Role of Fluoride in Formation of Urinary Calculi: Studies in Rats

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ABSTRACT The effect of fluoride on urinary caculi formation in young rats was investigated. Two studies, in which rats received diets that included either higher calcium (9 g/kg diet) or normal calcium (5 g/kg diet), were conducted. At each level of calcium, one group of rats received a high level of fluoride and another a low level of fluoride in the diet. Rats ingesting high fluoride diets exhibited a higher incidence of crystalluria and bladder stones compared with those receiving low fluoride diets. However, compared with higher calcium diets, normal calcium diets delayed the appearance of crystalluria and produced smaller calculi. Calcium and oxalate were the major components of the calculi. Calculi of rats fed the higher calcium and high fluoride diet contained relatively less protein and more calcium compared with calculi formed in rats ingesting the higher calcium and low fluoride diet. The concentration of fluoride in calculi from rats fed high fluoride diets was significantly higher than that of calculi from rats fed low fluoride diets. A significant positive correlation between calcium and fluoride concentration of calculi was observed in rats fed the higher calcium diet only. These studies indicate that ingestion of excess fluoride facilitates calcium oxalate crystalluria and promotes the formation of bladder stones in rats, under the experimental conditions used. J. Nutr. *112*: 1787–1795, 1982.

INDEXING KEY WORDS: fluoride · urinary calculi · crystalluria · bladder stones

Urolithiasis is a major health problem in Southeast Asia. This disease in India is characterized by endemic occurrence in a distinct geographical pattern (1, 2), the reasons for which are not clear. It was suggested that dietary patterns of the population may have an important role in the development of urolithiasis (3, 4). While investigating the role of nutrition on calculogenesis, researchers have emphasized the intake of major nutrients, vitamins, and their excretory pattern in urine. However, this approach has not been successful in completely explaining the underlying factors responsible for the endemicity of urolithiasis in specific areas in India. It is quite possible that apart from the macronutrients, intake of certain metal and nonmetal ions through food and water may also exert a profound influence in the formation and growth of urinary calculi. In vitro studies indicate that some trace metal ions such as cadmium, magnesium, iron and mercury may have significant roles in growth and stability of calcium oxalate crystals (5). However, so far no systematic study has been undertaken to investigate the role of trace elements in the etiology of urolithiasis. Therefore, studies of the role of various trace metals and nonmetal ions in the genesis of urinary calculi were initiated. While testing the effect of various ions on in vitro calcification of bovine Achilles tendon by the method of Thomas and Tomita (6), it was observed that fluoride caused a significant increase in the

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TABLE 1

Composition of experimental diets

Ingredients	Low fluoride diet	High fluoride diet
	g/kg of diet	
Casein	100	100
Sucrose	800	800
Refined peanut oil	50	50
Vitamin mixture[1]	10	10
Mineral mixture[2]	40 (a) or (b)	40 (a) or (b)
Sodium fluoride[3]	0	0.05

[1] Vitamin mixture (8): 1 g of vitamin mixture contains vitamin A, 1000 IU; vitamin D, 100 IU; vitamin E, 10 IU; vitamin K, 0.5 mg; thiamin, 0.5 mg; riboflavin, 1.0 mg; pyridoxine,0.4 mg; pantothenic acid, 4.0 mg; niacin, 4.0 mg; choline, 200 mg; inositol, 25 mg; *p*-aminobenzoic acid, 10 mg; cyanocobalamin, 2 μg; biotin, 0.02 mg; folic acid, 0.2 mg. Sufficient cornstarch was added to make 1 g. [2] Salt mixture of Hubbell et al. (7) was used with some modifications as shown in table 2. (a) Salt mixture with higher calcium concentration Ca:P = 4.5; (b) salt mixture with normal calcium concentration Ca:P = 2.3. [3] Fluoride (F^-) concentration: low fluoride diet = 1.0 mg/kg of diet; high fluoride diet = 23 mg/kg of diet.

rate of calcium uptake. Investigations were therefore extended to animals to evaluate the role of fluoride in calculogenesis.

