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# Duration of the Fluoride-Induced Urinary Concentrating Defect in Rats (39987)

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*Introduction*. In addition to its role as the major fluoride excretory organ, the kidney appears to be one of the first organs to show changes in function following acute exposure to high doses of fluoride. This may not be due to any particular sensitivity on the part of the kidney (1) but, rather, to the locally high fluoride concentrations which are established during urine formation (2).

Many studies on the acute renal toxicity of fluoride have used the single, high-dose technique and the time course of toxicity has generally not been followed beyond a few hours (3, 4). There are, however, reports which suggest that acute fluoride exposure may produce prolonged renal dysfunction. Gottlieb and Grant (5) noted a strongly alkaline urine which persisted for 1 week after a single iv dose of fluoride. Taylor *et al.* (6) noted increased urine output and protein excretion and decreased urine specific gravity and sugar excretion for several days after iv administration of near-lethal doses of fluoride. Other studies (7, 8) have described a chronic renal concentrating defect which may, in rare cases, persist for months following methoxyflurane anesthesia. There is considerable evidence to suggest that the immediate concentrating defect seen after methoxyflurane anesthesia is due to elevated body fluid fluoride concentrations (2, 9–11) which are derived from the biotransformation of the anesthetic molecule (12). These elevated fluoride concentrations, as well as the renal concentrating ability, usually return to normal within a week. The chronic concentrating defect has not been linked to a residual fluoride effect but this possibility cannot be discounted.

The present report examines the relationships between declining body fluid fluoride concentrations and renal tissue solute concentrations, urinary osmolality, glomerular filtration rate, and the excretion rates of sodium, potassium, and chloride.

*Methods. Experiment 1*. Female, 170- to 200-g rats of the F-344 strain were anesthetized with sodium pentobarbital, 40 mg/kg, ip. Surgical preparation consisted of bilateral cut-downs over the femoral veins. One vein was used for the constant infusion of the sustaining infusion at 25 $\mu$l/min. The other vein was used for the hydroxymethyl [$^{14}$C]inulin prime injection (0.13 $\mu$Ci in 0.20 ml of isotonic saline). Sustaining infusions consisted of 145 m*M* sodium and varying concentrations of chloride and fluoride. For the *F*(0)[1] (saline control) groups, the sustaining chloride concentration was 145 m*M;* for the *F*(125) group the sustaining chloride and fluoride concentrations were 140 and 5 m*M*, respectively; for the *F*(250) groups these concentrations were 135 and 10 m*M*, respectively. All infusates contained tracer hydroxymethyl [$^{14}$C]inulin. After starting the sustaining infusion and administering the [$^{14}$C]inulin prime dose, a 0.5-hr equilibration period was allowed. Three 0.5-hr clearance periods were taken and the infusions were then stopped. No other infusions were subsequently given to any group. Urine and plasma samples were collected as previously described (2).

Three *F*(0) and three *F*(250) rats were sacrificed at the end of the third 0.5-hr urine collection period. Following closure of the incisions, all other rats were returned to their cages. Three *F*(0) and three *F*(250) rats were brought to the laboratory 3 hr later. Two more 0.5-hr urine collections were made before sacrifice. At 22.5 hr after stopping the infusion, three *F*(0) and three *F*(250) rats were similarly studied, except that three 0.5-hr urine collections were taken. At 39 hr after stopping the infusion, four *F*(0), three *F*(125), and three *F*(250)

[1] The fluoride infusion rate is denoted by *F*(*x*), where *x* is the number of nanomoles of fluoride ion infused per minute.



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rats were similarly studied. A single, terminal blood sample was collected from each rat for plasma fluoride analysis. Upon sacrifice, the kidneys were rapidly removed, sectioned into cortex and inner medulla, and prepared for analysis as previously described (2).

Sodium and potassium were determined by flamephotometry, chloride by electrometric titration, osmolality by freezing point depression, and fluoride with the ion-specific electrode following HMDS diffusion as previously described (2). Hydroxymethyl [$^{14}$C]inulin was determined by liquid scintillation counting. All data are presented as the means ± SEM. Statistical analyses for differences between the means of the fluoride-infused and control groups were done using Student's $t$ test, two-tailed, unpaired. The significance level was set at $P < 0.01$.

