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Fluoride Uptake and Retention at Various Stages of Rat Molar Enamel Development

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Suckling rat pups were given intraperitoneal fluoride injections at selected ages so that we could study fluoride uptake in the enamel of the maxillary first molar at various stages of enamel development. Plasma fluoride levels in six-day-old and 11-day-old pups were monitored following the intraperitoneal injection of fluoride. The findings indicate that: (1) fluoride was more easily taken up and retained during the early stages of enamel formation, but fluoride uptake can occur during all stages of enamel formation; (2) when injections were started early in enamel formation, more fluoride was contained in the enamel of the maxillary first molar at 13 days of age; and (3) the same dose of fluoride per gram body weight resulted in greater exposure to elevated plasma fluoride levels in six-day-old pups than in 11-day-old pups.


Introduction.

There is uncertainty concerning the age at which systemic fluorides should be administered to achieve optimum caries resistance (Marthaler, 1979 — for review; Thystrup et al., 1979). In addition to a lack of definitive clinical studies, there is limited understanding of the timing and mechanisms of fluoride uptake in developing enamel. It has long been recognized that fluoride is incorporated into the lattice structure of apatite (Brown et al., 1977, for review), and it was reasonable to infer that most fluoride uptake would occur during the stage of rapid mineralization, relatively late in the process of enamel formation. However, studies on several species have shown that, when fluoride concentrations in the extracellular fluids are relatively constant, fluoride concentrations are established early in enamel formation and decline during the rapid mineralization (maturation) stage (Ahlborg et al., 1975; Deutsch et al., 1972; Deutsch et al., 1984; Deutsch and Pe' er, 1982; Speirs, 1975, 1978, 1980; Weatherell et al., 1975, 1977). In addition, there are conflicting reports on the occurrence of fluoride uptake in rat incisor and molar enamel during the maturation phase (Bawden et al., 1982; Hammarström, 1971; Speirs, 1980; Whitford and Lake, 1979).

The studies reported here were designed to determine the effects of fluoride administration at various times during enamel formation on the fluoride content of late-forming enamel in the molar teeth of rat pups.

Materials and methods.

Enamel fluoride uptake was studied using four experimental protocols:

In the first series, littermate seven-day-old rat pups were given intraperitoneal injections of either a water solution of sodium fluoride or an equal volume (0.04 mL) of saline solution. Three injections were given over an eight-hour period.

The total fluoride dose delivered was 0.05, 0.1, 0.25, or 0.5 μgF/g body weight. At 13 days of age, the animals were decapitated, the maxillary first molars dissected free, the enamel organs and pulps removed, and the enamel collected by careful scraping with the aid of a dissection microscope. The pooled enamel from the 10 pups injected at each dose level and from their 10 controls was air-dried for 48 hours, weighed, and analyzed for fluoride content according to the microdiffusion method of Taves (1968) as modified by Whitford and Reynolds (1979).

In the second series, 13-day-old rat pups were injected in the same manner and with the same fluoride/g b.w. doses or saline as described above. The animals were decapitated the next day. The enamel was collected from the maxillary first molars and assayed for fluoride.

In the third series, littermate pups were injected with either fluoride solution or saline three times a day (8 a.m., 1 p.m. and 4 p.m.), beginning at selected days of age and continuing through 12 days of age. The daily fluoride dose was 0.28 μg F/g body weight. Enamel was collected from the maxillary first molars at 13 days of age and assayed for fluoride content.

The fourth part of the study was conducted in a manner similar to the third, except that the daily fluoride or saline dose was given in a single intraperitoneal injection.

All doses for the enamel fluoride uptake studies were administered on a per-unit-weight basis to account for the different sizes of the pups resulting from variations in age. Such is the usual approach to standardizing fluoride exposure. We conducted studies to determine whether the method of administration resulted in similar concentrations of fluoride in the extracellular fluids of pups at different ages.

