METABOLIC TURNOVER OF FLUORIDE BY THE SKELETON OF THE RAT*

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The turnover of skeletal fluoride, a process which involves the acquisition and elimination of fluoride by bone, has not been completely studied. This topic is of current interest in relation to the programs of fluoridating communal water supplies as a means of partial control of dental caries in the human (1).

Skeletal tissues have a marked affinity for fluoride and the bones are known to accumulate fluoride when the intake of the element is increased (2, 3). Balance studies carried out with young human adults showed no significant retention of fluoride when the intake was 0.5 to 1.0 mg. (4) or 4.0 to 5.0 mg. (5) per day. Since the normal fluoride intake in regions of low fluoride-bearing waters can hardly exceed 1.5 mg. per day (4, 6), the results of the balance studies do not account for the observation that the fluoride content of the bones of the human increases progressively with age (2).

The only published study contributing significantly to the question of the rate and degree of elimination of skeletal fluoride is that of Glock et al. (2). These workers used rats which had been fed a ration containing over 200 parts per million of fluoride for 32 and 40 weeks. After withdrawal of the high fluoride-containing ration the fluoride concentration (i.e. per cent fluoride) in the fat-free long bones decreased markedly and progressively over a period of 14 weeks. Since Glock and coworkers recorded the data of fluoride analyses of the bones in terms of concentration rather than as in the absolute amount of the element present in a given bone, it can be understood that their results were influenced by skeletal growth as well as by actual elimination of fluoride from the skeleton. That is, skeletal accretion would have the effect of diluting the fluoride present in the skeleton, with the result that a decrease of concentration

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of the element in growing bone does not describe accurately actual fluoride elimination. The authors do not provide data by which the magnitude of skeletal growth of their animals can be judged, but it is known that many bones of rats continue to increase in mass and length until relatively advanced ages (7).

The present studies were prompted by the need to secure data as to the uptake and elimination of fluoride by the skeleton of rats in a manner such that skeletal accretion would not affect the quantitative significance of the results. To this end the absolute amounts of fluoride found in selected whole bones are reported.

In order to demonstrate quantitatively significant turnover of skeletal fluoride in a relatively short time the animals were given drinking water containing 20 p.p.m. of fluoride. At this level of fluoride intake an alteration of the pigmentation of the incisor teeth occurs, but abnormal non-dental effects are absent (8).

In the present studies young and mature animals were employed. In view of the long continued growth of the skeleton in the rat a somewhat arbitrary decision surrounds the question of maturity in this animal. Armstrong (9) noted by the use of radiographic methods that the rate of growth of the humeri of rats of the strain used in this study was greatly reduced by the time the animals were 132 days old, corresponding to a body weight of 300 gm. Accordingly this body weight was taken as the criterion of relative maturity for the purposes of the present study.

**EXPERIMENTAL**

The essential plan of these experiments was to sacrifice animals at intervals of time during (a) a regimen of increased fluoride intake and (b) after withdrawal of the fluoride supplement. Data in each instance were initially limited to a 60 day fluoride regimen, followed immediately by an equal post-fluoride period. However, the low degree of defluoridation of the bones after withdrawal of the fluoride supplement indicated the need for observations over a more prolonged post-fluoride period. Accordingly, the study involved the use of 232 male rats of the Sprague-Dawley strain divided into three groups, as follows:

**Colony A**—Weanling rats, 60 experimental and twenty control animals; initial age 27 days; initial weight $64 \pm 4.5$ (s.d.) gm.; animals sacrificed after 20, 40, and 60 days of fluoride regimen and at the same intervals of time after withdrawal of the fluoride supplement.

**Colony B**—Weanling rats, thirty-eight experimental and eighteen control animals; initial age 27 days; initial weight $63 \pm 6.4$ (s.d.) gm.; animals 60 days on fluoride regimen, sacrificed at 1, 90, 120, and 150 days after withdrawal of fluoride supplement.
Colony C  Mature rats, fifty-nine experimental, twenty-seven controls, and ten animals sacrificed at day zero; initial age 92 days; initial weight $328 \pm 15.9$ (s.d.) gm.; animals sacrificed after 0, 20, 40, and 60 days of fluoride regimen and at 20, 40, and 60 days after withdrawal of the fluoride supplement.

