

Effects of Acute Fluoride Intoxication on Rats¹ (40054)L. SINGER,² W. D. ARMSTRONG, AND R. H. OPHAUG*Department of Biochemistry, University of Minnesota, Minneapolis, Minnesota 55455*

The overall effects of acute fluoride poisoning are undoubtedly related to the concentration of fluoride in circulating plasma and in the tissues. The minimum concentration of fluoride in plasma that will result in death is probably dependent on a number of factors such as the mode of intake, duration of extreme elevation in fluoride concentration, age of the animal, the critical level that affects enzymes, and the relation of the fluoride concentration to that of other ionic constituents in the plasma and tissues.

The lethal concentration of fluorine in plasma of fasted rabbits (1), acutely poisoned by gastric intubation of a solution of sodium fluoride, (100-140 mg/kg body weight) was higher (≤ 28 ppm) than that reported by De Lopez *et al.* (2) for young rats given 50 mg/kg of body weight (10-12 ppm). The highest plasma fluoride concentration (35 ppm) observed by Hall *et al.* (1) was found 2 hr after a rabbit was fatally poisoned with 140 mg of sodium fluoride per kg of body weight. Fujimoto and Okuda (3) found levels of 20 ppm fluorine in the plasma of rabbits one hr after the oral administration of a single dose of 220 mg sodium fluoride and at the end of the 24-hr period the concentration had returned to normal levels. A decrease in total calcium concentration in plasma was observed while fluoride levels increased following the application of 25 mg sodium fluoride per kg of body weight to the buccal mucosa of young rabbits.

In order to establish the range of plasma fluoride concentrations that occur as a consequence of acute fluoride poisoning, young male rats were employed in this investigation to study the uptake and distribution of parenterally administered high fluoride doses. The effects of these treatments on the ionic

and total calcium concentrations in plasma were also examined since acute fluoride poisoning may produce alterations in plasma calcium levels and interference with the role of calcium in metabolism.

Methods. Male rats weighing approximately 200 g were given 3, 4 or 5 mg of fluoride (15, 20 or 25 mg/kg body weight) as sodium fluoride intraperitoneally in 1 ml of solution. The rats were fed Purina Laboratory Chow (20-60 ppm F⁻) and tap water (1 ppm F⁻) throughout the experimental period. A few select animals were given inert fluoride labeled with radioactive fluoride (¹⁸F), a positron emitter with a half-life of 109.7 min and sacrificed 10, 30 or 60 min following the dosage. All animals receiving 3 or 4 mg of fluoride were bled under ether anesthesia by heart puncture at 10 min and 1, 4 and 24 hr after the dosage. Select animals receiving radioactive fluoride were also bled after 30 min. To test the possibility that food residues present in the intestinal tract and stomach of animals given access to food might function as a significant compartment for distribution of intraperitoneally administered fluoride or modify the toxic effect of acute doses of fluoride, some of the animals were removed from food and water 16 to 18 hr prior to being given the fluoride challenge dose labeled with radiofluoride (¹⁸F). The animals given 5 mg of fluoride immediately became moribund and blood could be obtained only at 10 and 60 min after the dosage since most animals did not survive for longer periods. Anesthesia was generally not required for animals receiving 5 mg of fluoride. In addition to plasma, the humeri, tail tendon and muscle were taken for analytical and for radiochemical analyses from animals receiving labeled fluoride. Control animals that did not receive the fluoride treatment were sacrificed to provide baseline information on the normal concentration of fluoride in plasma and the selected tissues.

The total fluorine concentration of blood

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plasma from each animal was determined by microdiffusion and colorimetric analyses (4). Ionic fluoride levels were measured in plasma ultrafiltrates with the Orion ion-specific electrode (5) when sufficient volume of plasma was available. The total calcium levels in plasma were determined using a modified calcium fluorimetric technique (6). The ionic calcium concentrations were determined with an Orion flow-thru calcium ion-activity electrode. Radiofluoride concentrations in the plasma and other tissues were determined with a well-type crystal scintillation counter. All radioactivity measurements, after correction for background counts and volume, were adjusted for radioactive decay to a single point in time.