Six-day-old littermate Sprague-Dawley rat pups were injected intraperitoneally with a solution of NaF in de-ionized water containing 0.1, 0.25, or 0.5 μg F/g b.w. in 0.04 mL. Two pups from each litter were injected with a similar volume of saline to serve as baseline controls. Two animals were anesthetized with ether, and blood samples were obtained by cardiac puncture at 15, 30, 60, and 120 minutes post-injection. Blood was collected from the two baseline animals in the same manner, with heparinized syringes. The samples were centrifuged, the plasma recovered, and the fluoride content was determined. The experiment was repeated on three litters at each dose, providing six values for each observation time at each of the three doses. The entire protocol was repeated on 11-day-old pups. The data were statistically analyzed using the t test.

Results.

The results of the first experiment on enamel uptake of fluoride are shown in Fig. 1. Each group of animals was injected with the respective fluoride dose or with saline at seven days of age, and the enamel was collected from the maxillary molars at 13 days of age. It can be seen that the saline-injected rats had low fluoride concentrations in their enamel and that the values were quite similar from one control group to the other. The concentration of fluoride in the enamel of the fluoride-
Fig. 1 — Fluoride concentrations in maxillary first molar enamel following intraperitoneal injection of fluoride at seven days of age. Enamel was collected at 13 days of age.

Fig. 2 — Fluoride concentrations in maxillary first molar enamel following intraperitoneal injection of fluoride at 13 days of age. Enamel was collected at 14 days of age.

Fig. 3 — Fluoride concentration in maxillary first molar enamel when fluoride injections were started on various days of age and continued through 12 days of age. Enamel was collected at 13 days of age. The total daily dose was 0.28 µg F/g body weight and was given in three injections over an eight-hour period.

Fig. 4 — Fluoride concentration in maxillary first molar enamel when fluoride injections were started on various days of age and continued through 12 days of age. Enamel was collected at 13 days of age. The daily dose of 0.28 µg F/g body weight was given in a single injection.

injected rats increased in a linear fashion in relation to the dose. Even at the highest dose (0.5 µg F/g b.w.), the enamel F concentration was still rather low.

The results of the second series are shown in Fig. 2. Although the relationship between dose and the enamel fluoride concentration was not as consistent as that found in the first experiment, the general trend was that increasing fluoride doses resulted in increased enamel fluoride concentrations. The absolute increases in the enamel fluoride concentrations were similar to those shown in Fig. 1.

The results of the third phase of the study are shown in Fig. 3. Fluoride injections, three times a day, were started on the indicated days and continued through day 12. It is clear that the earlier the fluoride injections were started, the more fluoride was contained in the enamel at 13 days of age. The data presented in Fig. 4 show that similar results were achieved when the daily dose was given as a single injection instead of being divided into three injections. The divided-dose regimen appeared to result in higher fluoride concentrations in the enamel at 13 days than did the single daily injections, but statistical evaluation of the differences was not possible.

The values shown for each group in each of the experiments represent a single fluoride assay on pooled enamel collected from 10 animals. The amount of enamel collected from each
animal was so small, especially in the younger animals (from 0.5 to 1.0 mg), and the fluoride content so low that it was necessary to pool enamel from 10 animals to obtain reliable fluoride assay results. The collection of enamel was so time-consuming and tedious that it was not feasible to run duplicate groups routinely. In all of the experiments, control values for fluoride in enamel at 13 days of age were consistently from 0.8 to 1.2 ng/mg.

The plasma fluoride concentrations from six-day-old and from 11-day-old pups are compared following 0.1, 0.25, and 0.5 μg F/g b.w. doses in Figs. 5a, b, and c, respectively. The highest observed mean plasma fluoride concentrations were at 15 min for all doses in animals of both ages. At 30 min, concentrations had fallen to approximately half of the mean values at 15 min. At one hour, they were close to baseline except for the highest dose, which approached baseline values at two hours.

