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FLUORIDE INCORPORATION INTO DEVELOPING MOLAR TEETH OF RATS

Rosa Anita ROCCA*
Sérgio Miguel ZUCAS**
Orlando Ayrton de TOLEDO*

ABSTRACT: A quantitative analysis of fluoride was made in molar teeth of rats aged 10, 20, 30 and 60 days. The animals were daily injected subcutaneously, since birth to sacrifice, with 0,15 mg/NaF (0.067 mg/F) or 0.30 mg/NaF (0.134 mg/F). The comparative study of the data with those observed in molar teeth of rats without any treatment evidenced that: the administered fluoride dosis did not affect the molar teeth development or even the somatic growth of the animals as showed by weight evaluation; the molar teeth of rats exhibit gradual reduction of the capacity for fluoride fixation durind mineralization process; and the percentual fixation of injected fluoride presented similar results for both tested dosis.

KEY WORDS: Fluoride, tooth mineralization.

The mechanism of the anticariogenic effect of fluoride has been investigated but none of the presented suggestions received the support of convincing proofs. However, the analysis of epidemiological studies provide suficient evidence for the acceptance that full benefit for dental prevention is achieved when it is proportionated during the stage of enamel matrix formation and mineralization.

Only few studies were made on the uptake and distribution of fluoride in developing teeth starting from the first stages of their formation and mineralization. The data collected by the authors in rat incisors (DEUTSCH *et al.*, 1972; WEATHERELL *et al.*, 1975), bovine molars (DEUTSCH *et al.*, 1974) premolars and molars of domestic pigs (SPEIRS, 1975) evidenced that considerable amounts of this ion are present in forming enamel but fell rapidly as their mineral content approached that of mature tissue.

WEATHERELL *et al.* (1975) suggest that this phenomena could be due to dilution by the subsequent deposition of relatively fluoride-free enamel and it seems possible that some of the halogen might be free or bound to the organic matrix and perhaps be lost as part of that during enamel mineralization. SPEIRS (1975), however, stated that about this event several important questions must be taken for consideration; for example, he pointed out the functional significance, if any, of the high fluoride levels in the early stages of mineralization and the nature of the apparently labile fluoride.

An extensive review focusing the assimilation of fluoride by enamel throughout the life of the tooth enamel was presented by WEATHERELL *et al.* (1977), pointing out the importance of the subject and the lack of information in several areas where the situation is not clear and where the results must be regarded as preliminary or not yet completely proved.

In this work our purpose was to analyze quantitatively the fluoride fixation in molar teeht of young rats, maintained under aconstant and controlled dosis administered since birth.

* Disciplina de Odontopediatria. Faculdade de Odontologia de Araraquara, UNESP, São Paulo, Brasil.
** Departamento de Alimentos e Nutrição Experimental. Faculdade de Ciências Farmacêuticas, USP, São Paulo, Brasil.

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MATERIAL AND METHOD

Forty-eight newborn albino rats of both sexes near 7 g in weight were used. They were obtained from rats matched on 3: 1 female and male proportion for about a week. The dams were then housed in individual cages during pregnancy and lactation periods.

All the animals received *ad libitum* a laboratory diet (Testevita, Moinho Fluminense S/A, Rio de Janeiro) and fluoridated drinking water (0.7-0,8 ppmF) from public supply of Araraquara city (SP).

Twenty-four hours after birth the litters were weighted and separated into three experimental groups constituted by sixteen animals each.

Control rats (group I) did not receive treatment and the experimental ones were injected subcutaneously with a daily dosis of 0.15 mg/NaF (0.067 mg/F, group II) or 0.30 mg/NaF (0.134 mg/F, group III). Representative animals of treated groups were weighted and then killed by inhalation of ethilic ether after 10, 20, 30 and 60 days of treatment and always twenty-four hours after the last injection. Simultaneously, same aged control rats (group I) were sacrificed by using similar procedure.

The heads of the animals were immediately removed for obtaining the molar teeth. All the molars of each animal were dried at 105°C up to reach a constant weight and deffated in a Soxhlet for 16 or 24 hours using ethilic ether as solvent. After that, they were newly submitted to drying as previously described and reduced to powder for fluoride analysis.

The fluoride was isolated using the diffusion vessel of ZUCAS & LAJOLO (1968) and analyzed quantitatively by the spectrophotometric method of MEGREGIAN (1954). Standard errors of the means were calculated according to MANTEL (1951).

The models of multidimensional variance and of linear correlation for two variables were applied to analyze statistically the data reffering to the fluoride concentration (ppm) in the rat molar teeth of both control and treated groups. The significance level adopted was of 0,05.

RESULTS AND DISCUSSION

The data were summarized in Table 1.

The comparative analysis of the data concerning to mean body weight of control (group I) and treated rats (groups II and III) served as basis for admission that fluoride dosis tested in the present work did not produce toxic effect thus being within the physiological limits even for neonatal period of growth.