In order not to obscure the effects of the fluoride supplement, it was necessary to feed the animals a ration with the lowest possible fluoride content. All animals received *ad libitum* the semisynthetic ration described as Diet 1 by Estremera and Armstrong (10). The fluoride content of this ration was found not to be in excess of 0.6 p.p.m., the fluoride present in the Wesson (11) salt mixture accounting for about one-third of the total amount. The fluoride content of this ration is definitely lower than that of commercial animal food biscuits, which have been found to contain 16 p.p.m. (12) and 3.0 p.p.m. (13) of fluoride. Each animal was given twice weekly 2 drops of an improvised concentrate of the fatsoluble vitamins. The average daily intake of the fat-soluble vitamins was 0.1 mg. of vitamin E, 0.05 mg. of vitamin K, 870 i.u. of vitamin A, and 165 i.u. of vitamin D.

The young rats of Colonies A and B were started on the experimental regimen immediately on receipt from the dealer. The mature animals in Colony C were fed the low fluoride-containing diet over a 3 week preexperimental period. The fluoride supplement was introduced into the regimen when the body weight of the lightest animal in this group was 300 gm., at which time the animals were 92 days old. The rats were randomized within each colony so as to give three to five control and ten experimental animals for each of the subperiods.

The fluoride supplement given the animals of the experimental groups during the 60 day fluoride regimen was supplied in the drinking water, which was prepared by adding sodium fluoride to distilled water to give a fluoride concentration of 20 p.p.m. The drinking water was supplied *ad libitum* and the volume of apparent fluid intake of each experimental animal was recorded by use of graduated containers attached to the drinking fountains. It is recognized that the fluoride consumption of the rats calculated from the data of the intake of the fluoridated water represent maximum values, since spillage and evaporation of the fluid, while controlled in so far as possible, could not be prevented entirely. The animals on the fluoride regimen were given distilled water for 1 day before being sacrificed in order to clear the mouth of fluoride and to reduce the fluoride content of the body fluids. Distilled water was supplied to the control animals during the entire period of the study and to the animals of the experimental groups during the post-fluoride regimen.

1 The sodium fluoride specified in the Wesson salt mixture was omitted.
An attempt was made in the post-fluoride period to select animals for each experimental sublot with equal apparent fluoride intakes and so that the mean body weight of the animals constituting the sublot would approximate that of the entire experimental group at the time the animals of the sublot were sacrificed.

After sacrifice the right humerus and the incisor teeth of each animal were removed, cleaned, and rendered fat-free by extraction in a Soxhlet apparatus for 72 hours with an equal part mixture of ether and alcohol. The bones of the entire skeletons of four selected animals were isolated by the use of the same procedures. The specimens were then dried at 110° and weighed to give the dry, fat-free weights. In some cases the enamel and dentin of the pooled incisor teeth were separated by the method of Manley and Hodge (14). All specimens were ashed at 650° to constant weight. The fluoride content of the samples was determined by the use of methods previously described (15). The fluoride in each sample was distilled twice to avoid interference caused by phosphate, and the original distillates were concentrated in platinum ware. Specimens from the same group were pooled previous to analysis when a low fluoride content was anticipated, as in the case of the enamel of the teeth and with some of the humeri of the control animals.

**DISCUSSION**

No retardation in rate of growth attributable to fluoride was manifested by comparing the curves of body weights, determined weekly, of the control and experimental animals. The absence of deleterious effects produced by the fluoride supplement on skeletal growth (8) was corroborated by the data of ash weight of the humeri, as shown in Fig. 1. These data emphasize that the accumulation of mineral matter in the humeri of both control and experimental animals was progressive, attesting the long continued skeletal growth in the rat.

