Abstracts of Steinman

Compiled by the International Academy of Microendocrinology

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Ralph R. Steinman: He was born in Asheville, N.C. in 1910. He graduated from Emory University, School of Dentistry in 1938 and served 31/$_2$ years in the Army Air Corps as a dentist during World War II. He graduated with a M.S. from the University of Michigan in 1953. He has been teaching and doing research on dental caries at Loma Linda University, School of Dentistry since 1953. With Dr. John Leonora he is a co-discoverer of the Hypothalamic-parotid gland endocrine axis which controls the rate of fluid movement in the dentin. This axis plays a major role in the physiological activity in the tooth and when it fails, the tooth is subject to dental caries. He has published several articles on this and related subjects.

CARIES AND CELLULAR NUTRITION, RALPH R. STEINMAN/DENTAL PROGRESS, VOL. 2; #3 APRIL 1962

The mere presence of bacteria on the surface of a tooth does not necessarily mean that caries will follow. Whether the microorganism penetrates the tooth depends in part upon that toath's metabolism Only when the metabolism is impaired can noxious material enter and encourage further ingress of more noxious material until gross disease is present. To understand the pathogenesis of dental caries, one must realize that many of the metabolic processes observed in teeth are similar to those of other organs. The chemical composition of the liquid parts of dentin is similar to that of interstitial fluid. It contains amino acids, glucose, manganese ,iron, calcium, phosphorus, copper, and sodium. There is a rapid exchange between this fluid and other fluids of the body, and this exchange rate varies.

The first line of defense both of the body in general and of the teeth specifically is an impermeable barrier to external noxious materials. The tooth, like the skin, does have a mechanical barrier; but the tooth also depends upon metabolic "shields". Some of these shields are **ATP,** ADP, acetylcholine, coenzyme A, ATPase, cholinesterase, and sulf-hydryl. It has been shown that some of the metabolic shields were absent in dentin adjacent to pulp at the first evidence of pathology. Similarly, animal studies have shown that when some of these factors are blocked, methylene blue can penetrate through both enamel and dentine; and an excess of sugar has the same effect as a deficiency of phosphorus in this regard.

What is more, stress, by altering electrolyte balance, can also selectively alter the permeability of cell membrane and tooth structure.

There are other metabolic factors. Hamsters which develop spontaneous diabetes also develop rampant caries—even on a non-cariogenic diet. It has been reported that where

thyroxin is deficient, caries incidence is higher. Pyridoxine-deficient rats, hamsters, and monkeys have more decay than do animals receiving adequate amounts of pyridoxine. Castration of animals decreases caries incidence, perhaps because of a lowered serum serum sodium level. Heredity apparently is another factor. Other studies have shown that nutritional elements alter the incidence of caries.

The logical approach, then, to caries control is a way of life which includes a sound nutritional program and freedom from stress. For many individuals this would represent a changed way of life. But there is much evidence that the answer to caries lies in nothing less.

A POSSIBLE PHYSIOLOGICAL MECHANISM OF RESISTANCE TO DENTAL CARIES. RRS, DDS, MS
REPRINTED FROM THE JOURNAL, SOUTHERN CALIF. STATE DENTAL ASSN., VOL. XXXIII, NO. 11, NOV. 1965, JUNE 28, 1965

The problem has been to find some physiological process which is related to metabolism which will influence the tooth from the pulp to the surface of the enamel. Furthermore, this metabolic process must be able to counteract some attacking agent of microbial origin which may be an essential agent in the production of dental caries.

Anaerobic growth is known to occur in the protected areas of the dental plaque and in the depth of pits and fissures. Preliminary measurements of these protected areas would suggest that they have a low electrical potential. No doubt the gaseous phase of these areas contains significant amounts of carbon dioxide as this is the end product of considerable microbial metabolism.

The metabolic activity of cells greatly influences the redox potentials of the environment by reducing the oxygen tension and shifting the potential toward a more negative value. Histochemically it has been shown that a concentration of metabolic activity is occuring in the odontoblastic layer of the pulp whose processes extend to the enamel.

It is apparent that the artificially applied negative electrode to the pulp cavity of the tooth in this study maintained the integrity of the enamel in the destructive carbon dioxide atmosphere. One way in which metabolism could effect resistance of the tooth to the surface of the enamel is by maintaining the correct electrical potential. If resistance is to be effective and if it is related to metabolism then there must be some process by which that metabolism must affect the tooth to the surface of the enamel.

THE PHYSIOLOGICAL BASIS FOR CARIES SUSCEPTIBILITY AND RESISTANCE RRS, REPR. FROM THE JOURNAL, SOUTHERN CALIFORNIA STATE DENTAL ASSN., VOL. XXIX, NO. 7, JULY 1961

As yet, there is no evidence to suggest that hardness or density of tooth structure confer immunity to caries. Clinically, it is observed that even hypoplastic areas do not necessarily decay. The striking effect of stress in increasing caries susceptibilty would suggest that there are systemic factors which have a marked influence upon caries susceptibility. Among other effects upon the system, this produces an altered electrolyte balance with a concomitant change in osmosis. The teeth of the mandibles placed in the solution of methylene blue showed a very marked penetration of the cusp tips and a uniform penetration of the enamel.

The methylene blue showed no penetration through the cusp tips of the molars of rats from 17 days of age onward. This is in marked contrast to the penetration found in the teeth in the removed mandibles. The only area which does show penetration is opposite the food in the depth of the grooves. Animals 40 days of age or older on the noncariogenic diet usually fail to show penetration even in this area. Those on the cariogenic diet obviously continue to show penetration in this area.

Those animals on the non-cariogenic diet which received the Hydrogen Peroxide whether by mouth, stomach tube, or by intraperitoneal injection, showed a very marked penetration through the tip of the cusp. This was evident after 48 hours of treatment. There was also a uniform penetration of the enamel of these teeth.

Some of the animals received Hydrogen Peroxide from 21 to 26 days of age and then were placed on water. Most of their teeth showed a marked recovery from the harmful effects of the peroxide.

The failure of the dye to penetrate the cusp tip of even the 17 day-old animals as well as its failure to penetrate at an older age in contrast to the marked penetration into the teeth of the mandibles removed from the animal, would suggest that prevention of penetration is a vital function of the tooth. The marked penetration of the teeth of animals receiving the Hydrogen Peroxide whether by mouth, stomach tube, or by intraperitoneal injection, would suggest that this vital process is systemic in nature, and if it has not progressed too far, recovery may occur. The localized penetration of the dye beneath the food and bacteria would suggest that this flow may also be influenced by external factors.

Isotope studies using small molecules show that molecules placed on the surface of the tooth may be picked up internally and those placed internally may be picked up on the surface of the tooth. Histologic examination of the teeth of patients receiving silver salts revealed a marked deposition throughout the dentin and in the deeper layers of the enamel. These studies suggest a two-way transport system within the dentin. If this transport system is functioning normally, it would appear that pathology does not occur; but if this transport is reversed by Hydrogen Peroxide, for example, very rapid pathology occurs. To better visualize this transport in health and disease, the following drawings are submitted:

In the normal transport, Figure 7, flow A is greater than B. This results in a flow force from within out. Flow C would then prevent any material from entering the tooth. Now if B is larger than A as in Figure 8, there would be a marked penetration of noxious material from without the tooth into the tooth. This material could reach the odontoblast and conceivably further reduce its ability to function properly.

This work would suggest that, as is true of other pathology, there is first an altered function of the tissue involved. In the tooth this altered function is characterized by an altered transport system through

the tooth structures. This altered transport system is largely controlled systemically, but can be altered by external factors which penetrate the enamel and are carried by this circulation into the deeper structures of the tooth. It is perhaps by this altered systemic function that the external noxious material arrives in the deeper structures to produce its pathology. This concept of transport in health and disease is in harmony with the findings of caries incidence as affected by the electrolyte balance as well as the marked effect of stress upon the incidence of caries.

Beneath the food on a broad front, a marked penetration of methylene blue was observed in animals on a cariogenic diet. This appears to be the result of a localised area of abnormal transport. McLeod and Gordon observed that oral streptococci produce Hydrogen Peroxide. Later work suggests that similar results may also be obtained when testing for the presence of Hydrogen Peroxide by reducing systems in the absence of air. It would thus appear that Hydrogen Peroxide or some closely related compound may be involved in the early pathosis of caries. This early pathology is characterized by an altered transport system within the tooth which allows noxious material to penetrate deeply into the tooth.

THE EFFECT OF PYRIDOXINE AND INJECTED CARBOHYDRATES ON INCIDENCE OF CARIES, DENTINAL CIRCULATION RELATED TO DIET. **RALPH R. STEINMAN, MERVYN HARDINGE, M.D.,**

PH.D., REPRINTED FROM JOURNAL OF DENTAL RESEARCH, ST. LOUIS, VOL. 37, #5, PG. 874-879, SEPT-OCT, 1958

A long-term experiment was carried out in which littermates were divided into two groups at weaning. One group was placed on Purina, the other on the synthetic diet previously described, cariogenic. Into all animals, 0.5 ml. of 2 per cent trypan blue was injected intraperitioneally once a week for 3 weeks.

At 25 days of age, gold crowns were placed on the lower right first molars of 3 rats in each group. After 12 weeks all animals were sacrificed and their teeth examined for concentration of the dye. The teeth of the animals on the Purina were significantly darker than those on the cariogenic diet. No color difference was observed between comparable crowned and uncrowned teeth in the same mouth.

The marked difference in the ability of dentin to concentrate trypan blue in animals on the noncariogenic and cariogenic diet would strongly suggest that the "circulation" differs in caries-resistant and caries-susceptible teeth. This postulate is further strengthened by the structural differences, that is, the ring formations in the secondary dentin of animals on the cariogenic diet which are not present in the dentin of animals fed Purina.

The bypassing of the oral cavity by parenterally administered monosaccharides (glucose and fructose) indicates that their cariogenic effect results from systemic action. It would thus appear that carbohydrates may increase the production of caries by both the well-established local action and the currently demonstrated systemic effect.

In a previously reported paper, it was demonstrated that the nutritional status as reflected by physical weights and measurements was found to be closely correlated with the incidence of caries. In general, as the animals deteriorated from the first through the third generation, there was also a corresponding deterioration of the teeth. In two groups there was a deterioration from the first and third generation. The teeth in these groups paralleled the nutritional state, poor teeth in the second generation and good teeth in the first and third generations. Those groups in which the teeth remained uniformly good through the 3 generations were found to be in a uniformly good physical state.

From a review of the literature, it is clear that evidence indicates that the production of caries in the posteruptive period is affected by factors which may influence circulation, nutrition, and metabolism

Brewer, Muhler, and Fischer demonstrated that the decreased vascularity resulting from ligation of the major vessel to the teeth caused a significant increase in caries. The chewing of hard substances was found by Neumann, Lefkowitz, and DiSalvo to reduce the incidence of caries. This might be explained by the resultant stimulation of circulation.

Several factors affecting both metabolism and caries have been reported. As already mentioned, the incidence of caries in hamsters and monkeys can be influenced by the concentration of pryidoxine in the diet. Bavetta and McClure, McClure, and others found that L-lysine deficiencies increased the incidence of caries. X-ray therapy was shown by Schlack and Effinger to augment the caries process in experimental animals. Del Regato has also observed this clinically. Whether these latter observations are a result of disturbed circulation or metabolism has not been resolved. Bixler, Muhler, and Shafer found androgens increased caries in rats. Workers in this group also demonstrated that by altering the concentration of circulating thyroxin the caries process could be retarded or augmented. Mitchell and Johnson observed that the administration of acetazoleamide (Diamox) significantaly increased caries in hamsters. Whether the role of this compound as a carbonic anhydrase inhibitor is implicated in its cariogenic action has not been established.

INTRAPERITONEAL INJECTIONS OF CARBOHYDRATES TO SUCKLING RATS, AND SUBSEQUENT DENTAL CARIES. RALPH R. STEINMAN, MELVIN HALEY, AND PAT O'DAY, REPRINTED FROM JOURNAL OF DENTAL RESEARCH, ST. LOUIS, VOL. 37, NO. 4, PG. 719-721, AUGUST, 1958.

Previous work has shown that when a carbohydrate is given to caries susceptible rats during the suckling period in addition to mother's milk, a subsequent increase in caries occurs. Since the molars had not errupted in the mouth until the end of the suckling period, this effect must have been other than local. This investigation was carried out to determine whether this increase in caries was the result of a digestive disturbance or mainly systemic in nature.

The injection of a mixture of glucose and fructose to suckling rats 3 times a day during the suckling period produced a subsequent increase

in caries. This increase in caries by injection of various monosaccharides is similar to the increase produced by the oral administration of the same carbohydrate.

It would appear that the injection of a monosaccharide ultraperitoneally during the calcification of the molars caused a subsequent

increase in caries over that in littermates not so treated. This increase in subsequent caries by injection is comparable to the increase in subsequent caries following the oral administration of a carbohydrate over that in littermates not so treated.

The administration of a carbohydrate by mouth is subject to variations which are not encountered by intraperitoneal injections. The amount of carbohydrate administered is of importance. In a study in which an artificial formula was fed to the young during the last third of the suckling period, an increase in the amount of a given carbohydrate caused a corresponding increase in caries. The milk of rats contains about 3 per cent of carbohydrate.

