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Determination of Fluoride in Urinary Calculi Using a Quantitative Microdiffusion Method

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Summary. A microdiffusion method for the separation of fluoride from other ions in urinary calculi has been developed, tested and assessed. The procedure involves digestion at 75 °C of samples with silicone-impregnated mixtures of nitric and perchloric acids in a specially designed diffusion cell and determination of the diffused fluoride with an ion-sensitive electrode. Several test samples were used to assess the recovery, accuracy and reproducibility of the procedure. Results for 20 stones of Indian origin are presented and discussed.

Key words: Urinary calculi – Trace elements – Fluoride – Diffusion – Ion selective electrode

Introduction

The role of fluoride in the genesis of urinary calculi has been addressed by many investigators over the past few years. Some researchers have reported an increase in the percentage of calcium oxalate stones in communities receiving a fluoridated water supply [14, 16, 23] while others have found significantly higher fluoride levels in urinary calculi from patients living in endemic fluorotic areas [11, 13]. Animal studies have shown that excess fluoride ingestion facilitates calcium oxalate crystalluria and promotes the formation of bladder stones [1]. Contrary to these reports, other human and animal investigations have failed to establish a clear relationship between fluoride bearing water and urinary calculi [9, 28]. Indeed, protection by fluoride against calcification [6, 17], and an inhibitory effect towards calcium oxalate and hydroxyapatite growth [7, 18] has been reported for fluoride at certain concentrations. Nevertheless, the presence of fluoride in urinary calculi has been clearly demonstrated both in the above studies and in other investigations [2, 10, 26]. Moreover, its concentration in stones appears to be related to the type of calculus involved [2, 10, 11, 26]. Thus it seems likely that fluoride is involved in some way in the genesis of urinary calculi.

In order to characterise the pathogenic role of fluoride, it is essential that accurate and reliable methods be available for the qualitative and quantitative determination of this element in urinary calculi. Accordingly, we developed a procedure which involves a microdiffusion method [3, 24] for its separation from other ions and its measurement with the fluoride ion sensitive electrode [3, 5]. Recovery, accuracy and reproducibility of this procedure were assessed with several test samples and it then applied to the analysis of 20 urinary calculi from India.

Materials and Methods

Diffusion cells were manufactured from propylene rods according to the design shown in Fig. 1. Acids used were prepared from HNO_3 (70%) and $HClO_4$ (72%) (both BDH, Aristar). Aliquots of 5 M stock solutions of 1:1 (v/v) acid mixtures were impregnated with silicone by shaking one tenth of their volume of either hexamethyldisiloxane (HMDS, Ega-Chemie) or 1 cSt 200 Fluid (Dow Corning) for 5 min.

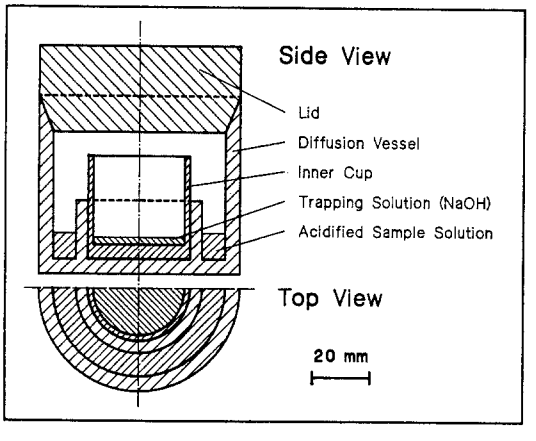

Fig. 1. Diffusion vessel

The supernatant was discarded and the acid solution was saturated by shaking with the same amount of original Si-organic liquid for a further 5 min. About 50 ml of the mixture was prepared for each experiment.

Sodium hydroxide solutions (1 M) prepared from Merck AR grade NaOH were found to be low in fluoride concentration and were used for the trapping solutions.

Five milliliters of acid mixture was found to be sufficient for digestion of 200 mg sample. Two milliliters of NaOH wetted the bottom of the inner cup completely. After pipetting this volume into the inner container, weighed portions of sample were introduced into the outer compartment of the diffusion chamber. HMDS saturated acids were added and the vessel was closed immediately. Steel clamps were used to hold the lids in position. Diffusion was carried out at ca. 75 °C for at least 3 to 4 h but in some cases this was extended to 16 to 24 h. Solutions were then allowed to cool and the pH was adjusted to between 5 and 6 with HNO_3. Thereafter these were quantitatively transferred to 5 ml volumetric flasks, and diluted to volume with de-ionized, distilled water. Solutions were poured back into the inner containers of the diffusion cells, stirred and cell potential was measured with the fluoride electrode. In most cases 0.5 ml of TISAB III (Orion) was added to correct for differences in pH and ionic strength and electrode potential was re-determined. As a third independent procedure, a standard addition method was applied.