Results. Rats receiving 3 or 4 mg of fluoride (15 or 20 mg/kg body weight) exhibited extremely high mean plasma fluoride levels after 10 min which declined rapidly thereafter (Table I). By 24 hr plasma fluorine concentrations had returned to nearly preexperimental levels (0.12 ± 0.009 , 12 animals). After 10 min those animals given the 5 mg dose exhibited plasma fluorine levels as high as 57 ppm. The concentration of fluorine found in plasma, tendon and humeri of a limited number of the animals that received the dose labeled with radiofluoride and were sacrificed at 10, 30 and 60 min following the challenge are displayed in Table II. These tissues and the muscle were analyzed for radiofluoride. Calculated fluoride levels were obtained based on the ^{18}F determination of the tissue

and the specific activity ($\text{cts}/\mu\text{g } ^{19}\text{F}$) of the administered solution. The fluoride concentration in the muscle of these animals and the relation between the muscle and plasma fluoride contents are presented separately in Table III.

The concentrations of ionic and total calcium in the plasma are presented in Fig. 1 as bar diagrams. The number at the top of each bar indicates the number of animals in the mean value. The total height of the bar indicates the total plasma calcium concentration (mg %) and the horizontal line near the center of the bar denotes the mean concentration of ionic calcium (mg %). The number in the clear area of the bar is the % of the total calcium in the ionic state.

Discussion. Animals receiving 3 or 4 mg of fluoride (15 or 20 mg/kg body weight), despite having very high plasma fluorine levels (>30 ppm) 10 min after receiving the challenge dose, invariably lived and exhibited a rapid return toward the preexperimental plasma total fluorine level (0.12 ppm). Approximately 70% of the plasma total fluorine was ionic.

The animals given 5 mg of fluoride did not always have higher plasma ionic fluoride levels than those receiving the 4 mg dose. This is particularly evident in the results at 60 min. There is no simple explanation why most of the animals given the 5 mg dose died within 60 min whereas all the animals treated with 4 mg of fluoride survived. It is indeed possible that much higher levels than 49 and 44 ppm

TABLE I. FLUORIDE CONTENT OF PLASMA AFTER INTRAPERITONEAL ADMINISTRATION OF FLUORIDE.^a

Time of collection	Fluoride dose ^b		
	3	4	5
<i>10 minutes</i>			
Total	29.5 ± 1.58 (12)	35.1 ± 2.52 (12)	48.0 ± 2.10 (11)
Ionic	22.9 ± 1.07 (9)	29.2 ± 2.45 (9)	37.6 ± 2.83 (8)
<i>60 min</i>			
Total	6.4 ± 0.75 (11)	15.1 ± 1.08 (14)	9.0 ± 2.49 (9)
Ionic	5.2 ± 0.90 (11)	11.9 ± 0.079 (8)	4.17 ± 1.85 (4)
<i>4 hr</i>			
Total	0.72 ± 0.108 (10)	1.41 ± 0.253 (10)	
Ionic	0.43 ± 0.020 (4)	1.44 ± 0.112 (4)	
<i>24 hr</i>			
Total	0.19 ± 0.031 ^c (11)	0.33 ± 0.105 (8)	
Ionic	0.05 ± 0.006 (3)	—	

^a Parts per million ± SEM (number animals).

^b mg/200 g body weight.

^c Preexperimental levels 0.12 ± 0.009 (12).

