TOXICOLOGY AND APPLIED PHARMACOLOGY 50, 31–39 (1979)

Acute Fluoride Toxicity: Influence of Metabolic Alkalosis

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Received October 4, 1978; accepted March 5, 1979

Acute Fluoride Toxicity: Influence of Metabolic Alkalosis. WHITFORD, G. M., REYNOLDS, K. E., AND PASHLEY, D. H. (1979). *Toxicol. Appl. Pharmacol.* **50**, 31–39. Acute fluoride toxicity was induced in anesthetized rats by the continuous iv infusion of fluoride at 1.40 μmol/min. This infusion continued until the death of the animals. Early signs of toxicity, which developed in all animals during the first hour of fluoride infusion, included diuresis, falling urinary osmolality, and glomerular filtration rate at plasma fluoride concentrations of 0.4 to 0.5 mM. The animals were then assigned to three groups. Groups 1, 2, and 3 received intravenous infusions of isotonic saline (control), isotonic sodium bicarbonate, and isotonic sodium bicarbonate plus acetazolamide, respectively. Compared to group 1, groups 2 and 3 survived the high fluoride infusion longer (5.93 vs 7.61 and 10.27 hr, respectively) and tolerated more fluoride (47.8 vs 61.9 and 80.7 mg/kg, respectively). At any given time (dose) after starting the high fluoride infusion, plasma fluoride concentrations were lower and mean arterial blood pressures, glomerular filtration rates, fluoride renal excretions, clearances and fractional clearances, and blood and urine pH values were higher in animals from groups 2 and 3 compared to group 1. Further, group 3 was more resistant to fluoride toxicity than group 2. Terminal tissue-to-plasma fluoride concentrations for heart were lowest in groups 2 and 3 while there were no differences among the groups for brain. It is concluded that metabolic alkalosis can favorably influence the course of a developing episode of acute fluoride toxicity and that this maneuver should be an important addition to the currently accepted therapeutic regimen.

The toxicity of inorganic fluoride has received considerable research attention and has been characterized in terms of the doses involved, the signs and symptoms, the accompanying biochemical and physiologic disturbances, predisposing factors such as age and sex, the clinical course, and treatment (Hall *et al.*, 1972; Hodge and Smith, 1965; Horowitz, 1977; Leone *et al.*, 1956; Maynard *et al.*, 1951; Mörnstad, 1975).

A recent report from our laboratory identified the preexisting acid–base status of rats as an important factor in determining sensitivity to acute fluoride toxicity (Reynolds *et al.*, 1978). In that study, metabolic acidosis and metabolic alkalosis were produced by the preadministration of ammonium chloride and sodium bicarbonate, respectively. Fluoride was infused iv at a constant rate until death. It was found that the alkalotic rats survived approximately twice as long, tolerated nearly twice as much fluoride, died with higher plasma fluoride concentrations, and generally had higher glomerular filtration rates and vital signs at any given plasma fluoride concentration. It was also noted that tissue-to-plasma fluoride concentration ratios for heart and skeletal muscle were lower in the alkalotic animals.

It was concluded that the protection afforded by a preexisting metabolic alkalosis probably involved two mechanisms. First, by increasing urine pH, the renal clearance rate of fluoride was enhanced (Whitford *et al.*,

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1976; Harmon *et al.*, 1976) and, thus, fluoride was excreted more rapidly. Second, by expanding intracellular-to-extracellular pH gradients, intracellular fluoride concentrations were lower at any given plasma level. The hypothesis that the transcellular distribution of fluoride is determined by the diffusion equilibrium of hydrogen fluoride was advanced and developed in some detail.

The present work was designed to determine whether the course of a developing acute fluoride toxic episode in rats could be influenced by imposing a metabolic alkalosis. Several variables were evaluated including acid–base status, plasma, brain, and heart fluoride concentrations, renal fluoride excretion and clearance rates, glomerular filtration rates, plasma calcium concentrations, and cardiopulmonary parameters. The results support and extend the findings of Reynolds *et al.* (1978) and suggest that alkalosis may be an important addition to accepted measures in the treatment of acute fluoride toxicity (Smith and Hodge, 1965).

