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EXPERIMENTAL DENTAL CARIES

IV. Fluorine and Its Relation to Dental Caries

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In the first of a series of papers on mottled enamel, Black in collaboration with McKay (6) indicated that there was a lower incidence of dental caries in mottled enamel areas than elsewhere. Bunting, Crowley, Hard, and Keller (7) found the percentage of children with dental caries in a mottled enamel district

> "about the same as would be found in any other community, but although caries occurred in the mouths of most children, its extent and activity were remarkably limited. The great majority of cavities consisted of small pit and fissure lesions in the molars and seldom did caries extend beyond that stage. In this respect the behavior of dental caries in the mouths of these children is distinctly different to that which usually occurs."

McKay (25) in 1929 presented data showing definitely that mottled molars are less subject to dental caries than normal molars. The discovery of the causal relationship of fluorine to mottled enamel (8, 32) two years later led to numerous substantiating surveys of the fluorine content of water supplies and the resulting severity of mottled enamel, but the incidence of dental caries has not generally been reported. A notable exception is the observation by Arnim, Aberle, and Pitney (5) that of 1605 permanent incisors, 24 per cent having white spots in the enamel, none was carious. Masaki (26) and Erausquin (21) observed reduced incidence of dental caries in endemic mottled enamel areas. Ainsworth (1) described less caries in mottled enamel cases and of special interest found "12.9 per cent of deciduous teeth carious against 43.3 per cent in all districts." He learned of only one case in which deciduous teeth showed slight mottling.

Dean (15), from 1– previously unpublished data on dental caries in some of the cities surveyed for mottled enamel (16, 17, 18), from 2– his data of mottled enamel for South Dakota (19) correlated with

incidence of dental caries in the same areas (27), from 3– similar comparisons for 4 Colorado cities, and from 4– data on dental caries (27) and information from the Wisconsin State Board of Health on the fluorine content of the water supplies of 7 Wisconsin cities, concluded "that the severity of dental caries is, in general, lower in mottled enamel areas. . . ."

Dean, Jay, Arnold, McClure, and Elvove (20) compared the incidence of dental caries in 12, 13, and 14 year old children in Galesburg and Monmouth with that in Macomb and Quincy. These Illinois cities are comparable socially and meteorologically. Galesburg and Monmouth have water supplies with 1.7–1.8 p.p.m. of fluorine and 46.9 and 67.7 per cent of the children, respectively, have detectable mottled enamel. Macomb and Quincy have 0.2 p.p.m. of fluorine in drinking water and for the children 1.6 and zero per cent mottled enamel, respectively. The caries incidences are, in terms of carious teeth per 100 children: Galesburg, 201; Monmouth, 205; Macomb, 401; Quincy, 633. The respective percentages of caries-free children were 35.1, 35.1, 14.3, and 4.0. The distribution of children according to the number of *L. acidophilus* found in the saliva shows consistently more Galesburg children with the low numbers compared with Quincy. There was no significant difference in the amylolytic activities of the salivas from these latter 2 cities. The incidence of caries of interproximal surfaces of the 4 maxillary anterior teeth was extremely low in Galesburg and Monmouth, and mortality of the first permanent molars was about one-fourth that of Macomb and Quincy. The authors were convinced that water supplies affect dental caries incidence, but could not conclude that the higher fluoride content of the waters of Galesburg and Monmouth accounted for the better resistance to dental caries in these cities as compared with Macomb and Quincy because of other differences in the mineral composition of the water supplies.

Evidence of an entirely different kind for a beneficent rôle of fluorine was provided by Armstrong and Brekhus (2, 4), who reported the mean fluorine content of sound and carious human enamel as 0.0111 and 0.0069 per cent, respectively. They suggested a causal relationship.

Miller (28) inhibited caries development in rats by either 250 p.p.m. of sodium fluoride or 500 p.p.m. of calcium fluoride in a caries-produc-

ing ration. Miller's conditions, however, were entirely different from those concerned with the formation of caries-resistant teeth.

