Roles of Kidney and Skeleton in Regulation of Body Fluid Fluoride Concentrations[1] (35066)

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Absorbed fluoride is quickly distributed through body fluids. The concentration of total fluoride in plasma is normally low and studies with rats and the human (1–3) have demonstrated operation of mechanisms which counter and offset large or sustained rises of body-fluid fluoride content when the fluoride intake is markedly elevated. Regulation of plasma fluoride content is effected by skeletal sequestration of fluoride and by urinary fluoride excretion (4). These homeostatic mechanisms may, however, be overwhelmed by very large fluoride intakes as, for example, when rats are fed diets containing 100 ppm fluoride (5) or when they receive injections of 2 mg or more of fluoride. In these circumstances large elevations of plasma fluoride content occur.

In this investigation the effects of preloading the skeleton with fluoride on some physiologic responses to a large fluoride challenge dose were studied. Alterations of plasma fluoride content and quantity of the dose excreted in the urine or sequestered in the skeleton of rats were determined at intervals of time following intragastric administration of a large amount of fluoride. Female rats were used since it is possible to catheterize the urinary bladder of such animals. This allowed collection of urine over timed periods and assured that the specimens were not contaminated with fluoride from the cage, food, or feces.

Materials and Methods. Two groups of weanling female Sprague-Dawley rats were fed a low fluoride containing diet (6) and distilled water (Group I) or the same diet and distilled water to which 50 ppm of fluoride as sodium fluoride was added (Group II). When the animals weighed approximately 225 g (8–9 weeks of age), the urinary bladders were catheterized and the animals were placed in restraining cages constructed of screen-wire. Each animal was given approximately 2 ml of distilled water or water containing 50 ppm fluoride by gastric intubation, as was appropriate for its group, at 0 and at 2 hr. Urine was collected at intervals over a 5-hr preexperimental period to establish normal fluoride excretion patterns. The animals were then given 2.94 mg of fluoride as sodium fluoride in 2 ml of distilled water at time 0 and 2 ml of distilled water at 2 hr, recorded from the beginning of the experimental period. Urine was collected from the catheters at 30 min, 1, 2, 3, 4, 5, and 10 hr after the administration of the fluoride challenge dose. Some animals were recatheterized on the following days to obtain urine formed between 30–35 hr and 48–50 hr after the fluoride dose. Selected animals were sacrificed by cardiac puncture at several time periods and the plasma and humeri were analyzed for fluoride (7).

A single humerus of rats of widely different ages and history of fluoride intake has been shown (8) to contain, with a high degree of consistency, 2.3% of the total skeletal fluoride. Based on this observation the fluoride content of the entire skeleton of each animal was calculated from the fluoride content of a humerus.

Results and Discussion. All results are expressed as means with values of the SEM. The numbers in parentheses in Table I and

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TABLE I. Urinary Fluoride Excretion by Rats.

Exptl. period (hr)	Total (μg)[a]		Rate (μg/hr)		Percentage of dose excreted[d] (accumulative)	
Group:	I	II	I[b]	II[c]	I	II
0 – 0.5*	3.66 ± 1.35 (9)	11.6 ± 2.81 (8)	7.32 ± 2.70	23.2 ± 5.62	0.11	0.07
0.5– 1.0	20.4 ± 3.84 (9)	28.1 ± 5.51 (7)	40.8 ± 7.68	56.2 ± 11.02	0.80	0.69
1.0– 2.0*	57.5 ± 7.14 (9)	85.1 ± 11.01 (7)	57.5 ± 7.14	85.1 ± 11.01	2.73	3.27
2.0– 3.0	66.8 ± 8.11 (9)	90.0 ± 8.93 (7)	66.8 ± 8.11	90.0 ± 8.93	4.99	6.01
3.0– 4.0*	70.2 ± 7.42 (9)	107.4 ± 11.43 (7)	70.2 ± 7.42	107.4 ± 11.43	7.36	11.00
4.0– 5.0	89.4 ±17.25 (5)	69.6 ± 8.37 (5)	89.4 ±17.25	69.6 ± 8.37	10.4	12.8
5.0–10.0	208.5 ±18.94 (8)	230.3 ± 29.41 (6)	41.9 ± 3.78	46.1 ± 5.88	17.3	21.0
25 –30*	49.8 ±11.78 (9)	164.4 ± 19.22 (10)	9.96 ± 2.35	32.9 ± 3.84	—	—
48 –50	3.98 ± 0.77 (5)	33.7 ± 16.75 (4)	1.99 ± 0.38	16.9 ± 8.37	—	—