The reduction in subsequent caries in those animals receiving the vitamins C and B_6 in addition to the 20 per cent of glucose and fructose solution might indicate that the administration of the added carbohydrate alone was diluting the diet received by the young. Vitamin C has been shown to be necessary during the calcification of the teeth. Although rats are able to synthesize vitamin C, at this age this may not be possible in adequate amounts under adverse conditions. The milk of rats contains some vitamin C normally. A relationship between vitamin B_0 deficiency and dental caries has been proposed. Vitamin B_0 is also found in rats' milk.

EFFECT OF PREWEANING ADMINISTRATION OF LACTOSE ON SUBSEQUENT CARIES SUSCEPTIBILITY. RALPH R. STEINMAN AND MELVIN I. HALEY, PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 1958, V97, 274-275.

Previous work showed that administration of a 20% sucrose solution 3 times a day to suckling white rats during calcification of their molars produces a highly significant increase in the subsequent caries score as compared with unsupplemented littermates. This technic

which measures only the systemic effect of supplements apart from local effects thus appeared useful in studying the systemic effect that other carbohydrates may have on subsequent caries. The 10% difference in caries score between experimental and control animals was not significant. This is in marked contrast to results obtained when a 20% sucrose solution was administered in equal dosage to suckling rats. In previous work, 20% sucrose solution given under similar circumstances caused over a 100% increase in caries as compared with unsupplemented littermates.

CAN DENTAL CARIES SUSCEPTIBILITY BE AFFECTED BY THE INGESTION OF CARBOHYDRATES?

RALPH R. STEINMAN REPRINTED FROM THE JOURNAL OF THE INDIANA STATE DENTAL ASSOCIATION, VOL. 39; 130-134, APRIL 1960

Dental caries is one of the most prevalent diseases of civilized man. It would appear that there are three conditions which must exist before this disease can occur. Studies with germ-free animals, by Orland and associates, would indicate that bacteria are essential in this process. Further studies should reveal the specific organisms involved and the part each may play. It was observed, by Kite and associates, that when animals are fed only by means of a stomach tube no caries occurred. It would seem that food in the oral cavity, especially fermentible carbohydrate, is an essential requirement for this disease. It is a common observation that these two requirements can be present in the oral cavity and still no caries may result. Whether caries will be present or not depends upon a third condition—the susceptibility of the teeth.

It is becoming more apparent that the systemic environment present during the formation of the teeth largely determines their subsequent susceptibility to disease.

Certain vitamins are known to be essential during the calcification process of the teeth. Vitamin A is necessary in the formation of enamel. It would appear also that vitamin′ C is one of the essentials, along with vitamin D. Minerals must be present during this period in order for calcification to proceed properly. Calcium and phosphorus have been shown to be essential.

During calcification, metabolic activity is high throughout the whole tooth. It would, therefore, be reasonable to assume that the health and welfare of the forming tooth is intimately associated with the health and welfare of the individual as a whole.

Secondary nutritional factors, as well as primary nutritional factors, may affect with equal force the nutritional status of the individual. The correct foods may be ingested but the body may fail to utilize them properly. The complexity of this may be illustrated by the problems encountered in the absorption of calcium and phosphorus. The absorption of calcium and phosphorus is favored by an adid medium which helps maintain the salts in solution, and it is, favored by Vitamin C and **D** and by the presence of lactose. The calcium-phosphorus ratio should be correct to favor absorption. It would appear that not only must the foods be present but they must be available in the right proportions to be utilized efficiently.

In recent years some interesting observations, by Sognnaes, have been made with animals on a purified ration relative to the incidence of caries. These diets were stated to be complete in all known essentials but differ from natural diets in that they are very low in fat and all of the carbohydrate is furnished in the form of sucrose. With rats, hamsters, and mice, it was found that the offspring of mothers subsisting on this diet had a significantly higher incidence of caries than did the offspring of mothers subsisting on natural diets. Studies have been reported by Steinman and Haley concerning the possible role an excess of carbohydrates administered during the suckling period may have increasing caries susceptibility. In the early work reported by these workers various carbohydrates were administered by means of an eye dropper three times a day to suckling rats. When sucrose was the carbohydrate administered to one group of suckling rats and water was given to their litter mates, it was found that those receiving the sucrose had over twice as much decay subsequently as did the litter mates which received only water.

In such studies it was apparent that the greater the amount of a carbohydrate added to the formula the greater would be the subsequent increase in caries. As observed previously, the carbohydrate giving the least increase in subsequent caries was lactose. Sucrose, Karo, and dextro-maltose caused the greatest increase in subsequent dental caries.

In areas of the world which consume a high carbohydrate diet and yet have a low incidence of caries, like India and China, it is found that the carbohydrate is largely in the form of starch.

All known carbohydrates are broken down into simple sugars before absorption. From what is known of the metabolic cycle of sugars there is no significant difference in the way simple sugars are metabolized. There are two possible explanations as to why the added lactose caused the smallest increase in subsequent caries. It is known that lactose favors the absorption of minerals, such as calcium and phosphorus. The second possible explanation is that the lactose is absorbed into the blood much slower than the other sugars and as a result caused less change in the blood glucose level. Along with a rapid rise in the blood sugar, a decrease in serum inorganic phosphate was observed. This decrease in readily available serum inorganic phosphate is reflected in the developing tooth buds of the suckling rats. A decrease of approximately 10 per cent of the total phosphate content of the molar buds of these animals treated with the sugars was found. It could be that this altered phosphate content of the teeth causes the teeth to be more susceptible to caries.

It would appear that because lactose in the diet favors the absorption of certain minerals and because it is absorbed slowly, thereby affecting the phosphate level of the blood serum but slightly, it has the least effect in increasing caries susceptibility.

It is interesting to observe that in diets in general the carbohydrate portion makes up the bulk of its caloric content. Yet all diets of equal carbohydrate content do not result in the same amount of caries. When the high sugar content of synthetic diets is replaced by a polysaccharide (e.g., starch), there is a marked decrease in caries. It was also observed that when some of the carbohydrate in the diet was injected intraperitoneally that an increase in caries resulted. It could be that ingested sugar which is absorbed rapidly has a systemic effect in addition to the well known local effect. It might be suggested from this, since the bulk of the caloric content of the diet is carbohydrate, that it should be on the kind which is slowly absorbed, rather than the rapidly absorbed sugars.

The physiologic effect a given carbohydrate may have upon an animal probably depends upon many factors. It could be related to the amount of carbohydrate in the diet, the speed of absorption, the availability of phosphate for phosphorylation, endocrine balance, and the presence of the nutrients necessary for metabolism. This balance, then may be disturbed by a number of factors other than the amount and type of carbohydrate in the diet. A physiologic excess of carbohydrate may be produced by a sudden influx into the blood of some simple sugar from a diet which appears to be well balanced. Since caries susceptibility is somewhat related to a mineral balance, particularly in the formative period of the teeth, it would be wise to disturb this balance as little as possible. It also appears that this balance is disturbed more by a rapidly absorbed sugar than by one absorbed more slowly. The carbohydrate which should comprise the bulk of the caloric requirements of the individual should be one which is digested and absorbed slowly, namely starch.

During the suckling period the carbohydrate of choice is lactose, because it is absorbed slowly. If breast feeding is impossible, then the formula that is used should be one in which lactose in incorporated as the carbohydrate. There are several formulae on the market which have lactose as the carbohydrate.

EXPERIMENTAL CARIES WITH HUMAN FOODS. RALPH R. STEINMAN, MERVYN HARDINGS, M.D., PH.D., AND ROBERT WOODS, PH.D. REPRINTED FROM JOURNAL OF DENTAL RESEARCH, ST. LOUIS, VOL. 37, NO. 5, PG. 865-873, SEPT-OCT, 1958

A critical analysis of the data obtained from three generations of 11

rats fed on eight different combinations of human foods is presented. The nutritional status and physical well-being of all animals showed an inverse correlation with caries incidence. It would appear that with these diets, good nutritional statis is associated with a low caries incidence while nutritional deterioration is accompanied by an increase in caries.

THE POSSIBLE ROLE OF HYALURONIDASE IN THE INCIPIENT CARIOUS LESION. RALPH R. STEINMAN, REPRINTED FROM THE JOURNAL OF THE SOUTHERN CALIFORNIA STATE DENTAL ASSOCIATION, VOL. 28, NO. 7, JULY 1960

Nine animals receiving the antihyaluronidase died before the end of the experiment. The 31 surviving animals on the 1 per cent antihyaluronidase diet had an average of 0.8 incipient lesions per animal, whereas the 30 animals which did not receive the antihyaluronidase had an average of 4.09 incipient lesions per animal.

The antihyaluronidase as used appeared to be toxic as shown by the number of deaths occuring within the 10 day experimental period. It might be suggested that the material was also toxic to the bacteria and produced its results as a result of this toxicity. In spite of the deleterious effect upon the animals it seemed to significantly reduce the number of incipient lesions.

It is of interest to note that conditions which affect the permeability of other tissues affect the incidence of caries. For example, younger animals have more permeable tissues than do other animals. Undernourished animals have less permeable tissues than do animals receiving rations in adequate amounts. It is well known that younger animals and individuals have far more decay than more mature individuals. It is also well known that people in famine areas have less decay than do well-nourished groups.

The first line of defense of a tooth or the body is a relatively impermeable natural covering. The results of this study would suggest that hyaluronidase of bacterial origin may play a part in the production of the incipient carious lesion by making the first line of defense of the tooth more permeable to noxious bacterial agents. An antihyaluronidase administered in animals on a cariogenic diet has been found to significantly reduce the number of incipient carious lesions.

This finding would suggest the possible role of bacterial hyaluronidase in the carious process.

THE EFFECT OF STRESS UPON THE INCIDENCE OF DENTAL CARIES. RALPH R. STEINMAN. REPRINTED FROM THE JOURNAL OF THE SOUTHERN CALIFORNIA STATE DENTAL ASSOCIATION, VOL. 28, NO. 11, NOVEMBER, 1960.

The confined animals had a rather poor general appearance and on the average weighed about 22% less than the animals allowed to exercise. The confined animals which were exercised in general appearance were superior to the animals receiving no release from the confining cages.

As was found previously, the animals which were confined had significantly more caries than did the animals not confined. In the first experiment there were 179 lesions in the 12 confined animals, an average of 14.9 lesions per animal. In the animals which had exercise and freedom to more there were 71 lesions in the 12 animals, which is an average of 5.9 lesions per animal. The control group which were allowed to run free from 21 to 35 days of age in a large cage had 43 lesions, an average of 4.8 per animal.

All of these animals were on the same diet. All comparisons were made between litter mates. It is obvious that the confinement of the animals significantly increases caries susceptibility. In the first experiment two conditions were imposed upon the animals, one of stress and the other a lack of exercise. In the second experiment the one group of confined animals did have exercise, but even though this did improve the general appearance of the animals it did not significantly reduce their caries susceptibility. Perhaps in a longer period of time there might be found a significant difference. The results of this experiment would suggest that stress may be a significant factor in increasing caries susceptibility, at least in young animals.

THE INCIDENCE OF DENTAL CARIES AS ALTERED BY ELECTROLYTE AND WATER BALANCE.

RALPH R. STEINMAN. REPRINTED FROM THE JOURNAL OF THE SOUTHERN CALIFORNIA STATE DENTAL ASSOCIATION. VOL. XXIX, NO. 4, APRIL, 1961.

In recent experiments it was found that stress caused a marked increase in the incidence of the incipient carious lesion. Since the same food and water was available ad libitum to both groups of animals, it would appear logical to assume that increase in caries susceptibility produced by stress was probably of a systemic nature. It is well known that non-specific stress will cause an increased secretion of corticotropin. It is further known that the administration of corticotropin to normal animals will, among other effects, cause a retention of sodium chloride. Since the water transport system could be altered under these circumstances, the nutritive state of every cell in the body might be affected, for by means of water, all nutrition is carried to the cell and all waste products carried from the cell. The major electrolytes in the extracellular fluid which largely control osmotic pressure and water balance of the system are sodium and chloride. The nutritive state of the cell assumes importance in caries when it is recalled that ligating arteries to teeth increased caries in those teeth. This procedure would most certainly alter the nutritive state of the teeth so treated.

It was found that the saline caused a significant increase in the number of incipient carious lesions. The control group had a score of 6.77, whereas the experimental had a score of 10.34. A chi square analysis of the data gives 1000 to 1 that the observed differences were not due to chance. In the second experiment utilizing animals which were older, it was found that the drinking of the 0.9% saline had no affect upon the incidence of caries. These results are summarized in Table II.

In the third experiment it was observed that DOCA produced a significant increase in the incidence of caries. The control animals had a score of 5.81 while those receiving the DOCA injections had a score of 9.40. The chi square analysis of this date gives better than 1000 to 1 that the observed difference was not due to chance. Table III summarized the differences found in this experiment.

The fourth experiment revealed that the administration of Diuril, a diuretic, which causes the excretion of sodium chloride decreases the incidence of dental caries. The control group of animals had a score of 6.46 lesions per animal whereas those which received the Diuril 1 mg/1 gm ration had a score of 3.60 lesions per animal.