Sharpless and McCollum (31) observed no evidence of abnormality in the teeth of rats raised on a low fluorine diet.

The present authors, on evidence described in this paper, first suggested a beneficent rôle of fluorine in the formation of caries-resistant teeth in the published abstracts of the annual reports of the director of Mellon Institute (33) and elsewhere (10, 11, 22).

TABLE I

Milligrams per 4 grams of salt mixture

	SALT MIXTURE	
	Osborne and Mendel	Harris
	mg.	*mg.*
Sodium	137	136
Potassium	737	294
Calcium	496	273
Magnesium	64	58
Iron	12	24
Manganese	0.265	None
Aluminum	0.013	None
Phosphorus	310	409
Sulfur	27.7	76
Chlorine	478	113
Fluorine	(1.03)	None
Iodine	0.144	None
Ca/P	1.6	0.67

EXPERIMENTAL PROCEDURES

A ration of sucrose[1] 66, casein[2] 20, "Crisco" 10, and salt mixture 4[3] (See Table I) was used as the basis of the studies, except for diets 11–3 and 11–4.

The number of milligrams of each element contributed by 4 grams of each salt mixture to 100 grams of food is shown in Table I.

Merck's reagent sodium fluoride was added to the salt mixtures to provide the number of parts per million set forth in Table II. The basis of the addition was the 10.3 p.p.m. fluorine contributed by the sodium fluoride of the regular Osborne and Mendel salt mixture (30).

[1] Commercial refined cane sugar.

[2] Vitamin-free product of the Casein Company of America.

[3] Harris salt was purchased from The Harris Laboratories, Inc. The Osborne and Mendel salt (30) was made from C.P. reagents without the addition of sodium fluoride.

The data of Table I are based on the salts as added rather than by analysis.

Diets 11–3 and 11–4 were yellow corn meal 66, whole milk powder ("Klim", unirradiated) 30, alfalfa meal, powdered to pass 60-mesh sieve, 3, and C. P. sodium chloride 1.

During pregnancy, the animals on all diets except 11–3 and 11–4 received a daily supplement of 1 gram of fresh beef liver, 0.5 gram dried yeast, 0.5 gram of alfalfa, and 0.1 drop of Haliver oil with viosterol. They were given the same supplement during the lactation period with an addition of 1 gram each of yeast and alfalfa. The animals on diets 11–3 and 11–4 received distilled water *ad libitum*; the others were supplied with tap water containing 0.3 p.p.m. fluorine as shown by analysis.

The litters were reduced to 8 after three days and to 6 on the seventh day, preferably to 3 males and 3 females. The young were weaned at 21 days and placed on a diet of the same composition as diet 11–3, except ordinary sodium chloride was used.

All young were killed after 8 weeks on the corn meal caries-producing diet and the teeth were examined for occlusal and fissure caries.

TABLE II

Diets of rats during pregnancy and lactation and incidence of caries in their offspring

Diet Number	8–56	8–115	8–120	10–2	10–3	10–6	10–3+ 10–6	11–3	11–4
Number of Offspring	63	33	52	66	62	65	127	40	62
Salt Mixture[a]									
Harris	4	4							
Osborne and Mendel[b]			4	4	4	4			
Added Fluorine, p.p.m.	0	41.2	0	0	10.3	41.2		0	20.6
Occlusal Caries									
Mean Incidence	10.13	5.97	8.67	12.15	9.87	10.37	10.15	12.80	12.79
Standard Deviation	7.19	5.07	3.66	4.51	3.88	4.96	4.4	4.38	4.77
Fissure Caries									
Mean Incidence	16.79	11.76	16.77	16.98	16.71	15.84	16.27	19.00	19.77
Standard Deviation	3.46	5.12	3.45	3.95	3.56	4.55	4.12	3.12	3.13

[a] gm. per 100 gm. food.

[b] fluorine free.

RESULTS

The data relating to dental caries are shown in Table II. Table III (12) presents the interpretation by statistical analysis.

