Reprinted from Publication No. 19 of THE AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE, pages 68–73.

RESUME OF THE FLUORINE-CARIES RELATIONSHIP

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OUR series of investigations on the relation of nutrition to dental caries was started in November, 1933, as a study of the factors which would alter the rate of decay. We chose the rat as the experimental animal for these studies in spite of the fact that at that time it was believed the dental lesions produced in rats by coarse cereal particles were not analogous to dental caries in man. However, we reasoned that, if the lesions were first initiated by coarse corn meal and then the rat was placed on diets which did not contain this initiating factor, any further progress of the condition would be caused by a true carious process. This premise proved sound, for we readily observed in many cases that caries continued to develop after we had removed the animal from the ration of coarse corn meal.

Concurrently with these experiments in caries, we were investigating the effects of various diets on the entire life cycle of the rat. As reproduction and rearing of young was part of that experiment, we had many young rats in excess of our needs. As a matter of economy, we used many of these young rats in our dental caries experiments. We observed, particularly in the case of animals which had been fed a high meat diet, that their offspring were distinctly more resistant to dental caries. This fact suggested to us that the diet during pregnancy and lactation or, in other words, the diet during the period of the formation of the permanent molar enamel of the young rats, influenced subsequent susceptibility to corn meal caries. We accordingly set up a series of experiments to test the effect of various food factors in conferring relative resistance to caries in young rats.

Among other substances tested, we supplemented the diet of mothers during pregnancy and lactation with 0.5 gm of XXX liquor. This is a product of the Borden Company obtained as a residue, when all other useful products have been removed from milk. It is very rich in the B-group vitamins, and is saturated with its mineral constituents. Mothers fed the supplement of XXX liquor bore young with very high resistance to corn meal caries. In April, 1936, at the Washington Meeting of the American Institute of Nutrition, George R. Sharpless (1936) gave a paper indicating that the addition of aluminum salts to the diet of the rat in which fluoride was present prevented the formation of mottled incisors in rats. This suggested to us that XXX liquor contained aluminum salts, which were preventing the deleterious action of fluorine. Consequently, we set up an experiment to produce teeth which were quite susceptible to decay because of the high fluorine content in the mother's diet during pregnancy and lactation. It was then our intention to add aluminum salts to the diet to prevent the bad effects of fluorine. However, the results of this preliminary experiment were the most resistant teeth that we had observed at that time. Consequently, we changed our hypothesis regarding the relation of fluorine caries to the one that there is an optimum content of fluorine in the diet during the period of formation which will produce highly resistant teeth. It cannot be said that we sought such results; they came to us in an experiment designed to prove exactly the opposite of what we observed.

It may be pointed out that a certain series of fortunate events made possible this early experiment. The fellowship system of industrial research at the Mellon Institute

provided the accident that we were adjacent to the laboratory where studies of industrial uses for XXX liquor were under way. As we were studying the effects of single carbohydrates on dental caries, we used the vitamin-free casein of the Casein Company of America, because it contained a minimum of lactose. We were not interested in the fact that it was vitamin-free. Fortunately for us, it is also the casein with the lowest content of fluorine of any casein then or now on the market. Pittsburgh tap water, which we used, is almost free of fluorine, showing about 0.2 to 0.3 parts per million. We purchased a salt mixture (Harris) which we believed to be the Osborne and Mendel (1917) salt mixture. When we became interested in fluorine, we analyzed this salt mixture and found it to contain only one-fourth the fluorine expected of the usual Osborne and Mendel salt mixture. Inquiries of the manufacturer brought the statement that this was an older formula given to him by Osborne, and that there was no addition of sodium fluoride to the formula. It may be pointed out that subsequent analysis of the XXX liquor revealed that it contained adequate amounts of fluorine to explain the beneficial effects of the early experiments in which it was fed to the mothers.

The first experimental results, using sodium fluoride, were obtained in August, 1936 (Weidlein 1937). At that time the proponents of the toxic effect of fluorine were in their ascendancy and we (Cox *et al.* 1939a) did not at once reduce our results to formal publication.