Owing to the misleading nature of results expressed in terms of per cent fluoride in bones of changing mass, the analytical results are reported as the absolute amount (micrograms per bone) of fluoride in the humeri. The mean of these results obtained with the experimental and the control animals sacrificed at each of the subperiods is shown graphically in Figs. 2 and 3. A high initial fluoride uptake was shown by the humeri of both weanling and mature animals. The rate of increment of skeletal fluoride during the 60 day fluoride regimen diminished with continued fluoride ingestion in a manner such that straight lines with negative slopes were obtained when the logarithms of the fraction of ingested fluoride present in the humeri were plotted against time.

Calculations based on the assumption that one humerus contains 2.3 per
cent of the total fluoride present in the edentulous skeleton (Table II) showed that the skeletons of the young animals stored about 53 per cent of the apparent fluoride intake during the first 20 days of the fluoride regimen. The retention decreased to about 43 per cent of the total fluoride intake by the 60th day. The mature animals retained 36 per cent of the

![Graph showing fluoride uptake and elimination by right humeri.](http://www.jbc.org/)

**Fig. 1.** Mean weights of ashed right humeri of albino rats given drinking water containing 20 p.p.m. of fluoride for 60 days, compared with those of control animals.

**Fig. 2.** Fluoride uptake and elimination by the right humeri of young and maturing rats. The numbers at each of the plotted points indicate the mean apparent fluoride intake by the animals in mg. during the fluoride regimen.
apparent fluoride intake on a 20 day fluoride regimen and by 60 days on this regimen the retention had dropped to 29 per cent of the ingested fluoride.

The mean fluoride contents of the humeri of the control animals are also shown in Figs. 2 and 3. The sole source of fluoride available to these animals was the small quantity present in the ration. Nevertheless, it appears that the skeletal fluoride of the young control animals was augmented during the period of 120 days (Colony A) and 210 days (Colony B) over which they received the low fluoride-containing ration. However, the fluoride content of the humeri of the mature control animals (Colony C) was not significantly altered over a period of 120 days, during which the animals received the low fluoride-containing ration. The relatively high initial fluoride content of the humeri of the mature control animals was undoubtedly due to these animals having received, while in the dealer's colony, a diet with a higher fluoride content than that used in the present study.

During the post-fluoride period only a relatively minor degree of elimination of stored fluoride occurred over a 60 day period in both young and mature animals (Colonies A and C). The decrements of stored fluoride were of the order of 10 to 15 per cent of the total skeletal fluoride and were accomplished in the 40 day period immediately after the withdrawal of the fluoride supplement. The results obtained with Colony B (Fig. 2), which was followed over a 150 day post-fluoride period, confirm the con-
clusion that a large proportion of the skeletal fluoride was firmly fixed and not available for excretion in significant amount. These observations suggest that after fluoride enters the skeleton a part is rapidly turned over and excreted, but that the largest amount is eventually deposited in locations in the bones from which its mobilization and excretion are much retarded. A similar situation has been demonstrated with regard to skeletal fixation and turnover of radioactive calcium (16). The time required for the elimination of the radiocalcium in the labile skeletal fraction and the amount of the injected dose of the radioisotope becoming incorporated in the stable bone fractions were comparable to those which were found with fluoride in this study.

| Table I |

| Per Cent Fluoride of Rat Incisors |

All percentages are on a dry, fat-free basis.

