooth surface then the acid reacts with the hydroxyapatite crystals of the tooth enamel to form tricalcium phosphate and dicalcium phosphate. These phosphate compounds are more soluble than hydroxyapatite. When sufficient dissolution of the enamel has occurred a carious lesion is initiated (Nizel, 1972).

Host Factors

The occurrence of dental caries in some individuals who consume very little sugar and the lack of caries in some individuals who consume large amounts of sugar is a situation which proves the importance of tooth susceptibility in the cariogenic process (Koulourides et al., 1976). Navia (1979) has summarized the various host factors which may be involved. They include: the integrity and perfection of the enamel; the morphology of the

teeth; the size ratio of teeth to mandible; and the chemical and immunological composition of saliva and gingival fluids. Host factors may be genetically inherited or they may be nutritionally acquired (Shaw, 1959).

The role of saliva in prevention of dental caries has been verified by experiments on hamsters from which the salivary glands have been surgically removed (Finn et al., 1955). The desalivated animals developed more than 5 times the number of caries as the control group of animals that were not desalivated but were fed the same diet. Increased caries associated with decreased salivary flow due to various causes have been reported in humans (Stephen, 1971; Kermiol, 1975). No definite relationship has been found to exist between caries prevalence and salivary amylase, urea, ammonia, calcium or pH (Newbrun, 1977). Although saliva is known to contain various antibacterial factors, salivary content of lysozymes, lactoperoxidases, or immunoglobulins has not been correlated with dental caries (Newbrun, 1977).

The chemical composition of the tooth enamel has also been heavily implicated in dental caries susceptibility. In several studies no differences were found in the calcium, phosphorus, magnesium and carbonate contents of enamel from sound and carious teeth. These same studies reported significant differences in the fluoride content of sound and carious teeth (Shafer et al., 1974). The mechanism by which fluoride confers caries resistance to the enamel is not yet fully understood (Jenkins, 1967).

Control of Dental Caries

Present Methods

Knowledge of the most important elements of the dental caries disease process has led to the promotion of various solutions. The restriction of dietary sucrose both in frequency and quantity has long been recommended as a means of preventing dental caries (Morse, 1976). Oral hygiene procedures to remove bacterial accumulations from teeth have been strongly promoted. Fluoridation of community water supplies to decrease host susceptibility has been advised. Many experts believe that a conscientious application of these three solutions could solve the problem of dental caries (Sherp, 1971; Keyes, 1969; Prevention and Oral Health, 1973). Unfortunately, universal application of these solutions has not been possible.

Total elimination of sucrose from the diet is considered impossible (Finn and Glass, 1975; Winter, 1968). Sucrose is the least expensive source of calories since it requires the least amount of land per million calories (Stare, 1975). The economic status of a family may limit its ability to restrict sucrose intake (Shaw, 1959). Many population groups now require the calories from sugar as a staple part of their diet and will probably continue to do so (Winter, 1968). Sucrose is often consumed in combination with foods which supply other necessary nutrients

(Stare, 1975).

Even when economic or nutritional factors are not applicable, motivation to change dietary behavior is not easily achieved (Nizel, 1969). People like sweet foods and sweet foods are often the most conveniently available and well advertized foods (Prevention and Oral Health, 1973). Social pressures and individual appetites make the limitation of frequency of intake especially difficult for children (Bibby, 1965).

A similar situation has been encountered in the achievement of effective oral hygiene practices. It is difficult to achieve the proper motivation and knowledge required to mechanically control bacterial deposits on the teeth (Gibbons, 1975). The average time spent in brushing is one minute according to one study; this is not long enough to remove plaque from the tooth surfaces (Cohen et al., 1967). Evidence of one researcher indicates that the technical skill and perseverance required to continually maintain oral cleanliness exceeds the ability of the average human being (Löe, 1970).

The third aspect of the present solution to dental caries is the administration of fluoride to developing teeth. The most efficient way to achieve this is via water fluoridation. Emotional arguments and misunderstandings have limited the adoption of water fluoridation (Gibbons, 1975). As of December 31, 1969 only 43% of the U.S.

population had either natural or artificially fluoridated water (Nizel, 1972). Practical considerations prevent the adoption of water fluoridation in many developing countries (Greene and Suomi, 1977).

Although the combination of these three solutions is potentially very effective it has not been effective at the public health level. While these solutions are not being abandoned, the search for additional means of control continues.

Proposed Solutions

One area of research proposed for the solution of dental caries focuses on the cariogenic properties of foods. The study of possible modifications in texture and composition of foods to reduce their cariogenicity has been encouraged (Navia, 1979). It has been suggested that foods which are the most appetizing and also the most caries productive should be processed to make them less cariogenic (Bibby, 1965). The need for a chemical food additive that could achieve this goal while retaining the palatability and nutritious content of such foods has been expressed (Winter, 1968; Finn and Glass, 1975; Nizel, 1969; Navia, 1969). Suggested roles for these food additives include reduction in the amount of sugar available to bacterial breakdown, protection of the tooth surface, and modification of the tooth surface to increase its resistance to caries.

Since dental caries is the result of a bacterial infection it has been suggested that the most effective way to control the disease is by controlling the microbial agents involved (Fitzgerald, 1972). The National Caries Program of the National Institute for Dental Research has committed 50% of its total resources to research in combatting the microbial agent in dental caries (O'Brien, 1976). The search for antibacterial chemicals which could be effective against causative bacteria has been encouraged (Prevention and Oral Health, 1973). Many substances have been tested, however, the selection of such a chemical agent is hindered by the long list of requirements that it must fulfill. The ideal anticaries agent should possess the following characteristics: a narrow antimicrobial spectrum, preferably only cariogenic organisms; a high therapeutic index and low order of toxicity; a low potential for the induction of resistant microorganisms; a low potential for allergenicity; high stability under storage conditions; long lasting efficacy with relatively few applications; lack of general use for other therapeutic applications; lack of unpleasant organoleptic properties; and low susceptibility to inactivation by substances within the oral cavity (Fitzgerald, 1972).

A consideration of these requirements in the light of the suggestions concerning the alteration of food cariogenicity leads to the conclusion that the absolutely ideal

anticaries agent would be a substance which could reduce the cariogenicity of foods via antimicrobial effects. The possibility that such a substance exists is suggested by research concerning the role of fats in dental caries.

The Role of Fat in Dental Caries

Human Studies

Several early investigators observed an association between high levels of dietary fat in certain cultures and low incidence of dental caries in those cultures (Volker, 1956). Although these observations are correct the multitude of differences between the observed diets and more typical caries promoting diets prevent any conclusions regarding the isolated and specific influence of fat.

Observations of the low dental caries incidence in diabetic children who were consuming a 7:9:21 caloric ratio of protein:carbohydrate:fat stimulated Boyd et al. (1929) to study the possible influence of high dietary fat on dental caries in such children. They imposed a similar ratio of protein, carbohydrate, and fat on non-diabetic children hospitalized in an orthopedic ward. A total arrest of caries was observed. Caries incidence increased when the children returned to the ward diet. As part of the same study another group of normal children in their own homes were fed controlled diets high in vitamins and minerals and containing a ratio of protein, carbohydrate and fat that

was more balanced than the 7:9:21 ratio consumed by the diabetic children. Caries were also arrested in these children. Since an arrest in caries could be achieved by a more typical diet, strictly supervised, Boyd concluded that the arrest must have been due to metabolic influences of a diet high in required nutrients. Since the diabetic children were strictly supervised they were more certain to eat foods containing all needed nutrients than normal children who were more often allowed to choose foods according to personal preference. The results of this study do not negate the influence of fat. They do point out the complexities of the human diet and eating habits which make it hard to isolate the possible inhibition of caries by fat.

Boyd (1944) continued the investigation of the influence of fat on dental caries incidence in diabetic children whose diet was modified from 70% of the calories from fat to 35% of the calories from fat. Children were studied for at least three years. The low incidence of caries was not changed when the fat level was cut in half even though carbohydrates were doubled at the same time. Boyd interpreted these results as proof that fat did not have a protective effect. However, the study did not consider the fact that there may be a level of fat which gives maximum protection, or conversely a level of dietary sucrose which does not increase caries incidence.

Several studies conducted to determine the influence of specific supplements on dental caries produced results which pointed to an inhibitory action due to the fat contained in the supplement. The Committee for the Investigation of Dental Disease of the National Research Council of Great Britain administered supplements to children in three different institutions (Rosebury and Karshan, 1939). Cod liver oil, olive oil, and treacle (a type of molasses) were administered. Cod liver oil was found to be the best protective agent. Olive oil was found to be more protective than treacle. The sugar content of the molasses prevented it from being a proper control for the olive oil. The differences in caries could have been due to higher sugar intake in the treacle group rather than higher fat intake in the olive oil group.

McBeath and Verlin (1942) studied the anticaries action of vitamin D and discovered that the vitamin D contained in cod-liver oil had a greater anticaries effect than similar or even larger quantities of vitamin D administered as irradiated ergosterol. Although these authors did not conclude that the differences were due to differences in fat content of the administration vehicle another author has suggested that the local action of the fat in the cod liver oil preparation may have contributed to the lower caries in individuals who received the vitamin D in this form (Volker, 1956).

There have been no controlled human dietary studies relating the fat content of a diet to dental caries incidence. Human subjects do not readily volunteer to consume a strictly controlled diet for the length of time necessary in the study of dental caries incidence (Newbrun, 1977). Measurements of related parameters other than actual caries have been used to infer some information about the role of dietary fat in dental caries. The amount of acid formed due to the presence of food in the mouth and the amount of a food adhering to the teeth after eating were selected as factors which might determine the caries promoting ability of a food. Research was conducted to determine the characteristics of a food which could influence the retention of the food in the mouth and the production of acid which resulted from the presence of the food. A human study conducted by Bibby et al. (1951) showed no consistent differences in acid production due to the fat content of the foods tested; however, the retention of a food in the mouth was significantly reduced as the fat content of the food was raised. A further study of carbohydrate clearance from saliva after consumption of bread with and without butter indicated faster, but not significantly faster, oral clearance when fat was consumed with the bread (Lanke, 1965).

