

Fluoride Absorption: Independence from Plasma Fluoride Levels¹ (42291)

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Abstract. The concept that there are physiologic mechanisms to homeostatically regulate plasma fluoride concentrations has been supported by results in the literature suggesting an inverse relationship between plasma fluoride levels and the absorption of the ion from the gastrointestinal tract of the rat. The validity of the relationship was questioned because of possible problems in the experimental design. The present work used four different methods to evaluate the effect of plasma fluoride levels on the absorption of the ion in rats: (i) the percentage of the daily fluoride intake that was excreted in the urine; (ii) the concentration of fluoride in femur epiphyses; (iii) the net areas under the time-plasma fluoride concentration curves after intragastric fluoride doses; and (iv) the residual amounts of fluoride in the gastrointestinal tracts after the intragastric fluoride doses. None of these methods indicated that plasma fluoride levels influence the rate or the degree of fluoride absorption. It was concluded that, unless extremely high plasma fluoride levels are involved (pharmacologic or toxic doses), the absorption of the ion is independent of plasma levels. The results provide further evidence that plasma fluoride concentrations are not homeostatically regulated. © 1986 Society for Experimental Biology and Medicine.

The general metabolism of ionic fluoride has received considerable research attention because of its importance in public health and clinical dentistry and its use in the treatment of certain skeletal diseases such as osteoporosis. Twenty-five years ago, the concept that plasma fluoride concentrations were homeostatically regulated was advanced (1). It was based on data which showed nearly identical fluoride levels in ashed plasma of persons from communities with widely differing water fluoride levels. The authors suggested that the renal, skeletal, and gastrointestinal systems operated together in the homeostatic process.

It is now generally accepted that plasma fluoride levels rise in proportion to intake (2, 3) which indicates that they are not homeostatically regulated. The original data which suggested homeostasis was obtained with an analytical method which measured ionic fluoride and nonionic compounds of fluoride (4, 5).

The concept of fluoride homeostasis was supported, however, by subsequent research on the handling of the ion by the gastrointestinal tract. Stookey *et al.* (6) proposed that there was an inverse relationship between the

rate of fluoride absorption and plasma fluoride levels in rats. The plasma levels were varied acutely by nephrectomy or chronically by giving 1.0 mg of fluoride daily by gastric intubation for several months.

A careful reading of the experimental design used in the work of Stookey *et al.* (6) suggested that their main conclusion may have been drawn because of the lack of appropriate control groups of rats. The present work was done to further examine the relationship between plasma fluoride levels and the absorption of the ion for the purpose of clarifying the nature of the absorptive process.

Materials and Methods. Weanling female Sprague-Dawley rats ($n = 24$) were randomly assigned to three groups of eight rats each. They were housed in pairs in plastic metabolism cages throughout the 30-day experiment. The groups were given free access to drinking water containing 0, 10, or 50 ppm fluoride which was added as sodium fluoride. Body weights, 24-hr water consumption, and urine volume determinations were made every 5 to 7 days. Daily food consumption was determined three times during the last 2 weeks of the study.

The rats were fasted for 24 hr and given distilled water for 6 hr prior to sacrifice to reduce the residual amount of fluoride in the stomachs and intestines. Following anesthesia

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using pentobarbital (40 mg/kg ip), an endotracheal tube (PE190) and a carotid artery cannula (PE50) were placed in each rat. After the collection of a control blood sample, each rat received 2.0 mg fluoride/kg (as NaF) by gastric intubation. Additional blood samples (0.20 ml each) were taken from each rat at 20, 40, 60, 90, 120, 180, 240, and 300 min after the fluoride dose. The animals were then killed by decapitation. The femur distal epiphyses were removed, dried at 100°C for 2 days, weighed, and analyzed for fluoride and phosphorus. The stomachs and first 30 cm of intestine from each rat were removed and rinsed through with 30 ml of distilled water.

Urine and drinking water fluoride concentrations were determined following dilution with equal parts of an acetate buffer using the ion-specific electrode (7). The fluoride concentrations of food, bone, GI tract rinses, and plasma were determined with the electrode following diffusion using the acid-HMDS method of Taves (8) as modified by Whitford and Reynolds (9). A portion of the acid containing the dissolved epiphysis in each diffusion dish was analyzed for phosphorus using the method of Chen *et al.* (10). The net areas under the time-plasma fluoride concentration curves were determined using the trapezoidal rule. The data are expressed as the means \pm SEM throughout. Comparisons of group mean values for statistically significant differences were made using the *t* test, two-tailed, unpaired. A significance level of $P < 0.01$ was selected.

Results. There were no statistically significant differences in body weight among the three groups. The initial and final average body

weights were 42.1 ± 1.0 and 160.6 ± 2.1 g, respectively. Similarly, the groups were not statistically different with respect to daily food and water intake or 24-hr urine output.