It can be seen that the plasma fluoride concentrations were lower in the 11-day-old animals at every observation time except at 15 min post-injection at the highest dose (0.5 μg F/g b.w.). All differences were statistically significant (p < 0.05) except for the values at 15 min for the two highest doses and at two hours for the lowest dose. Standard errors at two hours were too small to illustrate in the Figs.

Discussion.

It is not clear at which stages of enamel formation the net uptake and retention of fluoride occur and in what relative amounts. The question is of clinical importance if one wishes to achieve optimum fluoride concentrations in enamel. Because it has long been known that fluoride ion is readily incorporated into the lattice structure of the apatite crystals, it was assumed that most of the fluoride was taken up into developing enamel during the later (maturation) stage of enamel formation, when most of the mineral deposition occurs (Marthaler, 1979). Human clinical studies on the question have been equivocal because of the difficulties in conducting relevant, well controlled prospective clinical trials.

From the biologic point of view, studies on several species indicate that when the extracellular fluid fluoride concentration is relatively constant, enamel fluoride concentrations are highest during the secretory, transition, and, perhaps, the early maturation stages, and decrease as rapid mineralization proceeds (Ahlberg et al., 1975; Deutsch et al., 1972; Deutsch et al., 1984; Deutsch and Pe’er, 1982; Speirs, 1975, 1978, 1980; Weatherell et al., 1975; Weatherell et al., 1977). An autoradiographic study by Hammarström (1971) indicated that 18F uptake occurs in developing rat molar and incisor enamel during the secretory and transition phases. No uptake could be detected during the maturation phase when 45Ca uptake was the highest. Using similar autoradiographic methods, Bawden and co-workers could not detect 18F uptake in maturation stage rat molar enamel, but results from quantitative in vitro methods indicated that some tracer uptake does occur during enamel maturation (Bawden et al., 1982). Whitford and Lake (1979) and Speirs (1975, 1978, 1980) have also published data indicating that fluoride uptake can occur during the maturation phase, if the extracellular fluid fluoride concentration is increased.

Crenshaw and co-workers have shown that the early enamel matrix proteins selectively bind large amounts of fluoride and that much of this binding capacity is lost as mineralization progresses (Crenshaw et al., 1978; Crenshaw and Bawden, 1981). They have speculated that, when the extracellular fluid fluoride concentration is relatively constant, most of the fluoride is taken up during the secretory and transitional phases of enamel formation, bound by the matrix proteins and released to the growing apatite crystals as the proteins are hydrolyzed and lost from the mineralizing enamel (Bawden et al., 1982). If this hypothesis is correct, the highest fluoride concentrations in maturing enamel should result from systemic fluoride administration when the enamel is in the early stages of formation. In addition, the water content of secretory phase and transitional phase enamel is relatively high, and there is a grad-

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**Fig. 5 (a,b,c)** — Plasma fluoride concentrations in six- and 11-day-old rats following intraperitoneal injection of fluoride at three doses.
ual loss of water from the matrix, beginning with the late secretory or early maturation phase. When the water content is high, diffusion of fluoride through the matrix should occur with relative ease. As the water content declines, the enamel should become less permeable to the diffusion of fluoride ions, thus reducing the potential for fluoride uptake as the maturation stage proceeds.

Maxillary first molar enamel in rats six, seven, and eight days of age is chiefly in the secretory phase (Kurahashi et al., 1968). At nine, 10, and 11 days, various phases are represented, with a progressive shift to the maturation stage. At days 12 and 13, the maturation stage predominates.

In all phases of the studies on fluoride uptake in enamel reported here, the fluoride content of developing rat molar enamel in control animals was low, because rat milk contains low concentrations of fluoride (Drinkard et al., 1985). In the first series, it was shown that, when the rats were given increasing doses of fluoride during the secretory phase of enamel formation, and the enamel was collected at 13 days of age, the enamel fluoride content was related to the dose. The same was true in the second experiment when the dose was injected at 13 days of age and the enamel collected the next day. The absolute increases in the enamel fluoride content were similar whether the animals were injected at seven or 13 days of age.