Data relating dietary fat consumption to dental caries incidence in humans is not extensive and not conclusive.

Research with laboratory animals has produced much more convincing evidence that caries are reduced by dietary fat.

Animal Studies

The earliest animal studies relating decreases in dental caries to increases in dietary fat were not conducted for that purpose. They were conducted to determine the effect of calcium, phosphorus and vitamin D on dental caries incidence in rats (Rosebury et al., 1935). A basic diet containing brown rice and white potato dextrin was supplemented by weight with 2 and 5% cod liver oil; 2 and 5% corn oil containing a dilution of viosterol adjusted to have the vitamin D potency of cod liver oil; and 2 and 5% unsupplemented corn oil. The oils were added to the dry diets of rats twice a week. Dental caries incidence in the fissures of the rat teeth was found to be significantly reduced by all three supplements. It appeared that the oil itself was exerting an anticariogenic effect. The authors made the suggestion that the fat might have exerted a protective effect in the environment of the teeth rather than contributing to the mineralization process. This possibility could not be proven by their data but it could not be excluded either.

The same authors conducted a second series of experiments to separate the anticaries action of vitamin D from that of corn oil and to define more precisely the anticaries action of fats (Rosebury and Karshan, 1939). In the first

experiment vitamin D was administered to rats as viosterol in a minimal amount of corn oil equal to .15% of the diet. One group of rats received the supplement by mouth. Another group of rats received a comparable amount of the supplement mixed with the diet. In the second experiment, 8 groups of rats were fed either 0%, 0.5%, 2% or 5% corn oil by weight in either a rice or a cornmeal diet. Five groups of rats were fed fats other than corn oil at the 5% level by weight in a rice based diet. The rice diet contained brown rice which had been ground to pass a sieve having 10 meshes per linear inch. The corn meal diet contained coarse yellow corn meal which passed a 20 mesh sieve but was retained on a 30 mesh sieve.

The results of the first experiment indicated that vitamin D had an additional caries inhibiting effect that was not dependent on the corn oil in which it was contained. The results of the second experiment demonstrated that increasing levels of corn oil caused decreasing levels of dental caries. In the rice diet the reduction was seen at both the 2% and 5% levels, however, the 5% corn oil in the cornmeal diet was not found to be more effective than the 2% level. Caries were inhibited more in the rice diet than in the cornmeal diet at all levels of corn oil additions. The amount of total fat was higher in the cornmeal diet due to a higher level of fat in corn than in rice, therefore, the reduction in dental caries due to fat was thought to be due to the free fat in the mouth rather than the physically

bound fat. Olive oil, Wesson oil, Crisco^R and lard were found to inhibit caries as well as corn oil did. Paraffin oil did not cause significant inhibition. Diets used in this study consisted of coarsely ground particles. The authors did not consider the fact that the cornmeal diet was less coarse than the rice diet. The greater inhibition by fat in the rice diet may have therefore been related to particle size. The authors suggested that the oil-fat effect was a local effect exerted in the mouth. They proposed that the fat may have lubricated the teeth or may have produced a film on either the food particles or the tooth surface which may have prevented penetration of enzymes into the food particles or the penetration of acid to the tooth surface.

Several early studies demonstrated the caries inhibiting properties of lard but provided limited information on the possible mechanisms whereby fat could inhibit dental caries. Schweigert et al. (1946) added 10% lard at the expense of 2.25 grams of carbohydrate per gram of lard to the diets of cotton rats which were being fed various carbohydrates. Caries were reduced significantly in all groups; however, the effect of the reduction of total carbohydrate in the diet could not be distinguished from the effect of the addition of fat. Shaw (1950) confirmed the reduction of dental caries with increasing percentages of lard in the diets of albino rats. As in the above study, the caries inhibition

by fat in this study was also inseparable from the inhibition effect of a reduced level of carbohydrate in the diet.

The proposed ability of fat to protect the teeth of cotton rats from the effects of a cariogenic diet was challenged by Constant et al. (1954). In this study the cariogenic effects of a basal diet of 50% oatmeal, 32% dried whole milk, and 18% sucrose were compared with the cariogenic effects of a diet containing 50% oatmeal, 8.5% vitaminized casein, 18% sucrose, 14.5% dextrin and 9% corn oil. All ingredients were expressed as percent by weight. Caries incidence and extent were found to be higher in the diet containing 9% fat. The authors failed to acknowledge the additional cariogenic challenge of 26% lower protein and 23.5% higher carbohydrate that was present in the second diet. Since the diet containing fat was not compared with a suitable control the results of this study did not contradict the possibility of caries inhibition due to fat.

Another series of experiments by the same authors (Constant et al., 1954) compared the effects of natural fat versus added fat in the diets of cotton rats. In the first experiment oatmeal was subjected to ether extraction. Three and a half percent of the oatmeal was extracted. The remaining, de-fatted oatmeal was fed to cotton rats in a diet containing 3.5% corn oil and other ingredients. Dental caries incidence was found to be slightly reduced in the rats fed the diet with added fat when compared to

dental caries incidence in rats fed a diet without added fat containing a comparable amount of non-extracted oatmeal.

In a second experiment 50% dried whole milk was replaced with dried skim milk and either 7 or 14% by weight corn oil or 14% by weight butterfat. A reduction of caries was observed in the rats fed the diets containing skimmed milk plus added fats. The 7% level of corn oil was found to afford maximum protection from caries. The authors concluded that added fat protected the teeth from dental caries more than fat found in food naturally. This conclusion supports previous studies which suggested a local rather than a systemic influence by fat.

A comparison of the effects of two different fats was conducted by McClure et al. (1956). Thirteen percent Crisco^R or 13% butterfat by weight were substituted for cornstarch in the cariogenic diets of white rats. Crisco^R was found to significantly reduce both the incidence and severity of caries. Butterfat was not found to reduce the incidence of caries significantly and caused only a marginally significant reduction in the severity of caries. The different levels of reduction by the two different fats proved that the effect of the added fat was not only due to a reduction of carbohydrate in the diet but was probably due to a characteristic of fat which varied in the two fats tested. The authors suggested that the reduction of caries was due to a change in the physical properties of the diet

which affected the capacity of the diet to be retained on the tooth surfaces.

The possibility that the caries inhibiting properties of various fats could vary depending on the level of unsaturation of the fat was investigated by Gustafson et al. (1953). Seven percent cocoa fat, olive oil, and poppy seed oil were added by weight to a high sucrose diet and fed to golden hamsters. The cocoa fat was low in unsaturated fatty acids, the olive oil had a medium content and the poppy seed oil was high in unsaturated fatty acids. After eating the diets for 110 days, the hamsters displayed no significant differences in dental caries.

Further investigation by the same group of researchers (Gustafson et al., 1955) was conducted to determine whether a difference in physical properties of fats would correlate with a difference in caries inhibiting properties of the fats. Arachis oil and lard both had melting points below body temperature and were compared to hydrogenated arachis oil which had a melting point above body temperature. The fats were added to cariogenic diets at the level of 25% by weight and the diets were fed to hamsters for 150 days. There was a large reduction of caries with all three fats, however, significant differences in the caries inhibiting properties of the three fats was found. The hydrogenated arachis oil with the higher melting point caused the

greatest inhibition. The arachis oil and the lard caused very similar reductions in caries. The later two fats have similar melting points but differ in content of unsaturated fatty acids. Since they provided practically the same inhibition of caries the lack of a correlation between caries inhibition by a fat and the level of unsaturation of the fat was confirmed. A positive correlation of physical properties such as melting point of the fat to caries inhibiting properties of the fat was suggested by the results.

A simple study on the diets containing the three fats mentioned above was conducted to investigate a possible mechanism for caries inhibition by dietary fat. It had been suggested that the fat added to a cariogenic diet forms a film of fat around the food particles and protects them from penetration by mouth fluids (McCollum, 1939). The solubility of sucrose in the diets containing the three fats was determined by measuring the amount of sucrose dissolved from each diet in 15 seconds following the addition of water. Whereas 86.7% of the sucrose was dissolved from the fat free diet, significantly less sucrose was dissolved from the fat containing diets. The solubility did not correlate with the relative caries inhibitory properties of the fats. There is some implication that fats may reduce the availability of sugar in food mixtures by affecting solubility but a correlation with the

amount of caries inhibition was not found in this study.

Alam et al. (1973) proposed that a higher content of linoleic acid in diets with increased content of certain fats was contributing to lower caries susceptibility by altering the composition of the teeth. Soybean oil, cottonseed oil, Crisco^R, and butter were each tested at the 3% and 9% level in the diet of rats. The increase to 9% was at the expense of cellulose. The cumulative caries score was lower in rats consuming 9% than for rats consuming 3% of each fat tested. Cottonseed oil was the only fat for which the reduction was significant. Although analysis of the fatty acid composition of the teeth revealed a higher content of linoleic acid in the teeth of rats fed diets containing a higher content of linoleic acid the reduction in caries could not be attributed to this change in tooth composition.

Several studies have appeared to contradict the theory that dietary fat inhibits dental caries in rats, however, results of such studies were not actually conclusive. Harris et al. (1965) reported that the addition of 5.19% hydrogenated lard by weight to a diet which was already cariogenic significantly increased the cariogenicity of the diet. The authors suggested that the particles of lard became impacted in the pits and fissures of the teeth and therefore increased the caries in those areas. Results of the experiment do not indicate whether there were any

changes in caries on the buccal or lingual or proximal surfaces of the teeth. The authors conclude that fats could not be cariostatic agents. These results should not have been applied to all fats. There may be differences which make individual fats more or less cariostatic.

Osborn et al. (1966) reported that fat in the diet of rats had no influence on either the development or the prevention of dental caries. Ten percent and 25% fat by weight were added to the diets of rats and no change in dental caries was observed. The particular kind of fat used in this study and the additional ingredients other than casein and gluten were not reported, therefore, interpretation of these data are difficult.