Our only studies in dental fluorosis were in an entirely different set of experiments, and were not directly concerned with caries. We fed a geometric series of daily doses (up to 256 micrograms) of fluorine directly to suckling rats, terminating this regimen at the time the rats were weaned at 21 days of age. We produced mottled enamel in the first two permanent molars (Cox *et al.* 1939b). Later, incidental to another study (Dixon and Cox 1939), we observed mottled enamel in the third molars only of rats that had been fed high fluoride diets from 21 days of age. None of the teeth of our main experiments *on the relation of fluorine to dental caries was mottled.*

Many authors have failed to notice the sharp difference of our experimental condition from those of Miller (1938). He fed very high doses of fluorides directly to experimental animals, and the feeding was *after the eruption of the teeth.* It may also be pointed out that since approximately two-thirds of Miller's diet consisted of coarse particles, which were not penetrated by the fluoride added to the diet, the effective concentration which he had employed was about three times that which he reports. Obviously, the conditions employed by Miller are far different from those which exist in areas where very low concentrations of fluoride in the drinking water result in an increased resistance to caries.

In our procedure, the fluorine reached the young animal by strictly physiological routes; namely, placental and mammary transmission. The dosage was a sub-mottling one. No fluorine was given to the rats after their teeth were erupted. Furthermore, the result is due to fluorine alone. It is the only variable of significance between the control and the experimental animal. The amount of sodium added as sodium fluoride is insignificant compared to the total sodium present in the diet, but the fluoride was a significant addition.

These conditions are different from those in observations on children where criticism can be levelled at conclusions that fluorine is the sole factor, inasmuch as other minerals of the waters vary. Our experimental demonstration of fluorine prevention of caries serves to bolster the conclusions in the human caries field that fluorine is indeed the active factor.

We do not believe that the prevention of caries in rats is due to fluorine in the saliva. This would imply that a certain amount of fluorine has been stored in the tissues of the rat, presumably in the bones, and released for secretion into the saliva to act in some anti-caries manner. We have (Cox 1940) pointed out that if fluorine acts through the medium of saliva, then one must expect to

find a higher fluorine content in the saliva of children in districts with fluorine in the water, and where a lower incidence of caries is observed. In our own meager experiments (Cox 1940) we did not find that difference to exist between Galesburg and Quincy, Illinois, children. This finding, wholly inadequate to prove the point, has, however, been substantiated by the findings of McClure (1941a).

The data of Bibby and Van Kesteren (1940) indicate that it takes very substantial quantities of fluorine to produce a marked effect in the lowering of the acid producing activities of bacteria. Furthermore, the viability of the bacteria was unaffected. Unfortunately, their data do not apply to saliva as their control medium, as shown by our calculations (Cox 1940) and subsequent analysis of their medium supplied to us by Bibby, had a fluorine content of 0.3 parts per million. This is three times the normal content of fluorine in the human saliva (McClure 1941a). It is not safe to extrapolate data of this order in a physiological way, as substances which inhibit at one level may promote activity at another. However, we have made experiments of a preliminary nature on media containing approximately 0.1 part per million of fluorine, and found that the addition of very small amounts of fluorine at that level does have remarkable inhibitive effects on acid production. It remains, however, to be shown that there is actually a difference in the fluorine content of the saliva between caries-free and caries-susceptible individuals.

It may be pointed out that the data of McClure (1941a) have to do with the average content of fluorine in the saliva, compared with the average caries, or average fluorine content of the water. If, however, for each individual there were determined the current fluorine level in the saliva, the count of acidogenic organisms, and the extent of active caries, and these data were analyzed then by the usual methods of correlation, it is not unlikely that some relation would be found between the fluorine content saliva and the activities of caries-associated organisms.

It must be pointed out that since in the experiments of Miller (1938) there were very high concentrations of fluorine present in the oral cavity in soluble form, and hence high concentrations were to be expected in the saliva and even to be resecreted into the saliva, still *some caries did exist.* We have observed this in rats to our own satisfaction, in a repetition of the work of Miller (Dixon and Cox 1939). It seems safe to conclude, therefore, that with present data there is no indication that fluoride, acting in the oral fluid, can inhibit dental caries.

We have concluded, from critical examination of the data of others, that the fluorine in the teeth does not act in any antibacterial manner to prevent caries. Armstrong and Brekhus (1938) have shown that human mottled enamel decays, even though it contains five times the fluorine of ordinary carious enamel. However, enamel with its fluorine content only moderately elevated is resistant to caries. Now the anti-bacterial action of fluorine would be expected to be a chemical one, and consequently active in proportion to the concentration of the fluorine. As highly fluorosed enamel is subject to decay, it does not seem that the anti-caries action can be a chemical one.