MATERIALS AND METHODS

The effect of high fluoride intake on urinary calculi formation was studied in Wistar strain albino rats fed either higher or normal calcium diets in two separate experiments.

Experiment 1: higher calcium diet (Ca:P ratio = 4.5:1)

Twenty-four weanling male rats, with an average body weight of about 39 g, were distributed equally into two groups so that rats in one group were littermates of those in the other group. Both groups of rats received a basal diet containing 10% casein, sucrose (80%) as a source of carbohydrate, and adequate amounts of minerals and vitamins. The composition of the diet is given in table 1. The mineral mixture used in this study was basically that of Hubbell et al. (7), with slight modification (table 2). The composition of the vitamin mixture conformed to the recommendations of the National Academy of Sciences, National Research Council (8). Calcium content of this diet was 9 g/kg of diet and the Ca:P ratio of this diet on analysis was found to be 4.5:1. One group of rats received only the basal diet, which contained a low level of fluoride (1 mg/kg of diet). This level is normally present in many mineral mixtures used for experimental rats. Additional fluoride as sodium fluoride was included in the diet fed to the second group of animals to raise the level of fluoride to 23 mg/kg of diet. This diet was designated as a high fluoride diet.

Experiment 2: normal calcium diet (Ca:P ratio = 2.3:1)

Fifty weanling male rats were divided by littermate distribution into two groups of 25 rats each with a mean body weight of about

TABLE 2

Composition of salt mixture

Formula	Study 1 salt mixture (a)	Study 2 salt mixture (b)
	g	*g*
Potassium aluminum sulfate ($K_2SO_4Al_2(SO_4)_3$)	0.18	0.18
Calcium carbonate ($CaCO_3$)	560.0	312.0
Potassium phosphate (KH_2PO_4)	230.0	230.0
Potassium chloride (KCl)	112.0	112.0
Sodium chloride (NaCl)	69.0	69.0
Magnesium carbonate ($MgCO_3$)	35.0	35.0
Magnesium sulfate ($MgSO_4 \cdot 7H_2O$)	20.0	20.0
Ferric phosphate ($FePO_4 \cdot 4H_2O$)	21.0	21.0
Cupric sulfate ($CuSO_4 \cdot 5H_2O$)	0.9	0.9
Manganese sulfate ($MnSO_4 \cdot H_2O$)	0.4	0.4
Potassium iodide (KI)	0.08	0.08
Sodium fluoride (NaF)	0.04	0.04
Sucrose	0	250.00
	g/kg diet	
Total calcium	9.0	5.0
Total phosphorus	2.2	2.2
Ca:P	4.1:1	2.3:1
Magnesium	0.43	0.43

TABLE 3

Body weight, urinary pH and urinary oxalic acid:creatinine concentration in rats fed high and low fluoride diets, at the end of the experiment[1]

Group	No.	Initial body weight	Final body weight	Urine	
				pH	μg oxalic acid/ml / μg creatinine/ml
		g	*g*		
Higher calcium diet					
High fluoride group	12	38.8 ± 1.18	121.8 ± 5.95	6.4 ± 0.16	0.10 ± 0.007
Low fluoride group	12	39.8 ± 1.83	123.8 ± 7.49	6.3 ± 0.12	0.13 ± 0.013
Normal calcium diet					
High fluoride group	25	43.5 ± 1.27	131.3 ± 3.28	6.4 ± 0.07	0.09 ± 0.01
Low fluoride group	25	42.4 ± 1.18	131.6 ± 4.00	6.2 ± 0.08	0.08 ± 0.012

[1] Values represent means ± SEM.

43 g. Experimental diets of this study were similar to those used in the earlier study except for the mineral mixture (table 1). Calcium content of these diets was normal (5 g/kg of diet) and the Ca:P ratio was 2.3:1. As in the first study, one group of rats received a high fluoride diet (23 mg/kg of diet), and another, a low fluoride diet (1 mg/kg of diet).

