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Fluoride-induced Diuresis:

Renal-tissue Solute Concentrations, Functional, Hemodynamic, and Histologic Correlates in the Rat

Gary M. Whitford, Ph.D., Donald R. Taves, M.D., Ph.D.†*

Groups of anesthetized rats received graded doses of fluoride in isotonic solutions by continuous, iv infusions. Resultant plasma fluoride concentrations included those known to occur in patients following methoxyflurane anesthesia. Graded increases in urine-flow rate were not accompanied by increases in sodium or potassium excretion or osmolar clearance. In some experiments, sodium or potassium excretions were reduced, as were urinary osmolality and tubular free-water reabsorption. Glomerular filtration rates tended to be reduced in the fluoride-infusion groups, while ^{131}I-hippuran clearance was not altered. The inner medullary sodium concentration was inversely related to plasma fluoride concentration. Increased renal-tissue water content accounted for a portion of slightly reduced medullary potassium concentrations. Histologically, glomeruli from fluoride-infused rats contained less than half the erythrocytes of control rat glomeruli. Outer medullary vessels appeared hyperemic. These results suggest that the fluoride-induced concentrating defect results from increased medullary blood flow, increased permeability to water, and/or decreased sodium reabsorption, in the ascending limbs of Henle's loops. These findings are consistent with the hypothesis that fluoride is responsible for the concentrating defect following methoxyflurane anesthesia. (Key words: Fluoride; Concentrating defect; Methoxyflurane nephrotoxicity.)

* Assistant Professor of Oral Biology–Physiology. Present address: Medical College of Georgia, Dept. of Oral Biology, Augusta, Ga. 30902.

† Associate Professor of Pharmacology and Toxicology.

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THE LITERATURE contains references to the effects of inorganic fluoride on both kidney structure [1-4] and kidney function,[2, 4-8] but these effects have not been studied in detail. This lack is probably due to the fact that renal effects are not a prominent feature of fluoride toxicity when fluoride is ingested orally, the usual route of exposure. More information about parenterally administered fluoride is needed in order to evaluate the hypothesis [9] that fluoride released from the anesthetic methoxyflurane, $CH_3OCF_2CHCl_2$, is responsible for the high-output renal failure occasionally seen after anesthesia.

Taves *et al.*[9] advanced the hypothesis after finding a much higher plasma fluoride concentration (275 μM) in a patient with high-output renal failure than in two other patients who were free of nephrotoxic symptoms (<30 μM).‡ Subsequent studies by Fry *et al.*[10] indicated the usual plasma fluoride concentration to be 20–60 μM in both man and rat the day after methoxyflurane anesthesia, while Mazze *et al.*[11] reported that maximum values averaging 105 and 190 μM were associated with laboratory and clinical evidence of changes in renal function. There were, however, no data to demonstrate that the plasma fluoride concentrations seen clinically could produce comparable renal effects in animals when other methoxyflurane metabolites [10, 12] were not present. Thus, the hypothesis rested on a tenuous base.

Three recent reports have partially filled this hiatus. Whitford and Taves presented preliminary data [13] from anesthetized rats infused with fluoride indicating that urine-flow rate tripled when plasma fluoride concentration was raised from approximately 1 μM to 300 μM, which is appropriate to the hypothesis. They also suggested that the basis of the diuresis was

‡ Plasma fluoride concentrations of healthy adults drinking fluoridated water (1 ppm or 52 μM) are approximately 1 μM.

TABLE 1. Time Courses of Urine-flow Rates (μl/min) for Various Fluoride-infusion Rates*

	Urine-flow Rates (μl/min)				
	F, 0	F, 50 nmol/min	F, 100 nmol/min	F, 500 nmol/min	F, 1,000 nmol/min
0–30 min					
Mean	2.15	2.16	2.10	3.45	4.31
SEM	0.12	0.20	0.13	0.25	0.73
Number	37	10	24	41	5
30–60 min					
Mean	2.44	2.80	2.74	4.68	5.04
SEM	0.15	0.26	0.18	0.30	1.42
Number	37	10	24	39	5
60–90 min					
Mean	2.59	3.68	3.73	5.76	7.88
SEM	0.11	0.31	0.32	0.34	2.17
Number	37	10	24	39	5
90–120 min					
Mean	2.97	4.17	3.94	6.71	6.93
SEM	0.16	0.39	0.31	0.42	1.07
Number	37	10	24	38	5
120–150 min					
Mean	3.04	4.29	4.54	7.27	5.84
SEM	0.14	0.43	0.33	0.42	0.66
Number	37	10	24	39	5
150–180 min					
Mean	3.10	4.66	4.88	7.98	5.91
SEM	0.13	0.39	0.41	0.58	1.30
Number	37	10	24	39	5
180–210 min					
Mean	3.19	4.77	5.23	8.75	5.31
SEM	0.16	0.33	0.43	0.76	1.83
Number	35	10	24	35	5
210–240 min					
Mean	2.98	4.93	6.62	10.58	—
SEM	0.41	0.43	0.84	1.63	
Number	7	9	9	8	