*Experiment 2.* The results of Experiment 1 suggested the possibility that the glomerular filtration rates of the $F(125)$ and $F(250)$ groups continued to be depressed long after plasma fluoride concentrations had returned to control levels. This experiment was designed to test that possibility. Nine rats of the same strain, sex, and body weight were prepared for study as described in Experiment 1. Three rats were assigned to each of the fluoride infusion rate groups [$F(0)$, $F(125)$, and $F(250)$]. The animals were primed with [$^{14}$C]inulin and infused as described previously. Following three 0.5-hr clearance periods, all rats received a fluoride-free isotonic saline infusion containing [$^{14}$C]inulin for 3.5 hr. During the last hour of this infusion, plasma and urine samples were taken for two more clearance determinations. All rats were then returned to their cages. Twenty-two hours later, the rats were again anesthetized, primed with [$^{14}$C]inulin, and infused with isotonic saline containing [$^{14}$C]inulin at 25 μl/min. Following a 0.5-hr equilibration period, three 0.5-hr clearance periods were completed. In this experiment, only the clearance of [$^{14}$C]inulin was studied.

*Results. Experiment 1.* Figure 1 presents data from the three 0.5-hr clearance periods taken during the initial infusion. The top bar for each infusion rate group is the mean value for the first 0.5-hr period. The middle and bottom bars are for the second and third 0.5-hr periods, respectively. Each fluoride group showed a modest but not statistically significant increase in urine flow rate ($\dot{V}$) compared to the $F(0)$ control group. Urine osmolalities for the $F(0)$ group tended to increase slightly with time, while those of the fluoride groups showed dose-related decreases. The reductions were statistically significant in the $F(250)$ group. At the end of the initial infusion period, the mean renal inner medullary sodium concentrations for the $F(0)$ and $F(250)$ groups were 238 ± 10 and 94 ± 1 m$M$, respectively. The corresponding renal cortical sodium concentrations showed no difference at 77 ± 2 and 75 ± 1 m$M$. Cortical and inner medullary chloride concentrations paralleled those of the sodium concentrations. These data indicate that the concentrating defect was established during the brief fluoride exposure.

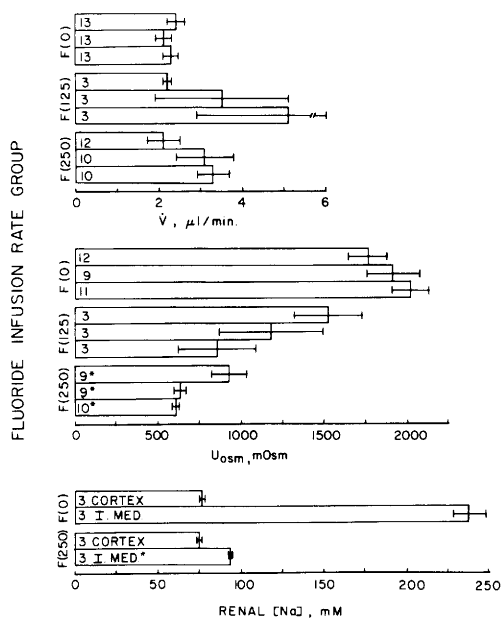


FIG. 1. Urine flow rates ($\dot{V}$), urine osmolalities, and renal tissue sodium concentrations during the fluoride infusion period of Experiment 1. Top, middle, and bottom bars are data (means ± SEM) from the first, second, and third clearance periods, respectively. Numbers in the bars indicate the observations. Asterisks indicate $P < 0.01$ compared to the $F(0)$ group.