METHODS

Twenty-two random-bred female Wistar rats (199 ± 4 g) were anesthetized with pentobarbital sodium, 35 mg/kg, ip. Surgical preparation included placement of an endotracheal tube, cannulation of an iliac vein for intravenous infusions, and cannulation of a carotid artery for the anaerobic collection of blood at the midpoint of each urine collection period and monitoring of systemic blood pressure, heart rate, and respiration rate (Statham P-23 Gb pressure transducer connected to a Beckman R411 dynagraph recorder). Urine was collected from an indwelling bladder catheter at 30-min intervals.

The experiment began with the iv injection of a priming dose of hydroxymethyl ^{14}C-inulin (1.5 μCi in 0.3 ml isotonic NaCl) and the infusion of an isotonic sustaining solution (2.5% mannitol, 75 mM NaCl, and tracer ^{14}C-inulin) at 25 μl/min.[1] Following a 1-hr equilibration period, two 0.5-hr control urine collections were made. At the beginning of period three, the fluoride-free sustaining infusion was replaced with one containing 2.5% mannitol, 15 mM NaCl, 56 mM NaF, and tracer ^{14}C-inulin. This infusion delivered fluoride at 1.40 μmol/min and was continued until the death of the animals. Two more 0.5-hr urine collections were made and at the end of this hour when early signs of fluoride toxicity were evident, the animals were divided into three treatment groups. In addition to the high fluoride infusion, group 1 (saline control, $n = 8$) received 150 mM NaCl, group 2 ($n = 8$) received 150 mM $NaHCO_3$, and group 3 ($n = 6$) received 150 mM $NaHCO_3$ and acetazolamide (2 ng/μl). These solutions were infused at 0.50 ml/min for 4 min and at 25 μl/min thereafter. Thus, during this latter part of the study the volume infusion rate was 50 μl/min.

Immediately upon the death of each animal, the heart and brain were removed. These were weighed, homogenized in doubly deionized, distilled water, and the supernatants were analyzed for fluoride as described below. The fluoride concentrations of these tissues were expressed in terms of tissue water assuming total water contents for heart and brain of 74 and 75%, respectively (unpublished observations).

Blood and urine pH, pCO_2 and pO_2 determinations were made using a Radiometer BMS 3 Mark 2 system. Bicarbonate concentrations were calculated using the Henderson–Hasselbach equation. Urinary osmolality was determined using a Wescor vapor pressure osmometer (Model 5100A). Total plasma calcium was determined using the method of Gindler and King (1972). ^{14}C-Inulin was determined by liquid scintillation counting and external standardization, and fluoride was analyzed in acetate buffered samples with an Orion ion-specific electrode (Model 94-09A) as previously described (Whitford *et al.*, 1977). Glomerular filtration rates were calculated from the renal clearances of ^{14}C-inulin. All data are expressed as mean ± SE and significant testing was accomplished using Student's *t* test, two-tailed, unpaired.

RESULTS

Table 1 shows that the sodium bicarbonate and the sodium bicarbonate plus acetazolamide treated groups survived the lethal fluoride infusion 28 and 73% longer than the saline-infused control group, respectively. The mean cumulative fluoride doses associated with death (Table 1), expressed as milligrams per kilogram, were 47.8 for group 1, 61.9 for group 2, and 80.7 for group 3. The terminal plasma fluoride concentrations for

[1] Mannitol, NaCl, $NaHCO_3$ and NaF were A.C.S. Certified and purchased from the Fisher Scientific Company, Fair Lawn, N.J. Hydroxymethyl ^{14}C-inulin (radiochemical purity 99%) was purchased from the Amersham Corporation, Arlington Heights, Ill. Acetazolamide (Diamox) was purchased from Lederle Laboratories, Pearl River, N.Y.

TABLE 1

SURVIVAL TIMES AND QUANTITIES OF FLUORIDE INFUSED AFTER STARTING LETHAL FLUORIDE INFUSION AND TERMINAL PLASMA FLUORIDE CONCENTRATIONS IN RATS

	Group[a]		
	1	2	3
Survival time (hr)	5.93 ± 0.32 (8)[b]	7.61 ± 0.51 (8)[c]	10.27 ± 1.49 (6)[c]
F Infused (μmol)	498 ± 27 (8)	639 ± 43 (8)[c]	862 ± 125 (6)[c]
Terminal $[F]_P$[d] (mM)	1.814 ± 0.163 (8)	2.202 ± 0.150 (8)	1.860 ± 0.114 (5)

[a] Group 1, saline-infused control; group 2, sodium bicarbonate treated; group 3, sodium bicarbonate and acetazolamide treated.