In 1933 Moore suggested that the ingestion of large amounts of NaCl might be a contributing factor in the large amount of dental caries among civilized groups. He suggested that its effect may be due to an altered nutritive state of the tooth. In a recent report it was found that those individuals with a large amount of chloride ion in the saliva had, in general, a higher incidence of caries. The part the chloride ion was thought to play in the increase in caries was local. It is well known that the chloride ion is necessary for amylase activity. From this it was suggested that the chloride ion in the saliva would facilitate the breakdown of starch to acid within the mouth. However, in the present work, this could not be a factor since the cariogenic diet used contained no starch. The increase in chloride ion in the saliva might be a reflection of an increase in chloride ion in the plasma. It is generally recognized that newly erupted teeth are significantly more caries susceptible than are the teeth of older animals. It is also recognized that the metabolic activity within newly erupted teeth is higher than in more mature teeth. The difference in caries response between the older and younger animals given the 0.9% saline to drink might suggest that the added saline interferes with the normal metabolism and maturation of the teeth.

It is generally thought that the increase in caries accompanying the removal of the major salivary glands of the rat is the result of a local action upon the surface of the tooth. In this connection it is well to note that the removal of the major salivary glands is accompanied by an increased adrenocortical activity. The adrenal cortex plays a major role

electrolyte balance. It has further been noted that these animals grow more slowly than do normal rats. There is the possibility that the salivary glands produce a hormone which may account for the slower growth of animals with the salivary glands removed. It is also known that some sex hormones cause a rise in the concentration of serum soduim. Could this explain the reduction in caries following the castration of young animals?

The amount of salt which is excreted would depend, in part, upon the available water supply. If the amount of water is limited, there would be corresponding reduction in the amount of salt excreted by the animal. This would suggest that the degree of hydration is a factor in caries susceptibility. It is possible that the increased concentration of NaCl within the body and the tooth could affect its health and welfare in two ways. Since the electrolytes (Na and Cl) play a major role in controlling osmosis and water transport, any interference with the normal function of this system could alter the nutritive state of the tooth and thereby alter maturation and resistance to disease. Any change in osmosis within the tooth might alter its relationship with its external environment. It is possible that under certain circumstances the normal osmotic pressure would repel noxious bacterial agents while under other circumstances of abnormal osmotic pressure it tends to encourage the ingress of noxious material into the tooth. Thus the electrolyte balance of the tooth may play a vital role in susceptibility or resistance to disease.

Many and varied are the alterations from normal physiology brought about by stress. Under stress the nutritive state of the animal may be altered by loss of appetite, by changes in digestion, and intestinal function. If, superimposed upon these alterations, a change in the water transport system is produced, the resulting changes could affect every cell and fiber of the system.

HEREDITY, ENVIRONMENT, DIET, AND CARIES IN CHILDREN. RALPH R. STEINMANN, D.D.S., M.S. AND ROBERT W. WOODS, PH.D. REPRINTED FROM THE JOURNAL, SOUTHERN CALIFORNIA STATE DENTAL ASSOCIATION, VOL. XXXII, NO. 5, MAY 1964.

In this study of the 278 children from four to twenty years of age, heredity seemed to play no part in determining the presence or absence of caries in these children. This is in harmony with the work of Schoenthal and Brodsky, but is at variance with the conclusions of Dahlberg and Dahlberg. Of considerable interest are the findings that those who had no decay ate less frequently of white bread and packaged cold cereals. They also ate more frequently of whole grain cereals than those who had decay. The statistical significance of these three differences varied from 90% to 99%. The agreement of these three items lends emphasis to the importance of whole grain cereals in the

diet. These results are in harmony with animal experiments which show beneficial effects of whole grain products in the diet. Primitive peoples usually had significantly less decay than the same racial stock who received the refined foods of civilization. This is usually suggested to be the result of the addition of sugar to the diet. No mention is made of the refined cereal products which are also added to the diet. From the present study one might suspect that refined cereals might be a factor in caries production.

The between meal eating habits of those who reported decay deserves attention. It would appear that more of those who had decay ate between meals more frequently than those who had no decay.

THE EFFECT OF COW'S MILK, SOY MILK, AND CHOCOLATE MILK UPON THE INCIDENCE OF CARIES IN THE RAT, RATHBUN, W. EUGENE, BOND, DELMER E. AND STEINMAN, RALPH R., D.D.S., M.S., REPRINTED FROM THE JOURNAL, SO. CALIFORNIA STATE DENTAL ASSN., VOL. XXXI, NO. 9, SEPTEMBER, 1963.

It is generally recognized that differences in diet may produce differences in growth rate and susceptibility to dental caries. The purpose of this study was to obtain quantitative information regarding the effects of cow's milk, soy milk, chocolate milk and a high sugar diet on the growth rate and caries incidence of an Osborn-Mendel strain of rats.

In all groups it will be observed that the chocolate milk produced about three times the incidence of decay as did the other milks or the cariogenic diet. Table I summarized the results of the first experimental group.

The growth rates of the three experimental groups were substantially less than the control group on a high sugar diet (cariogenic diet). The differences in the growth rates between the three groups was not of any significance. It is believed that there are two significant findings in this study. The first is that the rats fed chocolate milk developed a significantly greater number of carious lesions than the rats in any of the other groups. The second is that those rats given cow's milk developed a similar number of carious lesions to those on a high-sugar diet.

The factors responsible for the large increase in decay in those rats fed chocolate milk are not known. The amount of carbohydrate in the chocolate milk was much less than the carbohydrate content of the high-sugar diet. Chocolate milk contains about eleven percent carbohydrate and the control diet contains approximately 63% sucrose. Other factors in this increase in decay are not within the scope of this study.

It is of interest to note that those rats fed on cow's milk developed a similar number of carious lesions as those on the high-sugar diet.

However, it should be noted that these animals were placed on cow's milk at 15 days of age. It was shown by Anderson that under similar experimental conditions, rats fed milk fortified with the addition of iron, copper and manganese developed no dental caries. These elements were not added to the experimental group fed cow's milk in this study.

Admittedly, it is a poor nutritional practice to depend upon only one food, regardless of how good it may be, as the only source of nutrient. However, one would hardly suggest from these results that chocolate milk is a substitute for whole milk. Two questions might also be raised. Why should a food which has almost no fermentable carbohydrate in it, the cow's milk, produce decay? Why should a liquid food produce decay in an amount equal to that produced by a diet of solid foods?

THE POSSIBLE ROLE OF ACETYLCHOLINE ESTERASE IN RESISTANCE TO DENTAL CARIES. RALPH R. STEINMAN. REPRINTED FROM THE JOURNAL, SOUTHERN CALIFORNIA STATE DENTAL ASSN. VOL. XXX, NO. 11, NOVEMBER 1962.

Prominent among the enzymes and associated factors which are markedly reduced in the dentinal tubules almost to the pulp of the tooth beneath the first evidence of demineralization of the enamel is a choline esterase. In addition to its role as a chemical mediator of nerve activity the formation and breakdown of acetylcholine, a process which requires acetylcholine esterase may be related to selective permeability of cells. Considerable evidence suggests the role of selective permeability in resistance to dental caries.

An enzyme such as acetylcholine esterase cannot be completely inhibited in an animal without fatal results. The injection of an enzyme inhibitor probably reduces the activity of a given enzyme quite generally throughout the body of the animal. All tissues containing the enzyme in question would no doubt be effected.

The lesions in the animals on the non-cariogenic diet which had been given the antiacetylcholine esterase are very similar to the lesions found in the animals on the cariogenic diet. It would appear that the process of decay is similar in both cases. The local environment in the grooves of the teeth of the animals on the noncariogenic diet would be quite similar. Those animals which received the antiacetylcholine esterase most probably had decay because the resistance of the tooth was markedly lower by the administration of the antiacetylcholine esterase. The histochemical finding of the depletion of acetylcholine esterase in the dentin beneath the first evidence of demineralization in the enamel suggests its relationship to resistance to decay.

The production of caries in animals on a noncariogenic diet when given an antiacetylcholine esterase confirms the suggested retie of acetyl-choline esterase in resistance to decay. The formation and breakdown of acetylcholine esterase is known to be vitally connected with selective permeability. Histochemically many of the factors linked to the metabolic chain necessary for this process have been demonstrated in teeth and absent in the dentin beneath the first evidence of demineralization in the enamel. Many conditions which are known to increase the incidence of caries in animals such as stress, irradiation, diamox, and changes in the electrolyte balance are known to effect adversely this process. A deficiency of substrate to operate this system such as pyridoxine, pantothenic acid, phosphorus, and protein have under specific circumstances been found to be associated with an increase in caries. It would thus appear that selective permeability which involves among other metabolic factors acetylcholine esterase is related to resistance to caries and when this system is altered, if conditions are favorable, caries will occur.

It would appear that the term noncariogenic diet is a relative term and has significance only in terms of the resistance of the host. It further seems true that the determining factor is not necessarily the local environment, but the resistance of the host. It may not be what is upon the surface of the tooth that determines the course and extent of the disease, but rather what is allowed to penetrate into the tooth due to an altered selective permeability of the tooth. Specifically the metabolic process involved appears to include acetylcholine esterase.

MOLECULAR CHANGES OBSERVED IN DENTAL CARIES AND THEIR POSSIBLE NUTRITIONAL IMPLICATIONS, RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE JOURNAL OF APPLIED NUTRITION. VOL. 15, NO.'S 3 & 4, 1962. PAGES 188 TO 191.

Claude Bernard once stated, "The organism is an entity, a whole, from which no single part can be isolated." Could this be true of the teeth? Or are they an inert object left to the mercy of an unfriendly environment? Are they a living part of the whole? Resistance to infectious agents is usually considered to be a vital, systemic phenomenon. Is this true concerning the teeth? We often see what we are looking for. Perhaps I have looked too intently for the biological processes which may or may not be present in the teeth to correctly evaluate certain observations.

As an example of this process let us consider just two of the many molecules necessary for the life processes within the cell. Normally there is about four times the amount of sodium outside the cell as found inside, and there is about 18 times the amount of potassium inside the cell as found outside. These concentrations of sodium and potassium are maintained by what is often referred to as sodium pump. This pump moves the sodium from inside the cell to the outside of the cell.

The histochemical and radiographic findings can be summarized as follows: Beneath the first radiographic evidence of demineralization in the enamel in the dentin there appears to be a depletion or marked diminuation of cholinesterase, adenosinetriphosphatase, adenosinetriphosphate, sulfhydryl, and disulfide, and magnesium. This area of change extends practically the full length of the dentinal tubules reaching almost to the pulp. In the dentin more adjacent to the enamel beneath the first evidence of demineralization is also to be found an area of marked increase in reaction to ninhydrin, possibly denoting a breakdown of the protein matrix of this area. Thus it appears that caries is not a surface phenomenon but rather that it occurs in considerable depth. The deeper changes appear to be changes from normal metabolism as shown

by altered enzyme patterns and associated metabolites accompanied by a possible breakdown for the organic portion of the dentin in this same area but not extending to the pulp of the tooth.

Since it is to be observed that many conditions alter the incidence of caries, the control of this disease is not a simple matter that will respond to the administration of some pill, regardless of its content. If one considers the many factors which alter the incidence of caries, one must admit that the answer to this problem is a way of life. In this way of life one might include heredity, proper nutrition throughout the lifetime of the individual, freedom from undue stress, the use of water, sunshine, fresh air, exercise and any other environmental factor which is known to influence general health. This approach to the control of any disease is unpopular. Man desires to live as he pleases, to break all the laws of health but to avoid the consequences of his way of life. So far, science has failed to provide the answer to this kind of approach to health and disease. There is considerable scientific evidence that a program of prevention based upon sound biological principles is the answer to man's physical problems. Man desires to eat his cake and between meal snacks and have his teeth too. So far, it just cannot be done.

A DEMONSTRATION OF THE EFFECT OF HUMAN FOOD COMBINATIONS UPON DENTAL CARIES IN RATS, RALPH R. STEINMAN, D.D.S., M.S., MARSHA SAUNDERS, B.S., YVONNE GILLILAND, B.S., CAROL TAGUE, B.S. REPRINTED FROM THE JOURNAL SOUTHERN CALIFORNIA STATE DENTAL ASSN., VOL. XXXI, NO. 12, DEC. 1963.

In the first demonstration using the diets composed of the various combinations of human foods it was quite obvious that more decay was found in the groups receiving the refined foods. Although one could not point to any one food as being the controlling factor relative to the amount of caries, one could suggest that the addition of refined foods to the diet of the animals in some way increased the amount of decay experienced. Furthermore, the diet with the greatest amount of refined food in it (Group III) was associated with the poorest growth and the greatest amount of decay.

A characteristic difference between a cariogenic and a noncariogenic diet is that the non-cariogenic diet is usually composed of a wide variety of natural foods while the cariogenic diet is usually composed of a limited variety of foods some of which are highly refined. Primitive peoples subsisting upon natural foods usually are quite free from decay while the same racial stock subsisting upon the common foods of civilization have a significant amount of decay.

HISTOCHEMICAL ANALYSIS OF LESIONS IN INCIPIENT DENTAL CARIES. II. SULFHYDRL AND DISULFIDE RADICALS, RALPH R. STEINMAN, COLLEGE OF MEDICAL EVANGELISTS, LOMA LINDA, CALIFORNIA, REPRINTED FOR PRIVATE CIRCULATION FROM JOURNAL OF DENTAL RESEARCH, VOL. 39, NO. 5, SEPT.-OCT. 1960

In a previous report a number of histochemical changes preceding cavitation were shown to occur in the dentin just beneath the dentinoenamel junction. These included a cessation of oxidation reduction, an increased stainability of carbohydrate and protein, the presence of a silver nitrate-reducin gagent, and the loss of some mineral. The present paper extends these observations to a consideration of the possible presence of sulfhydryl and disulfied radicals in the dentinal tubules under normal and pathologic conditions.