<table>
<thead>
<tr>
<th>Colony</th>
<th>Subperiod</th>
<th>Experimental groups</th>
<th>Control groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Whole teeth</td>
<td>Dentin</td>
</tr>
<tr>
<td>B</td>
<td>60 days on F⁻, 1 day off</td>
<td>0.070</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>60 &quot; &quot; &quot; 90 days &quot; &quot;</td>
<td>0.0025</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 &quot; &quot; &quot; 120 &quot; &quot;</td>
<td>0.0088</td>
<td></td>
</tr>
<tr>
<td></td>
<td>60 &quot; &quot; &quot; 150 &quot; &quot;</td>
<td>0.0040</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0 day &quot; &quot;</td>
<td>0.011</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>20 days &quot; &quot; 1 day off</td>
<td>0.040</td>
<td>0.046</td>
</tr>
<tr>
<td></td>
<td>40 &quot; &quot; &quot; 1 &quot; &quot;</td>
<td>0.050</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>60 &quot; &quot; &quot; 20 days &quot; &quot;</td>
<td>0.057</td>
<td>0.056</td>
</tr>
<tr>
<td></td>
<td>60 &quot; &quot; &quot; 40 &quot; &quot;</td>
<td>0.030</td>
<td>0.034</td>
</tr>
<tr>
<td></td>
<td>60 &quot; &quot; &quot; 60 &quot; &quot;</td>
<td>0.008</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Rat incisor teeth grow throughout life and present a kymographic record of the chemical and histologic alterations produced in the teeth during the preceding 40 to 50 days (17). The mean results of the analyses of these teeth (Table I) of the experimental animals of Colony B show that they maintained to the end of the 150 day post-fluoride period a much higher fluoride content than that of the teeth of the control animals. During the 150 day post-fluoride period the animals in Colony B ingested only the small amount of fluoride present in the diet. Thus it appears that the major source from which the fluoride content of the teeth was maintained at higher than normal values was the fluoride of the skeleton. These results suggest that an intraskelatal turnover of small amounts of fluoride via the blood continues after fluoride excretion becomes very low in the late post-fluoride period. This circumstance would allow the continuously forming incisor teeth to be calcified from a medium with a fluoride content
higher than normal. The analyses of the enamel and dentin fractions of the incisor teeth of the animals in Colony C (Table I) showed that the dentin, during the fluoride regimen, was able to acquire more fluoride than the enamel. Also, when the fluoride supplement was withdrawn, the fluoride content decreased at a greater rate in the dentin.

In these studies of fluoride metabolism the humerus was selected for analysis on the assumption that the turnover of fluoride in this bone would be representative of that in the skeleton as a whole. In order to test the reliability of this assumption the bones of the entire skeleton of four rats of different ages and conditions of exposure to fluoride were examined. It was also desired to obtain information as to whether there exist in the rat different degrees of allocation of fluoride among the bone types, as has been claimed to be the situation in human cases of chronic fluoride intoxication (18).

Table II describes the four animals, gives their histories of fluoride ingestion, and shows the percentages of total skeletal fluoride and of total skeletal ash present in each of the skeletal components. Despite differences in age and in conditions of fluoride intake among the animals, a reliable degree of consistency of the proportion of skeletal fluoride present in one humerus was found. The mean value was 2.3 per cent, with a standard deviation of 0.12. The femurs also exhibited a moderate variation in the amount of skeletal fluoride present in these bones (range 8.8 to 10.1 per cent). The vertebrae (range 27.5 to 35.0 per cent), the thoracic skeleton2 (range 6.9 to 8.2 per cent), and the bones of the paws (range 6.9 to 11.0 per cent), however, showed much larger variations in their fractions of total skeletal fluoride.

As shown in Table II, there was a high degree of correlation between the percentages of total skeletal fluoride and ash in the skeletal components. These results indicate that the chief factor determining the deposition of fluoride in a bone is the mineral salt and that there are not large differences between bones in the availability of the mineral salts for fluoride incorporation. The situations in which the largest discrepancies between the proportions of skeletal fluoride and ash occurred, i.e. vertebrae, skull, paws, and thoracic skeleton, are probably related to differential rates of growth and calcification between these and the other skeletal components. While even a non-growing bone acquires appreciable quantities of fluoride, probably by exchange for hydroxyl or bicarbonate ions of the apatite crystals of the mineral phase (19), a growing bone would be expected to fix fluoride also by precipitation as the bone salt is formed. Also disproportionately large growth of a bone after the fluoride intake of the animal

2 Ribs, sternum, and clavicles.

4 Correlation coefficient = 0.99.
is reduced would cause a dilution of its fluoride content and produce the result of a relatively greater fraction of the total skeletal ash than of total skeletal fluoride in the bone. The last column in Table II shows the ratios of the ash weights of the skeletal components of Rat D to those of Rat A.

**Table II**

**Distribution of Fluoride and Ash in Components of Edentulous Skeletons of Rats**

The values in the columns under "Fluoride" and "Ash" refer to the fraction of total skeletal fluoride and ash (in per cent) found in each of the skeletal components.

