Renal Clearance of Fluoride.* (22641)

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The available evidence as reviewed by Hodge and Smith(1) indicates that rapid urinary excretion of fluoride is one of two major means by which the body prevents the accumulation of sufficient fluoride ion to reach toxic levels. Approximately half to two-thirds of the fluoride ingested is removed in this manner; the balance is deposited in the skeleton.

It would be unnecessary to assume that a special excretory mechanism were involved if the renal clearance of fluoride could account for the prompt appearance in the urine of a sizeable fraction of a small oral dose(1) and for the prompt disappearance of blood-borne fluoride after intraperitoneal(2) or intravenous(3) doses. Indications that this hypothesis is tenable were found in data from a fluoride balance experiment in rabbits(4); the tubular resorption of fluoride apparently was less efficient than that of sodium, chloride or water.

In this report renal clearance of fluoride by the female dog has been measured under a variety of conditions. Even at normal blood fluoride concentrations, in the microgram per 100 ml range, fluoride exhibited a rapid clearance, exceeding the simultaneously measured water or chloride clearances.

Methods. Renal clearance experiments were performed on 4 female mongrel dogs weighing 9.7 to 11.8 kg. These dogs were maintained on a stock fox chow ration (containing ca. 13 ppm F) and drank tap water containing 1.0 ppm F (artificially fluoridated). Since the experiments were designed to measure clearance and not balance, the exact fluoride intake was not determined.

During clearance periods the animals were unanesthetized and loosely restrained in the supine position. Urine was collected through Foley inflatable catheters; the collection was terminated by 2 bladder washings with distilled water and one with air. Food was withdrawn 24 hours prior to each experiment, but tap water was allowed *ad libitum* unless indicated. With normal fluoride intake and excretion rates, *i.e.*, no intravenous fluoride given, the clearance periods were 20 to 60 minutes. The periods were shortened to 10 minutes when fluoride was injected by vein.

Fifteen ml blood samples were withdrawn into heparin from a jugular vein at the midpoint of each clearance period. When fluoride was not injected, the blood contained so little fluoride that the plasma from 3 samples was pooled to provide an adequate sample for accurate analysis. Presumably this value reliably estimated the control blood fluoride concentration during the 3 hour test period.

Glomerular filtration rates were estimated by the clearance of endogenous creatinine. Hare's method(5), slightly modified, was employed. Flame photometry was used to measure sodium(6). The Fiske-Subbarow method was used for phosphate analyses(7). Fluoride in plasma and urine was determined by the methods of Smith and Gardner(8,9) and chloride by titration with mercuric nitrate (10).

Low rates of urine flow were obtained by withdrawal of water for 27 hours preceding tests. Diuresis was induced a) by administering tap or distilled water via stomach tube, and b) by administering hypertonic solutions of mannitol or salts. Intravenous injections and infusions were made into the jugular vein opposite that used for blood withdrawal.

Results. Ultrafilterability of Fluoride in Dog Plasma. As an indication of whether the fluoride in dog plasma is bound to non-

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filterable proteins, the fluoride concentration in an ultrafiltrate of plasma was measured. Samples of normal plasma and of plasma containing 1.4, 6.6 and 10.5 μg/ml added fluoride were ultrafiltered through seamless visking casing using the centrifuge type apparatus of Toribara(11). There was no difference between the fluoride concentrations in the ultrafiltrate and in the original plasma. Since fluoride moved through the visking membrane at the same rate as did water, the total plasma concentration can be used in calculating the renal clearance of fluoride.

TABLE I. Normal Fluoride Clearance in the Dog.