TABLE II. FLUORIDE CONTENT OF TISSUES^a OF RATS AFTER ADMINISTRATION OF LARGE DOSES OF FLUORIDE LABELED WITH RADIOFLUORIDE.^b

Fluoride ^c injected	Plasma		Tendon		Humeri	
	Predicted	Found	Predicted	Found	Predicted	Found
			10 min			
3	25.0	24.7	12.0	10.0	191	160
	21.1	21.8	11.1	9.6	181	172
4	37.7	34.3	13.9	11.4	203	174
	22.9	22.6	34.3	30.4	163	174
5	46.6	43.2	15.5	13.4	214	189
	54.6	49.1	13.5	12.3	227	228
			30 min			
3	15.1	14.1	19.5	20.0	245	232
	13.6	13.0	8.1	6.7	242	232
4	23.7	20.3	7.5	5.1	271	253
	21.3	20.7	13.1	11.3	263	301
5	27.6	27.6	18.5	21.6	288	243
	27.1	21.7	16.0	14.4	283	287
			60 min			
3	7.3	6.4	4.8	4.4	248	257
	2.5	2.6	2.8	3.0	197	188
4	14.1	12.5	8.7	7.6	305	271
	11.7	10.2	9.1	7.3	334	270
5	13.6	12.8	10.5	9.1	279	346
	17.1	17.8	12.5	12.6	269	316

^a Parts per million on fresh weight or volume basis.

^b The predicted values for fluoride contents were derived from the ¹⁸F contents of the plasma and tissue, the total ¹⁸F and ¹⁹F body contents and the dose of fluoride given the animals.

^c Milligram fluoride ion as sodium fluoride.

TABLE III. FLUORIDE CONTENT OF MUSCLE.^a

Time (min)	Dosage ^b					
	3		4		5	
	ppm	% Plasma ^c level	ppm	% Plasma level	ppm	% Plasma level
10						
1	5.1	20.4	6.8	18.0	9.0	19.3
	4.8	20.7	5.2	22.7	9.3	17.4
30						
1	4.2	27.8	6.8	28.7	10.0	36.2
	4.2	30.9	7.6	35.7	10.0	36.9
60						
1	2.7	37.0	5.2	36.9	8.5	62.5
	0.7	28.0	5.2	44.4	9.5	55.6

^a Based on specific activity of ¹⁹F solution injected and the ¹⁸F content of muscle.

^b Milligram/200 g body weight.

^c Determined level.

for total and ionic fluorine concentration, respectively, observed for individual animals may have existed prior to or shortly after the 10 min sampling period following the 5 mg dose of fluoride. A large excursion in fluoride content of body fluids could produce alterations in metabolism for which these animals were not able to fully compensate and, as a result, death occurred within 60 min. The *in vitro* activity of a number of enzymes has

been reported to be reduced to one-half or less by concentrations of fluoride between 0.2 and 19 ppm in the media (7). These concentrations are considerably higher than the ionic fluoride concentration found in plasma and soft tissues under normal circumstances (0.01–0.05 ppm) but in some instances are not higher than the levels attained after the large doses of fluoride. De Lopez *et al.* (2) used young animals that were fasted for 24

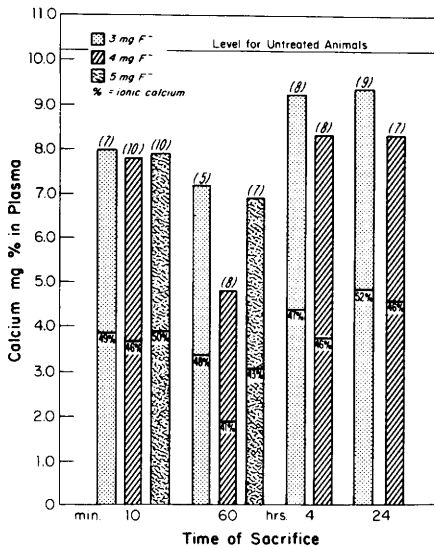


FIG. 1. Effect of acute fluoride toxicity on plasma calcium levels. The number at the top of each bar indicates the number of animals in the mean value. The total height of the bar indicates the total plasma calcium concentration (mg %) and the horizontal line near the center of the bar denotes the mean concentration of ionic calcium (mg %). The number in the clear area of each bar is the % of the total calcium in the ionic state.