Figure 2 shows the excretion rates of the major endogenous solutes, sodium, potassium, and chloride. The top bar for each

infusion rate group is the mean for the three 0.5-hr collections during the initial infusion. The second, third, and fourth bars for the $F(0)$ and $F(250)$ groups are the mean values observed for the collections taken at approximately 4, 24, and 40 hr after stopping the infusion, respectively. The bottom bar for the $F(125)$ group represents data obtained 40 h after stopping the initial infusion (there were no 4- or 24-hr collections for this group).

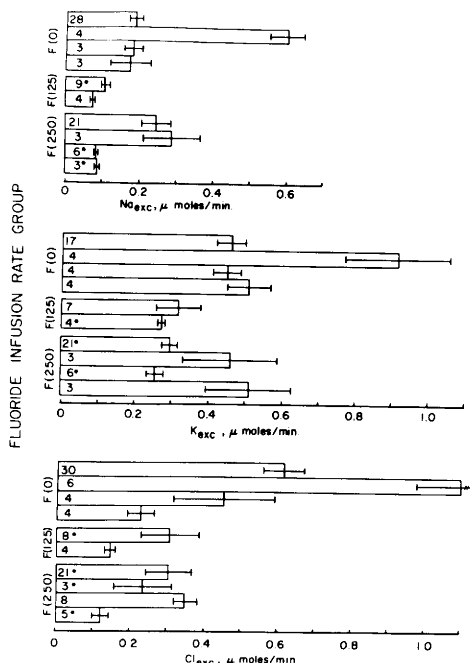

FIG. 2. Excretion rates of sodium, potassium, and chloride for each infusion rate group during the fluoride infusion period (top bars) and up to 40 hr (bottom bars) after stopping the fluoride infusion in Experiment 1. The second and third bars of the $F(0)$ and $F(250)$ groups represent data obtained at 4 and 24 hr after stopping the fluoride infusion, respectively. Means ± SEM; $N$ given in bars. Asterisks indicate $P < 0.01$ compared to the $F(0)$ group.

The data in Fig. 2 show generally depressed solute excretion rates in the $F(125)$ and $F(250)$ groups for each observation period. This depression was greater for sodium and chloride than for potassium. The reductions in sodium and chloride excretion attained statistical significance for the $F(250)$ group even at 40 hr after stopping the initial infusion. Urine flow rates of the fluoride groups at 4, 24, and 40 hr were not statistically different from those of the saline-infused control group.

Glomerular filtration rates observed during the initial infusion are shown in Table I. These rates were depressed in the fluoride groups in a dose-response manner.

Figure 3 shows the time course of inner medullary sodium concentrations, urinary osmolalities, and plasma fluoride concentrations. The large decrease in inner medullary sodium concentration of the $F(250)$ group present at the end of the initial infusion showed only a slight recovery 4 hr later even though the mean plasma fluoride concentration had fallen from 211 to 51 $\mu M$. Subsequently, there was a progressive return of inner medullary sodium concentra-

TABLE I. EXPERIMENTS 1 AND 2. TIME COURSES OF GLOMERULAR FILTRATION RATES (MICROLITERS PER MINUTE).

| | Experiment 1 | | | Experiment 2 | | | |
|---|---|---|---|---|---|---|---|
| | Fluoride infusion rate group | | | | Fluoride infusion rate group | | |
| Clearance period | $F(0)^a$ | $F(125)$ | $F(250)$ | Hours after $F^-$ infusion | $F(0)$ | $F(125)$ | $F(250)$ |
| 1 | 1282[b] | 1124* | 944* | 0[c] | 1196 | 1045 | 872* |
| | ±46 | ±22 | ±55 | | ±72 | ±99 | ±63 |
| | 13 | 3 | 11 | | 9 | 9 | 8 |
| 2 | 1300 | 999* | 780* | 3 | 1157 | 930* | 677* |
| | ±77 | ±53 | ±104 | | ±62 | ±35 | ±38 |
| | 12 | 3 | 12 | | 6 | 6 | 6 |
| 3 | 1260 | 795* | 645* | 24 | 1243 | 919* | 598* |
| | ±77 | ±66 | ±62 | | ±91 | ±58 | ±72 |
| | 13 | 3 | 11 | | 9 | 8 | 9 |

[a] $F(x)$ = fluoride infusion rate in nanomoles per minute.
[b] Mean, SEM, $N$.
[c] During fluoride infusion.
* Significantly different from $F(0)$ group at $P < 0.01$.