[b] Mean ± SE (n).

[c] $p < 0.02$ compared to group 1.

[d] $[F]_P$, plasma fluoride concentration.

these groups were 1.81, 2.20, and 1.86 mM respectively.

Table 2 shows that the three groups did not differ with respect to blood pH, pCO_2, pO_2, and bicarbonate concentration or urine pH and pCO_2 during the first period. In the saline control group (group 1), blood pH and bicarbonate concentrations fell when the animals were near death. In the other two groups, these parameters showed increases early in the study due to the infusion of bicarbonate and, late in the study, they remained approximately equal to the values observed in the first period. Relative to the first period, all groups showed decreases in blood pCO_2 and increases in blood pO_2 in period 6 and thereafter. The infusion of bicarbonate (group 2) or bicarbonate plus acetazolamide (group 3) resulted, as expected, in elevated urine pH and pCO_2 values. These changes were sustained well into the study. Urine pH in the control group increased slightly after period one while the pCO_2 declined resulting in only small changes in the calculated urinary bicarbonate concentrations.

No attempt was made to determine plasma fluoride concentrations in periods one or two (no fluoride infusion). According to previous results (unpublished), these values would have been less than 0.002 mM. Table 3 shows the plasma concentrations in the odd-numbered periods beginning with period 3 when the infusion of fluoride was begun. There were no statistically significant differences between the groups in this period and this was also the case in period 4 (0.404 ± 0.014 mM, $n = 22$). Beginning with the saline, bicarbonate, and bicarbonate plus acetazolamide infusions in period 5, however, the plasma fluoride concentrations of group 1 showed a more rapid rate of increase than those of groups 2 or 3. Further, the plasma levels of the animals in group 2 increased more rapidly than those of group 3. One rat in group 3 was sacrificed in period 31 (the 14th hour after starting the fluoride infusion) and, at that time, its plasma concentration was 0.95 mM. This value was not included in the terminal plasma values of Table 1.

There were no statistically significant differences between the groups for mean arterial blood pressure, heart rate, or respiration rate in period 1 (Table 3). These values remained relatively constant through period 5. Beginning in period 6 (not shown; the fourth 0.5 hr after staring the fluoride infusion), blood pressure began to decline in all groups. This decline was most rapid in group 1 and was considerably attenuated in groups 2 and 3. It is apparent that these differences in the rate of fall of blood pressure correlated well with the rates of increase in plasma fluoride concentration (Table 3). There was no evidence of reflex increases in

TABLE 2

TIME COURSES OF BLOOD pH, pCO_2, pO_2, AND BICARBONATE CONCENTRATION AND URINE pH AND pCO_2 IN RATS TREATED WITH FLUORIDE

	Blood								
	pH[a]			pCO_2[a]			pO_2[a]		
Period	1[b]	2	3	1	2	3	1	2	3
1	7.35 (8)	7.35 (8)	7.35 (6)	46.3 ± 2.2 (8)[c]	48.5 ± 1.5 (8)	45.3 ± 2.4 (6)	88 ± 4 (8)	89 ± 3 (8)	89 ± 3 (6)
6	7.41 (8)	7.46 (8)	7.48 (6)	40.0 ± 2.2 (8)	39.9 ± 1.8 (8)	38.7 ± 1.5 (6)	110 ± 3 (8)	111 ± 4 (8)	109 ± 3 (5)
10	7.37 (8)	7.47 (8)	7.49 (6)	36.9 ± 2.1 (8)	40.7 ± 1.9 (8)	39.0 ± 0.6 (6)	126 ± 2 (8)	112 ± 4 (8)[d]	105 ± 5 (6)[d]
15	7.14 (3)	7.45 (7)	7.49 (5)	39.9 ± 8.3 (3)	39.6 ± 2.7 (7)	33.5 ± 4.3 (5)	121 ± 10 (3)	124 ± 5 (7)	117 ± 6 (5)
18 and 22		7.46 (3)	7.39 (5)		39.6 ± 3.4 (3)	35.2 ± 0.5 (5)		131 ± 8 (3)	127 ± 4 (5)