Perry and Armstrong (1941) have shown that dietary fluoride can enter the posteruptive enamel of rats in significant amount. McClure (1941b) has also shown that if fluoride is added to the rat's diet, or is injected, it enters into the enamel of the permanent teeth. However, McClure did not find that such enamel is more resistant to caries. Armstrong, however, in this volume (page 62) has presented data on caries development contrary to those of McClure. Cheyne (1940), using our methods (Cox *et al.* 1939b) to produce mottled enamel in suckling rats, found that these rats developed numerous cavities on coarse corn meal diets, showing again that highly fluorosed enamel is subject to caries. Also, it may be pointed out in the Miller experiment (1938), where a high content of fluorine was present in the diet, that some of it must have penetrated into the enamel

(McClure 1941b; Perry and Armstrong 1941). Nevertheless, this fluorosed enamel was subject to decay, as even under the extreme conditions of Miller's experiment, with fluoride in the saliva as well, some caries resulted.

Dean (1941) and his associates observed that the teeth of children born at Bauxite, Arkansas, at about the time of the change in the water supply from a high fluoride water to one quite low in fluorine, were much more resistant to caries than those of the same aged children at Benton, Arkansas, only four miles away, but whose water supply had always been low in fluoride content. This indicates that there was a definite resistance to caries, due to the water used during the formation of the teeth.

Now, as it does not seem plausible that fluorine is acting in any bactericidal or bacteriostatic method, either through the enamel of the teeth or through the saliva, some other mode of explanation of this resistance to caries must be adopted.

Fluorine definitely alters enamel structure. In fact, as pointed out elsewhere in this volume by McKay (page 5), fluorine is the only known element which does alter the structure. Furthermore, it is known to alter structure only when it is present in diets during the formation of the enamel. Consequently, it is logical to invoke a structural explanation of the resistance to caries.

We may assign three regions of structures which shade imperceptibly into each other. First, there is the low fluorine enamel which, because of some structure, is highly susceptible to caries. Then there is the optimum region of fluorine, in which a resistant structure is produced. Third, there is that more highly fluorosed enamel which becomes brittle, or susceptible to destruction and is again susceptible to caries. The structure which is caries-resistant may be one in which fluorine takes an active part in the structure, or it may be one induced by the presence of fluorine at the time of the formation of the tooth. The level of fluorine in the enamel may be adventitious.

We have no data as to what this resistant structure may be, whether it may be crystalline, an alteration of the interprismatic substance, or only on the surface. The last seems highly probable. We suggest that some micro-contour of the surface may be found associated with this increased resistance to caries.

Having arrived at the conclusion that structure plays the dominant role in the prevention of the initiation of caries, we were then led to theorize on the cause of the initiation of caries. Now if structure prevents caries, obviously caries arises through a failure of structure. Failure of structure suggests a mechanical cause. In this case, the origin of rat caries, through the use of hard particles in the diet, lends considerable weight to a theory of traumatic origin of caries. The fact that teeth are traumatic agents is not contrary to this view.

It is obvious that caries does not suddenly appear as large cavities, but develops from some very small origin. It is our hypothesis that through forces of compression, tension, and a combination of these in shear, openings in the enamel appear of the order of size of microorganisms. These new crevices may then be colonized by bacteria. If these acidogenic organisms are present, and their food supply is sufficiently available, caries will ensue, whatever the fluorine content of the enamel may be.

It must be pointed out that these destructive forces of compression and of tension would be most concentrated at points of curvature. These points of high curvature coincide with the sites where tooth decay is known to be most prevalent. It is a coincidence that in these sites of maximum curvature, food stagnation also occurs, which supplies microorganisms with nourishment for excavation of tooth substance.

It must be pointed out that in this theory, the conditions must be favorable for caries development. If caries does not ensue, the micro-crevices may be colonized by non-acid forming organisms, they may be filled with debris, or they may be ground away in attrition. On this theory, people who are known to be relatively free of caries, may not be said to have teeth resistant to caries,

but that they are only not eating the foods which cause these incipient lesions to develop into macro-lesions.