Enamel Studies (In Vitro)

Most of the above animal studies implied that dietary fat had a role in the inhibition of dental caries. Several mechanisms were proposed. The suggestion was made that fat could be creating a film on the teeth and preventing the dissolution of the enamel (McCollum et al., 1939). Several investigators have tested this possibility in vitro with both dietary and non-dietary fats. Walsh et al. (1950) conducted a study to determine to what extent an intact tooth surface already wet with saliva could be covered with a protective film. Various fats were applied to human teeth that had been presoaked in saliva. After application of

the fat the teeth were again soaked in saliva for a set time and the amount of calcium in the saliva was measured as an index of decalcification. Olive oil, paraffin oil, cetyl alcohol in paraffin and 0.1% aqueous sodium lauryl sulphate were found to have no significant influence on the rate of decalcification. Butter, lard, and petroleum jelly gave a small level of protection. Oleic acid gave significant protection. Cetylamine at the 1% level in paraffin oil gave the greatest protection. At a pH of 4, cetylamine was found to reduce decalcification as much as 50% over a 20 hour period. The authors concluded that the cetylamine had a strong affinity for the tooth surface. Since cetylalcohol was ineffective, the authors suggested that the NH_2 group was probably adsorbed on the enamel allowing the hydrocarbons of cetylamine to form a bridge to paraffin oil. The paraffin oil then formed a film on the tooth. It was assumed that the affinity between the enamel and the amine caused the displacement of saliva and the establishment of the more viscous oils on the tooth surface. Since dietary fats such as butter and lard did not have a very strong effect it was assumed that their slight action was due to mechanical properties and not due to the formation of films.

A more recent study on the dissolution of hydroxyapatite crystals by medium chain fatty acids was conducted by Dirksen et al. (1973). Octanoic, decanoic, lauric, and myristic acids were tested for their ability to inhibit

dissolution of hydroxyapatite crystals in an acetate buffer. Fatty acids were prepared in 5% and 15% ethanol. None of the fatty acids reduced dissolution levels in the 5% ethanol. Lauric acid reduced dissolution in the 15% ethanol.

The above enamel dissolution experiments are difficult to apply to a dietary situation. They do imply that inhibition of dental caries by fat may be due to multiple mechanisms.

Antimicrobial Activity of Fats

Derivatives of fat have long been known to exhibit bactericidal properties (Kabara, 1979). Soaps, the salts of long chain fatty acids, are believed to exert an inhibitory action on bacteria by suppressing the surface tension in the bacterial medium. Bacteria are dependent on surface tension for many processes including the accumulation of nutrients on their surface membranes (Pelczar, 1965). A depression of surface tension often leads to bacterial cell death.

Research on the antimicrobial activity of fatty acids other than soaps has also been conducted. The search for an appropriate culture medium for lactic acid bacteria led Williams et al. (1947) to discover that oleic acid at certain concentrations is an essential growth factor for several lactic acid bacteria. At other concentrations oleic acid was found to inhibit growth. Other fatty acids,

especially unsaturated ones, were also found to have a toxic effect on the bacteria.

The ability of a series of fatty acids to support growth of oral microorganisms evaluated by Stephen (1950). The fatty acids evaluated were oleic, linoleic, undecylenic, and the short chain fatty acids from C_2 to C_{18} . The microorganisms tested were 25 strains of lactobacilli, several strains of staphylococci and streptococci and mixed oral flora of plaques and saliva. All of the microorganisms showed some susceptibility to the inhibitory effects of C_6 to C_{12} acids. Slight stimulatory effects were observed for some of the lactobacilli with oleic and linoleic acids. Fatty acids of carbon lengths 14-18 gave small inhibition or none at all.

These early studies indicated that antimicrobial activity of fatty acids did exist and varied in intensity depending on the chain length and degree of unsaturation of the fatty acid. More recent studies confirmed these results and provided more information on the relationship of the chemical structure of fatty acids and derivatives to their antimicrobial action. Kabara et al. (1972) tested the bacterial inhibition of 30 straight chain fatty acids and derivatives. Twenty microorganisms were studied. Many of the compounds tested were inhibitory. The minimum inhibitory concentration (MIC) for saturated fatty acids decreased from a maximum for the C_6 and C_{20} fatty acids to a minimum

for the C_{12} fatty acid. The greatest bacteriostatic activity against gram (+) microorganisms was found in lauric acid (C_{12}). Unsaturated fatty acids tested were C_{14} , C_{16} , and C_{18} . The bacteriostatic activity of these acids was increased by the addition of a cis double bond in a delta 9 position. The addition of a second double bond further increased the bacteriostatic action of C_{18} . Since lauric acid (C_{12}) was found to have the greatest bactericidal activity of the saturated fatty acids various derivatives of lauric acid were also tested. The alcohol and aldehyde were found to be similar in activity to the acid form against most organisms. The 1,3-dilaurin and trilaurin derivatives were found to be less active than lauric acid but the monolaurin proved to be more active than the free acid.

Kabara et al. (1977) confirmed these findings in a further study of 40 lipophilic substances, both natural and synthetic. Many of the substances exhibited antimicrobial effects. In general lower chain fatty acids were biologically more active when esterified to glycerol. C_{11} and C_{13} fatty acids as monoglycerides were less active than C_{12} as a monoglyceride. Lauric esters of other polyhydric alcohols were also active. Monolaurin was again the most active bactericidal compound screened.

The confirmation that fatty acids and derivatives did inhibit bacterial growth and that certain fatty acids and

derivatives were more effective than others implied that the more active compounds might be specifically applied to various situations where antimicrobial agents were required.

Dental Caries Inhibition By an Antimicrobial Lipid

The possible role of fat in dental caries inhibition has been previously discussed. Several mechanisms were proposed for the inhibition. Fat may have increased oral clearance of carbohydrates from the mouth, it may have decreased the solubility of sugars in the mouth, or it may have coated the enamel with a protective film. None of these mechanisms was proven. Knowledge of the antimicrobial properties of specific fats implied that inhibition by dietary fat could be due to a fourth mechanism. The content of antimicrobial fats in the dietary fats tested could be causing the inhibition. It was possible that a known antimicrobial fat could provide maximum inhibition of caries by exerting more than one mechanism.

Most cariogenic bacteria are gram (+) microorganisms. The monoglyceride of lauric acid had previously been shown to be the most effective antimicrobial lipid against gram (+) organisms. Since monolaurin was considered a generally recognized as safe substance (GRAS) by the Food and Drug Administration, monolaurin was studied for its anticariogenic properties.

Kabara et al. (1979) conducted an in vitro study of the activity of Lauricidin^R which was a 90% pure preparation of the monoglyceride of lauric acid. Various oral microorganisms were tested. Lauricidin^R had strong antibacterial effects on gram positive microorganisms. Streptococcus mutans was inhibited to the greatest extent of all the organisms tested.

The ability of monolaurin to inhibit the growth of Streptococcus mutans in the oral cavity of rats was studied by Whitlock et al. (1978) in conjunction with an analysis of dental caries incidence in the same rats. Inhibitory action of monolaurin was compared to the action of two other fats; Crisco^R which was considered non-bactericidal and sucrose ester (SSE) which was considered slightly bactericidal. The fats were incorporated at the 2% level by weight into diets containing 67% refined sucrose, 18% skim milk powder and several other ingredients. Rats were fed chow type rations for two days after weaning and then were inoculated with Streptococcus mutans and fed the test diets for four weeks. Streptococcus mutans colony forming units (CFU) in the oral cavity of rats fed the diet containing monolaurin were significantly lower than for rats fed either of the other two fats. A 59% reduction in dental caries in the group of rats fed monolaurin compared to the group fed Crisco^R was not significant due to the large individual differences within each group.

Feeding studies on rats were also conducted by Kabara et al. (1979). Lauricidin^R and Crisco^R were compared at the 2% level in the diet of rats. Animals in this study were weaned and immediately fed the test diets to insure adequate development of caries. After four weeks the animals were sacrificed and the microorganisms present in the oral cavity were counted. Wide variation between animals resulted in no statistical differences between the control and monolaurin groups for any of the groups of microorganisms studied. Teeth from these rats were analyzed for dental caries by Schemmel et al. (1979). A statistically significant reduction in smooth surface dental caries was found in the rats fed monolaurin (Lauricidin^R).

The previous experiment was repeated by the same group of investigators (Kabara et al., 1979). Most conditions were the same. Animals were sacrificed and analyzed for oral microorganisms and dental caries at the end of two, four, and six weeks. Since liver powder, an essential ingredient of the previous diets, was not added until the fifth week analysis of data from the two and four week experimental periods was difficult. Streptococcus mutans CFU, total CFU, and smooth surface caries were all significantly lower in rats fed monolaurin for six weeks than in control rats which did not receive monolaurin.

The action of monolaurin as an anticaries agent was promising but was in need of further verification under

various dietary challenges. Various dosages needed to be investigated to determine the lowest concentration that could be added to a food product to reduce its cariogenicity.

MATERIALS AND METHODS

Part I

Experimental Design

Thirty six female Osborne-Mendel (O.M.) rats were distributed by weight at 18 days of age among several treatment groups so that the combined weight of the rats in each treatment group was approximately the same. The rats were bred in the laboratory of the Department of Food Science and Human Nutrition (FSHN). The original O.M. stock came from the National Institutes of Health (NIH). The original NIH colony of Osborne-Mendel rats was Caesarian delivered and then infected with four organisms. These were rodent strains of two lactobacilli, an enterococcus and a bacteroid (Fitzgerald and Larson, 1971). Presumably, the animals in the Department of FSHN carried these same organisms since the colony is maintained under conditions which minimize entry of extraneous bacteria.