The studies using diets 8–56, 8–115, and 8–120 were completed in December, 1936, with respect to occlusal caries, the fissure caries scoring being done only recently. As the difference between diets 8–56 and 8–115 is solely the addition of sodium fluoride to the latter, and the chances are 1000 to 1 against the difference of caries incidence being due to chance, it may safely be concluded that the 41.6 p.p.m. additional fluorine has led to the greater caries resistance of the teeth.

The amount of sodium added is clearly negligible. It was on the basis of this experiment that we suggested (33) that fluorine may play a part in the formation of caries-resistant teeth.

We had purchased the Harris salt mixture as the Osborne and Mendel salt. But analysis during the course of the experimentation showed it to have only about one-fourth the expected fluorine. Correspond-

TABLE III

Odds that the occurrence of differences of mean incidence of caries is not due to chance

DIETS AND ADDED FLUORINE	CHANCES TO ONE	
	Occlusal Caries	Fissure Caries
8–56 vs. 8–115 0 41.2 p.p.m.	1000	2,500,000
8–56 vs. 8–120 0 0	5.3	—
8–115 vs. 8–120 41.2 p.p.m. 0	120	1,400,000
10–2 vs. 10–3 0 10.3 p.p.m.	480	0.47
10–2 vs. 10–6 0 41.2 p.p.m.	31	7
10–3 vs. 10–6 10.3 p.p.m. 41.2 p.p.m.	0.92	3.4
10–2 vs. 10–3 + 10–6 0	300	3.1
11–3 vs. 11–4 0 20.6 p.p.m.	—	3.4

ence with the manufacturer revealed that it was made by an older formula of Osborne and Mendel and contained no added fluorine. Therefore diet 8–120, containing no added fluorine, provided us with a salt mixture of entirely different composition (See Table I). The caries incidence between the diets with Osborne and Mendel salt mixture and the Harris salt does not differ significantly, but the

incidence on diet 8–120 is significantly different from that on diet 8–115. Thus this situation is somewhat analogous to that encountered by Dean, Jay, Arnold, McClure, and Elvove (20) as they could not conclude that fluorine was the factor responsible for difference in caries incidence between Galesburg and Quincy children because of the widely different composition of the mineral content of the drinking waters of these cities. Our experimental results are proof that fluorine alone plays a part in reducing caries incidence by its presence during the formation of the teeth.

The data of *fissure* caries on diets 8–56, 8–115, and 8–120 substantiate our conclusions based on *occlusal* caries, but the odds are far higher against pure chance having produced the caries distribution.

Diets 10–3 and 10–6 yielded rats with significantly greater resistance to *occlusal* caries than control diet 10–2. But *fissure* caries incidence was not significantly different. As diets 10–3 and 10–6 did not show a significant difference in occlusal caries, the data from these two diets were combined for comparison with the control ration. Again the difference is not to be ascribed to chance in the case of the occlusal caries; fissure caries incidence, though less for the diets with added fluorine, is still not significantly different from the control.

The control diets discussed were originally compounded for general studies on dental caries and materials were not specifically chosen for low fluorine content. We have not had satisfactory analyses of the control ration for fluorine and are in agreement with Armstrong (3), Dahle (14), and McClure (24) that the value of analyses for fluorine on materials which must be ashed is questionable. Considering that possibly a ration of natural materials might be used as a low fluorine diet, we have employed the corn meal caries-producing ration as the breeding diet in diets 11–3 and 11–4. According to Churchill, Bridges, and Rowley (9) and also McClure (24), this ration should be low in fluorine.

The breeders for the experimental rats were derived from animals fed diet 11–3 from the beginning of pregnancy and were raised on diet 11–3. Thus all the mothers for both 11–3 and 11–4 animals had lived all their lives on a low fluorine ration.