This, then, gives us a tripartite theory of the origin of caries. First, an inadequacy in the diet—fluorine being one factor—results in a caries-susceptible enamel. Second, through trauma, a crevice is developed in the enamel—an incipient lesion; and third, by chance invasion of that lesion or lesions, by aciduric organisms, with a plentiful supply of the proper food, caries results. If such a theory represents the true sequence of events, the logical way to prevent caries is to provide the preventive diets at the time of the formation of the enamel. The second stage, the traumatic induction of caries, cannot be avoided. The third stage, the development of the cavities under modern conditions is also practically unavoidable. If the teeth are susceptible to decay, the only practical means of control is restorative dentistry.

We have been led to this three-stage theory by other experimental evidence and by observation. It has been observed by many, but chiefly stressed by Lilly (1932), that rats, on high fermentable carbohydrate diets, do not develop caries, even though they be supplied with mouth washes containing aciduric organisms. We also have observed that only an occasional carious tooth developed in our stock animals, which were on diets containing 66 per cent of fermentable carbohydrates. These stock diets, incidentally, were very low in fluorine, as they were based upon the vitamin-free casein, Pittsburgh tap water, and the low fluorine salt mixture (p. 69). On the other hand, if caries was first induced in rat teeth, fermentable carbohydrates promoted a further enlargement of those cavities. This logically leads to the conclusion that the combination of fermentable carbohydrates and bacteria, which are constantly present in the mouth of the rat, is insufficient to induce caries in the rat. Trauma is necessary.

Bodecker and Ewen (1937) have studied unilateral caries in man, defined as caries in the proximal surfaces in adjoining teeth in which the condition is present in only one of the two contacting surfaces. They point out that the same bacteria and the same food, which impact there, are present in contact with both surfaces. Consequently, the bacteria and the impacted food cannot explain the origin of caries in only one side of the region. They explain this difference in unilateral caries by the difference in age of the teeth. The diets during the formation of such teeth and the post-eruptive exposures differ. This argument, however, breaks down with the central incisors. In this case, the adjoining teeth are practically of identical age, both as to pre- and post-eruptive conditions.

However, there are subtle traumatic influences which act on even adjacent teeth. Because of different positions of the teeth themselves, or of the teeth with which they come in occlusion, because of difference in biting habits, one of these teeth may come under stronger traumatic influence. The present position of those teeth may not be a true index as to their past traumatic history. They may have suffered accidental trauma, the single experience being sufficient to initiate the lesion.

In any pursuit of this hypothesis, very large numbers of pairs of incisors should be observed. It must be recalled that trauma may have been preeruptive, that migration of teeth may have changed conditions, that habits of chewing may have changed. In addition to study of central incisors, it may be noted how many malposed teeth, not subject to trauma, are decayed; also, how early the erupting teeth decay before they have come into full occlusion, and thus suffer trauma.

Conclusion

We have concluded, as a result of our experimental studies and the critical examination of the data of others, that caries-resistant teeth can result from preeruptive diets; that fluorine is *one* factor in the construction of such caries-resistant teeth; and that a posteruptive effect of fluorine, under normal conditions, is yet to be demonstrated.

Following such conclusions, we have recommended (Cox 1939) that studies be made, leading to the addition of fluorides to community water supplies, where that element is too low to provide protection against dental caries. On the other hand, we believe that complete removal of fluorine from drinking water should be avoided. When the mode of action of fluorine is known, and the results are generally accepted, and the optimum dosage, regulated for a condition of various temperatures, has been determined, then the fluorine content of community water supplies can be regulated to effect a mass prevention of dental caries. If the very significant reduction of dental caries observed in northern Illinois is because of the chance concentration of fluorine in the water, it seems that much better results can be obtained by seasonal control of the fluorine at the optimum level. Fluorine is the only element thus far proved to have a definite relationship to dental caries. It is not unlikely that other factors are required for the caries-resistant structure. We have ourselves observed much improved teeth in rats resulting from diets of 100 per cent meat; we have found rats coming from diets in which the mother had a high-fat diet, half being butter fat, suggesting that vitamin D may play a part if present during the period of the formation of the teeth. Conclusive proof of preeruptive factors other than fluorine, however, we regard as lacking. We have found no substance which acts through metabolic channels to alter the rate of dental decay if it is fed posteruptively. We believe that when all the facts are known, the toxic effects of fluorine will be far outweighed by the beneficial effects in the prevention of dental caries.

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Reprint #1000

To Margaret, co-author,
with 100% appreciation from
me who doesn't believe in
use of "100%" except in very
rare circumstances.

Gerald J. Cox