Three experimental groups of 12 rats each were fed diets containing 2% of a test fat. All other ingredients of the diets were identical (Table 1). Two of the groups were infected with Streptococcus mutans. One of these groups received 2% Lauricidin^R and one received 2% hydrogenated

Table 1. Composition of diet

<u>Ingredients</u>	<u>Percent by weight</u>
Skimmed milk powder ^a	28
Sucrose (6x) ^b	57
Brewer's yeast ^c	4
Alfalfa ^d	3
Liver, dessicated, defatted ^e	1
Sodium Chloride	2
Corn oil ^f	3
Test fat ^g	2

^aHigh heat, spray processed non-fat dry milk purchased from Valley Lea Dairies Inc., South Bend, Indiana.

^bBig Chief 6x powdered sugar (starch content not over 3%) purchased from Monitor Sugar Co., Bay City, Michigan.

^cBrewer's yeast purchased from Teklad Test Diets, Madison, Wisconsin. (Type 200-B; 54.7% protein; containing the following per gram: 200 mcg pantothenic acid, 30 mcg pyridoxine, 0.5 mcg biotin, 3600 mcg choline, 4000 mcg inositol, 20 mcg folic acid, 500 mcg niacin, 70 mcg riboflavin, 600 mcg thiamine; plus the other B complex factors naturally present in yeast.)

^dAlfalfa purchased from Ohio Blenders, Toledo, Ohio. (Composed of not less than 17% crude protein, not less than 1.5% crude fat, not less than 27% crude fiber.)

^eLiver, dessicated, defatted purchased from U.S. Biochemicals Corporation, Cleveland, Ohio.

^fCorn oil purchased from Nugget Distributors, Stockton, California.

^gTest fat was either Crisco^R purchased from Proctor and Gamble, Cincinnati, Ohio, or Lauricidin^R which was a 90% pure preparation of the monoglyceride of lauric acid and which was generously supplied by Medchem Laboratories, Monroe, Michigan.

vegetable shortening. The third group of rats was not inoculated and was fed 2% hydrogenated vegetable shortening.

Preparation of Diet

The diet is a modified form of the high sucrose NIH diet 2000 (Keyes, 1959). The diet was prepared a few days before the start of the experiment and again at four weeks. All ingredients were placed in a 5 kg. capacity Hobart mixer and allowed to mix for at least 30 minutes. Diet was stored under refrigeration at all times during the experiment.

Experimental Procedure

Immediately after all rats were assigned to treatment groups the rats of treatment groups 1 and 2 were inoculated with a pure culture of Streptococcus mutans 6715¹. Approximately 0.2 ml of the 30 hour working culture was placed in the mouth of each rat. Streptococcus mutans 6715 cells for inoculation were obtained from an 18-24 hour Trypticase soy broth culture and standardized to 25% transmittance at 550 nm with a spectronic 20². The rats were also given 5% sucrose water with 10 ml standardized culture per liter for 48 hours. After 48 hours drinking water was changed to pure distilled

¹The original culture was obtained by Drs. Mickelsen and Schemmel, through the generosity of Dr. Rachel Larson, NIDR. It has been carried through several generations in Dr. J.J. Kabara's laboratory.

²Bausch and Lomb.

and rats were given it ad libitum for the duration of the experiment.

Rats were housed in pairs in metal screen suspended cages. The right ear of the even numbered rats was clipped for identification. Rats in treatment group three which were not inoculated with S. mutans were housed on a rack other than the one used for the other two groups to avoid cross infection. The environment was controlled with 12 hours of light of each 24 hours with a temperature maintained at 23 ± 1 C. Rats were weighed in the afternoon after the first 48 hours and once a week thereafter.

Diet was available ad libitum for a period of 44 or 45 days. Food dishes for all rats were refilled every two or three days or whenever the level of food for any pair of rats was below one half cup. Porcelain cups were weighed when full and when taken from the cage for re-filling. Spillage was collected on newspaper beneath each cage. Spillage was separated from feces, weighed, and added to the weight of the cup when taken from the cage. Food intake for each feeding period was determined by subtracting the weight of the cup, remaining contents, and spillage from the original weight of the full cup. Food intakes were totaled for each week. Clean cups and water bottles were provided weekly.

After being fed the diet for 44 or 45 days, rats were anesthetized by ether inhalation and then decapitated. Six rats from each group were sacrificed on day 44. The remaining six

rats were sacrificed on day 45. After decapitation, the heads were divided along the sagittal plane and the molar teeth from the left mandibular and maxillary jaws were evaluated for both microorganisms and dental caries. The teeth from the right jaws were evaluated for dental caries only. The incisors were not studied because they are generally not considered to be involved in dental caries.

In order to quantitate the microorganisms from the left molars the teeth were aseptically removed from the jawbone retaining enough bone to keep the 3 molars together. The jaw section containing the teeth was transferred to 5 ml of reduced transport fluid (RTF). After sonication the teeth were removed from the fluid and air dried before being scored for dental caries.

To insure that the microbiological procedures were not damaging the left molars a different method of soft tissue removal was used on the right jaws. The right halves of the heads were placed in the MSU dermestid beetle colony. After removal from the beetle colony, the jaws were soaked in a 2% solution of ammonium hydroxide for 30 minutes, soaked in tap water for 30 minutes and allowed to air dry for 24-48 hours.

Scoring Carious Lesions

Jaws were coded before scoring to avoid possible bias during scoring due to knowledge of which treatment group the teeth were from. Three molars in each jaw quadrant

were scored for caries by the method of Keyes (1958). This method specifies four separate surfaces of each tooth to be examined. The buccal surface is the surface closest to the cheek; the lingual surface is the surface closest to the tongue. The buccal and lingual surfaces are the surfaces Keyes refers to as smooth surfaces. The proximal surfaces are those surfaces between the teeth where the teeth are touching or nearly touching. The sulcal surfaces are the inner surfaces sometimes called occlusal or fissure surfaces. The three molars in each quadrant are different sizes. Keyes has defined a basic linear unit and defined the number of these units on each surface of each tooth. The number of sulcal units was determined by viewing the sulci of sagittally sliced teeth. The depth of decay has been defined according to four categories. When water is allowed to evaporate off a tooth surface any decalcified enamel dehydrates more rapidly than sound enamel and appears as a chalky white area. If the surface is chalky but unbroken the caries involvement is said to be E. If the surface is slightly loose and flaky the involvement is Ds. If a probe can be inserted into the lesion the involvement is Dm. If the probe can be extended all the way to the pulp chamber the involvement is Dx. Each surface of each tooth is given a score which represents the number of linear units involved in E decay, Ds decay, Dm decay, and Dx decay. The total caries score for that surface of that tooth is the

sum of all the linear units involved at any depth. It is equivalent to a 2 dimensional (length vs. depth) score. Using this method it is possible to compare total caries per surface area per rat. It is also possible to compare enamel caries or Ds caries separately. Totals for buccal and lingual surfaces are usually added together and referred to as total smooth surface areas. Proximal surfaces have been included in smooth surface areas by many researchers but this study considered proximal areas separately. Smooth, proximal and sulcal areas are never added together.

Each jaw quadrant was soaked in water for a few minutes and then examined under a Bausch and Lomb dissecting microscope under low magnification (20x). As the water evaporated the buccal and lingual surfaces of the teeth were scored for caries. The typical buccal or lingual lesion is a long narrow horizontal area. Sulcal caries that have spread into the smooth areas were carefully excluded from the scores for the smooth areas.

After all the teeth were scored for buccal and lingual caries they were allowed to soak in a murexide³ stain for 24 hours. After 24 hours the stain was removed and the teeth were allowed to air dry for several days. The buccal and lingual scores were then verified under

³ Murexide (ammonium purpurate) purchased from Sigma Chemical Co., St. Louis, Mo. (60 mg murexide dissolved in 75 ml distilled water and 175 ml absolute alcohol and filtered through a vacuum).

magnification.

In order to examine the sulcal areas of the teeth each jaw quadrant was sliced⁴ into equal halves at an angle parallel to the buccal and lingual surfaces. Before slicing, the teeth were coated with clear nail polish to avoid loss of teeth during the slicing process. While being sliced the teeth were held with a small hemostat beneath a slow drip of water. The sliced teeth were air dried for 24 hours and then examined for sulcal and proximal caries.

The depth of the sulcal and proximal lesions was determined by the amount of penetration of the stain. For these surfaces the border between the enamel and the dentin can be seen. If the pink stain has not penetrated this border then the lesion is E only. If the stain is 1/4 way into the dentin then the lesion is Ds. If the stain is 1/4 to 3/4 of the way into the dentin then the lesion is Dm. If the dentin is totally stained then the lesion is Dx.

Proximal surfaces were scored in the same manner as sulcal, however, if no proximal lesions were visible a probe was used to separate the 1st molar from the 2nd molar and the 2nd molar from the 3rd molar to expose the entire proximal surface. This insured that proximal caries were not overlooked due to uneven slicing of the teeth.

⁴A steel disc saw, 0.004 inch thickness and 0.75 inch in diameter was mounted on a mandrel held in a straight handpiece of a standard dental engine.

Caries were recorded on the score sheet designed by Keyes. Morsel caries were not evaluated in this study.

Analysis of Data

Means and standard errors were determined for cumulative weight gains and cumulative food intakes for each group of rats. Means and standard errors were determined for the number of carious teeth per rat and for the total caries score per rat for each of the three tooth surfaces for each group of rats. Analysis of variance was performed by the CDC 6500 computer, Michigan State University.

Significant differences between mean values for caries scores was assessed by Dunnett's test (Dunnett, 1955) using treatment 1 (inoculated rats fed 0% Lauricidin^R) as the control. The Student's paired t-test (Navia, 1977) was used for comparison of mean values of total caries scores on the right and left side of the jaws for each group of rats. Student's paired t-test was also used to compare mean values of total caries scores on the maxilla and mandible of the teeth from each group of rats.