* Infusion of solutions started at zero time.

a reduction in renal inner medullary sodium concentration. Frascino [11] reported that a plasma fluoride concentration of approximately 400 μM in hydropenic dogs resulted in an increase in urine-flow rate, and decreases in urinary osmolality and renal medullary sodium concentration, with little or no change in glomerular filtration rate or sodium excretion rate. Mazze *et al.*[15] noted diuresis (unresponsive to vasopressin), urinary dilution, hemoconcentration, and weight loss in one strain of rats given methoxyflurane or fluoride, with plasma fluoride concentrations ranging to as much as 700 μM.

Additional information is needed concerning the effects of low plasma fluoride concentrations on renal function and the mechanism of action. The present experiments concentrate on plasma fluoride levels of clinical interest and the associated changes in renal-tissue composition, function, hemodynamics, and histology.

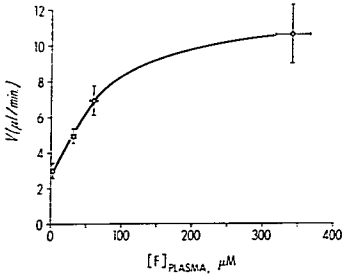

FIG. 1. Urine-flow rates and plasma fluoride concentrations (means ± SEM) during the eighth half-hour of fluoride infusion in experiment 2. *Left to right:* F, 0; F, 50 nmol/min; F, 100 nmol/min; F, 500 nmol/min.

Methods

Female, 200-g, Sprague-Dawley rats were anesthetized with sodium pentobarbital (40 mg/kg, ip). The basic experiment was to assign four rats randomly to different fluoride-infusion-rate groups. All infusates were isotonic, containing 145 mM sodium and various concentrations of chloride and fluoride. Additional, trace solutes were hydroxymethyl ^{14}C-inulin and ^{131}I-hippuran, depending upon the experiment. Solutions were infused continuously at a slow rate (20 µl/min) into the left iliac vein.

The urinary bladder was catheterized intraurethrally with polyethylene tubing. Urine was collected, usually for half-hour periods, by attaching a capillary tube of constant inside diameter and known volume to the catheter. Urine volumes were determined by measuring the urine column. This technique is more rapid than weighing, and the two methods were found to agree to within 0.5 per cent. Blood was collected in heparinized capillary tubes from the severed tail tip at the mid-points of urine-collection periods, or terminally from the tail tip or heart, depending upon the experiment.

In experiments which included analysis of renal tissue, both kidneys were removed simultaneously following the final urine collection. Each kidney was divided along its longitudinal axis. The polar thirds were cut away and discarded. A second longitudinal cut was made so that a wedge from the center of the kidney remained. This wedge was divided into cortex, outer medulla, inner medulla, and papilla in one experiment, while in two others the inner medulla included the papilla. Corresponding sections from each kidney were pooled for weighing, homogenization, and analysis. Homogenization was done in 0.70 to 1.00 ml of deionized, doubly-distilled water. The facing kidney half was similarly sectioned except for the papilla, then weighed, dried to constant weight in a 70-C oven and reweighed to determine water content. All renal-tissue analyses are expressed in terms of water content. In one experiment kidney sections were stained with hematoxylin and eosin and prepared for microscopic examination.

Sodium and potassium were determined by flame photometry, osmolality by freezing-point depression, and fluoride by electrode, as previously reported,[12] or with a modification of the Morin-thorium method of Taves [16] following fluoride isolation by hexamethyldisiloxane diffusion. Urinary *p*H was determined with narrow-range *p*H paper and systemic hematocrit by microanalytic technique: these values were not influenced by fluoride infusion. Hydroxymethyl ^{14}C-inulin [17] was determined by liquid scintillation. To obtain similar quenching, 100 µl of cold rat plasma from a common pool were added to each urine, standard, and background sample. ^{131}I-hippuran was counted in a well-type scintillation counter.