In both experiments the animals were housed individually in stainless-steel metabolic cages, and the diets were fed ad libitum for 10 weeks. Deionized, glass-distilled water was provided for drinking. During both studies, the temperature of the room housing the rat colony was regulated at about 25°. The rats were weighed regularly and the daily intake of food was recorded. Urine was collected over toluene for two consecutive days at the beginning of the experiment and subsequently at intervals of 2 weeks throughout the experimental period. Volume and pH were recorded immediately. By using the standard procedure (9), fresh urine was examined microscopically for crystalluria. Urine was analyzed for creatinine by the method of Clark and Thompson (10) and for oxalic acid by the method of Zarembski and Hodgkinson (11).

After 10 weeks, the rats were killed and the whole urinary tract was dissected out and examined for crystal deposits. All urinary concretions were removed, weighed and freeze-dried to a constant weight. For quantitative estimations of calculi components, the procedures described by Leonard and Butt (12) were followed. The calculi were analyzed for protein by the method of Lowry et al. (13), for calcium and magnesium by atomic absorption spectrometry, for oxalic acid by permangametry and for phosphorus by the method of Fiske and Subba Row (14). Fluoride was estimated by using a fluoride ion electrode (15) after ashing the calculi at low temperature.

RESULTS

All rats in both experiments gained weight throughout the experimental period, and the level of fluoride in the diet had no obvious effect on the growth rate of these animals (table 3). No change in urinary pH of rats ingesting different levels of calcium or fluoride was observed during the course of the experiment. In all the groups of animals, the pH of urine was around 6. Concentrations of urinary creatinine, oxalic acid and oxalic acid:creatinine toward the end of the experiment were not affected by the level of fluoride in the diets (in table 3 only the ratios of oxalic acid to creatinine are shown).

Crystalluria

Higher calcium diets. At the beginning of the experiment none of the animals on the higher calcium diet showed crystalluria. By the end of 2 weeks, 20% of rats ingesting a low fluoride diet and 70% of rats fed a high fluoride diet exhibited crystalluria. On chemical analysis, the crystals were found to be calcium oxalate. During the course of the experiment the incidence of crystalluria in

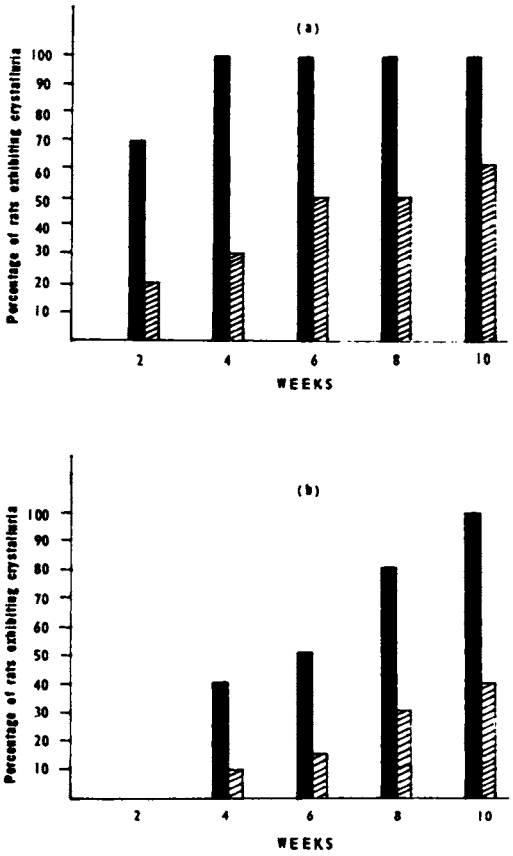

Fig. 1 Effect of fluoride on the incidence of crystalluria in rats fed higher calcium (*a*) or normal calcium (*b*) diets. Black bars: high fluoride; hatched bars: low fluoride.

both groups increased (Fig. 1*a*). By the end of the experiment the incidence of crystalluria was 60 and 100% in low and high fluoride groups, respectively.