hr before being given, by intubation, higher fluoride doses (40–70 mg/kg) than those employed in this study (15–25 mg/kg). The method of administering fluoride will influence the level of fluoride attained in the plasma. Interperitoneal administration of fluoride results in a rate of entrance of fluoride into the plasma which is only slightly slower than that obtained with intravenous administration. Fluoride given by intubation is evidently absorbed more slowly since lower peak plasma concentrations (10 ppm) were observed in De Lopez's study than in the present study. De Lopez *et al.*, however, reported plasma fluoride levels of approximately 4 ppm 12 hr after the administration of the dose, whereas in this study, levels of less than 2 ppm were observed within 4 hr. It is possible that a plasma fluoride level of 4–10 ppm for a 12-hr period is more toxic than higher levels for a considerably shorter period of time. There is little, if anything, known as to the *in vivo* effects of large increments in fluoride ion concentration on enzymatic activity. Numerous changes in the concentrations of glycolytic and citric acid cycle intermediates in the liver of intact rats given acute

doses of fluoride have been reported. There was no indication of enzyme inhibition in the kidney (8). With the exception of an increase in citrate levels in soft tissues, the changes were shown to be secondary to a primary effect of dietary fluoride on the amount and pattern of food intake (9). Recently, Ferguson reported that 10 ppm of fluoride provided in the drinking water for 12 weeks resulted in a decrease in serum alkaline phosphatase activity in rats, but no change in the activity of this enzyme in the liver or intestinal tissue (10). Acid phosphatase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase and leucine aminopeptidase activities in serum, liver, kidney or intestine were not influenced by this level of fluoride intake. In a similar study with rabbits little change, if any, in enzyme activities was noted (11).

To test the possibility that food residues in the intestinal tract and stomach might act as a significant compartment for distribution of the fluoride administered intraperitoneally, a group of animals not fed for 16–18 hr prior to experimentation were given the fluoride doses labeled with radiofluoride. The quantity of administered radiofluoride found in the combined contents of the stomach and intestinal tract of fed animals, which can be considered to contain a pool of inert fluoride, was small (less than 2.5% of the dose). It was therefore, concluded that the contents of the gastrointestinal tract was not an important factor acting to sequester fluoride administered intraperitoneally.

Within 10 min after the animals were given fluoride the total calcium level in the plasma was depressed in all dosage groups, although the % of the total calcium in the ionic form remained normal (Fig. 1). At 60 min a further reduction in total calcium in plasma was observed with all animals, but by four hr the trend was reversed for the animals dosed with 3 and 4 mg (15 and 20 mg/kg body weight) and calcium levels had risen considerably and plateaued at a level slightly below the normal concentration for untreated animals (10 mg %). The animals receiving 5 mg of fluoride did not survive for longer periods of time. The ionic calcium levels were severely depressed by 60 min. These results for the first 60 min are similar to the observations of Fujimoto and Okuda (3) who found that rabbits given a large oral dose of fluoride

(12.5 mg/kg body weight) had a slight decrease in plasma calcium levels after 3 hr which was still apparent 21 hr later. In their study, animals receiving 25 mg of fluoride per kg exhibited an immediate sharp decrease in plasma calcium concentration of approximately 25% by one hr which was followed by a slow progressive decrease for the remainder of the 24-hr period to reach a level 30% lower than normal. In the present study, however, the animals receiving 3 or 4 mg (15–20 mg/kg body weight) showed good recovery by 4 hr.