EXPERIMENT 1

Urine-flow rates and plasma fluoride concentrations from this experiment have been reported.[12] Fluoride-infusion-rate groups received 0 (isotonic saline solution), 100, 500, and 1,000 nanomoles/min.§ The saline-solution group had four rats and each of the others had five rats. Urine collections continued for 3½ hours, and then the kidneys were removed. The renal papilla was analyzed separately from the inner medulla.

EXPERIMENT 2

Fluoride-infusion groups received F(0), F(50), F(100), and F (500). These groups

§ Infusion-rate groups are designated by F followed by the nanomoles/min in parenthesis, *e.g.*, F(100) means 100 nanomoles/min of fluoride were infused.

TABLE 2. Sodium, Potassium, and Fluoride Concentrations in Kidney Sections (Means ± SEM)*

	Na (mM)					K (mM)					F (μM)			
	F, 0	F, 50 nmol/min	F, 100 nmol/min	F, 500 nmol/min	F, 1,000 nmol/min	F, 0	F, 50 nmol/min	F, 100 nmol/min	F, 500 nmol/min	F, 1,000 nmol/min	F, 50 nmol/min	F, 100 nmol/min	F, 500 nmol/min	F, 1,000 nmol/min
Experiment 1														
Cortex	66.2	—	74.0[b]	68.2	71.5	108.2	—	95.2[a]	89.0[c]	83.8[b]	—	189	968	1,612
	2.0		2.4	3.1	3.5	2.5		2.9	1.0	4.1		16	41	320
Outer medulla	88.5	—	102.5[a]	84.7	78.8	96.3	—	85.2[b]	78.2[c]	78.3[b]	—	300	1,284	2,056
	3.1		4.6	5.9	4.3	1.9		2.0	2.0	3.9		56	79	305
Inner medulla	204.3	—	141.8[b]	114.5[b]	102.1[c]	74.9	—	60.0[c]	64.8[c]	66.5[a]	—	360	1,812	2,783
	15.2		7.8	9.0	2.9	1.2		2.0	1.0	2.5		41	125	472
Papilla	369.1	—	191.4[c]	133.3[c]	110.2[c]	94.4	—	72.6[c]	70.6[c]	57.9[c]	—	726	2,405	5,836
	17.2		21.1	9.0	6.3	1.5		1.1	2.1	2.5		130	42	964
Experiment 2														
Cortex	79.6	81.4	79.8	75.4	—	100.2	102.5	96.5	85.3[b]	—	92	192	850	—
	1.2	3.0	2.5	2.2		3.0	3.0	3.6	4.3		9	21	165	
Inner medulla,	216.2	149.2[c]	117.2[c]	87.4[c]	—	61.7	60.0	58.8	56.4[a]	—	231	393	1,373	—
including papilla	7.8	11.4	5.7	2.1		1.3	2.2	2.0	1.8		35	65	309	

* Infusion of solutions started at zero time.
[a,b,c]: $P < .05$, $P < .01$, $P < .001$, respectively, compared with F, 0.

TABLE 3. Per Cent Water Content (Means ± SEM) of Renal Tissue Sections*

	Per Cent Water				
	F, 0	F, 50 nmol/min	F, 100 nmol/min	F, 500 nmol/min	F, 1,000 nmol/min
Experiment 1					
Cortex	74.6 0.5	—	76.9[b] 1.0	78.5[c] 0.8	79.0[c] 0.7
Outer medulla	78.4 0.5	—	81.3[c] 0.9	83.1[c] 1.2	83.9[c] 1.0
Inner medulla	83.8 2.5	—	86.6 2.5	87.9[b] 0.9	88.3[b] 0.9
Papilla	84.8 2.5	—	87.6 2.5	88.9[b] 0.9	89.3[b] 0.9
Experiment 2					
Cortex	76.1 0.5	75.3 0.3	76.7 0.4	79.2[b] 0.6	—
Inner medulla, including papilla	84.6 0.5	85.3 0.4	86.8[b] 0.3	88.3[c] 0.3	—

* Infusion of solutions started at zero time.
[b,c]: $P < 0.05$, $P < .01$, $P < 0.001$, respectively, compared with F, 0.

had eight, ten, ten, and ten rats, respectively. Urine collections were begun one hour before starting infusions and then continued for eight half-hour periods. The kidneys were removed during the ninth half hour of infusion. A single, terminal blood sample was taken. The renal papilla was included with the inner medulla.