Normal calcium diets. None of the rats ingesting normal calcium diets exhibited crystalluria at 2 weeks (Fig. 1*b*). By 4 weeks, only 10% of rats fed low fluoride and 40% of rats fed high fluoride diets developed crystalluria. At 10 weeks, the incidence of crystalluria was 40 and 100% in low and high fluoride groups, respectively. Thus the incidence of crystalluria in rats receiving the normal calcium diet increased progressively, whereas with higher calcium diets crystalluria increased sharply by 4 weeks, especially in rats on high fluoride intake, and remained at that high level at later times. Subtle but significant qualitative differences in crystalluria were observed between the two groups of rats fed the normal calcium diets. Urine of rats ingesting the low fluoride diet contained calcium oxalate crystals that were smaller (fig. 2) compared with crystals in urine of rats fed the high fluoride diet (fig. 3).

Urinary calculi

Higher calcium diets. Urinary concretions of different number, sizes and with various degrees of mineralization were found in bladders of 11 of 12 rats (91.6%) belonging to the high fluoride group (table 4). Besides bladder stones, bilateral kidney stones (sand-like deposits) were also found in two of these rats. On the other hand, only four rats (33.3%) fed the low fluoride diet developed bladder stones. The mean weights of bladder calculi developed in the high fluoride and low fluoride groups were 67.9 ± 17.34 (SEM) and 54.5 ± 18.52 (SEM), respectively. This difference was not statistically significant.

Normal calcium diets. At the end of the experiment, 18 of 25 rats (72%) on high fluoride intake and only 5 of 25 rats (20%) on low fluoride intake developed bladder stones (table 5). The mean weight of bladder calculi of the high fluoride group was 12.9 ± 1.66 (SEM) and that of the low fluoride group was 9.6 ± 2.42 (SEM). This difference was not statistically significant. No kidney stones were encountered in this experiment. In every rat only a single calculus was found in the bladder. These calculi were much smaller than those formed in rats ingesting higher calcium diets. The appearance of bladder calculi from these rats was also different. The calculi were more pliable and slimy. On the other hand, calculi that developed in rats fed higher calcium diets were bigger, granular, multilayered and multifaceted (fig. 4).

Composition of bladder calculi

Higher calcium diets. All the bladder calculi contained protein (table 5), and its proportion (9.0%) in calculi of rats fed a low fluoride diet was significantly higher ($P < 0.01$) than in calculi of rats fed a high fluoride diet (4.0%). Calcium and oxalic acid were the major constituents of these calculi.

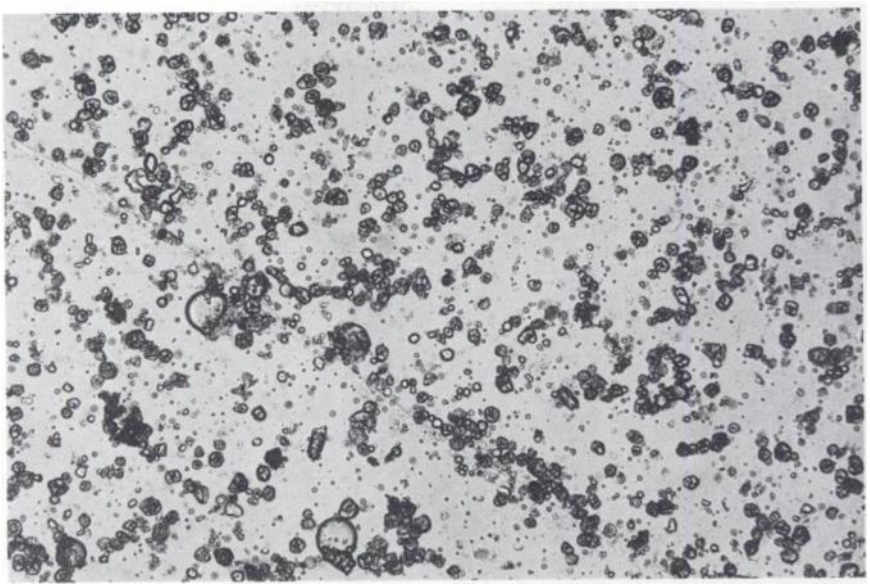

Fig. 2 Calcium oxalate crystalluria in rats ingesting the normal calcium and low fluoride diet. Note the smaller crystal size compared with figure 3. ×400.