Urine flow	GFR	C_{Cl^-}	C_{F^-}	Plasma F, μg/100 ml*
	Water *ad libitum*			
.10	32.1	.20	.73	60
.10	33.1	.12	.77	60
.10	35.0	.20	2.7	29
.12	29.0	.16	1.5	42
.13	32.0	.10	1.2	46
.15	35.6	.42	1.4	60
.17	33.5	.22	1.4	46
.20	37.6		1.8	28
.27	40.5		2.0	28
.33	39.1		2.0	28
.40	34.3	.29	1.6	46
.65	29.6	.15	1.2	61
.67	43.5	.41	4.0	34
Avg	35.0	.33	1.7	
	Adm. distilled water			
.23	34.7	.29	2.6	29
.40	28.3		2.0	(36)
.60	28.5	.05	2.5	(36)
.83	30.0	.04	2.3	(36)
1.07	42.0	.18	2.8	34
1.55	44.5	.30	4.1	34
1.70	33.4	.25	2.5	29
Avg	34.5	.17	2.9	
	Adm. tap water			
.12	29.6	.12	2.2	42
1.47	33.5	.12	4.5	42
1.84	29.2	.12	3.5	42
2.45	39.4	.11	2.1	61
2.92	34.3	.09	1.9	61
Avg	33.2	.11	2.8	
	Water withdrawn 27 hr			
.05	30.7	.15	4.3	15
.07	31.4	.12	3.2	12
.07	33.0	.22	4.1	15
.08	36.5	.26	7.2	15
.08	35.2	.16	2.9	12
.17	36.1	.14	4.1	12
Avg	33.8	.17	4.3	
Overall avg	34.4	.18	2.7	36
Range		.04-.42	.73-7.2	12-61

* Values within parentheses taken from pooled plasma samples.

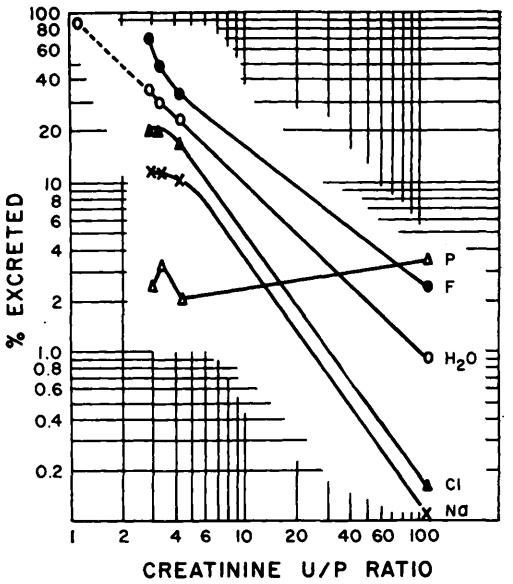

FIG. 1. Percentage of filtered ion and water excreted at various creatinine U:P ratios during hypertonic mannitol infusion.

In one instance where fluoride was added to plasma, chloride also was measured in the plasma and in the ultrafiltrate; no difference was found.

Normal fluoride clearance. A series of normal fluoride clearance measurements was made. The urine flow varied from 0.05 to nearly 3 ml min. (Table I). Collection periods each were 60 minutes. In the control group without special treatment (water *ad libitum*) fluoride clearances averaged 1.7 ml/min., with an average fluoride : chloride clearance ratio of 7 and fluoride : creatinine clearance ratio of 0.048. Thus, in this group, 4.8% of the filtered fluoride was excreted against a simultaneous chloride value of 95%. At elevated or reduced rates of urine flow established by the administration of distilled water or tap water or by water withdrawal, fluoride clearances increased to 2.9, 2.8 and 4.3 ml/min., respectively. Chloride clearances, on the other hand decreased to 0.17, 0.11, and 0.17 ml/min. Therefore in these 3 control groups, average fluoride : chloride clearance ratios (27, 25, 25) were considerably higher than in the water *ad libitum* group, as were also the average fluoride : creatinine clearance

TABLE II. Effect of Hypertonic Salt Infusion on Fluoride Clearance.