EXPERIMENT 3

Fluoride infusion rates were F(0) (n = 6) and F(100) (n = 9). An intravenous priming dose of hydroxymethyl ^{14}C-inulin (0.25 μCi in 0.20 ml isotonic saline solution) was given shortly after starting the sustaining infusion. Blood was collected during the third, fifth, sixth, and seventh half-hour urine collection periods.

EXPERIMENT 4

Fluoride-infusion-rate groups were F(0) (n = 9) and F(500) (n = 11). Otherwise this experiment was identical to experiment 3.

EXPERIMENT 5

Fluoride-infusion-rate groups were F(0) (n = 8) and F(450) (n = 11). An intravenous priming dose of ^{131}I-hippuran, in 0.10 ml isotonic saline solution, was given immediately after starting the sustaining infusion. Resultant plasma ^{131}I counts were at least five times background in 100-μl samples. Blood was collected as in experiment 3.

EXPERIMENT 6

This experiment involved three rats receiving F(100) and was designed to observe the time course of the reduction of inner medullary sodium concentration. Urine was collected in 15-minute intervals and single blood samples were taken from the tail tips terminally. Kidneys were removed 40, 70, and 100 minutes after the start of infusion. The inner medulla included the papilla.

EXPERIMENT 7

Five rats were used, two receiving F(0) and three receiving F(500) for six half-hour urine collections. Kidneys were then removed and prepared for sectioning and staining with hematoxylin and eosin. Glomerular erythrocyte numbers were counted. A glomerulus was included for cell counting only if it had been sectioned through its center, as judged by its area. Ten outer cortical glomeruli and ten inner cortical glomeruli from each rat were counted.

Student's t test for differences between control and fluoride-infused means was used for statistical analysis.

Results

Urine-flow Rate and Plasma Fluoride Concentration

Table 1 summarizes all urine-flow-rate data from experiments 1, 2, 3, 4, 5, and 7. Elevation of the mean urine-flow rates of the fluoride-infused groups attained statistical significance ($P < 0.001$) after the second half hour of infusion. This effect was found in all experiments except experiment 3, where urine flow rates for the F(100) group were lower than the control values for the first two half-hour collections. Subsequently, they became higher than control urine-flow rates, but not with statistical significance. The terminal, mean plasma fluoride concentration of the F(100) group was 78 μM. The F(1,000) group of experiment 1 showed a decline in urine-flow rates after the third collection, when the mean plasma fluoride concentration was about 500 μM.

Figure 1 plots the eighth half-hour urine-flow rate and corresponding plasma fluoride concentration for each infusion-rate group of experiment 2. The fluoride concentration of the F(0) group was not precisely determined but was less than 2 μM. The F(50) group had a mean fluoride concentration of 32 μM and a 64 per cent increase ($P < 0.01$) in urine-flow rate over the F(0) group. The F(100) and F(500) groups had plasma fluoride concentrations of 61 and 341 μM, respectively. Their increases in urine-flow rate over the F(0) group were 121 and 253 per cent, respectively ($P < 0.001$).

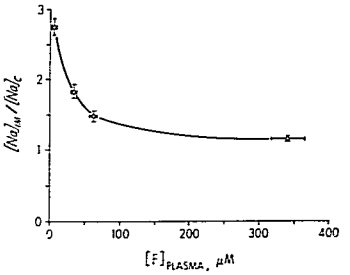

Fig. 2. Inner medullary/cortical sodium concentrations and plasma fluoride concentrations (mean ± SEM) during the ninth half-hour of fluoride infusion in experiment 2. *Left to right:* F, 0; F, 50 nmol/min; F, 100 nmol/min; F, 500 nmol/min.

Renal-tissue Sodium, Potassium, and Fluoride Concentrations

Table 2 shows the sodium, potassium, and fluoride concentrations in various renal sections for all rats of experiments and 1 and 2. The sodium concentrations of the inner medulla and papilla were inversely related to the fluoride concentrations. The potassium concentrations for all renal sections of the fluoride groups were generally reduced. A portion of this effect can be explained by increased water content (table 3). The renal fluoride concentrations, not determined for the F(0) group, ranged from 92 μM in the cortex of the F(50) group to 5.8 mM in the papilla of the F(1,000) group. A three- to fourfold concentration gradient (papilla/cortex) was present for fluoride.