[a] Conc (ppm) × vol (ml) = μg.
[b] Preexperimental (base line) = 0.59 ± 0.30 μg/hr.
[c] Preexperimental (base line) = 19.3 ± 1.67 μg/hr.
[d] These results were derived by subtraction of the amount of fluoride contained in the same volume of urine collected during preexperimental period by the same animal from the total amount of fluoride in the specimen.
* Indicates statistically higher ($p < 0.025$) results for total amount of fluoride and rate of fluoride excretion by Group II animals than by Group I animals.

II give the number of animals used for the determinations.

The skeletal fluoride concentration of Group II animals at the beginning of the experimental period was over 100 times that of Group I (Table II).

The plasma fluoride concentrations of both experimental animal groups increased to high levels (Fig. 1) within 0.5 hr after administration of the challenge dose. There was a generally downward trend in the results of plasma fluoride analyses after 0.5 hr particularly with the Group I animals. However, because of the high degree of variability of the results with individual animals at 1–3 hr there was no certain evidence that the plasma

TABLE II. Fluoride Uptake by Skeletons of Rats.

After administration of F (hr)	Ashed humeri (% F)		Total skeletal fluoride content[b] (mg)	% of total dose in skeleton[c]
Group:	I	II	I	I
0[a]	0.004 ± 0.0004 (9)	0.507 ± 0.0145 (8)	0.21 ± 0.023 (9)	
0.5	0.006 ± 0.0005 (6)	0.473 ± 0.0184 (6)	0.31 ± 0.025 (6)	3.4
1.0	0.009 ± 0.0005 (5)	0.473 ± 0.0286 (5)	0.38 ± 0.031 (5)	5.8
2.0	0.014 ± 0.0015 (6)	0.426 ± 0.0122 (6)	0.65 ± 0.082 (6)	14.9
3.0	0.016 ± 0.0028 (5)	0.425 ± 0.0155 (6)	0.79 ± 0.125 (5)	19.7
5.0	0.016 ± 0.0006 (6)	0.489 ± 0.0118 (6)	0.77 ± 0.020 (6)	19.0
30.0	0.029 ± 0.0012 (6)	0.512 ± 0.0468 (6)	1.62 ± 0.086 (6)	47.9
50.0	0.044 ± 0.0065 (6)	0.485 ± 0.0135 (4)	2.30 ± 0.368 (6)	71.1

[a] Preexperimental (base line).
[b] Calculated by multiplying the total amount of fluoride in a single humerus by 100/2.3 (see text).
[c] Corrected by subtraction of initial amount of fluoride present in bones prior to treatment, i.e., base-line animals.

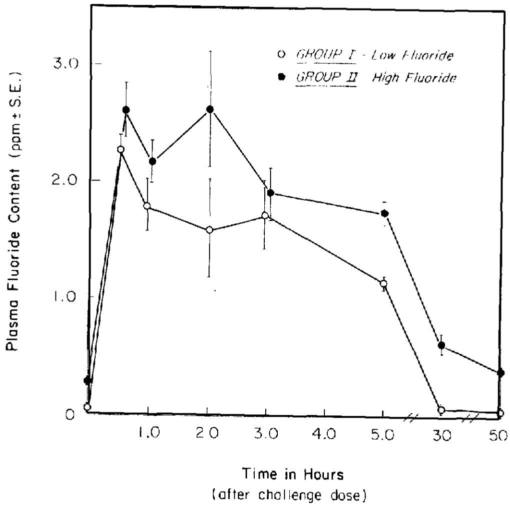

FIG. 1. Plasma fluoride concentrations of rats following intragastric administration of 2.94 mg of fluoride: Group I animals raised on diet of low fluoride content; Group II animals raised on same diet and water containing 50 ppm fluoride.