The concentration of calcium in stones of the high fluoride group was significantly higher ($P < 0.02$) than that in calculi of the low fluoride group. The stones also contained a small proportion of phosphorus and magnesium. The fluoride concentration was 0.36% in stones of the high fluoride group and only 0.053% in stones of the low fluoride group. This difference was found to be highly significant ($P < 0.001$). A significant positive correlation between fluoride and calcium concentration of calculi ($r = 0.66$; $P < 0.05$)

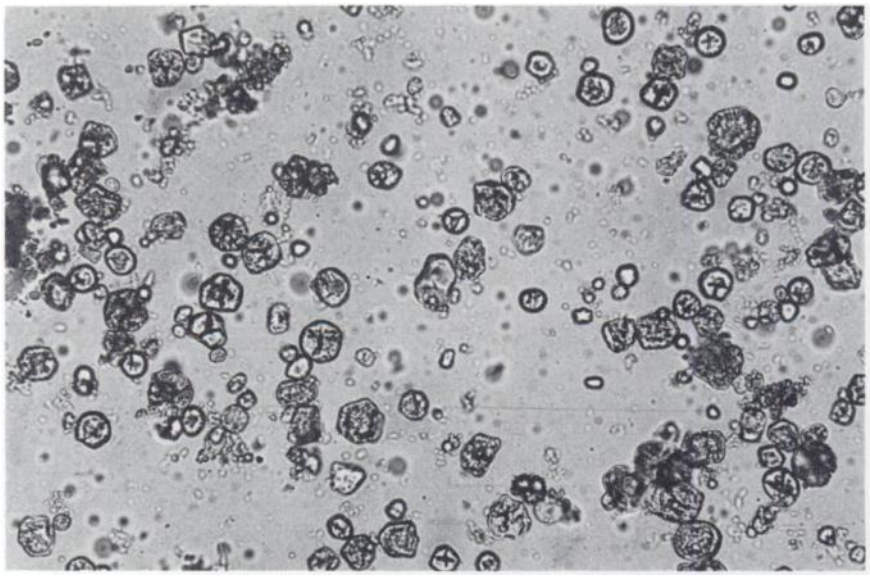

Fig. 3 Calcium oxalate crystalluria in rats ingesting the normal calcium and high fluoride diet. Note the bigger crystal size compared with figure 2. ×400.

TABLE 4

Effect of fluoride on incidence of crystalluria and bladder calculi in rats fed higher or normal calcium diets

Group	No.	Crystalluria incidence	Bladder calculi	
			Incidence	Fresh weight
		%	%	mg
Higher calcium diet				
High fluoride group	12	100	91.6	67.9 ± 17.34[1]
Low fluoride group	12	60	33.3	54.5 ± 18.52
Normal calcium diet				
High fluoride group	25	100	72.0	12.9 ± 1.66
Low fluoride group	25	40	20.0	9.6 ± 2.42

[1] Values represent means ± SEM.

was observed in both groups of rats ingesting higher calcium diets.

Normal calcium diets. Bladder calculi of rats on normal calcium intake (table 5) contained a higher proportion of protein (about 17%) and lower proportion of calcium and oxalic acid compared with the calculi of rats fed higher calcium diets. However, on normal calcium diets, no significant difference was observed in the concentrations of protein, calcium, oxalic acid, phosphorus or magnesium of calculi of high and low fluoride groups. The concentration of fluoride in calculi of the high fluoride group (0.24%) was significantly ($P < 0.001$) higher than that of calculi formed in the low fluoride group (0.02%). No correlation between calcium and fluoride concentration of calculi of rats on high and low fluoride intake was observed.

DISCUSSION

That ingested fluoride might have a role in the formation of urinary calculi was first suggested by Spira (16). Herman and Papadakis (17) were unable to confirm the role of fluoride in calculi formation in rats. Findings of the present investigation throw some light on the role of fluoride in calculogenesis.