TABLE 2—*Continued*

	Blood			Urine					
	HCO_3^- (mM)			pH			pCO_2		
Period	1	2	3	1	2	3	1	2	3
1	24.6 ± 0.7 (8)	25.7 ± 0.8 (8)	23.8 ± 1.4 (6)	5.52 (8)	5.60 (8)	5.82 (6)	51.2 ± 2.9 (8)	51.7 ± 2.9 (8)	55.1 ± 7.8 (6)
6	24.1 ± 1.0 (8)	27.6 ± 1.2 (8)	27.9 ± 0.7 (6)[d]	6.19 (8)	6.66 (8)	7.14 (6)	46.0 ± 2.1 (6)	58.8 ± 4.6 (6)	75.9 ± 5.0 (6)[d]
10	20.6 ± 1.2 (8)	28.4 ± 1.0 (8)[d]	29.0 ± 0.7 (6)[d]	6.29 (6)	6.84 (7)	6.77 (6)			
15	13.5 ± 4.0 (3)	26.9 ± 2.4 (7)	24.4 ± 2.3 (5)		6.48 (3)	6.97 (4)		53.9 ± 4.3 (5)	54.9 ± 4.5 (5)
18 and 22		27.3 ± 1.3 (3)	21.1 ± 2.7 (5)			6.45 (5)			

[a] pH values calculated as the negative log of the mean of the hydrogen ion concentrations (pCO_2 and pO_2 in mm Hg).
[b] 1, 2, and 3 refer to the saline control, bicarbonate, and bicarbonate plus acetazolamide groups, respectively.
[c] Data expressed as mean ± SE (*n*).
[d] $p < 0.01$ compared to group 1.

TABLE 3

TIME COURSES OF PLASMA FLUORIDE CONCENTRATION, MEAN ARTERIAL BLOOD PRESSURE, HEART RATE, AND RESPIRATION RATE IN RATS TREATED WITH FLUORIDE

	$[F]_P$[a]			MABP[a]		
Period	1[b]	2	3	1	2	3
1				131±3 (8)	146±5 (8)	126±11 (6)
	Begin lethal fluoride infusion					
3	0.156±0.026 (8)[c]	0.176±0.022 (8)	0.163±0.028 (6)	128±3 (8)	131±7 (8)	145±7 (6)
	Begin saline, bicarbonate, bicarbonate plus acetazolamide infusions					
5	0.629±0.019 (8)	0.601±0.043 (8)	0.558±0.031 (6)	129±5 (8)	127±4 (8)	140±9 (6)
7	0.679±0.028 (8)	0.680±0.092 (8)	0.535±0.020 (6)[d]	103±7 (8)	88±7 (8)	105±9 (6)
9	0.794±0.027 (8)	0.750±0.116 (8)	0.524±0.033 (6)[d]	84±5 (8)	84±6 (8)	116±8 (6)[d]
11	1.168±0.058 (8)	0.928±0.151 (8)	0.661±0.087 (6)[d]	57±5 (8)	80±7 (8)[d]	109±7 (6)[d]
13	1.475±0.111 (6)	1.176±0.216 (8)	0.894±0.243 (6)	37±5 (6)	66±10 (8)	90±12 (6)[d]
15	1.870±0.192 (6)	1.319±0.231 (7)	0.829±0.116 (5)[d]	24±6 (4)	63±12 (7)[d]	92±10 (5)[d]
17		1.414±0.224 (5)	0.983±0.195 (5)		58±11 (5)	70±11 (5)
19		1.610±0.311 (3)	1.216±0.284 (5)		49±9 (2)	87±6 (3)
21		1.294 (1)	0.973±0.168 (3)		40 (1)	73±9 (3)
23		1.628 (1)	1.145±0.262 (3)		35 (1)	56±6 (3)
25			1.315±0.283 (3)			41±7 (3)
27			1.469±0.301 (3)			75±4 (3)
29			0.800 (1)			60 (1)
31			0.953 (1)			54 (1)