fluoride concentrations declined appreciably between 1 and 3 hr after administration of the challenge dose. The large variations in results with both groups at 1–3 hr may have been a consequence of differences in rates of absorption of fluoride among the animals during this period. That fluoride absorption occurred over a protracted period is supported by the observation (Fig. 2) that the urinary fluoride concentrations did not reach maximum values until 2 hr after administration of the large dose of fluoride.

The results presented in Fig. 1 show consistently higher fluoride concentrations in the plasma of the animals of Group II (high fluroide pretreatment) than in Group I (low fluoride pretreatment). However, if the former results are adjusted for the initially higher plasma fluoride contents of the Group II animals in the preexperimental period (0.28 $\pm$ 0.024 ppm *versus* 0.05 $\pm$ 0.005 ppm fluoride), the increment of fluoride concentration in body fluids from the challenge dose becomes very nearly equal in the two groups of animals over the first 5-hr period.

The rates of urine formation by both groups of animals during the experimental period were slightly above those of the preexperimental period and the total urine volume produced in 10 hr by the animals of previous low fluoride intake was significantly higher than that of the animals raised on the high fluoride regimen (10.3 $\pm$ 1.18 ml compared with 7.3 $\pm$ 1.30 ml).

Figure 2 gives the findings of fluoride concentration in the urine of the two groups of animals collected during the preexperimental period (time zero) and at the indicated times after administration of 2.94 mg of fluoride. The fluoride concentration of urine of both groups rose rapidly and reached peak values at 2 hr after administration of the challenge dose when the urine-to-plasma fluoride concentration ratios were, respectively, 79 and 82 in Groups I and II. Concentration ratios near this range may represent the maximal attainable degree of urinary concentration of fluoride from plasma since the same ratio for Group II animals was 98 during the preexperimental period when the plasma fluoride concentration was 0.28 ppm. A plasma fluoride concentration of 0.28 ppm is distinctly above that of the animals fed a low fluoride containing diet (Group I) but is about 10 times lower than the highest plasma fluoride concentration found after administration of the large fluoride dose.

The urinary fluoride concentrations of the

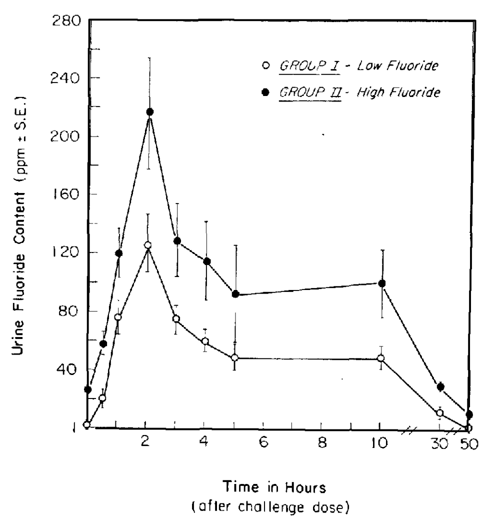

FIG. 2. Urine fluoride concentrations of rats following intragastric administration of 2.94 mg of fluoride; see Fig. 1 for other details.

Group II animals were higher than those of Group I following administration of 2.94 mg of fluoride in the animals. However, when the results for the former animals are adjusted for their higher base-line urinary fluoride contents the increments of urinary fluoride concentration, following the intragastric administration of fluoride, are brought very nearly into agreement between two groups of animals at most time periods.

Table I gives the quantities of urine fluoride and rates of urinary fluoride excretion over the indicated times during the experimental periods. There were no consistent significant differences between the two groups of animals in quantities of urinary fluoride or in rates of fluoride excretion in the 10 hr following administration of the large dose of fluoride. Only in the instances marked by an asterisk in Table I were these derived results statistically higher for Group II than for Group I. The last two columns of Table I present the calculated fractions of administered fluoride dose which appeared in the urine. These results were derived from the increments of urinary fluoride content over base-line quantities. The slightly higher values for the Group II animals in 5 instances are, however, of uncertain significance.