When fluoride intake was high, the incidence of crystalluria was found to be higher from the very early stages of the experiment, whether the rats were fed higher or normal calcium diets. On the other hand, in the corresponding groups of animals ingesting low fluoride diets, the appearance of crystalluria was delayed and its incidence was lower. These observations clearly indicate that high intake of fluoride plays an active role in facilitating urinary crystal formation. However, calcium levels of diets were also found to modify the effect of fluoride. Thus, when intake of both calcium and fluoride was high, the incidence of crystalluria reached a peak much faster, i.e., as early as 4 weeks. Whether or not these nascent crystals aggregate and grow to form a well-defined stone will depend on various factors. One of the condi-

TABLE 5

Effect of dietary fluoride on the composition of bladder calculi of rats fed higher or normal calcium diets[1]

Group	No.[2]	Protein	Calcium	Oxalic acid	Fluoride	Phosphorus	Magnesium
				%			
Higher calcium diet							
High fluoride group	11	4.0 ± 0.32	39.5 ± 1.24	54.6 ± 2.62	0.36 ± 0.032	2.8 ± 1.47	0.6 ± 0.08
Low fluoride group	4	9.0 ± 0.40[a]	29.8 ± 1.26[b]	58.0 ± 1.39	0.05 ± 0.019[c]	4.1 ± 2.40	0.4 ± 0.17
Normal calcium diet							
High fluoride group	18	17.5 ± 0.96	32.5 ± 1.62	44.0 ± 1.51	0.25 ± 0.021	2.0 ± 0.09	2.5 ± 1.42
Low fluoride group	5	16.2 ± 1.12	29.6 ± 2.23	46.8 ± 2.34	0.02 ± 0.010[c]	0.5 ± 0.05	0.9 ± 0.09

[1] Values represent means ± SEM. [2] No. = number of calculi analyzed. [a] $P < 0.01$. [b] $P < 0.02$. [c] $P < 0.001$.

Fig. 4 Bladder calculi from rats ingesting the higher calcium and high fluoride diet. Scale: 1-mm units. ×40.

tions conducive to calculi formation is increased concentration of precipitating substances (which constitute the calculi) in urine. In the present investigation, the calcium and fluoride intakes of rats were two important variables. Although the concentrations of these elements were not estimated in urine, it is reasonable to expect that their urinary excretion would vary depending on the level of intake. These changes in turn would exert a modifying effect on formation and growth of calculi. The relative importance of calcium and fluoride is clearly brought out in this study. When the Ca:P ratio of the diets was higher, and therefore inherently more conducive to calculi formation, the simultaneous increase in fluoride intake aggravated the situation by accelerating the growth of calculi. On the other hand, when the Ca:P ratio of the diets was more physiological, although high fluoride did increase the incidence of bladder calculi, these calculi were smaller than those that developed on the diet with a higher Ca:P ratio.

All calculi contained a significant amount of protein, which gave a positive periodic acid–Schiff test. This suggests the presence of a mucoprotein. Smaller calculi contained a relatively higher proportion of protein. This protein may be akin to the matrix protein described by Boyce (18). However, further investigations are under way to characterize the protein moiety found in the calculi of the rats.

The exact mechanism of fluoride action in calculogenesis is not clear. Fluoride may be acting in several ways. One strong possibility is that insoluble calcium fluoride present in urine might be acting as a potential initiator of crystal formation by aiding heterogenous nucleation. The fact that the appearance of crystalluria was accelerated by high fluoride diets supports this view.

Roginski and Mertz (19) had earlier reported a high incidence of urinary calculi in rats when the Hubbell, Mendel and Wakeman (HMW) salt mixture was included in diets containing 10% casein. These authors were not able to explain this phenomenon completely, even based on the Ca:P ratio of the HMW salt mixture used. It is quite likely that in addition to protein, calcium, phosphorus and magnesium levels of the diets used in their experiments, the high concentration of fluoride (18 ppm) in the HMW salt mixture used could have been one of the contributing factors for the high incidence of urinary calculi observed by these authors.