It is well known that sulfhydryl groups are essential in a number of enzyme systems. It was previously shown that there occurs a failure of the oxidation-reduction system within the area of early pathology. The evident diminution of reactive sulfhydryl groups in the incipient carious lesion extends the description of this phenomenon and further implicates the organic phase of the tooth in the pathogenesis of this disease. Further studies are being conducted on the chemical identity of the affected radicals and on the microbial agencies which apparently block them.

HISTOCHEMICAL ANALYSIS OF LESIONS IN INCIPIENT CARIES, **RALPH R. STEINMAN, C. GORDON HEWES AND ROBERT W. WOODS, SCHOOLS OF DENTISTRY AND MEDICINE, COLLEGE OF MEDICAL EVANGELISTS, LOMA LINDA, CALIF.** **REPRINTED FROM JOURNAL OF DENTAL RESEARCH, ST. LOUIS, VOL. 38, NO. 3, PAGES 592-605, MAY-JUNE 1959**

With the use of tissue specimens prepared in a manner to minimize post-mortem changes, and with the aid of histochemical technics to be described, it has indeed been possible to demonstrate unequivocally subtle but striking functional and structural changes at the site of anticipated gross carious lesions which occur very early after eruption of the teeth.

Sections of animals 28 days of age on the cariogenic diet showed marked changes in the dentin in the depth of some of the major grooves. These changes are confined to the area just beneath the enamel adjacent to the impacted food. The dentin in this area stained black by the technic of Dranko and also by the technic of Macallum. Fig. 4B shows a typical section of a 28 day old animal stained by the technic of Eranko. The dentinal matrix in this area showed a marked increase in stainable protein and a marked increase in stainable carbohydrates (Fig. 10B). As the animals became older this area progressed toward the pulp but did not seem to extend any further along the dentinoenamel junction.

The presence of an oxidation-reduction system was found uniformly throughout the dentin except in the dentin in the depth of some of the major grooves (Fig. 6, B).

Contact microradiographs of sections from these animals which had been on the cariogenic diet showed that this area of dentin in the depth of some of the major grooves is more radiolucent than adjacent areas in the same tooth (Fig. 7,B).

At 28 days of age, the sections of animals on the cariogenic diet showed marked changes in area 3 of Fig. 12. In this area, a marked increase in stainable protein, carbohydrate, and sulfur was found. There was, also, a loss of mineral content and a cessation of oxidation-reduction.

1. The lesion is characterized by: (a) Persistence of local enamel permeability to methylene blue in Area 2 beyond normal age of maturation. This is not restricted to lamellae but is along a broad front, which includes all of Area 2 (b) Diminished oxidation-reduction activity in Area 3. (c) Loss of mineral phase in Area 3 (d) Increased availability of carbohydrate and protein in Area 3—possibly from mucopolysaccharides of ground substance.

2. Whether these observed changes occur simultaneously or follow some pathogenetic sequence is as yet unknown.

3. Observations made in this study are consistent with the suggestion that the increased availability of carbohydrate and protein is the result of mineral loss.

4. This does not rule out the possible coincident action of a mucopolysaccharide hydrolyzing enzyme capable of including the observed changes. Mucopolysaccharides have been identified by other workers in the organic portion of the tooth. Crude toxins have been isolated from bacteria normally inhabiting the carious lesion. These toxins were found to be capable of hydrolyzing mucopolysaccharides. Diffusable food substances of cariogenic diet may account for reactive protein and carbohydrate in dentin of Area 3, but are hardly responsible per se for changes in oxidation-reduction potential or loss of mineral.

5. The loss of mineral may be the result of primary microbial agencies.

6. It may also be the secondary consequence of some induced endogenous cellular impairment suggested by (a) diminished local oxidation-reduction activity, and (b) structural differences in tubular terminal endings.

Further explanation for the character of the premonitory lesion herein described must await further experimental study.

RELATIONSHIP OF FLUID TRANSPORT THROUGH THE DENTIN TO THE INCIDENCE OF DENTAL CARIES, RALPH R. STEINMAN AND JOHN LEONORA. REPRINTED FROM JOURNAL OF DENTAL RESEARCH, VOL. 50, NO. 6, PART 2, NOV.-DEC. 1971

The rate of fluid movement in the teeth of 28-day-old rats is inversely related to the incidence of dental caries after 13 weeks of the same diet. The early hypomineralization found in the dentin at 28 days is a result of altered metabolism imposed by a reduced rate of fluid transport in the rats fed a cariogenic diet.

It is generally accepted that three conditions are essential in the oral cavity for dental caries to occur: a suitable microbe, a substrate for the microbe, and a susceptible tooth. Susceptibility or resistance to dental caries is usually thought of in terms of structure, which is determined, for the most part, during calcification. However, it is also plausible that susceptibility to dental caries may be related to altered metabolism within the tooth.

To maintain a rapid rate of metabolism through the EmbdenMeyerhof pathway in the avascular dentin, as reported by Bosia, Arese, and Pezolli, a rapid fluid transport system is essential for nutrient uptake and waste removal from the tooth structure. Any reduction in the rate of fluid transport through the dentin may have a deleterious effect on the health of the tooth. Under these conditions, the rate of metabolism would be altered and the end product of metabolism (lactic acid) might accumulate in the structure. The purpose of these experiments was to determine if the rate of fluid transport is related to the incidence of dental caries, to determine if the rate of fluid transport can be altered by systemic means, and to find what effect this alteration would have upon the incidence of dental caries.

Experiment 1—The rate of fluid movement (FM) through the dentin beneath the occlusal grooves was determined on the 28th day of life in rats fed either laboratory chow or a cariogenic diet, and in the anesthetized and unanesthetized state. Groups of ten or more rats were given an injection of the fluorescent dye, acriflavine hydrocholoride (50 mg/kg) intraperitoneally. The rate at which the fluorescent dye moved in and disappeared from the odontoblastic process was used as a criterion for the occurance of FM. The unanesthetized rats fed laboratory chow and the rats fed the cariogenic diet were killed at 15, 30, 45, and 60 minutes, and at 45, 60, 70, 90, 135, and 180 minutes, respectively.

The effect of long-term systemic administration of urea and citrulline on the incidence of dental caries is shown in Table 2. A highly significant reduction in dental caries was obtained with the higher doses of urea. The decrease in caries incidence obtained with citrulline was equivalent to that obtained with the lowest dose of urea, but it was not statistically significant. The decrease in the number of carious lesions was found to be a function of the total dose of urea.

The data show that the rate of FM through the odontoblastic processes is significantly effected by the dietary regime (Fig. 1). In contrast to the rate observed in rats fed Purina laboratory chow, a cariogenic diet significantly suppresses the rate of FM. Pentobarbital sodium anesthesia potentiates the suppressive effect of the high sugar diet. Correlation of the

FM ratio to the incidence of dental caries demonstrates that a high incidence of caries is associated with a suppressed FM. The common denominator between these two parameters is the cariogenic diet. A high FM ratio is correlated with an exceptionally low incidence of decay (Fig. 2).

Previous studies have shown that the suppressive effect of a cariogenic diet on FM is discernible as early as two days after the rats have been placed on this diet. However, in acute experiments that last only 45 minutes, the suppressive effect can be overcome by the systemic administration of urea or citrulline through intravenous or intra-arterial infusion (Table 1). With these chemicals, it is possible to establish FM at a level equal to or greater than that observed in rats fed laboratory chow. From the data, it is apparent that the intra-arterial infusion is significantly more effective in re-establishing FM than the intravenous infusion. This suggests that these compounds exercise their effect on FM by activating a mechanism in the central nervous system.

The idea that urea must exercise its effect through a systemic mechanism rather than through surface reaction is confirmed with the following observations. Others have shown that a reduction in food consumption is often associated with a decrease in dental caries. However, rats under stress may have a reduction in food consumption but have an increase in dental decay. A reduction in growth rate also is associated with an increase in dental decay. In our experiment, the rats receiving the highest dose of urea were under stress, as indicated by the increased adrenal and pituitary weights and the reduced thymus and body weights (Table 3). The kidneys were hypertrophied because of the increased stress from rapidly eliminating the urea load from the body fluids. In spite of stress, a significant decrease in the incidence of dental caries was achieved.

More evidence for the systemic effect of urea becomes apparent when the FM response of 28-day-old rats to plasma obtained from the urea-injected rats caused a significant rise in the FM ratio.

Evidence submitted elsewhere for publication shows that urea and other compounds of the ornithine cycle, and carbamyl aspartate of the pyrimidine cycle, exercise their effect on FM through the hypothalamus. Under the influence of urea, the hypothalamus secretes the hypo thalamic, parotid hormone-releasing factor, which stimulates the parotid gland. The latter gland secretes a parotid hormone that stimulates FM by acting on the odontoblasts. Surgical interruption of this endocrine axis. Evidence for the existence of the hypothalamic-parotid gland endocrine axis has been published previously.

The tooth is a unique structure, even when compared with bone, to which it shows certain structural similarities. Bone is a relatively

vascularized structure, whereas dentin is avascular, and is dependent

on a rapid fluid transport system for nutrient uptake and waste removal. Bone is surrounded by body fluid, and teeth are exposed to the

atmosphere. The aerobic environment may be of importance in influencing metabolic pathways in the teeth. Beneath the occlusal food plug, a more anaerobic environment can exist that could adversely affect the

metabolic pathways. In germfree animals on a high sucrose diet, the hypomineralization found in the enamel and dentin beneath the food plug might be explained as a consequence of reduced fluid transport, along with a shift to a more anaerobic metabolism and the accumulation of lactic acid in the dentin. These changes prepare the tooth for a successful attack by the unfavorable external environment.

The authors suggest that the early changes in teeth which eventually lead to carious lesions are the result of altered metabolism that includes a reduced rate of fluid transport and external conditions imposed by the food plug and its microbial inhabitants. These conclusions do not minimize the importance of the external conditions, but rather give added importance to them by showing how external conditions may influence metabolism within the teeth. Teeth will not decay in the absence of the microbe and its products on the external surface. The external factors

cannot be ignored, but for dental caries to be controlled, it might be well to consider how the altered internal metabolic process contributes to the problem of dental caries. Consideration of both the internal and external factors is a more complete approach to the prevention of

dental caries. Teeth decay because of an initial alteration in the internal metabolism. Reduction in the rate of fluid transport, as found in the teeth of animals maintained on the high sucrose diet, may be of primary importance in understanding the alterations in metabolism and pathologic sequelae.

THE MOVEMENT OF ACRIFLAVINE HYDROCHLORIDE THROUGH MOLARS OF RATS ON A CARIOGENIC AND NON-CARIOGENIC DIET, RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE JOURNAL, SOUTHERN CALIFORNIA DENTAL ASSN. VOL. XXXV, NO. 4, APRIL 1967

Through the Years, efforts to determine fluid movement in teeth have been made by means of various technics. Vital stains or dyes were used by Fish, Bodecker, Lefkowitz, and Steinman, to demonstrate the movement of dyes from pulp towards the enamel through the hard structures of teeth. Their work suggests that diffusion may occur through the hard structures of the tooth in two directions. Recently, Bodecker further suggested that the fluid within the hard structures of the tooth may play a role in resistance to dental caries.

More than 200 twenty-one-day-old rats were obtained commercially in lots of 25 to 50. These were divided randomly into two groups. One group of animals was placed on a high sucrose cariogenic diet. This group of animals were infected by swabbing their mouth three times with a 24-hour-old broth culture of streptococci FAL The FA1 stain was kindly furnished by Dr. Fitzgerald of NIH. The second group of animals were placed on a non-cariogenic diet of Purina laboratory chow.

To determine if metabolism were involved in the distribution of acriflavine HCl in the tooth and what effect two products microbial metabolism, CO_2 and lactic acid, may have upon the distribution of acriflavine HCl in the tooth the following experiments were conducted. The animals were anesthetized with sodium pentobarbital 25 mg/kilo body weight.

Preliminary observations revealed that the acriflavine HCl reached the odontoblastic layer of the pulp within 5 minutes following the intraperitoneal injection. Within 10 minutes or less some odontoblastic tubules fluoresce to the dentino-enamel junction. The dye gradually spreads into the dentin and through the enamel in about an hour. The dye gradually disappears over the next 7 to 10 days.

The application of lactic acid pH 2.3 to the cusps of upper molars had no effect upon the movement of acriflavine HCl into the odontoblastic tubules.

The speed of distribution and the pathway taken by the dye up the odontoblastic tubules would suggest fluid movement rather than passive diffusion through the tooth. The inhibition of acriflavine HCl movement by the applican KCN 10'M would suggest that energy is necessary for the movement of this fluid in the tooth. The lack of fluid transport in the dentin opposite the necrotic area would support this concept of a vital process being essential for fluid transport through the tooth.

The area of no dye penetration in the dentin beneath the occlusal grooves in animals on a cariogenic diet would suggest that one of the first changes occuring in the tooth in an area which will exhibit cavita-

tion several weeks later is a cessation of normal fluid transport. This alteration in fluid transport is probably brought about by some microbial product which may easily diffuse deeply into the tooth, furthermore it is probably an end product of carbohydrate metabolism. Lactic acid when applied to the external surface of the tooth does not alter the distribution of acriflavine HC1, while CO_2, may play a role in demineralization.

It would thus appear that one of the earliest changes which occurs in the tooth before cavitation is an alteration in the normal fluid transport

within the hard structures of the tooth. Thus dental caries may exhibit another typical change characteristic of pathology, an alteration in fluid transport within the tissue effected.