TABLE 3—*Continued*

	Heart rate[a]			Respiration rate[a]		
Period	1	2	3	1	2	3
1	396±14 (8)	369±10 (8)	366±30 (6)	54±2 (8)	55±5 (8)	57±7 (5)
	Begin lethal fluoride infusion					
3	398±15 (8)	362±8 (8)	382±9 (6)	52±3 (8)	53±5 (8)	56±5 (6)
	Begin saline, bicarbonate, bicarbonate plus acetazolamide infusions					
5	380±14 (8)	374±10 (8)	358±17 (6)	59±6 (8)	68±3 (8)	55±4 (6)
7	354±13 (8)	324±14 (8)	344±15 (6)	41±2 (8)	50±5 (8)	51±3 (6)[d]
9	330±17 (8)	335±14 (8)	372±20 (6)	41±3 (8)	52±3 (8)	49±5 (6)
11	289±23 (8)	300±18 (8)	366±25 (6)	43±5 (8)	51±4 (7)	43±3 (6)
13	230±23 (6)	270±27 (8)	326±23 (6)[d]	49±5 (6)	50±6 (7)	37±4 (6)
15	147±20 (4)	261±30 (7)[d]	350±14 (5)[d]	34±8 (4)	54±5 (7)	46±2 (5)
17		262±48 (5)	274±17 (5)		53±8 (5)	43±6 (5)
19		258±90 (2)	320±4 (3)		48±12 (2)	44±5 (3)
21		288 (1)	304±11 (3)		66 (1)	51±9 (3)
23		252 (1)	280±28 (3)		46 (1)	47±14 (3)
25			228±55 (3)			46±13 (3)
27			176±68 (3)			42±12 (3)
29			288 (1)			54 (1)
31			288 (1)			48 (1)

[a] Units: $[F]_P$ (plasma fluoride concentration), mM: MABP (mean arterial blood pressure) mm Hg; heart and respiration rate, min^{-1}.

[b] 1, 2, and 3 refer to the saline control, bicarbonate and bicarbonate plus acetazolamide groups, respectively.

[c] Data expressed as mean±SE (*n*).

[d] $p < 0.02$ compared to group 1.

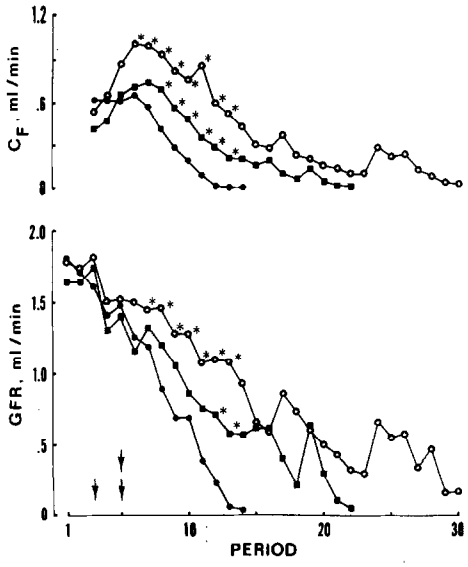

FIG. 1. Time courses of mean glomerular filtration rates (GFR) and fluoride renal clearances (C_F) in the saline-infused control group (circles), bicarbonate group (squares), and bicarbonate plus acetazolamide group (stars). The lethal fluoride infusion began in period 3 (arrow) and the saline, bicarbonate, and bicarbonate plus acetazolamide infusions began in period 5 (two arrows). Asterisks indicate $p < 0.05$ compared to the control group.

either heart rate or respiration rate associated with falling blood pressure. Heart rate tended to decline with blood pressure in groups 1 and 2 while, in group 3 (bicarbonate plus acetazolamide), it did not show a significant decrease until period 17. Respiration rates in group 2 remained stable through period 17 but, in general, they were depressed after period 5 in both groups 1 and 3.

Figure 1 shows the time courses for glomerular filtration rate (GFR) and fluoride renal clearance in each of the groups. During the first two clearance periods GFR values were similar in all groups and averaged 1.72 ± 0.05 ml/min ($n = 44$). In period 4, the second 0.5 hr after the fluoride infusion was begun, GFR was somewhat depressed in all groups (1.40 ± 0.09, $n = 22$) and the corresponding mean plasma fluoride concentration was 0.404 ± 0.014 mM ($n = 22$). After period 5, GFR generally trended downward in all groups. The occasional large increases in mean GFR shown in Fig. 1 were generally due to the death of an animal with a relatively low GFR in the previous period. As noted for blood pressure, GFR declined most rapidly in group 1 and least rapidly in group 3.

