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## Inhibition of Experimental Dental Caries in the Rat by Fluoride and Iodoacetic Acid.

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The initial lesion of dental caries appears invariably in the outer enamel surface of the tooth. Since inflammation cannot occur in the completely mineralized, avascular enamel the carious process differs from most pathological lesions by the absence of any noticeable tissue reaction. Therefore the initial lesion may be considered as a localized decalcification—the result of an interaction between the chemical constituents of the tooth surface and the *outer* adjacent environment. Most observers believe that the initial lesion begins under a plaque of bacteria. If this be so, it appears plausible that these bacteria may themselves cause the “localized decalcification” (1) by dissolving out minerals by acids normally produced in carbohydrate metabolism and thus *incidentally* causing the carious area, or (2) by dissolving out phosphate, *of necessity*, to maintain their normal metabolic processes. Either process would depend on enzyme systems capable of transferring the phosphate radical. That the phosphorylation mechanism for carbohydrate breakdown described by Embden, Meyerhof and others in muscle and yeast also operates in bacterial metabolism has been indicated by Nelson and Werkman.<sup>1</sup>

If the above hypothesis is correct then it might be anticipated that specific inhibitors of the phosphorylating and dephosphorylating enzymes, such as fluoride and iodoacetic acid, should delay the initiation or spread of dental caries. To test this hypothesis, the effects of small amounts of fluoride and iodoacetic acid added to the food and water have been observed in rats placed on a diet capable of producing extensive caries in the molar teeth. These compounds are of particular interest because, in addition to their specific effect on the phosphorylating mechanisms involved in carbohydrate breakdown, they inhibit the enzyme system postulated for the calcification (and presumably decalcification) of bone.<sup>2</sup>

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<sup>1</sup> Nelson, M. E., and Werkman, C. H., *J. Bact.*, 1936, **31**, 603.

<sup>2</sup> Robison, R., and Rosenheim, A. H., *Biochem. J.*, 1934, **28**, 684.

*Experimental.* The caries-producing diet consisted of the following: 630 g of brown rice (Comet brand); 280 g dried milk powder; 30 g alfalfa meal; 50 g "Crisco" (vegetable fat); 10 g NaCl.

The Crisco was warmed just to the melting point. The rice was thoroughly stirred into the warm Crisco until all the grains were completely and uniformly coated; the rice was then mixed with the other constituents of the diet. When fluoride and iodoacetic acid were added to the diets these compounds were dissolved in the warm Crisco and coated on the rice as described above. By this method of preparation, each particle of rice received a permanent coating of the fluoride or iodoacetic acid. Since rats eat one grain of rice at a time, and do all their chewing with the molars, the method of preparation of the diets ensured adequate contact between the chemical compounds and the surfaces of the teeth during mastication. Control diets were prepared exactly as above except for substitution of 630 g of dried rolled oats for the rice.

The animals were divided as follows:

*Group I.* Twenty animals on the diet of rice and pure H<sub>2</sub>O.

*Group II.* Twenty animals on the rice diet with fluoride added as follows: *Group II A.* 10 animals. Sodium fluoride, 250 mg per kilo of the complete diet. The fluoride was coated on the rice as described above. The water contained 4.2 mg NaF per liter. *Group II B.* 10 animals. Calcium fluoride, 500 mg per kilo of diet. Water containing 4.2 mg NaF per liter.

*Group III.* 10 animals. Iodoacetic acid, 200 mg per kilo of diet; 20 mg per liter of H<sub>2</sub>O. The diet for this group also contained 4 g powdered baker's yeast per kg to obviate the toxic action of iodoacetic acid on intestinal absorption.<sup>3</sup>

Groups of 10 animals each were given the control diets containing the rolled oats.

Female, albino rats, all about 28 days old and of the same stock were placed on the various diets at the same time. They were fed continuously for 100 days at which time all the animals were sacrificed, and their lower jaws were divided at the symphysis. A count of the carious lesions on each side was made. In one count, made before completion of a simplified technic by Dr. George Gomori of the University of Chicago, one segment of the lower jaw was ground down in the sagittal plane and each of the 3 molar teeth examined. Numerous planes were examined under a dissecting microscope,

<sup>3</sup> Laszt, L., and Verzar, F., *Pflüger's Arch. f. d. Gesamte Physiol.*, 1938, **236**, 693.

TABLE I.

Group	Diet and H <sub>2</sub> O	No. of animals	No. lower molar teeth examined	No. of lesions		
				Total	Per animal (X100)	Per tooth (X100)
I	Caries diet. Pure H <sub>2</sub> O	17	102	30	177	30
II	" " " plus NaF in food and H <sub>2</sub> O	9	54	3	33	6
III	" " " CaF <sub>2</sub> in food, NaF in H <sub>2</sub> O	10	60	9	90	15
IV	" " " iodoacetic acid in food and H <sub>2</sub> O	10	60	1	10	2

TABLE II.