<table>
<thead>
<tr>
<th>Rat</th>
<th>Rat A</th>
<th>Rat B</th>
<th>Rat C</th>
<th>Rat D</th>
<th>Rat A</th>
<th>Rat B</th>
<th>Rat C</th>
<th>Rat D</th>
</tr>
</thead>
</table>
| Rat A, weaned at 20 days; fed low F diet and water containing 20 p.p.m. of F for 37 days; F intake 10 mg.; no fluoride elimination period. Rat B, received from dealer at 71 days; given low F diet and distilled water for 21 days. Rat C, received from dealer at 71 days; given low F diet and water containing 20 p.p.m. of F for 20 days; F intake 14 mg.; no fluoride elimination period. Rat D, weanling on low F diet and water containing 20 p.p.m. of F for 60 days; F intake 20 mg.; given low F diet and distilled water during last 120 days of life.

These figures are indicative of the degree of accretion of mineral phase in the skeletal components during growth from a body weight of 200 gm. (Rat A) to 500 gm. (Rat D). On this basis the vertebral column exhibited the largest growth of the skeletal components. As shown in Table II, this part of the skeleton was found to have a large variation in its fractional content of the total skeletal fluoride and to exhibit the most varied relationship between fluoride and ash content. By the same line of reason-
ing a disproportionately small growth of a skeletal component during or after a period of fluoride intake would result in variations in the relationship between the fractions of total skeletal fluoride and skeletal ash among animals with different histories of fluoride intake. This circumstance is exemplified in the case of the pooled carpi, tarsi, and digits.

SUMMARY

Analyses were carried out for fluoride in the humeri and incisor teeth of young and mature rats sacrificed at intervals during (a) a 60 day regimen in which the animals received drinking water containing 20 p.p.m. of fluoride, and (b) after withdrawal of the fluoride supplement. The anatomical components of the entire skeletons of four rats of widely different age and history of fluoride intake were examined for fluoride and for ash content. The results permit the following conclusions.

1. The uptake and elimination of fluoride by the skeleton of rats is not accurately shown by fluoride analyses recorded in terms of fluoride concentration (i.e. per cent fluoride in the dry, fat-free or ashed bones) because of the long continued growth of the skeleton in this species. The correct situation as regards turnover of skeletal fluoride is depicted by records of the absolute amount of fluoride in a bone or in the skeleton as a whole.

2. During a 60 day regimen of increased fluoride intake the accumulation of fluoride in the humeri was progressive, but with decreasing increments with time in both young and mature animals. The young rats retained larger amounts of the ingested fluoride in the skeleton than the mature animals (43 versus 29 per cent).

3. In colonies of both young and mature rats withdrawal of the fluoride supplement resulted in the elimination of approximately 10 to 15 per cent of the skeletal fluoride in a period of 40 days. The remainder of the skeletal fluoride was so firmly fixed as not to be appreciably excreted during an additional 20 days (young and mature animals) or 110 days (young animals).

4. The fluoride content of the incisor teeth was found to be significantly higher in the experimental group 150 days after terminating the fluoride regimen than in the control group. This result is interpreted as indicating an intraskeletal turnover of small amounts of fluoride after fluoride elimination from the skeleton was no longer detectable.

5. A single humerus of rats of widely different age and history of fluoride intake

4 Presumably the same circumstance would apply to the fluoride content of bones of other species in situations of increasing skeletal mass. In the rat, skeletal growth would affect the interpretation of skeletal turnover of other substances whose skeletal contents are recorded in terms relative to the amount of bone.
ingestion was found to contain, with a high degree of consistency, 2.3 per cent of the total skeletal fluoride. This finding recommends the humerus as a representative bone in studies of fluoride turnover and permits the estimation of the total amount of fluoride in the skeleton without the labor of preparing the entire skeleton.

6. A high degree of correlation was found between the fractions of total skeletal ash in several skeletal components. This finding indicates that the principal factor determining deposition and fixation of fluoride in a bone is its mineral content.

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