It may be also observed that mean molar weight increased gradually up to 60 days in both control and treated groups.

A high concentration of fluoride (ppm) was verified in the molars of all 10-day-old rats but a statistically significant difference was observed between the values registered for control (112 ppm) and treated groups (714 and 820 ppm). With advancing age and independently of treatment, the fluoride content of rat molar teeth showed a pronounced and consistent decrease. In the treated groups (II and III) the rate of decrease was greater between 10-20 and 30-60 days of the experimental period. However, it was noted that while for group II (0.067 mg/F) the higher decrease occurred from 10 to 20 days, for those of group III (0.134 mg/F) the rate of reduction was more accentuated from 30 to 60 days of age. On the other hand, in the control group (I) the decrease was pronounced from 10 to 20 days, showed regular rate from 20 to 30 days and was little or null from 30 to 60 days. These findings indicate that the rate of decrease of fluoride content of these teeth was dependent of treatment and of daily dosis injected.

As reffered by SCHOUR & MASSLER (1963), the histology and physiology of the dental tissues of the rat molars are quite similar to those of human molars and they are

TABLE 1 — Values reffering to the body and molar teeth mean weight, percentual of molar teeth as related to body mean weight, and fluoride fixation in the rat molars.

Group	Treatment (days)	Total of injected fluoride (mg)	Mean Weight			Fixed Fluoride			Percentual of Injected	Fixation Fluoride
			Body (g)	Molars (mg)	Per cent of molars	Concentration (ppm)***	Total (μg)	Net (μg)	Total	Net
I	10	—	17.88 ± 0.25	21 ± 0.25	0.12	112 ± 9.75	2.35 ± 0.21	—	—	—
	20	—	41.85 ± 1.18	64 ± 1.25	0.15	26 ± 1.25	1.54 ± 0.23	—	—	—
	30	—	72.28 ± 1.13	93* ± 0.50	0.13	20 ± 2.75	1.86 ± 0.24	—	—	—
	60	—	165.75 ± 3.05	115** ± 2.00	0.07	19 ± 2.00	2.18 ± 0.26	—	—	—
II	10	0.67	17.88 ± 0.80	20 ± 1.50	0.11	714 ± 22.00	14.28 ± 1.34	11.79 ± 1.34	2.13 ± 0.20	1.76 ± 0.20
	20	1.34	33.85 ± 0.63	50 ± 0.25	0.15	139 ± 9.50	6.95 ± 0.46	5.32 ± 0.46	0.52 ± 0.04	0.40 ± 0.03
	30	2.01	64.17 ± 5.26	85 ± 7.00	0.13	126 ± 7.25	10.68 ± 1.17	8.82 ± 1.17	0.53 ± 0.14	0.44 ± 0.06
	60	4.02	141.23 ± 4.10	96 ± 3.25	0.07	51 ± 4.00	4.89 ± 0.26	2.71 ± 0.26	0.12 ± 0.01	0.07 ± 0.01
III	10	1.34	15.43 ± 0.83	19 ± 1.22	0.12	820 ± 22.00	15.82 ± 1.31	13.47 ± 1.31	1.18 ± 0.10	1.01 ± 0.10
	20	2.68	36.28 ± 0.93	49 ± 0.75	0.14	353 ± 6.00	17.29 ± 1.19	15.75 ± 1.19	0.65 ± 0.05	0.59 ± 0.04
	30	4.02	57.48 ± 1.28	69 ± 1.75	0.12	335 ± 0.00	23.38 ± 2.47	21.51 ± 2.47	0.58 ± 0.06	0.54 ± 0.06
	60	8.04	163.38 ± 9.10	94 ± 3.00	0.06	80 ± 2.00	7.49 ± 0.33	5.31 ± 0.33	0.09 ± 0.01	0.07 ± 0.00

* Statistically different of the Group III
** Statistically different of both Treated groups
*** Statistically different except for 60 day-old rats.

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limited in development chiefly to the first 125 days of life. The first molar, which is the largest of the three in each quadrant, begins its formation on the 20-21st day *in utero* and the second and third molars on the 1-2nd and 13-14th days respectively. First appearance in oral cavity is at the age of 19, 22 and 35 days for first, second and third molars respectively.

Relating these observations with our findings on the fluoride content in a ppm basis in the rat molars throughout experimental period, we assume that these teeth show a pronounced diminishing ability to store fluoride after the first stages of formation and mineralization. The stablished coefficient of linear correlation between the data of mean weight and fluoride concentration (ppm) of the molars was significant (- 0.71) and well explained by a decreasing linear equation.