The addition of sodium fluoride equivalent to 20.6 p.p.m. of fluorine did not significantly affect the caries susceptibility of the young. In

fact, fissure incidence was increased in the average but only as would be expected by chance once in four trials. The incidence of both occlusal and fissure caries is the highest that we have observed on any ration, suggesting that some deficiency existed in the corn meal diet that obscured the protective action of the fluoride. The incidence on diet 8–115 is the lowest we have observed from a casein-sucrose type ration. Furthermore, in the preparation of the breeders for the experimental animals a deficit of fluorine may have been created in the mothers that was not corrected by the relatively short time feeding of fluoride from the beginning of pregnancy so that fluorine might be passed to the young. We have preliminary data from another type of experimentation that indicate that the beneficent action of fluorine occurs early in tooth life.

In a preceding experiment we have observed a reduction in caries incidence in young derived from mothers who had been fed a supplement of "XXX liquor" during pregnancy and lactation. As "XXX liquor," a trade waste of the milk industry, is sufficiently concentrated to be saturated with fluoride from the original milk, it is reasonable to credit the improvement to fluorine. Analyses, though of questionable precision, have revealed sufficient fluorine to be effective.

In studies on arrest of caries we have used many rats raised on rations containing commercial casein. Non-seasonal variation in caries incidence has occurred in these animals. The statement of Hodge, Luce-Clausen, and Brown (23) that commercial caseins may contain comparatively large amounts of fluorine suggests that the variable incidence may be ascribed to the variation in fluorine content of successive lots of commercial caseins used in our rations, and in confirmation we have observed that the incisors of rats raised concurrently on diets with the commercial casein show evidence of fluorosis.

DISCUSSION

Our evidence that fluorine aids in the formation of caries-resistant teeth, linked with the findings of Armstrong and Brekhus (4) and of Dean, Jay, Arnold, McClure, and Elvove (20), shows that a very great reduction of the incidence of human caries can be obtained by supplying in food and water an optimum amount of fluorine during

tooth formation. Concordant evidence from 3 different approaches, with no adverse data, should be such sufficient proof of the value of fluorine in the prevention of dental caries that means of control of this element in the whole dietary of children should be undertaken. Control of the fluorine content of community water supplies, in most cases by addition of fluorides, provides an attractive means of mass reduction in dental caries, but prophylactic measures through other media, such as bottled waters, milk supply or the judicious use of fluoride-containing medicinals, are feasible.

Regulation of fluorine should be directed at an optimum intake of the element. In particular, the fluorine content of the water supply can be varied seasonally to compensate for varying water consumption. Climatic differences will make it necessary for each locality to find its own standards for addition of fluorides to the water supply. The economics of the required water treatment will also be local problems.

As brought out in this report, the rat is a satisfactory animal for the study of the relation of fluorine to dental caries. We have recently (13) produced mottled enamel in the *molars* of rats and thus provided a means of experimental study of the interrelations of fluorine to dental caries and mottled enamel in a single species. The lowering of incidence of dental caries in rats by fluorine in the formation of teeth, linked with the observations of Dean, Jay, Arnold, McClure, and Elvove (20), indicates that corn meal caries in rats is true caries.

Present evidence on fluorine and dental caries is sufficient to require that all future studies of dental caries must consider the influence of fluorine and that past investigations be scanned for the possible contribution of this element. For example, the survey of Mills (29) on the relation of dental caries incidence to latitude and drainage basins can be interpreted in terms of fluorine.

McKay (25) has used mottled enamel as a proof that structure has nothing to do with resistance to dental caries. We predict that a structure resulting from lack of fluorine will be found which will differ from the "normal" and that therefore the effect of fluorine is one of modification of structure to a more caries-resistant form. On the other hand, the data of Miller (28), though derived from experiments with very high concentrations of fluorine, suggest that the action of

fluorine may be through bactericidal or bacteristatic action. A still further possibility is that fluorine exerts its effects prior to calcification and that the higher fluorine content of sound enamel, as observed by Armstrong and Brekhus (4), is only a sequela of the continuing higher supply of fluorine.

Whatever is the mechanism of the relative prevention of dental caries by fluorine, whether the resistance is structural, chemical, organic or combinations of these factors, it seems evident that caries resistance can be built into enamel.

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