Fluoride renal clearance was not determined in periods 1 and 2. There were no statistically significant differences between the groups in periods 3 and 4 (Fig. 1) and, overall, the average value was 0.55 ± 0.03 ml/min ($n = 44$). After starting the saline, bicarbonate, and bicarbonate plus acetazolamide infusions in period 5, fluoride clearance rates increased in groups 2 and 3 while little change was noted through period 6 in group 1. The clearance rates trended downward in all groups beginning in period 7 or 8. Like GFR, fluoride renal clearance fell most rapidly in group 1 and least rapidly in group 3. After period 4, the fractional fluoride clearances (C_F/GFR) of groups 2 and 3 were consistently higher than those of group 1. These differences were statistically significant ($p < 0.05$) in 12 of the 18 periods in which comparisons were possible.

Other early indicators of acute fluoride toxicity, in addition to depressed GFR, were the falling urinary osmolalities and rising urine flow rates (Whitford and Taves, 1973). There were no statistically significant differences in urinary osmolality between the three groups in periods 1 or 2 and there was no difference between the mean values of periods 1 and 2. All values considered, urinary osmolality averaged 1824 ± 55 mOSM ($n = 44$) in the first two periods. In periods 3 and 4, the values were 1408 ± 84 ($n = 22$) and 832 ± 44 ($n = 22$), respectively. The corresponding urine flow rates in the first two periods averaged 7.0 ± 0.2 μl/min and increased to 10.5 ± 1.2 and 13.8 ± 1.0 μl/min in periods 3 and 4, respectively.

Table 4 presents the total plasma calcium concentrations which were determined only on the first, seventh, and terminal samples. There were no statistically significant differences between the groups in any given period although there appeared to be a rough

TABLE 4

TIME COURSES OF TOTAL PLASMA CALCIUM CONCENTRATIONS IN RATS TREATED WITH FLUORIDE

	Group		
Plasma	1	2	3
1	9.1±0.3 (8)[a]	8.6±0.3 (8)	9.7±0.3 (6)
7	6.5±0.5 (8)	6.9±0.3 (8)	7.2±0.3 (6)
Terminal[b]	3.6±0.4 (8)	2.8±0.2 (8)	4.2±0.5 (6)

[a] Mean±SE (*n*), (mg%)
[b] Obtained within 5 min of death.

relationship between plasma calcium and fluoride concentrations (see Tables 1 and 3).

Terminal tissue-to-plasma (*T/P*) fluoride concentration ratios for heart and brain are shown in Table 5. The *T/P* ratios for heart ranged from 0.399 to 0.485 and the values for groups 2 and 3 were significantly lower than those of group 1. In brain, the ratios ranged from 0.077 to 0.084 and no statistically significant differences were observed.

DISCUSSION

The toxic effects of inorganic fluoride are undoubtedly related to plasma and tissue fluoride concentrations (Hall, *et al.*, 1972). Fluoride is removed from these compartments almost exclusively by calcified tissue uptake and excretion by the kidneys. Insofar as it is possible to increase the rates of these processes, it should be possible to reduce the toxic effects of fluoride. Indeed, Maynard *et al.* (1951) and Mörnstad (1975) found younger animals to be more resistant to acute fluoride toxicity than older animals and this was attributed to the more extensive uptake of fluoride by the developing calcified tissues of the younger animals.

More recently, Reynolds *et al.* (1978) reported that the preexisting acid–base status of rats is an important variable in determining resistance to fluoride toxicity. They found alkalotic rats to be more resistant than acidotic rats and this effect was attributed to increased fluoride renal clearances associated with the higher urine pH values (Whitford *et al.*, 1976; Harmon *et al.*, 1976) and to lower intracellular fluoride concentrations at any given plasma fluoride level. The current study addressed a more clinically relevant point, that is whether imposing an alkalosis *after* the toxic episode had begun could provide a protective influence. The results indicate that such is the case.