Figure 2 shows the sodium concentration gradient (inner medulla/cortex) as a function of plasma fluoride concentration in experiment

Table 4. Experiment 6,* Time Courses of Reductions of Inner Medullary Sodium Concentration, Terminal Urine-flow Rate ($\dot{V}$), and Plasma Fluoride Concentration ($[F]_P$)

	Minutes of Infusion	$\dot{V}$ (μl/min)	Na (mM)		$[F]_P$ (μM)
			Cortex	Inner medulla	
Control data from experiment 2	240	3.0	79.6	216.2	—
Rat 1	40	3.6	101.8	241.9	18
Rat 2	70	9.3	87.8	157.2	34
Rat 3	100	13.5	91.1	153.5	—

* Rats 1, 2, and 3 received 100 nanomoles fluoride/min.

TABLE 5. Experiment 2, Sodium and Potassium Excretion Rates (Means ± SEM)*

	F, 0		F, 50 nmol/min		F, 100 nmol/min		F, 500 nmol/min	
	Na Excretion (nM/min)	K Excretion (nM/min)	Na Excretion (nM/min)	K Excretion (nM/min)	Na Excretion (nM/min)	K Excretion (nM/min)	Na Excretion (nM/min)	K Excretion (nM/min)
−60–0 min	187 16	484 40	268† 26	546 35	257 32	504 29	311† 44	565 43
60–90 min	285 41	816 76	434 83	954 95	239 29	726 65	270 28	798 103
120–150 min	390 63	972 70	520 136	928 74	303 32	912 70	430 43	801 84
210–240 min	472 67	832 113	668 125	894 73	574 90	949 92	625 144	743 124

* Infusion of solution started at zero time.
† $P < 0.05$.

2. The gradient reduction was statistically significant ($P < 0.001$) even for the F(50) group, whose terminal plasma fluoride concentration was 32 μM.

Table 4 contains data from experiment 6. Control data were from experiment 2 since the experiments were similar and the kidneys were sectioned identically. There was no reduction of inner medullary sodium concentration for the rat sacrificed at 40 minutes. The corresponding plasma fluoride concentration was 18 μM and the terminal urine-flow rate was not elevated. The fluoride effect was clearly present, however, in the rats sacrificed at 70 and 100 minutes. The plasma fluoride concentration of the 70-minute rat was 34 μM. Based on previous experimental results, the plasma fluoride concentration of the 100-minute rat would have been 40–50 μM. The terminal urine-flow rates of these two rats were elevated.

SOLUTE EXCRETION RATE

Preliminary experiments, not detailed here, indicated that sodium and potassium excretion rates of rats receiving F(200) and F(1,000) were approximately half those of rats receiving F(0). Table 5 shows the sodium and potassium excretion rates of experiment 2. These were essentially identical for all groups except during the pre-infusion period, when the excretion rates for the fluoride-infusion groups were, by chance, higher than that for the F(0) group.

Table 6 shows the sodium and potassium excretion rates, expressed in terms of 1 ml/min glomerular filtration rate, from experiments 3 and 4. The observed sodium excretion rates of the fluoride-infusion groups were lower by approximately half relative to the control groups in both experiments. Potassium excretion rates were also lower, but to a lesser degree. Correction of these excretion rates to units of glomerular filtration rate eliminated the differences in experiment 4 but not in experiment 3.

The influence of fluoride infusion on urinary osmolality is shown in table 7. During the pre-infusion period, urinary osmolalities were similar for all groups (approximately 2,000 mOsm). During the third period, the F(0) group's urinary osmolality increased slightly with little change thereafter. However, in each of the fluoride-infusion groups, urinary osmolality showed a consistent, nearly linear decline with time. The degree of reduction in a given period is directly related to the fluoride-infusion rate. The lowest mean urinary osmolality, 580 mOsm, occurred in the eighth period for the F(500) group. Mean plasma osmolality was 299 mOsm for each group.