Part II

Experimental Design

Forty eight male Osborne-Mendel rats were distributed by weight at 18 days of age among six treatments so that the combined weight of the rats in each treatment group was

approximately the same. Rats were bred from the same stock as those used in Part I. Six experimental groups of eight rats each were fed diets containing 2% of a test fat combination in one of the following ratios: 2% Lauricidin^R / 0% Crisco^R, 1% Lauricidin^R / 1% Crisco^R, 0.5% Lauricidin^R / 1.5% Crisco^R and 0% Lauricidin^R / 2% Crisco^R. Four of the groups were inoculated with Streptococcus mutans and each of these groups were fed one of the four fat ratios. Two of the groups were not inoculated. Rats in these groups were started on the experiment one day later than rats in the other four groups. One of the groups that was not inoculated was fed 2% Lauricidin^R and 0% Crisco^R. The other non-inoculated group was fed 0% Lauricidin^R and 2% Crisco. All other ingredients in the diet were identical to those used in Part I⁵.

Preparation of Diet

To insure uniform mixing of the fat with the remaining ingredients, diets were prepared in the following way:

1. All ingredients except corn oil and the test fat were measured and placed in a 5000 gram mixer.

They were mixed at the lowest speed for 10 minutes.

⁵The source of purchase of Brewer's yeast was not the same as Part I. Brewer's yeast for Part II was purchased from Schiff Bio-Food Products, Inc. Moonachie, N.J. (25 cal/7 grams: 45 to 48% protein, all essential amino acids, fortified with B₁₂; containing the following per oz.: 4.28 mg B₁, 1.14 mg B₂, 11.40 mg niacin, 1.14 mg B₆, 1.42 pantothenic acid, 2 mcg cobolamin, 90 mcg folic acid, 25 mcg biotin, 140 mcg PABA, 100 mcg choline, 100 mcg inositol, 540 mg potassium, 60 mg calcium, 2.5 mg iron, plus the rest of the B vitamins natural to yeast.)

2. Corn oil and the proper ratio of test fat were measured and blended in a bowl.
3. Portions of the pre-mixed ingredients from step 1 were added to the fat mixture and creamed together by hand. Dry ingredients continued to be added until the fat mixture was too stiff to blend.
4. The fat mixture was added to the rest of the ingredients in the 5000 gram mixer and mixed for 30 minutes.

Experimental Procedure

Identical to Part I except for the following:

1. Small metal food savers were placed in the food dishes to limit the amount of spillage.
2. Only the six teeth from the left sides of the jaws were scored.
3. The right sides of the rat heads were not defleshed by the beetle colony. They were autoclaved at 121-123 C for 5 minutes. Soft tissues were removed with instruments. The jaws were then soaked in a 2% solution of ammonium hydroxide for 5 minutes.
4. The smooth surfaces were examined under a stream of forced air.
5. The murexide stain was left on the teeth for 7 hours instead of 24 hours.

Analysis of Data

Means and standard errors were determined for the cumulative weight gains, the cumulative food intakes and the total caries scores on each of the three tooth surfaces for each group of rats. Analysis of variance was performed by the CDC 6500 computer, Michigan State University.

A Scheffé procedure (Gill, 1978) was used to analyze all possible comparisons among treatment means for cumulative food intakes. A Bonferroni-t statistic (Gill, 1978) was used to compare specific contrasts between treatment means for total caries scores on each of three tooth surfaces. The following contrasts were performed: 1 vs. 4, 2 vs. 4, 3 vs. 4, 5 vs. 6, 1 vs. 3, 1-4 vs. 5-6.

RESULTS

Part I

Body Weights

Mean body weight gains from 18 to 62 (63 days of age) are presented in Table 2. One animal in treatment group 2 died after five days on the experiment. Means in treatment group 2 were computed for the remaining 11 rats. The remaining animals in each of the treatment groups increased in weight over the six week experimental period. Final weights ranged from 169 ± 3 to 174 ± 4 . Rats which were not inoculated with Streptococcus mutans and not fed Lauricidin^R gained a mean weight of 137 ± 3 g. Rats which were inoculated and not fed Lauricidin^R gained a mean weight of 134 ± 2 g. Rats which were inoculated and fed 2% Lauricidin^R gained a mean weight of 136 ± 7 g. Differences between the groups were not significant.

Diet Consumption

Over the six week feeding period from age 18 to 62 days rats which were inoculated and not fed Lauricidin^R ate a mean of 1223 ± 29 g (Table 2). Rats which were inoculated and fed Lauricidin^R consumed a mean of 1152 ± 53 g. Rats which were not inoculated and not fed Lauricidin^R consumed a mean of 1215 ± 23 g. There were no

Table 2. Cumulative food intakes and cumulative weight gains of female OM rats fed cariogenic diets with and without Lauricidin R^a for 6 weeks^b

	Treatment Groups		Sign. Diff.
	Inoculated	Not Inoculated	
	1 (N=12)	2 (N=10)	3 (N=12)
Dose of Lauricidin R (%)	0	2	0
Dose of Crisco R (%)	2	0	2
Cumulative food intake per 2 rats (g)	1223 ^a ± 29	1152 ± 53	1215 ± 23
Cumulative weight gain per rat (g)	134 ± 2	136 ± 7	137 ± 3
Final weight per rat (g)	169 ± 3	170 ± 7	174 ± 4

^aMean ± SEM

^bRats fed experimental diets from weaning at 18 days of age

significant differences in mean food intake among rats in the three treatment groups.

Dental Caries Incidence and Severity

Right and Left Jaws. Means, standard errors, and statistical analysis of dental caries severity in the right and left jaws of rats in each treatment group are presented in Table 3. In all treatment groups on all three tooth surfaces there were no significant differences between the mean caries scores on the left sides of the jaws and the mean caries scores on the right side of the jaws.

Maxillary and Mandibular Teeth. Means, standard errors, and statistical analysis of dental caries severity in teeth from the maxilla and mandible of rats in each treatment group are presented in Table 4. Rats which were not inoculated did not develop caries on the buccal/lingual surfaces in the maxilla. Rats which were inoculated and not fed Lauricidin^R had a mean caries score of 42.5 ± 10.6 on the buccal/lingual surfaces of the teeth in the mandible and a mean caries score of 2.0 ± 0.7 on the buccal/lingual surfaces of the teeth in the maxilla. The differences in caries scores on the maxilla and mandible was statistically significant ($p < 0.005$). Percent of buccal/lingual caries in the maxilla was only 4.5.

Rats which were inoculated and fed 2% Lauricidin^R had a mean caries score of 19.6 ± 6.1 on the buccal/lingual surfaces of the mandible and a mean caries score

Table 3. Severity of dental caries in teeth from the right and left jaws in female OM rats fed cariogenic diets with and without Lauricidin

Treatment Group	Lauricidin (%)	Tooth Surface	Caries Scores			Sign. Diff.
			(Units/Rat) ^b			
			Left	Right		
<u>Inoculated</u>						
1 (N = 12)	0	Buccal/Lingual	21.6 ± 5.5 ^a	22.8 ± 5.9	n.s.	
		Sulcal	49.4 ± 1.7	48.3 ± 2.5	n.s.	
		Proximal	13.8 ± 1.2	14.6 ± 1.2	n.s.	
2 (N = 10)	2	Buccal/Lingual	12.6 ± 4.1	10.0 ± 2.6	n.s.	
		Sulcal	40.7 ± 1.7	38.6 ± 1.8	n.s.	
		Proximal	9.4 ± 0.5	10.3 ± 0.7	n.s.	
<u>Not Inoculated</u>						
3 (N = 12)	0	Buccal/Lingual	2.1 ± 0.5	1.9 ± 0.5	n.s.	
		Sulcal	50.6 ± 1.2	49.0 ± 2.7	n.s.	
		Proximal	4.6 ± 1.0	3.5 ± 0.7	n.s.	

^aMean ± SEM

^bValues represent an accumulated score which takes into account the width and depth of the lesions on all 12 teeth.

Table 4. Severity of dental caries in teeth from the maxilla and mandible of female OM rats fed cariogenic diets with and without Lauricidin R

Treatment Group	Lauricidin (%)	Tooth Surface	Caries Scores (Units/Rat) ^a		Caries Found in Maxilla (%)	Sign. Diff.
			Mandible	Maxilla		
			<u>Inoculated</u>			
1 (N = 12)	0	Buccal/Lingual	42.5 ± 10.6 ^b	2.0 ± 0.7	4.5	*** ^c
		Sulcal	61.3 ± 2.8	36.5 ± 1.5	37.3	**
		Proximal	14.3 ± 1.4	14.0 ± 1.1	49.4	n.s.
2 (N = 10)	2	Buccal/Lingual	19.6 ± 6.1	3.0 ± 0.8	13.3	**
		Sulcal	46.7 ± 2.3	32.6 ± 1.3	41.1	***
		Proximal	8.1 ± 0.6	11.6 ± 0.7	58.9	***
<u>Not Inoculated</u>						
3 (N = 12)	0	Buccal/Lingual	4.0 ± 0.7	0.00	0	***
		Sulcal	62.6 ± 1.8	37.0 ± 1.6	37.2	***
		Proximal	4.5 ± 0.7	3.6 ± 0.9	44.3	n.s.

^aValues represent an accumulated score which takes into account the width and depth of the lesions on all 12 teeth.

^bMean ± SEM.

^cStudents paired t-test. **p<0.01, ***p<0.005.

of 3.0 ± 0.8 on the buccal/lingual surfaces of the maxilla. The difference in caries scores on the maxilla and mandible was statistically significant ($p < 0.01$). The percent of caries found in the maxilla was 13.3.

There were no significant differences between caries scores on the proximal surfaces in the maxilla and mandible for either of the two groups that were not fed Lauricidin^R. Rats which were inoculated and fed Lauricidin^R had a mean caries score of 8.1 ± 0.6 on the proximal surfaces of the mandible and a mean caries score of 11.6 ± 0.7 on the proximal surfaces of the maxilla. The percent of proximal caries found in the proximal surfaces of the maxilla was 58.9. This was statistically significant ($p < 0.005$).