What is the role of this fluid transport? Was Bodecker correct in his suggestion that the fluid transport within the tooth played an important

role in resistance? These and other questions await further research before a definite answer can be given. However, this work would add emphasis to the suggestion of Bodecker made many years ago, that the fluid transport plays a role in resistance to dental caries.

1. If the rapid distribution of acriflavine through the tooth is taken as an indication of fluid movement through the tooth then the first change in the tooth which may be demonstrated before cavitation occurs is a marked decrease in fluid movement in the dentin.

2. A microbial product, CO_2 is capable of inhibiting fluid transport in the tooth.

3. The application of KCN 10 3M to the external surface of the tooth and the decrease in fluid transport would suggest that energy is essential to the movement of fluid through the tooth.

PHARMACOLOGIC CONTROL OF DENTINAL FLUID MOVEMENT AND DENTAL CARIES IN RATS,
RALPH R. STEINMAN, REPRINTED FROM JOURNAL OF DENTAL RESEARCH, VOL. 47, NO. 5, SEPT.-OCT. 1968

Parasympathetic stimulants were found to encourage the movement of fluid through the dentin from the pulp to the dentinoenamel junction. Regular treatment with a parasympathetic stimulant in rats that had all their major salivary glands removed significantly reduced the incidence of dental caries. Treatment with bradykinin in rats on a noncariogenic diet encouraged the movement of fluid into the tooth. When given regularly for 15 weeks, bradykinin caused significant dental caries.

Fluid movement through various organs such as the kidneys and liver has been studied by intraperitoneally injecting a fluorescent dye and observing the time sequence of fluorescence through the organ under observation.

Using acriflavine hydrochloride to demonstrate the movement of fluid through the dentin of molars of rats, Steinman reported that the dye would penetrate to the dentino-enamel junction in ten minutes. In rats

on a cariogenic diet, the fluid movement beneath the occlusal grooves was severely depressed weeks before cavitation. Furthermore, the movement of fluid required intact pulp and appeared

to require metabolic energy. The purpose of the present study was to determine if the movement of dentinal fluid could be altered by pharmacologic agents and if an alteration in fluid movement might have an effect on the incidence of dental caries in rats.

In the short-term experiments with rats on the cariogenic diet, these changes in dye distribution were observed: The upper teeth, to which the various parasympathetic stimulants had been applied, showed evidence of fluid stimulation; regions of inhibition and yellow-orange fluorescing material were no longer present in the dentin or enamel (Fig. 1A). A similar picture of dye distribution was observed in the rats given the parasympathetic stimulant intrapeitoneally. Atropine sulfate was found to reduce the amount of acriflavine dye movement into the tooth structure.

The results of the long-term treatment with bethanechol chloride in rats that had all their major salivary glands removed are given (Table 1). No first or second molars of the control rats were free from dental caries, but 23 first and second molars of the experimental rats were free from dental caries.

Intraperitoneally injected bradykinin (0.2 mg/kg) was found to encourage the movement of fluid from the external surface of the tooth toward the pulp. This was shown with rats on the noncariogenic diet, in which the yellow-orange material from the occlusal grooves was drawn into the dentin nearly to the pulp chamber.

Bradykinin, given regularly to rats on a noncariogenic diet for 15 weeks, was found to produce significant incidence of dental caries.

A number of technics have been used in attempts to determine the presence of fluid movement in teeth. Vital stains or dyes were used by Fish, Bodecker, Lefkowitz, and Steinman and Hardinge to demonstrate the movement of dyes through the hard structures of teeth. Radioactive isotopes have been used by Volker and Sognnaes, Armstrong and Barnum, Sognnaes et al, and others. Their work suggest that diffusion may occur in two directions through the hard structures of the teeth. Recently, in an in vivo study of human teeth, eBrgman and Linden demonstrated a centrifugal flow of fluid from the interior to the enamel surface.

The movement of fluorescent dyes through other organs has been taken as an indication of fluid movement through the organs under study. In the present investigation, and in a previous report, these points may be made: The fluorescent dye arriving from the intra-peritoneal injection in the pulp does not diffuse into the dentin en masse but moves up individual odontoblastic processes to the dentinoenamel junction. Vital pulp is essential for the dye movement. The movement of the dye may be inhibited by the application of potassium cyanide to the

teeth. Also, a para-sympathetic stimulant encouraged movement of dye from pulp to the dentinoenamel junction is a demonstration of active transport, although altered permeability cannot be excluded as an explanation. The removal of the salivary glands of rats on a cariogenic diet usually produces a significant increase in the incidence of dental caries. Parasympathetic stimulants cause an increase in salivation that might decrease the incidence of dental caries. In this experiment, the salivary glands were removed to increase the incidence of dental caries and to eliminate salivary gland stimulation as a factor in the reduction of the incidence of dental caries. Under these circumstances, any alteration in the incidence of dental caries could not be attributed to salivary gland stimulation and more logically could be attributed to an effect on the pulp of the tooth.

Bradykinin is a polypeptide that greatly increases capillary permeability and permits the release of peptides, and even protein, from the vascular system. Its action in causing the movement of fluid from the outside to deep within the tooth might be the result of a change in osmotic relationship between the pulp and the dentin.

The importance of normal fluid transport in the tooth is apparent from these studies. The first change to be demonstrated weeks before cavitation, in teeth of rats on a cariogenic diet is a failure of the fluid transport in the dentin. This failure of fluid movement is in relation to the microbial colony and thus may be influenced by some microbial product. Lactic acid has not been observed to alter fluid movement, but carbon dioxide appears to inhibit normal fluid movement. Also, the fluid movement may be stimulated by bethanechol chloride and other parasympathetic stimulants. The regular stimulation was found to produce a significant decrease of dental caries in rates on a cariogenic diet. Finally, a change in fluid movement into the tooth by the administration of bradykinin has been found to produce dental caries in rats on a noncariogenic diet. From these observations, it would appear that the health of the teeth depends in large measure on the normal fluid movement within the tooth. Similar to other biological tissues, the health and stability of the tooth, including the hard structures, appear to depend on a fluid transport. When this transport system fails, pathosis may result.

The results of the study indicate these conclusions: (1) The dentinal fluid movement may be altered by certain pharmacologic agents. (2) Parasympathetic stimulants appear to encourage the movement of fluid from inside the tooth toward the enamel. The stimulation was found to significantly reduce the incidence of dental caries. (3) Another drug, bradykinin, which encouraged the movement of fluid from outside the tooth toward the pulp, produced dental caries in rats on a noncariogenic diet.

THE RAT MOLAR IN HEALTH AND DISEASE, RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE JOURNAL OF THE CALIFORNIA DENTAL ASSOC. VOL. 37, NO. 2, APRIL 1961

The enamel, the dentin, and the pulp cannot exist alone. Each contributes to the welfare of the whole. Vital staining isotopes and fluorescent-labeled technique all show that there exists a vital connection between the circulation within the pulp and the hard structures of the tooth. Other studies have shown that there exists a "circulation" within the dentin. It would appear tha the enamel matures from within out and this process depends upon a vital pulp.

The integrity of the enamel seems to be dependent upon a healthy, functioning pulp and dentin. This dependence would be manifested by an interchange of material between these various tissues. By what means are such changes mediated between these two embryologically different tissues? This would necessitate the metabolic interaction between tissues separated by considerable distance. This type of metabolic interaction has been demonstrated in other tissues of the body. This process depends upon an electron transfer system, probably involving a sulfhydryl configuration. In the tooth this interaction, if present, would depend upon the odontoblast and its protoplasmic process which extends to the dentino-enamel junction. Histochemically, the presence of an oxidation reduction system in the dentin and the possible presence of sulfhydryl within the dentinal tubules have been demonstrated.

A number of important pathological changes occur in the molars of rats by 28 days of age. The first change to be noted is a marked increase in permeability beneath the food in the enamel. Also observed is a cessation of oxidation reduction in the dentin and a possible depletion of sulfhydryl within the dentinal tubules just beneath the enamel on a broad front. These are important changes involving function of these tissues.

About this same time changes in structure may be observed. These changes are characterized by a marked increase in reactive proteins by a marked increase in reactive carbohydrate and a partial demineralization of the dentin. These changes also occur on a broad front and follow the same pattern as the early metabolic changes. These changes, in three or more weeks, are followed by bacterial invasion and sloughing, or typical cavity formation. Thus, as is typical of disease processes in other tissues, it can be seen that caries occurs in a sequence of events which teminate in necrosis, bacterial invasion, and sloughing. As a typical of pathosis, these changes occur in depth, not as a surface phenomenon.

Recently it has been found that the stress of immobilization of young rats increase the incidence of incipient carious lesions almost three times. Since these animals were littermates and were on the same diet and water, it would indeed be difficult to explain these differences as being induced by other than systemic means.

Prominent among the changes induced by stress is an increased secretion of ACTH. It is known that this will induce an increased secretion of nitrogen, potassium and phosphorus, and a retention of sodium and chloride with a secondary water retention.

This could affect the flow of nutrients into the hard structures as well as alter the osmotic relationship of the tooth to its external environment.

Metabolic energy is required to pump the sodium to the outside of the cell. The higher the concentration of sodium outside the cell, the faster this metabolic pump must work to keep the sodium out of the cell. This places an added stress or load upon the cell and its carbohydrate metabolism. The degree of hydration also influences the rate of exchange of ions across the cell membrane.

It is generally thought that the increase in caries accompanying the removal of the major salivary glands of the rat is the result of a local action upon the surface of the tooth. In this connection, it is well to note that the removal of the major salivary glands is accompanied by an increased adrenocortical activity. It has further been noted that these animals grow more slowly than do normal rats. There is the possibility that the salivary glands produce a hormone which may account for the slower growth of animals with the salivary gland removed. It is known that some sex hormones cause a rise in the concentration of serum sodium. Could this explain the reduction in caries observed following the castration of young animals?

It is possible that the increased concentration of sodium and chloride within the body and the tooth could affect its health and welfare in two ways. Since the electrolytes (Na and Cl) play a major role in controlling osmosis and water transport, any interference with the normal function of this system could alter maturation and resistance to disease. Any change in the osmosis within the tooth might alter its relationship with its external environment. It is possible that under certain circumstances the normal osmotic pressure it would tend to encourage the ingress of noxious material into the tooth. Thus the electrolyte balance of the tooth may play a vital role in susceptibility or resistance to disease.

1. A tooth is a group of dissimilar, dependent tissues.

2. Its health depends upon the harmonious function of each of its individual tissues.

3. Disease is the result of disturbed function, in whole or in part.

4. The ultimate control of an infectious disease rests upon the resistance of the host.

THE HISTOCHEMICAL DISTRIBUTION OF MAGNESIUM IN DENTINAL TISSUES, RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE JOURNAL, SO. CALIF. STATE DENTAL ASSN., VOL. XXX, NO. 4, APRIL 1962.

The presence of Sulfhydryl, adenosine triphosphate, adenosine triphosphatase, and a cholinesterase has been demonstrated histochemically in the dentinal tubules of human teeth. These same molecules are absent or markedly diminished in the dentinal tubules almost to the pulp of the teeth beneath the first evidence of demineralization in the enamel. Since these molecules are intimately associated with metabolism, it would appear that one of the first changes in caries is in alteration from normal metabolism. Certain molecules, for example, phosphate and magnesium in addition to being an integral part of structure are associated with various metabolic processes in tissues generally. Magnesium is an activator of many enzymes of the glycolic systems. The integrity, the health, and the function of cells depend upon the proper function of these systems. Their importance is obvious. It was therefore thought important to determine the presence and distribution of magnsium in dentinal structures.

This technique shows the magnesium to be present throughout the length of the dentinal tubules. It is in greatest concentration in the tubules adjacent to the pulp. Beneath the first evidence of pathosis in the enamel magnesium appears to be markedly reduced or absent within the tubules extending almost to the pulp of the tooth.

Chemical determinations have shown magnesium to be present in the enamel and dentin in significant amounts. These determinations would include both the bound and unbound magnesium in the structure. That revealed by the histochemical method employed apparently is the free magnesium available for metabolism since it was found to be confined to the dentinal tubules. Furthermore, the distribution of the magnesium follows the observed distribution for sulfhydryl, adenosine triphosphate, adenosine triphosphatase, and a cholinesterase in the normally functioning dentinal tubules. The absence of magnesium beneath the first evidence of pathosis in the enamel also follows the depletion pattern of these molecules in the dentinal tubules beneath the first evidence of pathosis in the enamel

Thus is presented one more link in the chain of evidence which suggests that the first change in dentinal tissues to occur in the development of caries is a change in metabolism. This observation is in harmony with the observed changes in tissues other than dentinal during the development of infectious diseases.

It is sometimes difficult to correlate the many recent findings concerning dental caries in the over all picture. It is well to remember that although decalcification is a major feature of caries yet it does not resemble decalcification observed in patients who for example suck a lemon or take other acids. The work of Fitzgerald would indicate that since organisms which produce acid such as Lactobacillus are unable to initiate caries when introduced into a germfree environment other bacterial products are necessary to initiate dental caries.

THE POSSIBLE PRESENCE OF SULFHYDRYL, DISULFIDE RADICALS AND ADENOSINE TRIPHOSPHATE IN HUMAN TEETH. RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE JOURNAL, SO. CALIF. STATE DENTAL ASSN., VOL. XXIX, NO. 11, NOV. 1961.