Some direct or indirect evidence on the probable involvement of fluoride in calcu-

logenesis is available in the literature. High levels of fluoride were reported by Herman et al. (20) in the kidneys and bladder of subjects afflicted with urolithiasis. Fluoride content of kidney stones of subjects residing in areas with a high level of fluoride in water (2.6 ppm; 16 ppm) was found to be higher than that of stones from subjects living in low fluoride (<1 ppm) areas (21, 22). Further, incidence of urolithiasis was found to be highest where the fluoride content of water was higher (23). A significant increase in the percentage of calcium oxalate calculi was reported in a community after it received a fluoridated water supply (24). Further, when different types of calculi were analyzed, the calcium oxalate stones had the maximum concentration of fluoride (25). The above evidence suggests that, other conditions in urine being conducive to stone formation, excess of fluoride intake might aggravate the condition.

Bladder calculi found in rats of the present study were composed predominantly of calcium and oxalic acid, with minor amounts of phosphorus and magnesium. Roginski and Mertz (19), on the other hand, reported brushite ($CaHPO_4 \cdot 2H_2O$) as the primary component of the urinary stones found in their experimental rats. They also reported a possible presence of calcium oxalate in the calculi. These differences in composition of calculi observed in the two studies may not be due to experimental diets, because they were quite similar, but may be related to the location of calculi and pH of urine. The data on chemical composition presented in this paper pertains to bladder calculi that were the most predominant in the rats. Further, the pH of 24-hour urine, which was around 6.0, was more favorable to calcium oxalate stone formation. In fact, urine of these rats contained mostly calcium oxalate crystals. Roginski and Mertz (19) found calculi in the kidney and bladder as well as the ureter, and no information on either the type of calculi analyzed or the exact pH of the 24-hour urine was provided by these authors. Therefore, data on the chemical composition of calculi presented in these two studies are not strictly comparable.

It is interesting and important to note that Roginski and Mertz (19) observed that higher protein diets (14–18%) were noncalculogenic even though the HMW salt mixture was used. This useful information in fact allowed development of a suitable working model in rats that could simulate some of the dietary conditions encountered in populations with bladder stone disease. Surveys conducted in India have shown that bladder stone disease is prevalent predominantly in children belonging to a low socioeconomic group (26) whose diets contain 9–10% calories derived from proteins. The experimental diets used in this study had a similar protein calorie percentage.

Findings of the present study have practical significance. In India urolithiasis is endemic in certain areas (1, 2). Among several factors implicated for these regional differences, calcium and fluoride contents of diets and water may be two important determinants. Intakes of calcium and fluoride (and other minerals) of populations vary widely from region to region depending on the type of major cereals consumed and the quality of drinking water. In some of the endemic stone belt areas in India, fluoride content ($\bar{x}$ 16 ppm) of drinking water was found to be high (22, 27).

Based on the data discussed in this paper, it can be suggested that moderate intake of protein and high intakes of calcium and fluoride may be some of the important dietary factors responsible for urinary stone disease in some endemic areas. To test this hypothesis, a detailed dietary survey and biochemical and epidemiological studies are being undertaken in areas endemic in urolithiasis.

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LITERATURE CITED

1. McCarrison, R. (1931) The causation of stone in India. Br. Med. J. *1*, 1009–1015.
2. Andersen, D. A. (1968) Historical and geographical differences in the pattern of incidence of urinary