The osmolal clearances of the F(0) and F(50) groups approximately doubled after starting infusion. Tubular free-water reabsorption for these two groups increased by a factor of 2 (slightly less in the F(50) group). Osmolal clearance of the F(100) group in-

TABLE 6. Experiments 3 and 4, Observed and GFR-corrected* Sodium and Potassium Excretion Rates (Means ± SEM)

	F, 0				F, 100 nmol/min				F, 600 nmol/min			
	Na (mM/min)	Na' (mM/min)	K (mM/min)	K' (mM/min)	Na (mM/min)	Na' (mM/min)	K (mM/min)	K' (mM/min)	Na (mM/min)	Na' (mM/min)	K (mM/min)	K' (mM/min)
Experiment 3												
60–90 min	313	242	637	483	155	119	654	496	—	—	—	—
	98	72	105	74	22	19	58	45				
120–150 min	504	368	830	609	227	184	757	610	—	—	—	—
	211	125	102	34	27	22	42	28				
180–210 min	635	429	929	699	290	251	719	606	—	—	—	—
	196	122	140	60	22	23	66	48				
All of the above	484	346	808	573	210[a]	176[a]	707	566	—	—	—	—
	100	62	71	33	18	16	31	25				
Experiment 4												
60–90 min	425	240	919	542	—	—	—	—	185[b]	183	656	569
	84	30	140	73					18	20	72	29
120–150 min	398	221	1,008	557	—	—	—	—	201[c]	254	580[c]	661
	47	23	83	34					16	38	64	45
180–210 min	491	297	905	575	—	—	—	—	227[a]	305	631[a]	720[b]
	96	48	121	30					18	48	74	18
All of the above	438	253	900	558	—	—	—	—	204[c]	245	624[c]	649[a]
	43	22	66	28					10	23	40	22

* Na or K, observed excretion rates; Na' or K', corrected to units of GFR. Infusion of solutions started at zero time.
[a–c]: $P < .05$, $P < .01$, $P < .001$, respectively, compared with F, 0.

TABLE 7. Experiment 2. Urinary Osmolalities (Means ± SEM)*

	Osmolality (mOsm/kg)			
	F, 0	F, 50 nmol/min	F, 100 nmol/min	F, 500 nmol/min
−60–0 min	1,913	2,077	1,934	2,043
	76	88	90	59
60–90 min	2,087	1,813	1,505	930
	111	103	85	71
120–150 min	2,076	1,684	1,243	707
	78	125	53	60
210–240 min	2,072	1,517	971	580
	120	72	63	46

* Infusion of solutions started at zero time.

creased by about 75 per cent after the start of infusion, while the mean increase in tubular free-water reabsorption was by a factor of 1.5. The osmolal clearance of the F(500) group showed only a slight increase after the start of infusion, and tubular free-water reabsorption decreased progressively from a pre-infusion value of 12.3 to a final value of 5.0 μl/min.

GLOMERULAR FILTRATION RATE AND ^{131}I-HIPPURAN CLEARANCE

Table 8 contains glomerular-filtration-rate data from experiments 3 and 4, and ^{131}I-hippuran clearance data from experiment 5. The glomerular filtration rates of the fluoride-infusion groups, but not those of the control groups, decreased significantly during the experiments. By the end of the experiments, glomerular filtration rates were 80 and 50 per cent of control values in the F(100) and F(500) groups, respectively. Data from experiment 5 indicated that F(450) infusion for 3½ hours had essentially no effect on ^{131}I-hippuran clearance. Although there was a slight reduction over time, the differences between group means did not achieve statistical significance.

MICROSCOPIC APPEARANCE OF THE KIDNEY

Experiment 7 included microscopic examination of kidney sections from rats which had received F(0) and F(500) for three hours. There was no evidence of cellular damage in any section. However, the glomeruli of the sections from the F(500) group contained fewer than half the quantity of erythrocytes found in the glomeruli of the F(0) group. This was true for both superficial cortical glomeruli (49.5 ±3.1 *vs.* 22.7 ± 2.5 SE) and juxtamedullary glomeruli (31.3 ± 2.2 *vs.* 12.0 ± 1.2). The vessels of the outer medulla of the F(500) group, on the other hand, appeared hyperemic relative to those of the F(0) group. In the F(500) group there was also slight, but consistent, dilatation of the distal tubules in the cortex and intermediate juxtamedullary zone.

Discussion

The renal response of the normally hydrated rat to continuously elevated fluoride concentrations is consistent with the hypothesis that fluoride is responsible for the nephrotoxicity observed in patients after methoxyflurane anesthesia. A three- to fourfold increase in urine-flow rate with plasma fluoride concentrations of 200–300 μM has been observed in man [9, 10] and the rat. A statistically significant increase ($P < 0.01$) in urine-flow rate of 64 per cent occurred in rats when the plasma fluoride concentration was only 32 μM. The absence of increased solute excretion to accompany increased urine-flow rate, and the consequent reduction in urinary osmolality, are also similar for man [18, 19] and the rat. The results suggest a concentrating defect and are in agreement with the findings of Frascino in dogs [14] and Mazze *et al.*[15] in rats.