Recent experiments with animals would indicate that active metabolism is involved in preventing the ingress of noxious material through the enamel into the deeper portions of the tooth. When this metabolism fails, noxious material finds ready access into the tooth. Since the sulfhydryl radial is essential for a number of enzyme systems and adenosine triphosphate (ATP) is universally used by the cells of the body to support metabolic activities, the presence or absence of these factors is of considerable importance in the hard structures of the teeth.

When ATP is added to firefly latern extract (luciferase) a luminescence is produced. This reaction was used as the basis for a test to determine the presence of ATP in tooth sections. Saran

Wrap was used to separate the solution of luciferase from the emulsion of the film. The freshly prepared section was placed in this solution on the Saran Wrap. The solution and section were placed on a piece of Ansco Super Hypan film for 10 minutes, in the dark. The film was developed for 7 minutes. The presence of ATP was shown to be in different areas of the teeth. The greater the luminescence, the darker the area on the film and the greater the amount of ATP present in that area of the tooth.

The ability of sections of human teeth to decolorize methylene blue, the possible presence of sulfhydryl radicals, and the presence of ATP would suggest that active metabolism occurs in the teeth, particularly in the dentin. Furthermore, the failure of certain areas to decolorize methylene blue, the possible decrease in sulfhydryl radicals, and the absence of ATP would suggest that one of the early changes in the pathology of caries is an alteration in normal metabolism of the teeth. This early change appears to precede any change in the normal mineral content of these areas as shown by radiographs of these same sections. The content of these areas as shown by radiographs of these same sections. The concentration of ATP in the dentin adjacent to the pulp would suggest that this is the area of greatest metabolic activity.

The role that an active metabolism may play in the resistance of the teeth to disease is becoming apparent from animal experimentation. Evidence would suggest that the odontoblast is an osmotic pump which controls an active transport system within the hard structures of the tooth. When this fails or is altered, the incidence of caries is altered. This system may be altered by the administration of hydrogen peroxide, 2-4 dinitro phenol, or a mercurial compound such as mersalyl, by alterations in the electrolyte and water balance and by such nonspecific factors as stress. When this is done, the methylene blue placed in the mouth of such a rat will markedly penetrate the enamel and dentin of

the tooth. Other workers have noted the relationship between cell permeability to water and the integrity of the sulfhydryl groups. They found that mersalyl tied up the sulfhydryl groups of these cells thereby altering the metabolism and cell permeability to water.

The tooth must be considered as an organ, a functioning entity, not as an inert object left to the mercy of an unfavorable environment. This organ has an active metabolism which extends at least to the dentino-enamel junction. When this active metabolism is reduced or inhibited whether by external or internal factors, pathology may follow. As is true in other pathology, one of the first changes to occur in the tissue is a change in the metabolism of the structure involved in the teeth, it would appear that the hope for the control of this disease rests upon the external environment, important as that is, but upon the condition of the internal metabolism of the tooth. It would appear that the active metabolism of the tooth plays a vital role in preventing the ingress of noxious material into the deeper structures of the tooth. The resistance of the hosts is of paramount importance in the control of this disease or of any disease of bacterial origin. The resistance of the host may be influenced by many factors but the foundation of resistance rests upon adequate nutrition at the cellular level.

THE POSSIBLE PRESENCE OF ADENOSINE TRIPHOSPHATASE AND CHOLINE ESTERASE IN THE HUMAN TEETH. RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE J. SO. CALIFORNIA STATE DENTAL ASSN., VOL. XXIX, NO. 12, DEC. 1961.

The movement of the liquid phase of a tissue, although affected by the electrolyte balance, is also under the influence of the selective permeability of biological membranes. Considerable evidence that selective permeability depends upon the integrity of the sulfhydryl groups is given in a report by Belkin and Hardy. One of the enzymes involved in this vital process is that of acetylcholine esterase. Another possible enzyme in this system is adenosine triphosphatase (ATPase). Among the associated factors in this biological system would be (ATP). It has been found that the blocking of the sulfhydryl by mersalyl or with hydrogen peroxide, or the uncoupling of oxidative phosphorplation with 2-4 dinitro phenol would cause a marked ingress of methylene blue through the intact enamel and dentin of rats on a noncariogenic diet. It was therefore thought important to determine if human dentinal structures contained ATPase and a

cholinesterase and to determine its distribution in relation to any pathological changes which may be present. The adenosine triphosphatase appeared to be largely confined to the tubules. Its presence was observed throughout the dentinal tubules, but in greatest concentration adjacent to the pulp. It will be observed that beneath the occlusal groove in the dentin a diminution of reaction for the presence of ATPase is already apparent.

A number of metabolities and enzymes are essential in a biological system to perform selective permeability. Of the known factors, ATP, ATPase, cholinesterase and sulfhydryl have been demonstrated within the dentinal tubules of normal teeth and absent or markedly reduced in the dentinal tubules beneath the first evidence of pathologic change in the enamel It would appear from animal experimentation that the failure of this system allows noxious material which may be upon the surface to penetrate deeply into the tooth.

It is further recognized that the electrolyte balance as it affects osmosis also plays a part influencing the flow of the liquid phase through a tissue. The effect upon the incidence of caries by altering this balance lends support to this vital concept of functional resistance. With rats it was found that the retention of salt following the administration of DOCA (Desoxycorticosterone Trimethyl lactate) caused a significant increase in caries whereas the administration of Diuril which causes the excretion of sodium chloride resulted in a decrease in the incidence of caries.

It would appear that the presence of the noxious agent upon the surface of the tooth does not itself cause disease. It is only as this material is allowed to penetrate deeply into the tooth structure that disease occurs. Thus, as is true of other infectious diseases, whether the disease occurs depends largely upon the resistance of the host and not upon the mere presence of the external agent, as necessary as this may be. The penetration of the noxious agent into the tooth appears to be the result of a failure of one of the vital processes of tissue, a failure of the transport system.

Recent evidence would indicate that streptococci are the external causative agent in caries. Unfortunately, man does not develop life time immunity to this organism, but rather a transient immunity. However, man does overcome infections caused by this organism. Resistance to streptococci appears to be related to the less easily defined factors such as correct nutrition, the harmonious function of the endocrines, or the physical state as affected by fatigue or exposure. That this may be true relative to caries is shown by the many nutritional factors or endocrine factors which alter the incidence of this disease but which singly do not completely control the disease.

THE PRESENCE AND DISTRIBUTION OF SUCCINIC DEHYDROGENASE AND MONOAMINE OXIDASE IN THE PULP OF ADULT HUMAN TEETH. RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE J. SO. CALIF. DENTAL ASSN., VOL. XXX, NO. 12, DEC. 1962.

Succinic dehydrogenase is an iron containing flavoprotein found in the mitochondria. It is widely distributed in biological systems but often

is found in such small amounts that it can not be demonstrated histochemically. The concentration found in a given tissue depends upon the oxidative capacity of the tissue under observation. The dehydrogenases are the first members of a chain of enzymes which transfer H atoms or electrons from substrate to molecular oxygen.

The monoamine oxidases occur in a variety of animal tissues in small concentrations. A high amine oxidase activity is found in the liver and kidneys of some animals. A moderate amount has been found in the human heart and brain. Its function probably includes detoxification.

In single rooted teeth the greatest concentration of succinic dehydrogenase was observed in those odontoblasts whose processes extend to the enamel.

The enzyme appears to be in greatest concentration in the odontoblasts opposite the enamel.

Odontoblasts opposite a carious lesion appear to exhibit less activity than adjacent odontoblasts.

The distribution of the monoamine oxidase in the odontoblastic layer of the pulp chamber follows a pattern similar to that found with succinic dehydrogenase. This enzyme appears to be more sensitive to pathosis than the succinic dehydrogenase. The concentration of succinic dehydrogenase in the odontoblastic layer of the pulp, especially opposite the enamel, would suggest that this area has a rather high oxidative capacity.

The distribution of the monoamine oxidase in the odontoblastic layer of the pulp chamber follows a pattern similar to that found with succinic dehydrogenase. This enzyme appears to be more sensitive to pathosis than the succinic dehydrogenase. The concentration of succinic dehydrogenase in the odontoblastic layer of the pulp, especially opposite the enamel, would suggest that this area has a rather high oxidative capacity.

Although a quantitative determination was not made at that time ATP was present. This quantitative problem is being investigated further at the present time. Evidently considerable energy is being expended in the pulp of the adult human tooth. Nature does not needlessly expend energy. What is a possible function of this metabolic activity in the human tooth? There is considerable evidence for the presence of a transport system in the tooth which would require energy.

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Histochemically the necessary elements of an ion transport system are present. In certain other tissues of the body, for example, the eye, a fluid and ion transport system is known to exist and have been studied in considerable detail.

The concentration of dehydrogenase in the odontoblastic layer of the tooth is present. That the surface of the tooth, at least in rats, carries a reducing potential in the living animal is shown by the deposits of silver found on the teeth of rats given 1.5 parts per 1000 to drink over a period of several months. In the odontoblasts of the rat a high concentration of dehydrogenase can be demonstrated.

A transport system such as has been suggested requires energy to operate. In previous reports, with animals it was found that if the metabolism of the animals were inhibited with certain antimetabolites then dyes would penetrate into the tooth, whereas, if the animals were on a noncariogenic diet the dyes would not penetrate. Thus it appears that energy is required to prevent the ingress of noxious material into the tooth.

It is generally recognized that three conditions must exist in the mouth for decay to occur. There must be susceptible tooth, bacteria, and food for the microorganisms This present paper would suggest that resistance is not only a mechanical feature of the tooth but that it is metabolic also. When this metabolism is suppressed even the teeth of animals on a noncariogenic diet will decay. The purpose of this transport system is probably two fold. It prevents the ingress of noxious material into the tooth and it probably plays a role in the maintenance of the tooth.

Less is known about the specific function of the monoamine oxidase in the system than the succinic dehydrogenase. One of its suggested functions concerns the detoxification of certain noxious materials. Whether this occurs in the tooth has not been ascertained. However, it is interesting to note the areas in which it is present and the areas of depletion relative to pathology in the overlying enamel and dentin.

HISTOCHEMICAL AND RADIOGRAPHIC CHANGES OBSERVED IN HUMAN TEETH. RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE J. SO. CALIF. STATE DENTAL ASSN., VOL. XXIX, NO. 9, SEPT. 1961.

It appeared from this study that the disease, dental caries, occurs in depth and in a sequence of events which terminates in cavitation. Since it is quite well established that caries in rats and hamsters is caused by a streptococcal organism and since certain histochemical changes observed in rat dental tissues invivo can be duplicated by applying a streptococcal lysate to rat dental tissues invitro, it was therefore considered important to determine if similar histochemical changes could be demonstrated in properly prepared sections of human teeth.

All areas of the teeth did not show equal ability to decolorize methylene blue in absence of air and in the presence of suitable substrates such as sodium succinate in a phosphate buffer. The areas in the dentin opposite the deep grooves and opposite the contact point in which no demineralization may be observed appeared to decolorize the methylene blue more readily than adjacent areas.

Early incipient changes in the enamel often react markedly when treated for the presence of mucopolysaccharides. A marked increase in the dentin for mucopolysaccharides usually does not occur until the lesion becomes rather extensive.

In spite of the difference in size and difference in life span between human and rat teeth, the histochemical changes observed during pathology as observed in human teeth sections are similar to those observed in rat teeth sections. The human teeth being larger and existing over a period of years permit a separation between the histochemical changes which appear to occur simultaneously in the teeth of rats.

The histochemical changes observed in the human enamel seem to follow the enamel rods. This is quite noticable in those sections treated with silver nitrate. The reaction of the dentin to ninhydrin follows very closely the odontoblastic processes for considerable distance toward the pulp of the tooth. The histochemical reactions for mucopolysaccharides in the dentin seem to occur at a later time and seem to be less circumscribed. In general, the early pathologic changes seem to follow the organic pathways of the tooth toward the pulp. Once the pathology reaches the dentin histochemical changes may be noted far in advance of the demineralization as observed in the radiograph.

It was observed that oxidation-reduction as determined by the ability of the tooth structure to decolorize the methylene blue did not occur uniformly throughout the tooth. In the dentin beneath grooves in the enamel with no observable pathology, it would appear the oxidation-reduction occurs faster than in the adjacent areas of the same tooth. If histochemical changes are observed to the dentino-enamel junction, no oxidation-reduction would occur in the dentin for perhaps one millimeter and this area of no oxidation-reduction would be surrounded with an area of increased activity. Could it be that these changes in oxidation-reduction are a manifestation of resistance?

It is generally recognized that streptococci are involved in the production of caries in rats and hamsters. Certain histochemical reactions have been found to occur in the teeth of rats which are developing caries. Some of these histochemical reactions can be duplicated with rat's dental tissues in virtro. Since similar findings are observed histochemically in the teeth of humans having this disease, one would be led to suspect that caries in the human may also be caused by a streptococcal organism.

Since it is well known that lactobacilli produce acid and that they are unable to initiate a lesion in the teeth of rats or hamsters, one would suspect that the acid alone does not initiate the lesion. It is true that streptococci produce acid as well as toxins and enzymes. Decalcification is generally considered to be a surface phenomenon. It would be difficult to explain the changes which occur deep in the tooth as being the direct result of an acid which may be two or more

millimeters away from the area of histochemical change. In all probability, the early pathology is initiated by agents other than acid. After the lesion has been initiated, it may be that acid may play a major part in the sloughing process or cavitation, as it is commonly called. This sloughing process appears to occur after considerable histochemical changes have occurred in depth.