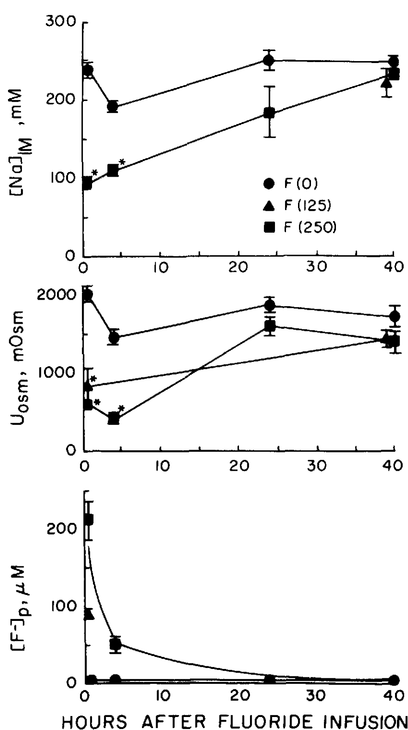


FIG. 3. Time course of renal inner medullary sodium concentration, urine osmolality, and plasma fluoride concentration relative to the period of fluoride infusion (0 time on the graph). Asterisks indicate $P < 0.01$ compared to the $F(0)$ group.

tion to control levels which, at 40 hr after fluoride exposure, remained slightly lower ($P > 0.1$) than that of the control group. For the $F(125)$ group, inner medullary sodium concentration was determined only at 40 hr and this value was not significantly different from that of the control group. Qualitatively similar results were observed for the time course of urine osmolality. The plasma fluoride concentrations of the $F(0)$ group were approximately 2 $\mu M$ throughout the experiment. All plasma, renal cortical and inner medullary fluoride levels for the $F(250)$ group are shown in Table II. At 40 hr for the $F(125)$ group, these values were $2.2 \pm 0.5$, $42 \pm 3$, and $127 \pm 37$ $\mu M$, respectively. Although renal tissue fluoride concentrations of the $F(0)$ group were not determined in this experiment, the 40-hr concentrations for the fluoride groups were within the range found for stock rats which had received no anesthesia or infusions (cortex = $36 \pm 9$ $\mu M$, inner medulla = $94 \pm 12$ $\mu M$; unpublished data).

*Experiment 2.* Table I presents the glomerular filtration rate time course. During the fluoride infusion period, the values were similar to those observed in Experiment 1 (Table I). During the 3rd and 24th hr after stopping the fluoride infusion, glomerular filtration rates were further reduced in the $F(125)$ and $F(250)$ groups. Compared to the control group and at 24 hr after the infusion, the reductions were 26 and 52% for the $F(125)$ and $F(250)$ groups, respectively. The data given above and in Table II suggest that the renal tissue and plasma fluoride concentrations at this time were approximately the same in each group.

*Discussion.* Two previous studies have investigated the influence of acutely elevated body fluid fluoride concentrations on the immediate changes in the urinary concentrating abilities of rats (2) and dogs (13). These studies reported qualitatively similar results which included increased urine flow rates, either a decrease or no change in the excretion rates of sodium, potassium, and chloride, and decreases in urinary osmolality, glomerular filtration rate, free water reabsorption, and inner medullary sodium concentrations. The fluoride-induced reduction in inner medullary solute concentration was viewed as the most probable primary event to account for the other features of the concentrating defect (2). Both reports suggested increased vasa recta blood flow

TABLE II. EXPERIMENT 1. TIME COURSE OF PLASMA AND RENAL TISSUE FLUORIDE CONCENTRATIONS (MICROMOLAR) FOR THE $F(250)$ GROUP.

| Hours after infusion | Plasma | Cortex | Medulla |
|---|---|---|---|
| 0 | 211[a] | 381 | 784 |
| | ±26 | ±14 | ±42 |
| | 10 | 3 | 3 |
| 4 | 51 | 152 | 525 |
| | ±10 | ±24 | ±180 |
| | 3 | 3 | 3 |
| 24 | 3.4 | 78 | 93 |
| | ±0.2 | ±24 | – |
| | 3 | 3 | 1 |
| 40 | 2.6 | 55 | 109 |
| | ±0.3 | ±8 | ±13 |
| | 3 | 3 | 3 |

[a] Mean, SEM, $N$. Renal tissue concentrations expressed in terms of tissue water.

and subsequent medullary solute washout as an underlying mechanism.