Mean caries scores on the sulcal surfaces of the teeth in the maxilla were significantly lower for rats in each of the treatment groups. Rats which were not inoculated and not fed Lauricidin^R had a mean caries score of 37.0 ± 1.6 on the sulcal surfaces of the teeth of the maxilla and a mean caries score of 62.6 ± 1.8 on the sulcal surfaces of the teeth of the mandible. The percent of the sulcal caries which were found in the maxilla was 37.2 ($p < 0.005$).

Rats which were inoculated and not fed Lauricidin^R had a mean caries score of 36.5 ± 1.5 on the sulcal surfaces of the teeth of the maxilla and a mean caries score of 61.3 ± 2.8 on the sulcal surfaces of the teeth of the mandible. The percent of sulcal caries which were found in the maxilla

was 37.3 ($p < 0.01$).

Rats which were inoculated and fed 2% Lauricidin^R had a mean caries score of 32.6 ± 1.3 on the sulcal surfaces of the teeth of the maxilla and a mean caries score of 46.7 ± 2.3 on the sulcal surfaces of the teeth of the mandible. The percent of caries found on the maxilla was 41.1 ($p < 0.005$).

Inoculated and Non-Inoculated Rats. Means, standard errors and statistical analysis of dental caries incidence and total dental caries scores for all three treatment groups are presented in Table 5. One of the rat jaws from treatment group 2 was lost during preparation for scoring. Data for treatment group 2 are presented for the remaining ten rats. Data for treatment groups 1 and 3 are presented for 12 rats in each group.

92% of the rats which were not inoculated with Streptococcus mutans and not fed Lauricidin^R developed caries on the buccal/lingual surfaces of the teeth. The mean number of carious teeth per rat in this group was 1.6 ± 0.3 . This means that for any one rat an average of 1.6 out of the 12 teeth scored was decayed. The location of the decayed tooth was similar for most rats in the group. 84.2% of the decayed teeth were mandibular 3rd molars. 15.7% of the decayed teeth were mandibular 2nd molars.

100% of the rats which were inoculated and not fed Lauricidin^R developed caries on the buccal/lingual

Table 5. Incidence and severity of dental caries in female OM rats fed a cariogenic diet with and without the addition of Lauricidin R for six weeks.

	Treatment Group				Sign. Diff. d,e
	1	2		3	
		Lauricidin R (%)			
	0	2		0	
	<u>Inoculated</u>				<u>Not inoculated</u>
Buccal/Lingual Surfaces					
Rats with caries (%)	100		90	92	
Cariou teeth per rat (N) ^a	5.4	0.7 ^c	4.7	0.9	1.6 0.3
Caries Score (Units/rat) ^b	44.5	10.9	22.6	6.1	4.0 0.7
					3<1
Sulcal Surfaces					
Rats with caries (%)	100		100	100	
Cariou teeth per rat (N)	11.9	0.1	11.9	0.1	11.9 0.3
Caries Score (Units/rat)	97.8	3.5	79.3	2.7	99.6 3.4
					2<1
Proximal Surfaces					
Rats with caries (%)	100		100	100	
Cariou teeth per rat (N)	11.0	0.4	10.0	0.3	5.4 0.7
Caries Score (Units/rat)	28.3	2.3	19.7	1.1	8.1 1.3
					3,2<1

^a12 teeth scored per rat

^bValues represent an accumulated score which takes into account the width and depth of the lesions on all 12 teeth.

^cMean $\pm$ SEM

^dStatistical significance from control (treatment 1) using Dunnett's test

$(\bar{Y}_i - \bar{Y}_c) / \sqrt{MSE (1/r_i + 1/r_c)} \pm t_{D\alpha, t, v}$ (Dunnett, 1955)

^e $p < 0.01$

surfaces of the teeth. The mean number of carious teeth per rat was 5.4 ± 0.7 . This was a statistically significant increase from the non-inoculated group ($p < 0.01$).

Rats which were not inoculated and not fed Lauricidin^R had a mean caries score of 4.0 ± 0.7 on the buccal/lingual surfaces of the teeth. Rats which were inoculated and not fed Lauricidin^R had a mean caries score of 44.5 ± 10.9 on the buccal/lingual surfaces of the teeth. The increase in caries on the buccal/lingual surfaces due to inoculation was 1112.5% ($p < 0.01$).

100% of the rats which were not inoculated and not fed Lauricidin^R developed caries on the sulcal surfaces of the teeth. The mean number of carious teeth per rat was 11.9 ± 0.3 . The mean caries score per rat was 99.6 ± 3.4 .

100% of the rats which were inoculated and not fed Lauricidin^R developed caries on the sulcal surfaces of the teeth. The mean number of carious teeth per rat was 11.9 ± 0.1 . The mean caries score per rat was 97.8 ± 3.5 . Differences in mean number of carious teeth per rat and differences in mean caries score per rat between the inoculated and non-inoculated rats was not significant.

100% of the rats which were not inoculated and not fed Lauricidin^R developed caries on the proximal surfaces of the teeth. The mean number of carious teeth per rat was 5.4 ± 0.7 . The mean caries score per rat was 8.1 ± 1.3 .

100% of the rats which were inoculated and not fed Lauricidin^R developed caries on the proximal surfaces of the teeth. The mean number of carious teeth per rat was 11.0 ± 0.4 and the mean caries score per rat was 28.3 ± 2.3 . On the proximal surfaces inoculation with S. mutans resulted in a significant increase ($p < 0.01$) in mean number of decayed teeth per rat and a significant increase ($p < 0.01$) in mean caries score per rat.

Lauricidin^R. 90% of the rats which were inoculated and fed diets containing 2% Lauricidin^R developed caries on the buccal/lingual surfaces of the teeth (Table 5). The mean number of carious teeth per rat was 4.7 ± 0.9 , and was not significantly different statistically from the mean number of carious teeth in the inoculated rats which were not fed Lauricidin^R. The mean caries score per rat in the rats fed Lauricidin^R was 22.6 ± 6.1 . This is a 50% reduction from the mean caries score per rat in the group of rats which were inoculated but not fed Lauricidin^R, but it is not statistically significant due to the wide variation in caries severity seen in both groups of rats. The caries scores for inoculated rats which were not fed Lauricidin^R range from 2 to 115 units. The caries scores for the inoculated rats which were fed 2% Lauricidin^R range from 0 to 62.

100% of the inoculated rats fed diets containing 2% Lauricidin^R developed caries on the sulcal surfaces. The

mean number of carious teeth per rat was 11.9 ± 0.1 and was not significantly different from the mean number of carious teeth per rat in the group of inoculated rats which were not fed Lauricidin^R. The mean caries score per rat for the group of rats fed Lauricidin^R was 79.3 ± 2.7 . The mean caries score per rat for the inoculated rats which were not fed Lauricidin^R was 97.8 ± 3.5 . The 19% reduction in sulcal caries due to 2% Lauricidin^R in the diet was statistically significant ($p < 0.01$).

100% of the inoculated rats fed diets containing 2% Lauricidin^R developed caries on the proximal surfaces. The mean number of carious teeth per rat was 10.0 ± 0.3 and was not statistically different from the mean number of carious teeth per rat in the group of inoculated rats which were not fed Lauricidin^R. The mean caries score per rat in the group of rats fed Lauricidin^R was 19.7 ± 1.1 . The mean caries score per rat for the inoculated rats which were not fed Lauricidin^R was 28.3 ± 2.3 . The 30% reduction in proximal caries due to 2% Lauricidin in the diet was statistically significant ($p < 0.01$).

Part II

Body Weights

All of the rats in the six treatment groups gained weight. Mean body weight gains and standard errors from 18 to 61-63 days of age are presented in Table 6. The

Table 6. Cumulative food intake and cumulative weight gains of female OM rats fed carcinogenic diets with various doses of Lauricidin R for 6 weeks.^a

Treatment Group	Lauricidin R (%)	Cumulative Food Intake per 2 rats (g)	Cumulative weight gain per rat (g)
		<u>Inoculated</u>	
1	2.0	1175 ± 41 ^b	130 ± 4
2	1.0	1200 ± 31	139 ± 5
3	0.5	1169 ± 29	131 ± 4
4	0.0	1227 ± 27 ^c	128 ± 6
		<u>Not Inoculated</u>	
5	2.0	1054 ± 20	129 ± 5
6	0.0	1046 ± 30	143 ± 4

^aRats fed experimental diets from weaning at 18 days of age.

^bMean ± SEM.

^cOne way analysis of variance indicated a statistical difference between treatment groups ($p < 0.001$). A Scheffe procedure (Gill, 1978) was used to compare all possible contrasts among treatment means. Test statistic:

$$f = \bar{q}_k^2 / \hat{V}(\bar{q}_k) \text{ compared with critical value } (t-1)f_\alpha$$

for each contrast where $\bar{q}_k = c_{1k}y_1 + c_{2k}y_2 + \dots c_{tk}y_t$, and where $\hat{V}(\bar{q}_k) = \sum_{i=1}^t (c_{ik}/r_i)MSE$. Significant differences were found between treatment group 4 and 5 ($p < 0.05$) and between treatment group 4 and 6 ($p < 0.05$).

range in mean body weight gain was from 128 ± 6 g in group 4 to 143 ± 4 g in group 6. Rats in group 4 were inoculated and fed diets containing no Lauricidin^R. Rats in group 6 were not inoculated and were fed diets containing no Lauricidin^R. One way analysis of variance indicated no significant differences among the treatment groups.

Diet Consumption

Since rats were housed two per cage and food intake was recorded per cage the mean cumulative food intake reflects the diet consumed by pairs of rats housed together. It is assumed that each rat consumed half of the food which disappeared. Mean cumulative food intakes and standard errors ranged from 1046 ± 30 for rats in treatment group 6 which were not inoculated and were fed diets without Lauricidin^R to 1227 ± 27 for rats in treatment group 4 which were inoculated and were fed diets without Lauricidin^R (Table 6). One way analysis of variance indicated a statistically significant difference among treatment groups ($p < 0.001$). A Scheffé procedure was used to analyze all possible comparisons between means. Significant differences were found between mean food intake for rats in treatment group 4 which were inoculated and not fed Lauricidin^R and mean food intake for rats in treatment group 5 which were not inoculated and were fed 2% Lauricidin^R. Significant differences were also found between mean food intake for rats

in treatment group 4 and mean food intake for rats in treatment group 6 which were not inoculated and were fed diets containing 0% Lauricidin^R. All other comparisons were not significant.