If one assumes that streptococci cause caries in human teeth, as well as in hamsters and rats, how might this change our concept of caries control? Unfortunately, we do not develop a life time immunity to streptococci. However, this organism is the major inhabitant of the mouth and in tissues other than the teeth does not produce pathology unless the resistance of that tissue is low. It is also observed that infections due to this organism are overcome by the body defences. Of great importance is the observation that if the resistance of the host is great enough, no infection will occur.

If this is true, it might be suggested that one of the first alterations in normal physiology may be concerned with this transport system. This would be in harmony with pathology in other tissues.

Susceptibility may vary from slight to complete. With a slight susceptibility, the tooth would probably decay in the depth of the grooves and perhaps interproximally. As susceptibility increases, other areas would become involved—such as buccal and lingual surfaces, until with no resistance, the whole tooth seems to break down. Caries seems to fit into the general picture of bacterial infections in that virulence of the organism, the number of organisms and the susceptibility of the host all may play a part.

CALCIUM AND PHOSPHORUS RELEASE FROM INTACT HUMAN ENAMEL UNDER ANAEROBIC CONDITIONS. RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM THE JOURNAL, SO. CALIF. STATE DENTAL ASSN., VOL. XXXII, NO. 10, OCT. 1964.

Previously, it was reported that proteolysis of intact human enamel occurred under anaerobic conditions and that the loss of the protein from the intact enamel increased markedly the porosity of the enamel. It is of interest to note that proteolysis of wool keratin also occurs more readily under reducing conditions.

If, as generally accepted, the first loss of structure of the enamel is the interprismatic material and the prism sheath, and since there is mineral as well as protein in this area, it was thought important to determine if the anaerobic atmosphere had any effect upon the minerals of the enamel.

Paper chromatography of some of these samples failed to show that fluoride had any effect upon the release of the proteins from the enamel under anaerobic conditions.

There was a consistent increase in calcium in the anaerobically treated specimens.

In the previous report, it was shown that the anaerobic environment causes a proteolysis to occur. By means of paper chromatography the marked increase of amino acids released from the enamel was shown to occur anaerobically. The present report shows that under these same anaerobic conditions the phosphorus and calcium are released in significantly greater amounts from the intact enamel than when under aerobic conditions. Preliminary histological observations would indicate that this loss of material is from the interprismatic area between the rods.

The findings herein reported and the previous report of the effect of the anaerobic environment upon the release of amino acids, might be interpreted as giving support to the proteolysis chelation theory of dental caries. It is certainly evident that a form of proteolysis did occur under the anaerobic environment. Chelation may indeed be occuring, the solutions

tested gave a pH of from 7.8 to 8.1. This would suggest that an acid is not involved. It would be very difficult to see how water plus enamel could produce any pH other than one on the slightly alkaline side of neutrality. It is not the purpose of this author to prove or disprove theories, but merely to report the findings.

Of considerable interest was the finding that the treatment of the sections of enamel with a saturate solution of sodium silico fluoride did decrease the release of phosphorus and calcium from the enamel under anaerobic conditions. It is also to be observed that the reduction in mineral loss is not as great as that found under aerobic conditions.

As we see it, the first problem faced by the microbe in producing dental caries is to effect the removal of the interprismatic material in considerable depth, thereby, producing a porous enamel. It would appear that this might easily be accomplished without acids or enzymes by an anaerobic atmosphere in the depth of developmental grooves or beneath the plaque. Whether this approach might lead to the eventual control of dental caries remains to be seen.

The treatment of an intact piece of enamel by an anaerobic atmosphere in the presence of water causes a marked increase in loss of phosphorus and calcium.

The phosphorus released under anaerobic conditions appears to be from the inorganic portion of the enamel.

The treatment of sections with fluoride reduces the amount of mineral lost under an anaerobic environment.

THE EFFECT OF WEANING ON THE INCIDENCE OF INCIPIENT CARIOUS LESIONS. RALPH R. STEINMAN, D.D.S., M.S. REPRINTED FROM JOURNAL OF DENTAL RESEARCH, VOL. 39, NO. 4, JULY-AUGUST 1960.

The following experiment was designed to determine the effect of sudden weaning upon the incidence of incipient carious lesions in the young rats. Throughout their lifetime all animals were on a cariogenic

diet. Half the animals in each litter were weaned at 20 days of age and continued on the cariogenic diet. Weaning littermates were kept together in separate cages. The other half of the animals remained with the dams on the same cariogenic diet. All animals were sacrificed at 30 days of age. The 30 animals weaned at 20 days of age had an average of 8.3 incipient lesions per animal, whereas the 30 littermates which were allowed to remain with the dams until sacrificed at 30 days of age had an average of 3.6 incipient lesions per animal.

THE IMMUNOHISTOCHEMICAL LOCALIZATION OF PLASMA ALBUMIN IN MURINE DENTINAL TISSUE, Jack D. Zwemer and Ralph R. Steinman. Reprinted from Journal of Dental Research, St. Louis, Vol. 38, No. 6, Page 1239, Nov.-Dec., 1959.

In view of the renewed interest in the histopathology of incipient dental caries and in the concept of a dental lymph suggested by the diffusion of diverse substanced through dentin (C.F. Bodecker in A Survey of the Literature of Dental Caries, Washington, D.C., 1952, National Academy of Sciences, National Research Council, Chapt. 5, p. 175), it seemed desirable to investigate the possible distribution of plasma albumin in intact dentin of the albino rat.

Specific fluoriscence referable to plasma albumin was readily demonstrable in the dentinal tubules for at least the proximal half of their course and in the pulpal margin of the dentinal matrix.

Staining was more pronounced in specimens taken from the younger animals. The controls did not exhibit the characteristic fluorescence. Further studies of plasma protein distribution in dentin—particularly of the globulins—should be of significance in assessing the dentinal tissue response to infectious agents.

CARIES AND CELLULAR NUTRITION, RALPH R. STEINMAN. REPRINTED FROM DENTAL PROGRESS, VOL. 2, NO. 3, APRIL 1962.

The mere presence of bacteria on the surface of a tooth does not necessarily mean that caries will follow. Whether the micro-organism penetrates the tooth depends in part upon that tooth's metabolism. Only when the metabolism is impaired can noxious material enter and encourage further ingress of more noxious material until gross disease is present.

As in any infectious disease, the resistance of the host is a vital factor. If this resistance is high, large numbers of bacteria can be present and there will be no disease. If resistance is low, disease follows.

The germ-free studies conducted by Orland and Fitzgerald indicate that streptococci are involved in caries. These organisms are probably the most versatile known. They produce a variety of toxins, enzymes, and acids. Since other organisms produce acids, acid alone cannot initiate

dental disease. But acid may chasten destruction of the tooth once metabolism has been altered.

To understand the pathogenesis of dental caries, one must realize that many of the metabolic processes observed in teeth are similar to those of other organs. The chemical composition of the liquid parts of dentin is similar to that of interstitial fluid. It contains amino acids, glucose, manganese, iron, calcium, phosphorous, copper, and sodium. There is a rapid exchange between this fluid and other fluids of the body, and this exchange rate varies.

The first line of defense both of the body in general and of the teeth specifically is an impermeable barrier to external noxious materials. The tooth, like the skin, does have a mechanical barrier; but the tooth also depends upon metabolic "shields." Some of these shields are ATP, ADP, acetylcholine, coenzyme A, ATPase, cholinesterase, and sulfhydryl. It has been shown that some of the metabolic shields were absent in dentin adjacent to pulp at the first evidence of pathology.

Similarly, animal studies have shown that when some of these factors are blocked, methylene blue can penetrate through both enamel and dentin, and an excess of sugar has the same effect as a deficiency of phosphorus in this regard.

What is more, stress, by altering electrolyte balance, can also selectively alter the permeability of cell membrane and tooth structure.

There are other metabolic factors. Hamsters which develop spontaneous diabetes also develop rampant caries—even on a non-cariogenic diet. It has been reported that where thyroxin is deficient, caries incidence is higher. Pyrioxine-deficient rats, hamsters, and monkeys have more decay than do animals receiving adequate amounts of pyridoxine. Castration of animals decreases caries incidence, perhaps because of a lowered serum sodium level. Heredity apparently is another factor. Other studies have shown that nutritional elements alter the incidence of caries.

The earliest changes observed in the tooth during the development of caries, then, are those related to metabolism, changes in enzyme activity, and associated factors. These changes are found beneath the earliest evidence of demineralization of the enamel. They are found in the dentin extending all the way to the pulp. They follow the organic pathways, the dentinal tubules.

The second change is in the organic portion of the tooth. With ninhydrin, there follows a marked increase in reactive proteins, probably a depolymerization of the protein and mucopolysaccharides of the dentin.

The third change is the demineralization of the hard structures of the tooth. But by the time the dentino-enamel junction is reached, histochemical changes that extend to the pulp can be observed.

Thus caries is a typical disease process in many ways. It is not a surface phenomenon but occurs in depth as a sequence of events leading

up to cavitation or necrosis and sloughing. The early events are characterized by metabolic alteration, changes in enzyme activity, changes in organic structure, and, finally, changes in the demineralization or cavitation.

Most important, the foundation of resistance to caries appears to rest upon the nutritional state of the host at the cellular level. The logical approach, then, to caries control is a way of life which includes a sound nutritional program and freedom from stress. For many individuals this would represent a changed way of life. But there is much evidence that the answer to caries lies in nothing less.

A PHYSIOLOGICAL BASIS FOR RESISTANCE TO DENTAL CARIES, **RALPH R. STEINMAN, JOHN LENORA, LOMA LINDA UNIVERSITY, LOMA LINDA, CALIFORNIA 92354.**

It is suggested that resistance to dental caries depends upon normal physiological processes operating in the tooth from pulp to enamel surface. These processes include metabolism and a fluid transport system which operates through the hard structures of the tooth to prevent the ingress of external noxious material. When these normal physiological processes are altered, resistance is lowered and dental caries may now occur.

In spite of tremendous scientific advances in the control of disease, dental caries remains one of the major health problems. Could it be that Jenkins was right when he said, "The comparative or complete failure of all methods of caries prevention on a community scale implies that their basis is wrong or that they have failed to consider all important factors"? A neglected factor is the possibility that resistance to dental caries could be systemic and mediated through the tooth.

Three conditions are generally considered to be essential in the oral cavity before dental caries will occur. The first essential is that the proper bacteria must be presented in sufficient numbers. Germ-free studies show that not just any bacteria which produce acid can cause dental caries but that only certain strains of streptococci are primarily responsible. The second essential is that bacteria must have food which includes carbohydrates among the essential nutrients. The third essential is that the teeth must be susceptible to the disease.

For the purpose of this article resistance will be divided into two parts. (1) structure of the teeth and (2) physiological factors within the teeth. Included under structure are any and all conditions which either alter or influence structure, while the teeth are being calcified and which may later influence the resistance or susceptibility of the teeth. Vitamins, minerals, trace elements and protein have all been shown to be important during the calcification of the teeth. This is well documented in the dental literature and will receive no further emphasis in

this article. Our emphasis; however, will be on the second part of resistance, namely certain physiological factors operative within the teeth in the post eruptive period. When these factors are functioning normally, resistance is effective, but, when operating abnormally, resistance is low. This is an area which has too long been ignored. There is some information on this subject

scattered throughout the dental literature in conjunction with the work done in our laboratory during the past several years. We, along with Sharpenak, believe that the evidence supports the concept that the teeth are destroyed because something has gone wrong inside of the teeth. That is, a physiological failure preceeds a structural failure.

The changes in the tooth associated with dental caries occur in depth. The first evidence of demineralization is seen beneath the surface of the enamel, and may also be evident in the dentin. At the same time that these early changes are observed in the enamel, the presence of inflammatory cells have been observed in the pulp opposite the changes in the enamel. Changes in depth are also observed in the molars of rats placed on a cariogenic diet. The evidence suggests that the carious process occurs in depth.

The nature of the carious process suggests that the major problem facing the tooth is to prevent the ingress of external destructive material. We would suggest that this is accomplished by certain physiological processes occuring in the enamel, dentin, and the pulp.

There is a movement of fluid from inside the tooth outward to the surface of the enamel. The absence in vivo of inward marginal leakage around fillings compared with that observed in vitro in teeth from the same person or animal would support this concept. Methylene blue placed in the mouths of rats fed Purina laboratory chow did not penetrate through the enamel from outside while animals of the same stock on a cariogenic diet for one week had penetration through the enamel in copeous amounts. Purina-fed animals are usually quite free of caries while those which remain on the cariogenic diet have significant amounts of dental caries. Thus, the first line of defense for the tooth may be physiological, a movement of fluid through the enamel from inside to the outside surface of the tooth which prevents the penetration of destructive material into the tooth.

The presence in the dentin of all the glycolytic enzymes, in significant amounts gives it the capability for rapid metabolism which would make a transport mechanism manditory. Many investigators from the days of Fish have demonstrated the movement of fluid through dentin. The rate of fluid transport through the dentin is under the control of the hypothalamic-parotid gland endocrine axis. This transport is suppressed in animals maintained on a high sucrose cariogenic diet compared to that observed in Purina-fed animals. Stimulation of the suppressed fluid transport mechanism in animals fed a cariogenic diet results in a signif-

icant reduction in dental caries. The administration of bradykinin to Purina-fed animals resulted in a reversal of fluid transport which produced an incidence of dental caries equivalent to that seen in sugar-fed rats. Fluid transport within the dentin is essential for maintaining active metabolism and for support fluid movement through the enamel.