The current studies support the above findings and, further, demonstrate that the depression of renal function persists for many hours after plasma fluoride concentrations have returned to control levels. During the brief exposure to fluoride, urine flow rates were elevated, although not with statistical significance, and urinary osmolalities and inner medullary sodium concentrations were reduced (Fig. 1). These major indicators show that the acute fluoride-induced concentrating defect was established during the 2-hr fluoride infusion period. Further, glomerular filtration rates were reduced in a dose- and time-related manner (Table I), while the excretion rates of the major endogenous solutes were variably reduced (Fig. 2, top bars). The changes in solute excretion can, in large part, be accounted for by changes in glomerular filtration, as has been reported (2).

The persisting effects of acute fluoride exposure are shown in Figs. 2 and 3 and Table I. In spite of the rapidly declining plasma fluoride levels, the reduced inner medullary sodium (and chloride) concentrations and urinary osmolalities in the *F*(250) group showed essentially no change at 4 hr and the solute excretion rates were depressed relative to the *F*(0) group. At 24 and 40 hr after the fluoride infusion, the inner medullary sodium concentrations and urinary osmolalities of the fluoride groups remained lower than those of the *F*(0) group, although the differences were not statistically significant, and the solute excretion rates remained generally depressed.

The glomerular filtration rates of the *F*(125) and *F*(250) groups did not recover but, in fact, continued to decline for at least 24 hr after the fluoride infusion (Table I). The data in Table II indicate that these several persisting effects were not related to continuously elevated plasma or renal tissue fluoride concentrations.

The failure of inner medullary solute concentrations, solute excretion, and urinary osmolality to recover in proportion to declining plasma and renal tissue fluoride concentrations may be related to continuously depressed glomerular filtration rates. As discussed elsewhere (2), the generation of a hypertonic inner medullary interstitium is dependent on the delivery of an adequate solute load to the ascending limb of the loop of Henle, among other factors. However, the initial decrease in medullary solute concentration need not be related to decreased glomerular filtration rate (2).

The mechanism whereby fluoride depresses glomerular filtration rate remains unknown. A preliminary report (14) described up to 50% reductions in rat glomerular filtration rate, while simultaneously determined systemic arterial blood pressure, renal blood flow and para-aminohippuric acid extraction showed insignificant changes. The possibilities of a fluoride-induced chronic alteration in the permeability characteristics of glomeruli and/or the distribution of intrarenal blood flow must, therefore, be considered.

*Summary*. The changes in rat renal function produced by acute exposed to graded doses of fluoride were measured over 2 days. During the 2-hr iv infusion, urine flow rate increased moderately in the fluoride groups, while urinary osmolality, inner medullary sodium and chloride concentrations, glomerular filtration rate, and the excretion rates of sodium, chloride, and potassium decreased in a dose-response manner. Four hours after terminating the fluoride infusion, plasma fluoride concentrations had fallen 75% (peak value, 211 $\mu M$). This decline was not accompanied by recovery in either urinary osmolality or medullary sodium and chloride concentrations, while at 24 and 40 hr, these values showed a gradual return toward control values. Solute excretion rates, especially of sodium and chloride, were depressed even at 40 hr. Glomerular filtration rate continued to fall throughout the first day after stopping the fluoride infusion. These results suggest that the delayed recovery in urinary osmolality and medullary solute concentrations, as well as the continued depression in solute excretion, are related to the prolonged fluoride-induced decrease in glomerular filtration rate.

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