The most striking and consistent effect of fluoride, and the likely cause of the concentrating defect, was the reduction of the inner medullary sodium concentration. The data from experiments 2 and 6 indicate that a significant reduction occurs when plasma fluoride concentration is as low as 32 μM.

The mechanism whereby fluoride reduces the inner medullary sodium concentration cannot be precisely determined, but certain possibilities can be excluded. There are five possible mechanisms: 1) increased tubular fluid flow rate [20, 21, 22]; 2) delivery of inadequate quantities of sodium to Henle's loops; 3) inhibition of sodium pumping from the ascending limbs of Henle's loops; 4) increased permeability to water of the ascending limbs of Henle's loops; 5) increased medullary blood flow.

It is doubtful that the first factor is important. Urine-flow rates usually did not ex-

TABLE 8. Time Courses of Glomerular Filtration Rate and ^{131}I-hippuran Clearance (ERPF) (Means ± SEM)*

	Experiment 3 GFR (ml/min)		Experiment 4 GFR (ml/min)		Experiment 5 ERPF (ml/min)	
	F, 0	F, 100 nmol/min	F, 0	F, 500 nmol/min	F, 0	F, 450 nmol/min
60–90 min	1.31 0.07	1.32 0.03	1.74 0.11	1.15[c] 0.10	2.74 0.15	2.81 0.34
120–150 min	1.34 0.12	1.24 0.08	1.82 0.10	0.90[c] 0.09	2.37 0.19	2.10 0.10
150–180 min	1.45 0.08	1.15[a] 0.07	1.66 0.08	0.92[c] 0.10	2.11 0.09	1.99 0.17
180–210 min	1.49 0.10	1.19[a] 0.04	1.63 0.11	0.85[c] 0.09	2.42 0.22	2.10 0.17
All of the above	1.40 0.05	1.22[b] 0.02	1.71 0.05	0.96[c] 0.05	2.41 0.09	2.23 0.11

* Infusion of solutions started at zero time.
[a,b,c]: $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, compared with F, 0.

ceed 12 μl/min, glomerular filtration rates tended to be reduced, and net sodium reabsorption was not inhibited. Atherton *et al.*[21, 22] observed marked changes in medullary solute concentrations during diuresis in rats, but urine-flow rates were well in excess of 100 μl/min.

Although a reduction of nearly half in glomerular filtration rate (table 8) might diminish the inner medullary sodium concentration, data from the other fluoride groups indicate that this is not the primary mechanism. During the third half hour of infusion at F(100), the reduction in glomerular filtration rate was only 7 per cent. In experiment 6, after 70 minutes of F(100) infusion, the inner medullary sodium concentration was definitely reduced. Moreover, it would be expected that F(50) would have even less effect on glomerular filtration rate, but this fluoride-infusion rate produced a statistically significant reduction in the inner medullary sodium concentration.

The failure of fluoride to promote increased sodium excretion suggests that sodium reabsorption from the ascending limbs of Henle's loops may not have been markedly reduced. However, inhibition of sodium reabsorption by the proximal tubule and the loop of Henle without increased sodium excretion, indicating increased reabsorption in more distal segments, has been demonstrated.[23, 24] Thus, decreased sodium reabsorption cannot be excluded.

The absence of increased sodium excretion is noteworthy. Yoshida *et al.*[25] demonstrated a 60 per cent *in-vitro* inhibition of Na–K–ATPase in the presence of 0.6 mM fluoride, which is one tenth the maximum papillary and less than the maximum cortical, fluoride concentration observed here. Martinez-Maldonado *et al.*[26] unilaterally infused digoxin, a potent inhibitor of Na–K–ATPase, into the renal artery of the dog. A profound, unilateral natriuresis and markedly increased osmolal clearance resulted. Na–K–ATPase activities in both the cortex and inner medulla of the digoxin-infused kidney were reduced by half. However, Walser [27] has described certain diuretics which inhibit Na–K–ATPase *in vitro* but fail to do so *in vivo*, so the apparent discrepancy between the *in-vivo* and *in-vitro* actions of fluoride is not unique.