Dental Caries Incidence and Severity

Proximal Surfaces. 100% of the rats in each of the six treatment groups developed caries on the proximal surfaces. The mean number of carious teeth per rat ranged from 3.3 to 5.8 (Table 7). Inoculated rats fed each of the three doses of Lauricidin^R had significantly fewer carious teeth per rat than inoculated rats that were not fed Lauricidin^R. All other differences in number of carious teeth were insignificant (Table 7).

The mean caries scores per rat for rats inoculated with S. mutans were 13.3 ± 0.7 for rats fed diets without Lauricidin^R, 6.3 ± 0.7 for rats fed diets containing 2% Lauricidin^R, 7.8 ± 0.9 for rats fed diets containing 1% Lauricidin^R, and 5.8 ± 1.1 for rats fed diets containing 0.5% Lauricidin^R. Mean caries scores for rats fed diets containing each of the three doses of Lauricidin^R were found to be significantly different from mean caries scores for rats fed diets not containing Lauricidin^R ($p < 0.005$). The mean caries score for rats fed diets containing 2% Lauricidin^R was not significantly different from the mean caries scores for rats fed diets containing 0.5% Lauricidin^R.

Table 7. Incidence and severity of dental caries on the proximal surfaces of the teeth of female OM rats fed various doses of Lauricidin R.

Treatment Group (N=8)	Lauricidin R (%)	Carious Rats (%)	Carious Teeth per rat (N) ^a	Caries Score (units/rat) ^b
		<u>Inoculated</u>		
1	2.0	100	4.6 ± 0.5 ^c	6.3 ± 0.7
2	1.0	100	4.5 ± 0.5	7.8 ± 0.9
3	0.5	100	4.1 ± 0.8	5.8 ± 1.1
4	0.0	100	5.8 ± 0.5 ^d	13.3 ± 0.7 ^e
		<u>Not Inoculated</u>		
5	2.0	100	3.3 ± 0.9	3.3 ± 0.3
6	0.0	100	3.6 ± 0.7	5.6 ± 0.9

^aSix teeth scored per rat.

^bValues represent an accumulated score which takes into account the width and depth of the lesion.

^cMean ± SEM.

^dOne way analysis of variance indicated statistically significant differences among the treatment groups ($p < 0.001$). The Bonferroni t-statistic $t_B = \bar{q}_k / \sqrt{\frac{\sum_{i=1}^k c_{ik}^2 / r_i}{k-1}}$ $MSE \pm t_B$, $\alpha/2, m, v$ (Gill, 1978) was used to compare the following contrasts; $q_1 = 1$ vs. 4; $q_2 = 2$ vs. 4; $q_3 = 3$ vs. 4; $q_4 = 5$ vs. 6; $q_5 = 1$ vs. 3; $q_6 = (1-4)$ vs. (5-6). Significant contrasts were q_1 ($p < 0.01$), q_2 ($p < 0.005$) and q_3 ($p < 0.005$).

^eSame procedure as in d. Significant contrasts were q_1 ($p < 0.005$), q_2 ($p < 0.005$), q_3 ($p < 0.005$) and q_6 ($p < 0.025$).

The mean proximal caries scores for rats which were not inoculated with S. mutans were 3.3 ± 0.3 in the group of rats fed diets containing 2% Lauricidin^R and 5.6 ± 0.9 in the group of rats fed diets not containing Lauricidin^R. The difference in mean caries scores in these two treatment groups was not statistically significant.

The reduction in mean proximal caries score for inoculated rats fed diet containing 2% as compared to 0% Lauricidin^R was contrasted with the reduction in mean proximal caries scores for non-inoculated rats fed diets containing 2% compared to 0% Lauricidin^R. This contrast was statistically significant ($p < 0.025$).

Sulcal Surfaces. One hundred percent of the rats in each of the six treatment groups developed caries on the sulcal surfaces (Table 8). All of the six teeth which were scored in treatment groups 1,2,3, and 4 had caries on the sulcal surfaces. The mean number of carious teeth per rat was 5.6 ± 0.7 in treatment group 5 in which the rats were not inoculated and were fed diets containing 2% Lauricidin^R, and 5.9 ± 0.4 in treatment group 6 in which the rats were inoculated and fed no Lauricidin^R.

The mean sulcal caries score for inoculated rats fed diets not containing Lauricidin^R was 40.1 ± 1.5 . The mean caries scores for inoculated rats fed diets containing 2%, 1%, 0.5% Lauricidin^R were 30.3 ± 2.3 , 28.3 ± 4.1 , and 25.9 ± 2.5 respectively. The mean caries scores for

Table 8. Incidence and severity of dental caries on the sulcal surfaces of the teeth of female OM rats fed various doses of Lauricidin R.

Treatment Group (N=8)	Lauricidin R (%)	Carious Rats (%)	Carious Teeth per rat (N) ^a	Caries Score (units/rat) ^b
<u>Inoculated</u>				
1	2.0	100	6	30.3 ± 2.3 ^c
2	1.0	100	6	28.3 ± 4.1
3	0.5	100	6	25.9 ± 2.5
4	0.0	100	6	40.1 ± 1.5 ^d
<u>Not Inoculated</u>				
5	2.0	100	5.6 ± 0.7	29.4 ± 2.4
6	0.0	100	5.9 ± 0.4	41.0 ± 3.3 ^d

^aSix teeth scored per rat.

^bValues represent an accumulated score which takes into account the width and depth of the lesion.

^cMean ± SEM.

^dOne way analysis of variance indicated statistically significant differences among the treatment groups ($p < 0.001$). The Bonferroni t-statistic $t_B = \bar{q}_k / \sqrt{\frac{1}{n} \frac{c^2}{r_i}}$ $MS_F \pm t_B, \alpha/2$, m, v (Gill, 1978) was used to compare the following contrasts; $q_1 = 1$ vs. 4; $q_2 = 2$ vs. 4; $q_3 = 3$ vs. 4; $q_4 = 5$ vs. 6; $q_5 = 1$ vs. 3; $q_6 = (1-4)$ vs. (5-6). Significant contrasts were q_1 ($p < 0.05$), q_2 ($p < 0.025$), q_3 ($p < 0.005$) and q_4 ($p < 0.025$).

rats fed diets containing each of the three doses of Lauricidin^R were found to be significantly less than the mean sulcal caries score for rats fed diets containing 0% Lauricidin^R ($p < 0.05$ for 2% vs. 0%, $p < 0.025$ for 1% vs. 0%, and $p < 0.005$ for 0.5% vs. 0%). The mean caries score for rats fed diets containing 2% Lauricidin^R was not significantly different from the mean caries score for rats fed diets containing 0.5% Lauricidin^R.

The mean sulcal caries score for rats which were not inoculated with S. mutans was 41.0 ± 3.3 for rats fed diets not containing Lauricidin^R and 29.4 ± 2.4 for rats fed diets containing 2% Lauricidin^R. The 28% reduction between these two groups was found to be statistically significant ($p < 0.025$).

The reduction in mean sulcal caries scores for inoculated rats fed diets containing 2% as opposed to 0% Lauricidin^R was contrasted with the reduction in mean caries scores for non inoculated rats fed diets containing 2% as opposed to 0% Lauricidin^R. This contrast was not found to be statistically significant.

Buccal and Lingual Surfaces. All of the inoculated rats which were not fed Lauricidin^R developed caries on the buccal/lingual surfaces (Table 9). The addition of the various doses of Lauricidin^R to the diets of the inoculated rats resulted in a marked reduction in the number of rats which developed caries on the buccal/lingual

Table 9. Incidence and severity of dental caries on the buccal and lingual surfaces of the teeth of female OM rats fed various doses of Lauricidin R

Treatment Group (N=8)	Lauricidin R (%)	Carious rats (%)	Carious Teeth per rat (N) ^a	Caries score (units/rat) ^b
<u>Inoculated</u>				
1	2.0	37.5	1.1 ± 0.7 ^c	3.5 ± 2.4 ^c
2	1.0	12.5	0.3 ± 0.3	1.8 ± 1.8
3	0.5	25.0	0.5 ± 0.3	1.5 ± 1.2
4	0.0	100.0	2.6 ± 0.6	16.4 ± 4.2 ^d
<u>Not Inoculated</u>				
5	2.0	0.0	0	0
6	0.0	62.5	0.9 ± 0.3	2.8 ± 1.4

^aSix teeth scored per rat.

^bValues represent an accumulated score which takes into account the width and depth of the lesion.

^cMean ± SEM.

^dThe presence of a large number of caries scores which were zero necessitated transformation of the data. A factor of 10 was added to each caries score before statistical analysis. A one way analysis of variance indicated statistically significant differences among the treatment groups. Bonferroni contrasts were computed as in footnote d, table 8. Significant contrasts were q₁ (p<0.005), q₂ (p<0.005) and q₃ (p<0.005).

surfaces. Only 25% of the rats fed diets containing 0.5% Lauricidin^R developed caries. Only 12.5% of the rats fed diets containing 1% Lauricidin^R developed caries, and only 37.5% of the rats fed diets containing 2% Lauricidin^R developed caries. The mean caries scores for inoculated rats fed 2%, 1%, and 0.5% Lauricidin were 3.5 ± 2.4 , 1.8 ± 1.8 , and 1.5 ± 1.2 respectively. This was a highly significant ($p < 0.005$) reduction of 78% to 90% from the mean caries scores of 16.4 ± 4.2 for inoculated rats which were not fed Lauricidin^R.