The rate and the direction of fluid transport in the dentin significantly affects dental health.

The hydrostatic pressure in the pulp of teeth is higher than found in most other tissues of the body. If the blood vessels to the teeth are severed, the hydrostatic pressure in the pulp will immediately fall to zero. Ligation of the blood vessels was found to produce a significant increase in dental caries in the rat. Without the normally high hydrostatic pressure of the pulp, it is doubtful if fluid could be moved through the dentin and enamel. The pulp might be considered as a second back-up-system for the movement of fluid through the enamel from the inside to the surface of the enamel. Animals fed Purina laboratory chow usually do not have appreciable dental caries. However, if these animals are irradiated, given hydrogen peroxide, or administered bradykinin the incidence of dental caries will increase similar to that found in animals eating a high sucrose cariogenic diet. And what is the microbe eating?—Purina. Conversely, caries may be reduced 95-100% in animals maintained on a high sucrose diet if the fluid transport in the dentin is stimulated. Again, what is the microbe eating?—a high sucrose diet. In either case the external environment is apparently destructive in terms of acid but whether dental caries occurs is largely dependent upon the host, and the normal physiological activity within the tooth. In health, intricate control mechanisms operate in harmony, but in disease disharmony is found. The defense of the tooth depends upon these systems operating in harmony. When disharmony

occurs, dental caries will occur if the external oral environment is unfavorable, and the destructive material is permitted to penetrate deep into the tooth. Carlos has well observed, "We tend to assume that if all the conditions outside the tooth are favorable for caries initiation, then the solubilizing agents will more or less naturally get out. I suspect that these assumptions are not tenable . . ." We also suspect they are not tenabel, but that the physiological activity within the tooth either permits or prevents the ingress of destructive material and thus determines the health of the tooth. The attack on the tooth is launched from the external surface, whether it succeeds or not is determined by the tooth.

EVIDENCE SUGGESTING THE EXISTENCE OF A HYPOTHALAMIC-PAROTID GLAND ENDOCRINE AXIS. RALPH R. STEINMAN, JOHN LEONORA, DEPT. OF PHYSIOLOGY AND BIOPHYSICS, SCHOOL OF MEDICINE AND DEPT. OF ORAL MEDICINE, SCHOOL OF DENTISTRY, LOMA LINDA UNIVERSITY, LOMA LINDA, CALIF. 92354, VOL. 83, P. 807-815, OCTOBER 1968.

Evidence of the existence of a hypothalamic-parotid endocrine axis was demonstrated with a fluorescent dye technique. The intraperitoneal injection of the dye, acriflavine hydrochloride, provided a simple method to study the movement of fluid through the odontoblastic tubules in the dentin of rat molars. Hypothalamic extract HE) injected iv into intact rats stimulated the movement of fluid through the odontoblastic tubules in the dentin. Extracts of other tissues and antidiuretic horomone failed to duplicate the effect observed with HE. Fluid movement was not obtained in sialoadenectomized rats. Fluid movement was obtained only when the HE was administered to rats with intact parotid glands or when parotid tissue extract was given iv to parotidectomized rats. These results suggest that the hormonal stimulation of fluid movement through the odontoblastic tubules is directly dependent upon the parotid. Fluid movement was obtained in hydrophysectomized rats, which indicated that the hypothalamic factor acted directly on the parotid gland rather than through the anterior pituitary gland. Evidence was obtained that the hypothalamic factor is not the recently isolated sialogen. (Endocrinology 83: 807, 1968)

For the past 80 years data have been published which have been interpreted to suggest that the salivary glands may possess, in part, an endocrine function, or that their morphological and physiological state is dependent upon hormones from various endocrine glands. (1). Clinically, it has been reported that the parotid glands in certain patients become swollen after each successive pregnancy and lactation.

The hormone-like substance, called parotin, was isolated in a crude form from bovine parotid glands and finally prepared in a "homogeneous crystalline form" in 1949. A few of the reported biological effects of parotin were: 1) decreased serum Ca level in the rabbit, 2) enhanced calcification of teeth and bone, and 3) increased uptake of labeled inorganic phosphorus in the incisors and femurs of rats. However, this work has not been widely accepted; at best, it has been considered suggestive.

In the present study, evidence is presented which suggests that the parotid gland does function in part as a endocrine gland and that it is under the direct control of the hypothalamus. The parotid hormone(s) stimulates the movement of the fluid through odontoblastic tubules in the dentin of rat molar teeth.

Odontoblastic cells are located in the pulp adjacent to the dentin. Extending from the odontoblast, through the dentin to the base of the enamel are protoplasmic extensions of the odontoblasts within small tubules. These tubules are known as odontoblastic tubules.

When the fluorescent dye arrived in the pulp of a tooth from an intraperitoneal injection, it did not diffuse into the dentin an masse.

Rather, it moved up the individual odontoblastic tubules toward the dentino-enamel junction.

Fluid movement did not occur when saline or sodium sulfite-sodium tetrathionate solution was given alone. This suggested that the principle in the HE which stimulated fluid movement was not inhibited or depressed by the presence of ADH in the extract.

Fluorescent dyes have been used to study fluid movement through the kidney and liver. Using a similar technique to study the movement of fluid through the odontoblastic tubules in the dentin of teeth, we have obtained evidence suggesting the existence of a hypothalamic-parotid gland endocrine axis.

The data show that the hypothalamic extract contains a factor which exerts a direct action on the parotid gland. The parotid gland in response to stimulation from the hypothalamic factor synthesizes and/or secretes a hormone (s) which is carried by the circulation to the teeth. The parotid hormone stimulates the movement of fluid through the odontoblastic tubules in the dentin of the teeth.

The interpretation that the parotid hormone stimulates fluid movement in the odontoblastic tubules is based on the following observations. First, the administration of acriflavine hydrochloride does not result in a mass diffusion of the dye throughout the dentin. Rather, Steinman has shown that when the dye penetrated the dentin, it moves up specific pathways to the dentino-enamel junction. Also, the movement of the dye is inhibited with the application of KCN on the teeth. It would appear unlikely that the application of KCN would inhibit passive diffusion on the dye through the dentin. More likely, the parotid hormone(s) either stimulates an active transport of fluid up the odontoblastic tubules or it may alter the permeability of the odontoblastic cell membrane, which then permits the movement of fluid in the tubules. At present, the function or composition of this fluid is unknown. Our data show that neither saline, sodium sulfite-sodium tetrathionate solution, nor extracts of brain, cardiac muscle, liver, pituitary, or thyroid induce the movement of fluid in the odontoblastic tubules. Pitressin is also ineffective. Fluid movement is not obtained when the HE is administered to parotidectomized rats.

Fluid movement is obtained only when the HE is administered to rats with intact parotid glands or when parotid tissue extract is given to parotidectomized rats. Since fluid movement is observed in rats in which all of the salivary ducts are completely ligated, one must conclude that the parotid hormone apparently diffuses from the capillaries to exert its action on the odontoblasts located in the dentino-pulp junction.

The administration of HE to hypophysectomized rats stimulates fluid movement. However, the sensitivity of the parotid gland to the HE under these conditions decreases markedly, since a dose 18 times the minimal effective dose in intact rats is required to give a good demon-

stration of fluid movement. These results suggest that the hypothalamic factor which stimulates the parotid gland exerts its action directly on the parotid gland, and that the hypothalamic-parotid axis is dependent upon an intact endocrine system for optimum performance. This probably is the first evidence suggesting that a hypothalamic factor bypasses the pituitary to activate another endocrine gland to synthesize and/or release another hormone (s) which has an effect elsewhere in the body.

Comparing, on a protein basis, the effectiveness of the HE with the parotid tissue extract in stimulating fluid movement in the odontoblastic tubules, it becomes apparent that the HE is considerably more effective in stimulating the parotid gland than the parotid extract is in acting directly on the odontoblasts. This suggests that the available active hormone level in the parotid gland must be low or the hormone may exist in a higher concentration as an inactive precursor which is readily activated by the hypothalamic factor over a short period of time.

There is one difference between the parotin activity discussed in this paper and the parotin isolated by the Japanse investigators. They claimed to have isolated from the submaxillary gland a hormone fraction which they called S-parotin and that it possessed all the biological effects

ascribed to parotin from the parotid gland. When we administered PRF to rats having only the submaxillary gland present, no fluid movement was obtained. This suggests that the submaxillary gland does not contain the same hormone present in the parotid gland which stimulated fluid movement.

THE ACIDOGENIC POTENTIAL OF CARIOGENIC AND NONCARIOGENIC DIETS IN THE RAT. RALPH R. STEINMAN (NOT YET PUBLISHED).

In a previous study it was shown that the cariogenic potential of a high sucrose diet could be nullified by suitable dietary supplements. In particular, supplementation with carbamyl phosphate, egg shell meal and the trace elements chromium, molybdenum and zinc reduced the incidence of dental caries to levels observed in Purina fed animals.

THE EFFECT OF INFUSING SELECTED CHEMICAL COMPOUNDS ON DENTINAL FLUID MOVEMENT IN THE RAT. Ralph R. Steinman. (Not yet published)

Parotid hormone is responsible for the activation of the fluid movement mechanism in the odontoblasts of the sugar suppressed rat. This concept has been confirmed in data to be published elsewhere which demonstrates that carbamyl-DL-aspartic acid is effective in stimulating fluid movement only when the hypothalamic-parotid endocrine axis is intact. If the axis is interrupted either by placing a lesion in the hypothalamus

or if the parotids are bilaterally extirpated, carbamyl-DL-aspartic acid is ineffective in stimulating fluid movement. This suggests that the control of fluid movement is primarily under hormonal control.

DENTAL CARIES AND THE EXTENAL ENVIRONMENT. RALPH R. STEINMAN. (NOT YET PUBLISHED)

Through the years a number of bacteria have been suggested as the possible external agent in the initiation of dental caries. At one time the major emphasis was upon the lacto baccillus but in recent years the emphasis has shifted to streptococci. This shift was brought about by the germ-free studies which showed the ability of certain strains of streptococci to initiate dental caries when introduced into germ-free animals which were on a cariogenic diet. The evidence showed that certain strains of streptococci from human carious material produced dental caries in the germ-free animals. Not only does this link the bacteria with the disease but it also shows the same etiological agent in both human and animal caries.

The plaque serves two essential purposes in relation to the disease, first it provides a localized home base for the microorganisms. Second the plaque serves as a retention depot for food and for the destructive bacterial products. If the destructive material were not held in close relation to the tooth it would be quite harmless.

Acid production has been studied in a number of ways. For example the salvia from a person or animal may be incubated with various foods at 37 C for a period of time. The pH of the resulting incubation is measured and the amount of acid determined by titration. The bacteria need other nutrients in addition to sugar. This may explain why in these incubation studies usually the more nutritious mixtures in terms of vitamins, minerals etc. will produce the lowest pH and the most acid. Although acid may be essential in the caries process, its production in these mixtures gives no indication of the amount of dental caries to be expected when these same foods are fed to experimental animals. Another method is to collect the food from the occlusal grooves of teeth and measure the amount of acid in this material. In this study no significant difference was observed in the amount of acid produced in cariogenic and non-cariogenic foods.

All of these various experiments on the possible acid production in the external environment would lead one to conclude that in terms of acid the external environment is

destructive regardless of the diet. It is further concluded that no relationship was found between the amount of acid produced by a food and the amount of dental caries that food will produce if fed to experimental animals. This should not be interpreted to mean that acid is of no significance in the carious process, but rather that even though it may be essential if the teeth are physiologically normal they will survive despite the external environment.

Since the application of acid to the external surface of a vital tooth usually results in erosion rather than dental caries it is possible that something in addition to acid is involved in the carious process. Evidence has been presented that a change in electrical potential may be involved.

The removal of the salivary glands has been observed to increase the incidence of dental caries. This is usually interpreted as showing the importance of salvia on the external surface of the teeth. This conclusion is not valid in that the removal of the salivary glands also removes any endocrine factors the salivary glands may have. For example, the hypothalamic-parotid gland endocrine axis would no longer be present. This has been shown to be of primary importance in the stimulation of fluid transport in the dentin. The importance of the salvia on the external surface of the teeth was shown by the only study of its kind in the literature to be of no value. It was shown that when the salivary glands were removed an increase in dental caries occurs. If saliva was added to the water of the desalivated animals further increase in dental caries was observed.

It is obvious that the removal of the plaque would dramatically reduce the incidence of dental caries. Why then has not this approach shown more favorable results? We suggest that the removal of plaque is difficult at best and impossible in pits and fissures. We would not suggest that oral hygiene be abandoned, but rather that in addition to oral hygiene that due thought be given to the systemic factors involved in resistance to dental caries.

It is well known that in the manufacture of sugar, white flour, and similar products that the major portion of vitamins, minerals, and trace elements are removed. Of the total caloric value of the average American diet it is estimated that roughly 50% is composed of these impoverished foods. One of two approaches may be suggested. The first and best approach would be to replace these impoverished foods by more nutritious ones, to dramatically reduce the use of refined sugar. A marked improvement could be made by eating no sweet sugary material between meals, by reducing the total amount of sugar in the diet to perhaps one fifth of what is used today and lastly by replacing the refined cereal products with whole grain products. This would in effect, increase the nutritive value of the diet. It would also increase the amount of fiber in the diet which appears to be of importance. In short, the whole body would benefit from this program as well as the teeth.

An approach of this kind would improve the general health as well as the oral health. For a healthy body, including the teeth, feed it well.