The alkalotic groups survived the lethal fluoride infusion 28% (Group 2, bicarbonate) and 73% (Group 3, bicarbonate plus acetazolamide) longer than the saline-infused control group. The protection afforded by alkalosis was related to more slowly rising plasma fluoride concentrations (Table 3) and this was, at least in part, due to the higher renal clearance rates of fluoride (Fig. 1). Moreover, mean arterial blood pressure, heart and respiration rates (Table 3), glomerular filtration rates (Fig. 1), and plasma calcium concentrations (Table 4) declined

TABLE 5

TISSUE-TO-PLASMA FLUORIDE CONCENTRATION RATIOS FOR HEART AND BRAIN IN RATS TREATED WITH FLUORIDE[a]

	Group		
Tissue	1	2	3
Heart	0.485±0.022 (8)[b]	0.399±0.012 (7)[c]	0.406±0.015 (6)[c]
Brain	0.077±0.009 (8)	0.082±0.016 (7)	0.084±0.010 (6)

[a] Tissue fluoride concentrations expressed in terms of tissue water.
[b] Mean±SE (*n*).
[c] $p<0.02$ compared to group 1.

less rapidly in the alkalotic groups. The *T/P* fluoride concentration ratios for the heart were significantly lower in the alkalotic groups compared to the control group (Table 5). All of these findings are in agreement with those reported by Reynolds *et al.* (1978) and they undoubtedly contributed to the protective influence afforded by alkalosis. Also in agreement with Reynolds *et al.* (1978), who found no group differences for cerebrospinal fluid-to-plasma fluoride concentration ratios, we found no differences in brain *T/P* fluoride ratios between the groups (Table 5). This suggests that differential permeabilities of the blood–brain barrier between the groups were not involved in the resistance to fluoride toxicity provided by alkalosis. It also suggests that the integrity of the blood–brain barrier remains intact in the presence of lethal fluoride concentrations.

The addition of acetazolamide to the bicarbonate infusate was much more effective than bicarbonate alone in reducing susceptibility to fluoride toxicity. The explanation for this appeared to be mainly related to the enhanced renal clearance of fluoride (Fig. 1) associated with the higher urine pH values (Table 2). As a result, plasma fluoride concentrations in group 3 increased less rapidly and glomerular filtration rates, blood pressure, and the other vital signs decreased more slowly. The terminal *T/P* ratios of groups 2 and 3, however, were not different for either brain or heart (Table 5) which suggests that the transcellular pH gradients were similar in each group (Reynolds *et al.*, 1978).

The bicarbonate–acetazolamide group died with a mean terminal plasma fluoride concentration which was essentially the same as that of the saline control group and which was lower than that of the bicarbonate group (Table 1). Further, using Tables 1 and 5, it can be calculated that group 3 had the lowest mean heart fluoride concentration (0.76 vs 0.88 mM in groups 1 and 2). This does not necessarily mean that acetazolamide sensitized the animals to fluoride toxicity. Rather, it might indicate that there is a time component, in addition to the concentration component, in fluoride toxicity. That is, group 3 survived high fluoride concentrations longer than either of the other two groups.

Whether acetazolamide alone can protect against fluoride toxicity is unclear. By alkalinizing the urine, it should enhance the excretion of fluoride. However, this would occur at the expense of extracellular bicarbonate which would tend to produce a systemic metabolic acidosis. As discussed in detail by Reynolds *et al.* (1978), intracellular to-extracellular pH gradients would then be expected to decline thus allowing higher intracellular fluoride concentrations at any given plasma concentration. The desirable increase in the urinary excretion of fluoride might, therefore, be offset by a redistribution of fluoride in vital tissues. Further research is indicated to clarify these relationships.

The recommended treatment of acute fluoride toxicity includes gastric lavage with calcium chloride solutions to remove unabsorbed fluoride, intravenous infusion of glucose, calcium gluconate, and plasma or whole blood, oxygen as required, and keeping the patient warm (Hodge and Smith, 1965). Treatment should be performed as soon after exposure as possible. The current findings, and those of Reynolds *et al.* (1978) strongly suggest that the administration of bicarbonate or bicarbonate with acetazolamide should be incorporated into the currently accepted therapeutic regimen.

ACKNOWLEDGMENTS

This work was supported by Grants DE-04332 and DE-04832 from the National Institute of Dental Research.

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