It is possible that increased medullary blood flow is the primary event responsible for reduced medullary sodium concentration. These possible causes of increased medullary blood flow are either direct or indirect. A direct effect of fluoride on the medullary vasculature is reasonable, since single, large doses of fluoride cause peripheral dilatation.[28] In general, renal cortical fluoride concentrations are higher than those of plasma by a factor of 3, while the inner medullary factors range from 4 to 7. Smith [29] has summarized data which show the

concentration of radioactive fluoride in the kidney to be 2½ to 4 times higher than that in skeletal muscle for as long as nine hours after dosing. Thus, direct vasodilatation localized in the kidney, especially the inner medulla, is possible.

A possible indirect mechanism could involve afferent–efferent shunting throughout the cortex. The effect of fluoride on glomerular filtration rate and the paucity of glomerular erythrocytes suggest afferent arteriolar constriction. The lack of a fluoride effect on hippuran clearance, indicating normal cortical blood flow rate, and the observation of increased numbers of erythrocytes in the vessels of the outer medulla suggest afferent–efferent shunting. Medullary blood flow would be expected to increase, since the pressure drop which normally occurs across the glomerular vascular tuft would be avoided.

Increased permeability to water of the ascending limbs of Henle's loops might explain such shunting. The resultant delivery of a more nearly isotonic fluid to the macula densa may be a critical factor in the control of glomerular filtration rate, as suggested by the juxtaglomerular apparatus feedback theory of renal autoregulation,[20] possibly by afferent–efferent shunting of blood. Increased permeability to water of the ascending limbs would also directly result in a diminished ability to concentrate sodium in the medulla. If increased permeability to water of the ascending limb is the basis of the fluoride-induced decrease in medullary sodium concentration, then the fluoride-intoxicated kidney would have difficulty forming hypotonic urine. Frascino's data [14] suggest dilution is not markedly affected, but neither blood nor renal fluoride concentration was reported, so the critical experiments remain to be done. Moreover, a direct effect of fluoride on the juxtaglomerular apparatus, resulting in afferent arteriolar constriction, cannot be excluded.

The decreases in osmolal clearance for the fluoride-infusion groups suggest that the decline in urinary osmolality was proportionately greater than the increases in urine-flow rate. Fluoride tends to decrease glomerular filtration rate. This reduction, accompanied by either no change or an increase in fractional solute reabsorption (table 6), can explain decreased osmolal clearances. Concurrent increases in urine-flow rate result in decreased tubular free-water reabsorption. In addition, reduced medullary interstitial hypertonicity can account, at least in part, for both increased solute reabsorption and decreased water reabsorption, the former through a lower transtubular gradient for ion back-diffusion, and the latter through the reduction of transtubular osmotic gradients.

The increased water content of the kidney sections of the fluoride-infusion groups can be partly explained by increased distal tubule and collecting-duct volumes. An additional factor might be increased medullary blood flow, in which case the countercurrent exchanger function of the vasa recta would be compromised and more water would be carried into the medulla.

After correction of renal-tissue potassium concentrations for increased water content, a slight reduction persisted for the fluoride-infusion groups. The basis for this effect is unknown, but it is not peculiar to flouride infusion. Heller *et al.*[21] noted a similar result in diuretic rats, as did Atherton *et al.*[21,22] Thus, decreased renal-tissue potassium concentration appears to be frequently associated with increased urine-flow rate in the rat.

There is a growing body of experimental evidence supporting the hypothesis that fluoride is the cause of post-methoxyflurane renal dysfunction. Our preliminary results with rats,[13] Frascino's results with hydropenic dogs,[14] the findings of Mazze *et al.* with rats receiving methoxyflurane or fluoride,[15] and the current results all point to a fluoride-induced concentrating defect which closely resembles that seen in post-methoxyflurane patients. The present experiments have examined renal changes associated with plasma fluoride concentrations of clinical importance and lead to the conclusion that the basis of the concentrating defect is a reduction in medullary solute concentration. The suggestion of reduced collecting-duct permeability to water as the cause [14,15] does not seem likely. The reduction of medullary solute concentration is sufficient to account for the functional changes, while decreased collecting-duct permeability to water cannot explain the reduction in medullary solute concentration. Further experiments on the mechanism of the renal effects of fluoride should be directed toward changes in in-

trarenal hemodynamics, permeability to water, and sodium transport in the ascending limbs of Henle's loops, or altered function of the juxtaglomerular apparatus.

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