	Time, min.	Urine flow	GFR	C_{Cl^-}	C_{F^-}	C_{F^-}/C_{Cl^-}	C_{F^-}/GFR
Hypertonic NaCl	0						
	20	.01	28.5	.062	3.9	63	.14
	27	Began infusion of 5% NaCl at 8.5 ml/min. T.V. = 255 ml					
	34	Washed bladder. Start of exp. periods					
	44	1.5	34.8	3.08	6.7	2.2	.19
	54	5.0	33.7	7.8	4.7	.6	.14
	64	8.8	42.8	13.1	9.8	.75	.23
Hypertonic NaCl c fluoride	0	Began infusion of 5% NaCl containing 100 mg F/ml at 10.0 ml/min. T.V. = 350 ml					
	15	Start of exp. periods					
	25	4.5	45.3	7.0	21.8	3.1	.48
	35	6.7	49.2	—	28.0	—	.57
	45	6.1	44.6	8.2	24.5	3.0	.55
Hypertonic $NaNO_3$	0	Adm. 300 ml distilled water by stomach tube					
	30	Start of control period					
	50	.09	48.5	.04	2.5	62.5	.05
	51	Began infusion of 2.6% $NaNO_3$ at 5 ml/min. T.V. = 280 ml					
	65	Washed bladder. Start of exp. periods					
	85	1.7	46.0	2.4	2.8	1.17	.06
	105	5.4	48.3	6.7	3.2	.48	.07
Hypertonic Na_2SO_4	0	Adm. 350 ml distilled water by stomach tube					
	10	Start of control period					
	30	.15	26.1	.04	7.7	192	.30
	35	Began infusion of 2.8% Na_2SO_4 at 7 ml/min. T.V. = 425 ml					
	50	Washed bladder. Start of exp. periods					
	70	7.3	31.0	.39	7.1	18.2	.23
	90	7.5	32.2	1.49	4.9	3.3	.15

ratios (0.081, 0.087, 0.126). Plasma concentrations of fluoride averaged 36 μg/100 ml with a range of 12-61. The blood level of fluoride was lower after water was withdrawn for 27 hours. Water was provided *ad libitum* for one control period in each of the water diuresis experiments; these data are listed in the "water *ad libitum*" group and account for the greater number of these periods.

Effect of Mannitol Diuresis on Fluoride Excretion. The experimental technique was as follows: 250 ml of a 25% mannitol solution containing 125 μg F^-/100 ml was infused i.v. at a rate of 6-7 ml/min., and urine was collected for three 10 min. periods following a 12 min. equilibration. Infusion of hypertonic mannitol evoked a considerable diuresis, with maximal urine production approximating one third of the glomerular filtration rate. Under these conditions the composition of the urine approaches that of the fluid entering the distal tubules(12). A representative mannitol diuresis experiment is plotted in Fig. 1. The percentage excretion of filtered substance has been plotted on a log-log scale *vs.* the creatinine U : P ratio. In these experiments the minimal creatinine U : P ratio was 2.9 and the maximal ratio was over 100. Phosphate excretion was practically independent of creatinine U : P ratios; 2 to 4% of the phosphate was excreted in each test. As the creatinine U : P ratio approached 1.0, the percentage of sodium and of chloride which escaped tubular reabsorption was less than that for water. This approach has led to presumptive evidence for the active proximal tubular reabsorption of sodium(12) and calcium(13). The fluoride excretion curve lies roughly parallel to that for water but above it. At all urine flow rates, fluoride was excreted with a clearance much greater than that of water, sodium, chloride or phosphate.

Effect of Salt Diuresis on Fluoride Excretion. Osmotic diuresis using hypertonic NaCl, $NaNO_3$, and Na_2SO_4 was employed to observe its effect on fluoride excretion. In addition

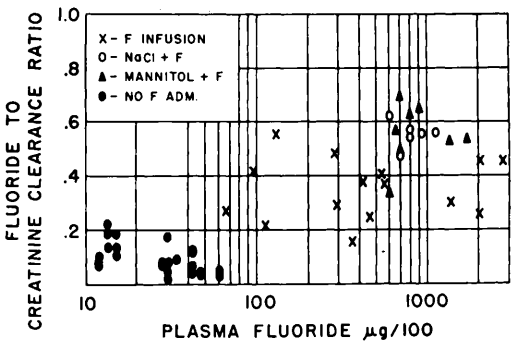

FIG. 2. Fluoride : creatinine clearance ratios at various fluoride plasma concentrations.

to increasing the urinary rate, the high plasma concentrations of such anions might be expected to interfere with or enhance fluoride clearance. The data are shown in Table II and Fig. 2. No significant increase of fluoride clearance above control levels followed the infusion of hypertonic $NaNO_3$ or Na_2SO_4 even though a large diuresis of water, sodium, chloride, and presumably infused anion occurred. Hypertonic NaCl did increase fluoride clearance appreciably, although the chloride clearance exceeded that of fluoride in the last 2 experimental periods. When a fluoride-containing hypertonic NaCl solution was infused, the fluoride clearance was actually greater than if only fluoride (NaF) had been used to raise the plasma fluoride (Table II). This tendency for a more rapid excretion of fluoride during NaCl diuresis was similar to that seen with mannitol diuresis.