Caries were present on the buccal/lingual surfaces in 62.5% of the non-inoculated rats which were not fed Lauricidin^R. There were no buccal/lingual caries in any of the non-inoculated rats which were fed Lauricidin^R. The reduction due to Lauricidin^R in the non-inoculated rats was not statistically significant due to the very low caries scores of the non-inoculated rats that were not fed Lauricidin^R.

DISCUSSION

Lauricidin^R caused significant reductions in incidence and severity of dental caries in rats inoculated with Streptococcus mutans 6715 and in rats which were not inoculated. Since Streptococcus mutans is one of the bacteria highly implicated in human dental caries, caries which resulted after inoculation with this organism were assumed to be similar to human caries. The ability of Lauricidin^R to inhibit dental caries which occurred after inoculation in rats implied an ability to inhibit dental caries in humans, however, Lauricidin^R would have to be tested in a human population for conclusive evidence.

It was previously demonstrated that several dietary fats inhibited the production of dental caries (Rosebury and Karshan, 1939; Gustafson et al., 1955). In the present studies diets containing Lauricidin^R were compared with diets containing equivalent amounts of Crisco^R. The greater ability of Lauricidin^R to inhibit dental caries production showed that Lauricidin^R had a specific inhibiting action beyond that of another fat. It is possible that Lauricidin^R inhibited the growth of S. mutans and therefore inhibited dental caries that result from infection

with S. mutans. This will only be determined after the microbiological data collected in this study have been analyzed.

Reduction of dental caries in rats has been associated with a reduction in the frequency of food intake (Larson et al., 1962). In the present studies frequency of food intake was not recorded or controlled. Since the only significant differences in cumulative food intake between treatment groups were between groups which were not compared for dental caries and since there were no significant differences in cumulative weight gains among the treatment groups it is assumed that the rats in the various groups experienced equal exposure to the cariogenic diet.

Inhibition of Dental Caries by Lauricidin^R on the Various Surfaces of the Teeth

Incidence and severity of carious lesions were recorded and totaled separately for three types of surfaces found on the teeth, namely, buccal/lingual surfaces, interproximal surfaces and sulcal surfaces. Smooth surface lesions are caries found on the buccal, lingual and interproximal surfaces of the rat molars. Buccal and lingual caries are those found on the surfaces of the teeth exposed to the cheek and tongue respectively. Interproximal lesions are caries which are found at or below the contact points between two molars. Sulcal lesions are caries found on the fissures of the occlusal surfaces of the rat molars.

Proximal and sulcal sites differ from buccal/lingual sites because they are less exposed to the oral cavity, they are more retentive sites for food debris, and they may form a special ecological niche for development of specific micro-organisms (Navia, 1977). Proximal and buccal/lingual sites, however, are more dependent on the development of plaque than sulcal caries are (Shafer et al., 1974). Because of these important differences in etiology a separate discussion of caries activity in the proximal, buccal/lingual, and sulcal sites is essential.

Sulcal Caries

In both Study I and Study II rats which were not inoculated with S. mutans had mean caries scores in sulcal sites which were not statistically different from the mean caries scores in the same sites in inoculated rats. This is consistent with the observation that the normal flora of Osborne-Mendel rats cause some fissure caries (Newbrun, 1977). Factors responsible for caries in the occlusal sulci of the rat molars were neither hindered nor stimulated by the addition of S. mutans. The added S. mutans was able to effect a symbiotic relationship with the native oral flora of the rats (Newbrun, 1977).

The addition of Lauricidin ^R to the diet of uninoculated rats in Study II caused a significant reduction in sulcal caries. This proved that Lauricidin ^R could

inhibit caries which were due to factors other than S. mutans. The addition of Lauricidin^R to the diets of inoculated rats in both Study I and Study II caused a significant reduction in sulcal caries. The inhibition in this case, however, was not greater than the inhibition seen in non-inoculated rats so any specific action of Lauricidin^R on caries that were the result of inoculation could not be identified.

Interproximal Caries

In both Study I and Study II rats which were not inoculated developed proximal caries. In both studies mean caries scores for proximal caries due to inoculation with S. mutans were approximately double the scores for rats that had not been inoculated. Although proximal caries are similar to sulcal caries because both types of caries may be partially caused by the trapping of food particles, proximal caries are also stimulated by the presence of dental plaque (Shafer et al., 1974). S. mutans has been frequently isolated from the plaque of caries active population (Hoerman et al., 1972) and is therefore suspected of being one of the plaque forming organisms in humans.

The fact that there was an insignificant reduction in proximal caries when various doses of Lauricidin^R were fed to uninoculated rats could be due to the very low mean

caries scores of rats in the group that was not inoculated. The levels of caries may already have been so low that addition of an inhibitory agent could not produce a significant effect.

The addition of Lauricidin^R to the diets of inoculated rats caused significant reductions in mean caries scores on the proximal surfaces. A specific inhibition of those proximal caries which were caused by the presence of S. mutans was implicated.

Buccal and Lingual Caries

Rats which were not inoculated with S. mutans developed very few buccal/lingual lesions in Study I and developed no carious lesions in Study II. Inoculation with S. mutans resulted in large increases in buccal/lingual caries in rats in both studies. The occurrence of caries on buccal/lingual surfaces is similar to the occurrence of caries on proximal surfaces. Both are smooth surfaces and caries on smooth surfaces are known to be dependent on plaque formation (McDonald, 1977; Newbrun, 1977). S. mutans is one of the only bacteria that has been successfully used to produce smooth surface caries in animals (Newbrun, 1977). Although rats do not develop grossly visible amounts of plaque when infected with S. mutans, it is assumed that caries that result due to the presence of S. mutans are due to the initiation of plaque formation (Navia, 1977).

The addition of Lauricidin^R to the diet of inoculated rats in Study I caused a 50% reduction in buccal/lingual caries. The reduction was not significant due to wide variation in the extent of caries in both Lauricidin^R fed rats and control rats. A large coefficient of variation frequently occurs in rats fed cariogenic diets and inoculated with S. mutans but to be sure that it was not due to the possible unequal exposure of rats to Lauricidin^R, in Study II special attention was paid to the mixing of all diet ingredients to be sure that both the Lauricidin^R and the control fat were well distributed throughout the diets. The addition of Lauricidin^R to the diets of inoculated rats in Study II caused from a 62% to an 87% decrease in the number of rats which developed buccal/lingual caries. The mean caries score per rat was 78% to 90% lower and all reductions were statistically significant ($p < 0.005$).

Effect of Various Doses of Lauricidin^R

Reduction in mean caries scores was found to be as great in rats fed a diet containing 0.5% Lauricidin^R as in rats fed the same diet containing 2% Lauricidin^R. This was true on the proximal, buccal/lingual, and sulcal surfaces of the teeth. The fact that a diet containing 0.5% Lauricidin^R was as effective in reducing caries as a

diet containing 2% Lauricidin^R may be due to the existence of a critical dosage similar to that demonstrated by Kabara et al. (1979) in an in vitro study of the inhibition of S. mutans by Lauricidin^R. In that study it was found that the killing index of Lauricidin^R was more dependent on time of exposure to Lauricidin^R than on concentration of Lauricidin when the concentration of Lauricidin^R was increased beyond a critical level.

Distribution of Caries in the Right and Left Jaws

The fact that no significant differences were found between mean caries scores on the right and left sides of the jaws of rats in each of the treatment groups in Study I confirmed the data of Schemmel et al. (1979). This finding is consistent with human studies (Shafer et al., 1974) and it indicated that the sonication procedure used on the jaws did not damage the teeth or lead to an alteration in detection of caries.

Distribution of Caries in the Maxilla and Mandible

The fact that rats in all 3 treatment groups had significantly more buccal/lingual and sulcal caries in the mandible than in the maxilla is consistent with previous rat studies (Shafer et al., 1974). Although humans usually have a greater number of carious teeth in the maxilla (Shafer et al., 1974), the mandibular molars develop

carious lesions sooner than the maxillary molars and attain maximum caries development by age 20 more often than maxillary molars (Rowe et al., 1976). The fewer number of teeth with caries in the mandible is due to the relative immunity to caries of the human mandibular incisors (Shafer et al., 1974). Since the present study was concerned only with the molar teeth of the rats, the higher percentage of buccal/lingual and sulcal caries in the mandible was indicative of a similarity between dental caries production in the rats in this study and dental caries development in humans.

Since the occurrence of proximal caries in the maxilla was not significantly different from the occurrence of proximal caries in the mandible in either of the groups of rats which were not fed Lauricidin^R, proximal caries are probably caused by a combination of factors which are equally present in the maxilla and mandible.

Reduction of Caries by Lauricidin^R in the Maxilla and Mandible

Reduction in buccal/lingual caries due to the addition of 2% Lauricidin^R to the diet occurred exclusively in the mandible. Reduction of sulcal caries due to the addition of 2% Lauricidin^R to the diet occurred predominantly in the mandible. Reduction of proximal caries due to the addition of 2% Lauricidin^R to the diet was greater in the

mandible although there was a significant reduction in both the maxilla and mandible.

Although previous studies with Lauricidin ^R have not demonstrated this tendency, Lauricidin ^R showed a definite tendency in the present study to inhibit caries on all 3 surfaces more in the mandible than in the maxilla. It is possible that other factors were already exerting a maximum inhibitory action in the maxilla and therefore Lauricidin ^R was ineffective as an addition to those factors.

CONCLUSIONS

A food grade lipid, Lauricidin^R, inhibited the severity of dental caries by 25% to 35% on the sulcal surfaces, by 42% to 56% on the proximal surfaces, and by 78% to 90% on the buccal/lingual surfaces of the teeth when included in a cariogenic diet fed to rats that had been inoculated with Streptococcus mutans. Lauricidin^R also inhibited the severity of dental caries on the sulcal but not the smooth surfaces of the teeth of rats which were not inoculated with Streptococcus mutans. The inhibitory effect of Lauricidin^R at a dietary level of 0.5% was not significantly different from that at the 2% level.

Conclusions regarding the inhibition of dental caries by Lauricidin^R in humans cannot be made. However, the present studies suggest that its potential for inhibition of caries in humans should be tested.

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