Since only about two-thirds of the enamel matrix has been deposited on day 7 (Lange and Hammarström, 1984), uptake and retention of fluoride in the enamel that had been laid down must have been relatively high to achieve the concentrations found in the enamel at 13 days.

We have shown previously that secretory enamel matrix proteins selectively bind large amounts of fluoride, and uptake of relatively large amounts of fluoride by the early enamel can be explained on that basis (Crenshaw et al., 1978; Crenshaw and Bawden, 1981). However, retention of what must have been a sizable proportion of the fluoride until the enamel was collected six days later is difficult to understand. The data on plasma fluoride concentrations following fluoride injections show that plasma fluoride concentrations were significantly elevated for only one hour. Since the protein binding of fluoride is reversible in vitro (Crenshaw and Bawden, 1981), one would expect that most of the fluoride bound by the organic matrix at peak extracellular fluid concentrations would be lost as plasma levels fell to baseline. Some fluoride would be incorporated into newly deposited and growing apatite. But mineral deposition is comparatively slow during the secretory stage, and a small increment would be deposited during the time the extracellular fluid fluoride concentration was elevated. Fluoride taken up in the skeleton would be released subsequently at a relatively slow rate. It is improbable that such release would cause increases in the plasma fluoride concentration sufficient to explain the fluoride contained in enamel at day 13 when the animals were injected at seven days of age.

The third and fourth experiments showed that the earlier the fluoride injections were started, the higher were the fluoride concentrations in enamel collected at 14 days of age. This was true whether the fluoride was given in a single daily injection or when the daily dose was divided into three injections over eight hours. Of course, the younger the animals were when they were started on daily injections, the greater the total dose of fluoride received during the course of the experiment. That in itself would account for at least part of the observed effect. In addition, the plasma studies indicated that exposure of developing enamel to elevated fluoride levels in the extracellular fluids was greater in younger animals, even though the dose per unit body weight was constant throughout the experiment.

As mentioned above, our data as shown in Figs. 1 and 2 indicate that the fluoride is taken up more readily in the early stages of enamel formation. All of these factors could account for the findings shown in Figs. 3 and 4.

The findings concerning fluoride uptake in enamel should also be considered in the context of the data on plasma F levels in pups following intraperitoneal injection. The baseline plasma fluoride levels were approximately one order of magnitude less in the pups than in their mothers. This again reflects the fact that the fluoride concentration in milk of our mother rats is consistently <0.01 ppm. The data indicated that the observed peak plasma fluoride concentrations were achieved within 15 min post-injection, and that they returned nearly to baseline values in one or two hours. Thus, intraperitoneal injections of fluoride exposed the developing enamel system to significantly elevated fluoride concentrations in the extracellular fluids for relatively short periods of time. In addition, the same dose on a per-unit-body-weight basis resulted in significantly higher plasma fluoride concentrations at 15 min for the 0.1 μg F/g b.w. dose in the six-day-old pups, and the return to baseline in the six-day-old pups was slower at all three doses. Data points were not frequent enough, particularly at the early times post-injection, to do meaningful area-under-the-curve calculations or other pharmacodynamic analyses. We sought only to test the assumption that the same dose per unit body weight results in the same changes in plasma fluoride concentrations in rat pups of different ages. Obviously, it does not. More extensive studies will be required to define the pharmacodynamics of the respective situations.

However, it is clear that the early enamel was exposed to somewhat higher and more prolonged increases in the extracellular fluid fluoride concentration. This may also be a factor in the relatively high uptake of fluoride in the secretory stage enamel which involved the entire first molar crown at the early ages. Whether similar age-dependent plasma uptake and clearance patterns occur in other species is unknown.

REFERENCES