Table I shows the daily fluoride intake with water, the daily urinary fluoride excretion, and the percentages of the excreted amounts relative to the amounts that were ingested with water. The 10 ppm water group excreted an overall average of 26.9% of the fluoride ingested with water and the 50 ppm group excreted an average of 23.7%. The average amount of fluoride ingested with food during the last 2 weeks of the study was 52 μ g per day. When this quantity was added to the intake with water for the last three measurement periods, which were made during the last 2 weeks of the study, the former group excreted an overall average of 23.8% and the 50 ppm water group excreted 24.3% of the amounts of fluoride ingested daily with the water and food. The distilled water group excreted an average of 13 μ g/day which was 24.7% of the fluoride ingested with the food. The differences in these percentages among the groups were not statistically significant.

The phosphorus concentrations of the femur distal epiphyses, on a dry weight basis, for the 0, 10, and 50 ppm fluoride water groups were 9.0 ± 0.15 , 9.1 ± 0.17 , and 9.1 ± 0.20 g%, respectively. The corresponding fluoride concentrations were 96.1 ± 3.4 , 470.1 ± 5.0 , and 2029 ± 82.3 ppm.

Five hours after the intragastric doses of fluoride on the final day of the study, the stomachs and first 30 cm of intestine of the 0, 10, and 50 ppm groups contained 2.24 ± 0.45 , 4.40 ± 2.18 , and $4.17 \pm 1.33\%$ of the admin-

TABLE I. DAILY FLUORIDE INTAKE WITH WATER AND DAILY URINARY FLUORIDE EXCRETION BY GROUPS OF RATS WITH DRINKING WATER FLUORIDE LEVELS OF 0, 10, OR 50 ppm

Measurement period	F intake with water, μ g			F excretion in urine, μ g			Ratio, %	
	0 ppm	10 ppm	50 ppm	0 ppm	10 ppm	50 ppm	10 ppm	50 ppm
1	—	319 \pm 5	1832 \pm 92	11 \pm 1	88 \pm 5	426 \pm 28	27.6	23.3
2	—	341 \pm 35	1904 \pm 24	14 \pm 2	85 \pm 6	388 \pm 31	24.9	20.4
3	—	321 \pm 8	1620 \pm 106	13 \pm 1	89 \pm 5	421 \pm 47	29.6	26.0
4	—	369 \pm 15	2057 \pm 59	14 \pm 1	100 \pm 7	501 \pm 48	27.1	24.4
5	—	347 \pm 8	1935 \pm 74	12 \pm 1	95 \pm 10	475 \pm 25	25.1	24.5

Note. Data expressed as means \pm SEM ($n = 4$). Values are per cage; each cage contained two rats. The ratios are the percentages of the excreted amounts relative to the amounts ingested with water.

istered fluoride doses, respectively. These values represent the net amounts of fluoride that were obtained after subtracting the amounts of fluoride found in 30 ml distilled water rinses of the tissues that were taken from rats that had been provided with water containing 0, 10, or 50 ppm fluoride for 9 days. The quantities recovered from the stomachs and first 30 cm of intestine from these rats, of which there were four per group, were 1.8 ± 1.0 , 1.9 ± 0.4 , and 2.3 ± 1.1 μg , respectively. As in the main study, these rats were fasted for 24 hr and given distilled water to drink for 6 hr prior to death.

Table II shows the plasma fluoride concentrations of the three groups as a function of time before and following the intragastric dose of 2.0 mg F/kg body wt. The control plasma levels reflected the levels of fluoride intake during the study. The peak concentrations occurred at 20 min. The rates of decline in the plasma fluoride concentrations were similar after 20 min although, during the last 3 hr, the concentrations were higher in the 10 and 50 ppm water groups which simply reflected their higher control plasma levels.

Table III contains the net areas under the time-plasma fluoride concentration curves. To obtain the net areas, the concentration of the plasma sample taken prior to the intragastric fluoride dose was subtracted from each subsequent concentration for each rat. There were no statistically significant differences among the groups for these data.

TABLE II. PLASMA FLUORIDE CONCENTRATIONS AFTER THE INTRAGASTRIC ADMINISTRATION OF 2.0 mg F/kg BODY WEIGHT

Minutes after dose	Group		
	Distilled water	10 ppm F water	50 ppm F water
0	0.42 \pm 0.03	1.6 \pm 0.2	5.9 \pm 0.5*
20	56.5 \pm 3.8	52.1 \pm 5.0	54.5 \pm 6.3
40	42.1 \pm 3.9	44.4 \pm 3.6	48.8 \pm 6.6
60	30.0 \pm 3.0	36.0 \pm 3.5	40.8 \pm 5.0
90	22.3 \pm 5.2	22.0 \pm 3.8	27.5 \pm 4.2
120	15.5 \pm 3.9	19.3 \pm 3.0	25.3 \pm 3.5
180	9.3 \pm 2.3	13.3 \pm 2.7	18.0* \pm 1.8
240	6.5 \pm 0.9	9.8 \pm 1.6	13.8* \pm 0.6
300	4.8 \pm 0.9	7.5 \pm 1.6	12.3* \pm 0.6

Note. Concentration units are micromolar. Data expressed as means \pm SEM ($n = 8$). * $P < 0.01$ compared to the distilled water group.