The results with respect to increment of plasma fluoride content and those of urinary fluoride excretion do not indicate a lesser ability of the animals with initially elevated skeletal and plasma fluoride contents (Group II) to dispose of a single large fluoride dose than was exhibited by the animals of low preexperimental fluoride intake (Group I). Thus, the main controlling mechansims of body-fluid fluoride content, skeletal sequestration of fluoride, and urine fluoride excretion, operate as efficiently and rapidly in rats with previous exposure to fluoride as in animals with very low tissue fluoride contents. These conclusions involve the assumption that the rate of fluoride absorption from the intestine by the Group II animals during the 10-hr experimental period was not lower than that of the Group I animals. This assumption is supported by the observations of near agreement of increments of plasma fluoride contents and parameters of urinary fluoride excretion by the two groups of animals. If intestinal absorption of fluoride by Group II animals had been depressed, a coincidental counterbalancing reduction of fluoride deposition in the bones of these animals would have been required to account for the agreement of the results with the two groups of animals in respect to plasma fluoride contents and urinary fluoride excretion.

The sustained higher quantities of fluoride and rates of excretion of fluoride into the urine in the 20–30-hr period by the animals of Group II, compared to the results with Group I, was possibly a result of remobilization of fluoride initially deposited in the bones of high fluoride content with consequent longer maintenance of elevated urinary fluoride output.

Table II gives information with respect to the bones. The calculations of total skeletal fluoride load and of the fraction of administered dose present in the skeletons of the Group I animals were performed as indicated in the test and in Table II footnote. Similar calculations of increment of skeletal fluoride load could not be made for Group II animals since the administered dose (2.94 mg of F) was small in comparison to the 20–30 mg of fluoride present in the skeletons of the animals at the beginning of the experimental period with the result that the concentration of fluoride in the ashed humeri was not significantly affected by whatever amount of fluoride was added to the bones.

The skeletal fluoride load of Group I animals increased rapidly and linearly over the period 0 to 3 hr and in the period 5–50 hr the increment of skeletal fluoride, representing a balance between uptake and mobilization, continued but at a rate approximately 0.2 that in the first 3–5 hr. By 50 hr the skeletal fluoride load of Group I animals had increased by 10 times through uptake of approximately 70% of the administered fluoride dose.

The results with Group I animals show that the skeletal regulation of the body-fluid fluoride content following the ingestion of a large dose of fluoride is faster and quantitatively larger than regulation by the kidney. Over 70% of the administered dose was sequestered by the skeleton of these animals in 50 hr (Table II) and most of the remainder of the dose was excreted in the urine (Table I).

Even though fecal excretion of fluoride was not measured, the amounts of the fluoride of challenge dose excreted in the urine and deposited in the skeletal tissues indicate that at least 29% of the fluoride challenge dose was absorbed by the Group I animals at the end of the 5th hour after administration of the dose and about 88% at the end of the 50th hour (Tables I and II).

Summary. An acute dose of 2.94 mg of fluoride, given by gastric intubation to mature female rats with or without previous high fluoride intakes, was rapidly absorbed and distributed in the body fluid compartments. Limited excursions of plasma fluoride contents were observed since the mechanisms establishing equilibrium of distribution of fluoride between blood and bone and those of urinary excretion of fluoride functioned rapidly. There was no evidence that animals with initially elevated skeletal fluoride contents were deficient in ability to control elevations of plasma fluoride contents or to dispose of fluoride in the urine. Results from the animals with a previous low fluoride exposure showed that skeletal regulation of the body fluoride was faster and quantitatively larger than excretion of fluoride by the kidney. At least 29% of the fluoride administered was absorbed by these animals in 5 hr. At 50 hr at least 88% of the administered dose was absorbed and 70% of the dose had been deposited in the skeletons.

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