For a well comprehension of this phenomena the total fluoride (microgram) contained in a determined amount of mineralized mass was also calculated. Thus, it was verified characteristical results for both control and treated groups. The total fluoride levels decreased between 10-20 and 30-60 days but increased from 20 to 30 days. Another noted event was that the total fluoride content in the molars of rats treated for 10, 20 and 30 days are markedly greater if compared with those observed in same aged control rats. However, when compared the values founded at 60 days the magnitude of the difference decreases considerably.

Similar situation was verified with regard to the net total fluoride, that was determined by considering the values of present total fluoride in the molar teeth of control rats as standard for correction.

These observations and the results concerning to the percentual retention of injected fluoride confirm our preliminary hypothesis on the rapid loss of capacity of rat molar teeth for fluoride fixation when it is administered during the mineralization period.

Experiments *in vitro* (BLAIR & HENRIQUES), 1968; HOFFMAN *et al.*, 1969) and autoradiographical studies in 8-10 days aged rats (ERICSSON *et al.*, 1960; HAMMARSTROM, 1971) revealed that the uptake and distribution of fluoride in dentine and enamel vary with the mineralization degree, suggesting that the organic components are involved in this event. As founded by others (DEUTSCH *et al.*, 1972, 1974; SPEIRS, 1975; WEATHERELL *et al.*, 1975), the flouride content of fully mineralized dental structures is lower than those in the forming stage. According to WEATHERELL *et al.* (1975), the concept that part of the fluoride acquired during the early developmental stage is withdrawn together with the organic matrix seems to have greater experimental support than those of fluoride dilution by increasing weight of mineral.

The different methodology used in the present study does not permit a direct comparison with the findings reported as yet in the literature.

It seems opportune to remember that according numerous reports (BROWN, 1965; QUINAUX & RICHELLE, 1967; TERMINE & POSNER, 1967; POSNER, 1971; TERMINE & EANES, 1972; STEVEBOCCIARELLI *et al.*, 1973; EANES, 1976), the mineral first deposited during the mineralization process is of an amorphous nature, probably octacalcium phosphate, which subsequently acts as a metabolic precursor of crystalline apatite or hydroxylapatite. After development, an inverse proportion of this two-phased nature of the mineral were found in bones (POSNER *et al.*, 1965; TERMINE & POSNER, 1966, 1967; EANES & POSNER, 1968; POSNER, 1971). In teeth these events appear poorly studdied but TERMINE & POSNER (1966) that reported that samples of mature human enamel had 100 per cent crystalline apatite while the dentine of the same teeth had 65 to 70 per cent crystalline material.

Based on our data, which revealed that the fluoride was fixed at high levels in the

early developmental stages of rat molar teeth (10 days) and in a reduced rate up to 60 days of treatment, we thought it seems reasonable to accept the hypothesis that this ion acts as a catalyst on amorphous crystalline transformation, as suggested by BROWN (1965). According the reffered author "this would account for remarkable efficacy of a small amount of fluoride in preventing dental caries, because only a relatively small number would be necessary to initiate hydrolysis step and thus preclude accidental retention of the more soluble octacalcium phosphate in the crystal".

The interpretation of our findings perhaps can be made also by considering the probable influence of fluoride on stabilization of tooth apatite (MORIWAKI *et al.*, 1975), by increasing the size and/or decreasing the crystal imperfections, thus reducing the effective surface areas of the mineral and therefore their reactivity for ions exchange as reported by some authors in X-ray diffraction studies on bones (SCHRAER *et al.*, 1962; ZIPKIN *et al.*, 1962; POSNER *et al.*,1963; BAUD & MOGHISSIBUCHS, 1965).

By acceptance of this hypothesis, it seems permitted to infer that indeed do not occur fluoride loss during the mineralization stage but a diminishing ability of dental structures for fluoride uptake resulting a dilution of the previously fixed amount into mineralized mass which increases as the tissue matures.

CONCLUSIONS

The administered fluoride dosis did not affect consistently the somatic growth of the animals or even the molar teeth development as showed by weight evaluation: the molar teeth exhibit gradual reduction of the capacity for fluoride fixation during mineralization process; and the percentual fixation of injected fluoride presented similar results for both tested dosis.

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ROCCA, R.A., ZUCAS, S.M. & TOLEDO, O.A. Incorporação de flúor em molares em desenvolvimento em ratos.

RESUMO: Foi efetuada a análise quantitativa do flúor presente nos molares de ratos com 10, 20, 30 e 60 dias de idade, que receberam, desde o nascimento, doses diárias de 0,15 mg/NaF ou 0,30 mg/NaF, injetadas subcutaneamente. O estudo comparativo dos dados obtidos, com aqueles observados nos molares de ratos da mesma idade, não tratados, evidenciou que: 1) as doses de flúor administradas não afetaram, de maneira consistente, o crescimento somático dos animais, nem dos molares; 2) os molares do rato perdem gradualmente sua capacidade para acumular flúor durante o processo de mineralização e 3) o percentual de flúor fixado mostrou comportamento similar para ambas as doses testadas.

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