TABLE III. NET AREAS UNDER THE TIME-PLASMA FLUORIDE CONCENTRATION CURVES AFTER THE INTRAGASTRIC ADMINISTRATION OF 2.0 mg F/kg BODY WEIGHT

Minutes after dose	Group		
	Distilled water	10 ppm F water	50 ppm F water
20	561 \pm 39	506 \pm 50	486 \pm 60
40	1540 \pm 110	1440 \pm 128	1401 \pm 180
60	2254 \pm 175	2200 \pm 182	2178 \pm 278
90	2888 \pm 466	2656 \pm 408	2666 \pm 618
120	3196 \pm 616	3222 \pm 495	3299 \pm 713
180	4172 \pm 786	4092 \pm 632	4305 \pm 847
240	4628 \pm 904	4673 \pm 727	4916 \pm 869
300	4949 \pm 976	5082 \pm 794	5379 \pm 873

Note. Units are micromoles \cdot min \cdot liter⁻¹. Data expressed as means \pm SEM ($n = 8$).

Discussion. The protocol for the present work included four different methods for evaluating the hypothesis that there is an inverse relationship between plasma fluoride concentrations and the gastrointestinal absorption of the ion. A 14-fold difference in plasma levels was achieved between the low-fluoride group and the group whose drinking water contained the ion at 50 ppm. In spite of this difference, there were no indications that the absorption of fluoride differed among the groups as judged by (i) the percentages of the daily fluoride intakes that were excreted in the urine; (ii) the concentrations of fluoride in the femur epiphyses; (iii) the net areas under the time-plasma fluoride concentration curves after an intragastrically administered dose; and (iv) the residual amounts of fluoride in the gastrointestinal tracts 5 hr after the intragastric dose.

The absence of an effect of different plasma fluoride levels on the rate or degree of fluoride absorption differs from the findings of Stookey *et al.* (6). They concluded that acutely nephrectomized rats absorbed fluoride at a slower rate than did *unoperated* control rats. They attributed the difference to the lack of renal function and higher plasma fluoride levels. However, abdominal surgery would have diminished gastrointestinal blood flow, motility, and secretion, effects that would generally depress absorption. The inclusion of a sham operated group probably would have shown the effects of the surgery.

In the other part of their study (6), rats were fed a low-fluoride diet with one-half of the rats each receiving an additional 1.0 mg fluoride per day by gastric intubation for several months. On the final day of the study, *all* rats were given 1.0 mg of fluoride by gastric intubation and 4 hr later their "entire gastrointestinal tracts were removed and analyzed for fluoride." No correction was made for the previously administered fluoride that was contained within the lumen or in the mucosa and the underlying tissue. The fluoride contained in the stomach and intestines of the rats that had been previously exposed by daily intubation may have been sufficient to cause the investigators to conclude that less of the fluoride dose had been absorbed.

Unlike other halogens and most other substances, fluoride is readily absorbed from the stomach particularly when the administered solution or the stomach contents have a pH of approximately 4.0 or less (11). This was attributed to the formation and diffusion of HF, a weak acid with a pK_a of 3.4. This molecule appears to be the form in which fluoride permeates a variety of cell membranes or epithelia including the renal tubule (12–14), urinary bladder (15), hamster cheek pouch (16), isolated rat diaphragm (17), isolated rat hepatocytes (18), certain bacteria (19–22) and lipid bilayer membranes (23). In the latter study, the permeability coefficient of HF was determined to be more than 10^6 times greater than that of ionic fluoride. The ion appears not to freely permeate these membranes because of its charge and large hydrated radius. An exception to this has been found in recent studies using segments of the small intestines of rats *in vivo* and *in vitro* (Whitford, unpublished). In these studies, the flux of fluoride has failed to show a pH dependence apparently because there is little difference between the intestinal mucosal reflection coefficients for HF and ionic fluoride.

In-so-far as passive diffusion is the mechanism for the absorption of fluoride, whether as HF or the ion, it would be theoretically possible for plasma fluoride levels to influence the rate of fluoride absorption. This would be experimentally apparent as plasma fluoride levels increased to approach or exceed those of the gastrointestinal mucosal interstitial fluid. However, plasma fluoride levels of humans

consuming drinking water fluoridated at 1 ppm ($52.6 \mu M$) and a diet prepared with this water are approximately $1 \mu M$ (2, 3) with slight variations during each day as determined by dietary patterns and the use of fluoride-containing products such as toothpastes ($52,000 \mu M$) or mouthrinses (11,900 to 47,400 μM). Therefore, unless pharmacologic or toxic plasma fluoride levels are involved, it is unlikely that plasma fluoride concentrations could be high enough to measurably influence the absorption of the ion.

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