Discussion. Renal clearance of fluoride at normal plasma levels in the dog, *viz.*, 12-61 µg F/100 ml (0.0065-0.033 mEq./l.) was rapid. For example, average normal excretion of filtered fluoride was 7.7% with extreme ranges of 2.3 and 13.9%. Few physiologically occurring anions are cleared by the kidney with such rapidity.

The fluoride clearance values (0.73-7.2 ml/min.) can be compared with available data on such anions as chloride, bromide, and phosphate. The normal chloride clearance is less than 1% of the glomerular filtration rate (averaging 0.5% in this study). Average clearance ratios of 0.6 for bromide : chloride (14) have been reported for the dog, a value much smaller than that for fluoride : chloride. However, an interdependence of bromide with chloride excretion was demonstrated, chloride loading increasing bromide : chloride excretion. In this respect these halogens, fluoride and bromide, are similar in that diuresis increased fluoride excretion. Nearly quantitative renal resorption of phosphate(14) occurs at normal plasma levels. The mechanism of its active resorption has a limited capacity and can be saturated, whereupon its clearance approaches that of creatinine. Osmotic diuresis should not affect the excretion of this ion. Fig. 1 shows that phosphate excretion did not vary with the creatinine U/P ratio. Fluoride, in contrast to chloride, bromide, and phosphate, demonstrated a high rate of renal clearance at the relatively low plasma concentrations attained upon fluoride administration. For example, the fluoride : creatinine clearance ratios, with only one exception, lay between 0.2 and 0.7 at plasma fluoride levels between 58-2883 µg/100 ml (Fig. 2). The highest fluoride : creatinine clearance ratios were obtained during mannitol diuresis (Fig. 2). Since fluoride clearance was always well below the creatinine clearance, there was no evidence in these experiments that tubular secretion occurred. A similar conclusion was drawn from estimations of the fluoride clearance in rabbits(4).

Summary. Fluoride in dog plasma was completely ultrafilterable through visking membranes when tested using the centrifuge type apparatus. In dogs with a normal water load, the average normal renal fluoride clearance was 2.7 ml/min.; the fluoride : chloride clearance ratio, 19; and the fluoride : creatinine clearance ratio, 0.077. Fluoride clearance was more rapid than that of simultaneously measured sodium or chloride clearances or urine flow. During mannitol diuresis, fluoride was cleared at a rate higher than that of sodium, chloride, or phosphate. Fluoride clearance, although greater than urinary flow, varied directly with the flow under mannitol osmotic diuresis. Intravenous infusion of hypertonic $NaNO_3$ or Na_2SO_4 solutions, creating a salt diuresis, did not affect fluoride excretion. Hypertonic NaCl infusion did increase

the fluoride clearance. Although fluoride : creatinine clearance ratios were raised greatly by intravenous fluoride, the highest ratios were attained during fluoride infusion plus osmotic diuresis with hypertonic mannitol or NaCl. No evidence for a tubular secretion of fluoride was obtained.

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Rapid Diagnosis of Human Influenza Infection from Nasal Smears by Means of Fluorescein-Labeled Antibody.*† (22642)

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Laboratory diagnosis of influenzal infection is usually carried out by means of retrospective serological reactions or by isolation of the virus from throat washings. Attempts have been made to shorten the time for diagnosis. Hummeler *et al.*(1) used the complement fixation test with known serum and the soluble antigen from primary isolation of the virus in chick embryos. He reported that typing of the virus was successful in 48 to 72 hours after collection of specimens. Fazekas de St. Groth(2) found that the "inhibitory index" to influenza virus of human nasal mucus was significantly increased during the acute stage of infection, allowing an early presumptive diagnosis of influenzal infections.

In the study of influenzal infection in ferrets by means of fluorescein-labeled antibody, it was possible to make a rapid diagnosis of influenzal infection during the acute stage of illness by detecting specific viral antigen in the desquamated nasal epithelial cells and macrophages in the nasal smears(3). This paper reports the application of this method to human influenzal infection. The results indicate that, although the staining of nasal smears by fluorescein-labeled antibody is less

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