Group	Diet and H <sub>2</sub> O	No. of animals	No. lower molar teeth examined	No. of lesions		
				Total	Per animal (X100)	Per tooth (X100)
I	Caries diet. Pure H <sub>2</sub> O	17	51	16	96	31
II	" " " plus NaF in food and H <sub>2</sub> O	10	30	1	10	3
III	" " " CaF <sub>2</sub> in food, NaF in H <sub>2</sub> O	10	30	3	30	10
IV	" " " iodoacetic acid in food and H <sub>2</sub> O	10	30	0	0	0

magnification 30  $\times$ , and definite areas of decalcification or actual cavitation were recorded. Later, the other half of the lower jaw was stained and cleared by the more exact Gomori technic (unpublished) and the 3 molars examined for carious areas. The upper molar teeth which are much less susceptible to experimental caries were not examined.

*Results.* The total numbers of lesions found by the 2 methods are grouped in Table I, and indicate that the animals fed sodium fluoride or iodoacetic acid, in addition to the caries-producing diet, show a very low incidence of caries as compared with the animals in Group I. The inhibiting-power of the calcium fluoride appears to be less marked than that of sodium fluoride or iodoacetic acid.

The results of the count performed by Dr. Gomori are recorded in Table II where the inhibitory effect of iodoacetic acid and sodium fluoride on the development of the rat caries is very strikingly demonstrated. This count represents one-half of the teeth recorded in Table I, but is probably equally significant because the improved histological method was employed. Both counts exhibit the same trends in each group, as shown by a comparison in Tables I and II.

The control animals on the oatmeal diet with or without addition of fluoride or iodoacetic acid did not develop any carious areas in the 100-day period.

The animals on each diet grew at a normal rate. Those fed sodium fluoride developed distinct mottling of the *incisor* teeth, and the ones given calcium fluoride slight mottling.

The results indicate a marked inhibition of experimental caries by both iodoacetic acid and sodium fluoride. Even though the numbers of animals studied are small it seems likely that the very great inhibition of caries caused by the iodoacetic acid and sodium fluoride represents significant results. The effect of calcium fluoride is less definite. (Calcium fluoride was tested in the hope that this insoluble fluoride would adhere to the teeth more tenaciously than sodium fluoride and act more effectively than the latter.)

The inhibitory compounds act probably by contact either (1) between the food and the flora of the tooth surface and/or (2) between the saliva and the tooth surfaces. The additional possibility that inhibiting quantities of iodoacetate or fluoride were incorporated into the tooth substance seems unlikely since the rat molars are rather completely developed and calcified by 28 days, the beginning of the experiment. Since knowledge of the action of fluoride and iodoacetic acid on protoplasm and cellular reproduction is still incom-

plete it is impossible to state definitely that the inhibitory effect of these compounds on dental caries results from specific effects on discrete enzyme systems, such as phosphorylation.

*Summary.* Sodium fluoride and iodoacetic acid added to a caries-producing diet fed to albino rats diminished greatly the incidence of carious lesions in the molar teeth.

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## Phospholipids and Complementary Activity.

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In a previous communication,<sup>1</sup> it was demonstrated that prolonged extraction of active, dehydrated complement with organic solvents, *i. e.*, alcohol, ether, etc., resulted in no reduction of complementary activity. In fact, the recovered lipids often exhibited anticomplementary properties when returned to the extracted residues prior to titrations.

Recently, Bloor and Snyder<sup>2</sup> have noted that a buffered suspension of oxidized phospholipids oxidizes a sensitive methylene blue preparation.

In view of earlier work from this laboratory,<sup>3</sup> which revealed an intimate connection between complementary powers and oxidation-reduction phenomena, it was decided worthwhile to study the effect of oxidized and unoxidized cephalin and lecithin on complementary activity.

Purified preparations of the 2 phospholipids were employed throughout these experiments. The oxidized phospholipids were prepared according to the technic of Bloor and Snider and complement was titrated by the method advocated by Ecker, Pillemer, Wertheimer and Gradis.<sup>4</sup>

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<sup>1</sup> Ecker, E. E., Pillemer, L., and Grabill, F. J., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **38**, 318.

<sup>2</sup> Bloor, W. R., and Snider, R. H., *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 215.

<sup>3</sup> Ecker, E. E., Pillemer, L., Martienssen, E. W., and Wertheimer, D., *J. Biol. Chem.*, 1938, **123**, 351.

<sup>4</sup> Ecker, E. E., Pillemer, L., Wertheimer, D., and Gradis, H., *J. Immunol.*, 1938, **34**, 19.