The total and ionic plasma calcium concentrations found 30 and 60 min following the dose would not seem to be compatible with life and normal neuromuscular activity on the basis of accepted concepts. Nevertheless, all the animals given 3 or 4 mg fluoride survived these circumstances. Surprisingly, animals receiving 5 mg of fluoride exhibited higher total and ionic calcium levels in their plasma at one hr than the animals given 4 mg of fluoride. A possible explanation for the fact that animals receiving 4 mg of fluoride were able to survive and rebound from their lethargy and altered calcium status is that their general metabolism was so severely affected, but not irreversibly, that their life processes were operating at a minimal rate. Under such circumstances, energy production and muscle contraction would be greatly reduced and obvious tetany would not be observed. Tetany was not observed in this study. By contrast, the animals that received 5 mg of fluoride had proceeded past the point of reversibility and were so severely poisoned that life soon ceased. The inhibition of critical enzyme system(s) may have been responsible for death, but this, like the explanation above, is only a speculation.

There was good agreement in the predicted (i.e., calculated) and determined total fluoride values in all tissues taken from the animals given radiofluoride with the possible exception of the concentration found in the humeri at 60 min (Table II). Undoubtedly, the agreement between the results for fluoride contents obtained by the two procedures is influenced by the fact that the fluoride contents were very much elevated over the normal concentrations and any bound or nonexchangeable fluoride was a relatively small part of the total fluorine content of the tissues. To some degree this concordance of results lends con-

fidence to the reliability of the analytical procedures applied to plasma and tissue containing high fluoride levels. These data indicate that a rapid equilibrium of fluoride between these tissues and the plasma occurred within 10 min after a large dose of fluoride was given intraperitoneally. This would imply that essentially all of the fluoride of bone had exchanged in a short period of time.

In the animals receiving the radiofluoride the highest concentrations of fluoride in plasma were attained by 30 min, and possibly as early as 10 min, and then decreased rapidly with time. This was not the time sequence for tendon, muscle or bone. The fluoride in tendon appeared to be high for at least 30 min and then decreased rapidly in the next 30 min, whereas the fluoride content of humeri and muscle peaked by 30 min and did not show any loss at 60 min.

Based on the specific activity of the injected fluoride solution, the concentration of fluoride in the entire body prior to receiving the dose and the amount of radioactivity in the muscle, the fluoride concentration in muscle 10 min after animals were given 3, 4 or 5 mg of fluoride was equal to approximately 20% of the fluoride concentration in plasma and was near the maximum level observed at the 60 min experimental period. By 30 min the concentration in the muscle was 30–35% of that in plasma. There was little change in this relation at 60 min for those animals receiving 3 or 4 mg of fluoride. However, those animals given the 5 mg dose had reached a concentration in muscle of approximately 50% of the fluoride level in plasma by 60 min. The muscle obviously attained a high fluoride concentration (7 and 9 ppm) by 10 min which was maintained for at least 60 min by those animals receiving 4 or 5 mg of fluoride respectively, whereas those animals receiving 3 mg of fluoride exhibited a sharp decline in concentration between 30 and 60 min from a maximum concentration of 5 ppm attained by 10 min.

Summary. Young male rats (~200 g) were employed to study the distribution of parenterally administered inert and radiolabeled fluoride. Animals receiving 25 mg of fluoride/kg body weight did not live longer than one hr and the plasma fluoride level reached a concentration of 48 ± 2.1 ppm. Other animals receiving 15 or 20 mg of fluoride/kg

had plasma fluoride levels greater than 30 ppm 10 min after the dose but returned to preexperimental levels (~0.10 ppm) by 24 hr. Total and ionic calcium levels in plasma were severely depressed in all animals following the fluoride administrations. The calcium levels at 30 and 60 min did not seem compatible with life although animals receiving 20 mg or less of fluoride/kg survived 24 hr. Radiofluoride concentrations in plasma, muscle, tendon, bone and gastrointestinal tract indicated the relative distribution and retention of the dose as well as the fluoride concentration at 10, 30, and 60 min after the dose was given. Muscle fluoride levels at all dose levels were 5–9 ppm after 10 min. These levels rapidly decreased in animals receiving 15 mg/kg but remained relatively constant for the other animals.

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