stones considered in relation to possible aetiological factors. In: Renal Stone Research Symposium (Hodgkinson, A. & Nordin, B. E. C., eds.), pp. 7–13, J & A Churchill Ltd., London.
3. Andersen, D. A. (1962) The nutritional significance of primary bladder stones. Br. J. Urol. *34*, 160–177.
4. Andersen, D. A., Sriramachari, S. & Khandagale, M. K. (1963) Investigations into relationship between bladder stones and malnutrition. Indian J. Med. Sci. *17*, 617–644.
5. Eusebio, E. & Elliot, J. S. (1967) Effect of trace metals on the crystallization of calcium oxalate. Invest. Urol. *4*, 431–435.
6. Thomas, W. C., Jr. & Tomita, A. (1967) Mineralization of human and bovine tissues in vitro. Am. J. Pathol. *51*, 621–628.
7. Hubbell, R. B., Mendel, L. B. & Wakeman, A. J. (1937) A new salt mixture for use in experimental diets. J. Nutr. *14*, 273–285.
8. Campbell, J. A. (1963) Method for determination of PER and NPR. In: Evaluation of Protein Quality, Publication No. 1100, pp. 31–32, National Academy of Sciences, National Research Council, Washington, DC.
9. Bradley, G. M. & Benson, E. S. (1969) Examination of the urine. In Todd-Sanford Clinical Diagnosis by Laboratory Methods (Davidsohn, I. & Henry, J. B., eds.), pp. 30–101, W. B. Saunders Company, Philadelphia.
10. Clark, L. C. & Thompson, H. L. (1949) Determination of creatine and creatinine in urine. Anal. Chem. *21*, 1218–1221.
11. Zarembski, P. M. & Hodgkinson, A. (1965) The fluorometric determination of oxalic acid in blood and other biological materials. Biochem. J. *96*, 717–721.
12. Leonard, R. H. & Butt, A. J. (1955) Quantitative identification of urinary calculi. Clin. Chem. *1*, 241–248.
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with the Folin phenol reagent. J. Biol. Chem. *193*, 265–275.
14. Fiske, C. H. & Subba Row, Y. J. (1925) The colorimetric determination of phosphorus. J. Biol. Chem. *66*, 375–400.
15. Singer, L. & Armstrong, W. D. (1968) Determination of fluoride in bone with the fluoride electrode. Anal. Chem. *40*, 613–614.
16. Spira, L. (1956) Urinary calculi and fluorine. Exp. Med. Surg. *14*, 72–78.
17. Herman, J. R. & Papadakis, L. (1960) The relationship of sodium fluoride to nephrolithiasis in rats. J. Urol. *83*, 799–800.
18. Boyce, W. H. (1968) Organic matrix of native human urinary concretions. In: Renal Stone Research Symposium (Hodgkinson, A. & Nordin, B. E. C., eds.), pp. 93–104, J. & A. Churchill Ltd., London.
19. Roginski, E. E. & Mertz, W. (1974) Development and reversibility of urolithiasis in rats by mineral mixtures. J. Nutr. *104*, 599–604.
20. Herman, J. R., Mason, B. & Light, I. (1958) Fluoride in urinary tract calculi. J. Urol. *80*, 263–268.
21. Zipkin, J., Lee, W. A. & Leone, N. C. (1962) Fluoride content of urinary and biliary tract calculi. In: Fluoride Drinking Waters (McClure, F. J., ed.), Public Health Service Publ. No. 825, pp. 435–437, U.S. Dept. H. E. W. (P. H. S.), Washington, DC.
22. Jolly, S. S., Sharma, O. P., Garg, G. & Sharma, R. (1980) Kidney changes and kidney stones in endemic fluorosis. Fluoride *13*, 10–16.
23. Juuti, M. & Heinonen, O. P. (1979) Incidence of urolithiasis leading to hospitalisation in Finland. Acta Med. Scand. *206*, 397–403.
24. Summers, J. L. & Keitzer, W. A. (1975) Effect of fluoridation on urinary tract calculi. Ohio State Med. J. *71*, 25–27.
25. Auermann, E. & Kuhn, H. (1971) Fluoride content of kidney stones. Fluoride *4*, 150–151 (abs.).
26. Ramalingaswami, V. & Aurora, A. L. (1961) Nutritional aspects of calculus disease of the urinary tract. Fed. Proc. *20*, Suppl. *7*, Part *III*, 317–322.
27. Singh, A., Jolly, S. S., Devi, P., Bansal, B. C. & Singh, S. S. (1962) Endemic fluorosis (an epidemiological, biochemical and clinical study in Bhatinda district of Panjab). Indian J. Med. Res. *50*, 387–398.