Three different test samples were used to assess the recovery, accuracy and reproducibility of the procedure: aqueous sodium fluoride standards; independent aliquots of uric acid, ammonium acid urate and calcium oxalate monohydrate to which small quantities of sodium fluoride had been added; and a large urinary calculus composed of struvite, apatite and calcium oxalate monohydrate.

It was anticipated that when digesting calculi containing uric acid, urates, calcium oxalate or carbonate apatite, the evolving carbon dioxide would compete with the fluoride for the trapping alkali. To test for possible loss of fluoride in the spray from CO_2 trapped in the NaOH solution, 70 mg aliquots of uric acid (Merck), ammonium acid urate (BDH), and calcium oxalate monohydrate (BDH) samples were digested and diffused after addition of 25 µg of fluoride ion to each test solution.

After establishing that both the precission and the accuracy of the procedure were highly statisfactory, the nuclei of 20 paediatric urinary calculi from India were analysed for their fluoride content using the same method. These samples were previously subjected to x-ray powder diffraction analysis to identify major and minor constituent phases.

Results

Figure 2 shows recovery of 0.1 mg fluoride (added as 100 mg/l NaF solution) as a function of diffusion time (T = 80 °C) for the two different silicone fluids employed. Figure 3 depicts the effect of higher temperatures on the diffusion process. Figure 4 displays calibration graphs. Curve 1 depicts the calibration line obtained by standardising the electrode assembly in aqueous sodium fluoride standards. The "pure electrode" slope S of this curve was subsequently employed in the calculation of fluoride concentrations ($c_{unknown}$) by the standard addition method according to the equation [19]

$$c_{unknown} = (p \cdot c_{standard}) \cdot [\pm\{1 - (1 + p) \cdot 10^{\Delta E/S}\}]$$

where p is the ratio of standard volume to sample volume and ΔE the difference in the electrode potential before and after addition of the standard. Since this curve deviates from linearity at very low fluoride concentrations, a quadratic expression (Table 1) was fitted to the experimental points [12], and the slope at the different concentrations was calculated from the derivative. Curve 2 was obtained by subjecting 1 ml of different aqueous fluoride standards to the entire diffusion procedure as described above. Curve 3 represents mV readings obtained after addition of 0.5 ml

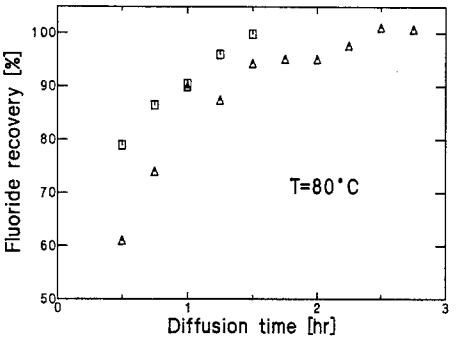

Fig. 2. Recovery of fluoride as a function of diffusion time and silicone fluid employed. △ = 2 ml NaOH (0.5 M) trapping solution, pH adjusted to 5.5 with HNO_3, made up to 10 ml final volume (incl. 0.5 ml TISAB III); digestion with 5 ml HNO_3 + $HClO_4$ (1 : 1 (v/v), 70% Aristar), saturated with 1 cSt 200 fluid; addition of 1 ml 100 mg/l aqueous NaF standard (0.1 mg fluoride). □ = 2 ml NaOH (0.5 M) trapping solution, pH adjusted to 5.5 with HNO_3, made up to 10 ml final volume (incl. 0.5 ml TISAB III); digestion with 4 ml HNO_3 + $HClO_4$ (1 : 1 (v/v), 70% Aristar), saturated with HMDS; addition of 1 ml 100 mg/l aqueous NaF standard (0.1 mg fluoride). (all points mean of three determinations)

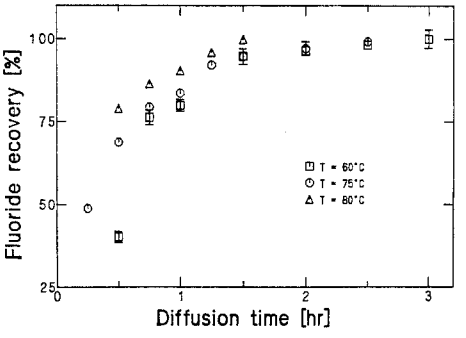

Fig. 3. Recovery of fluoride as a function of diffusion time and temperature

Table 1. Regression coefficients for aqueous sodium fluoride standard calibration curve (electrode slope parameters), computed by least squares fit of a second order polynomial in $lg(c_F)$ [mg/l] as independent variable x and E [mV] as dependent variable y: $y = a_0 + a_1x + a_2x^2$ (Fig. 4, curve 1)

a_0 =	16.0608
a_1 =	−55.7015
a_2 =	−2.2538

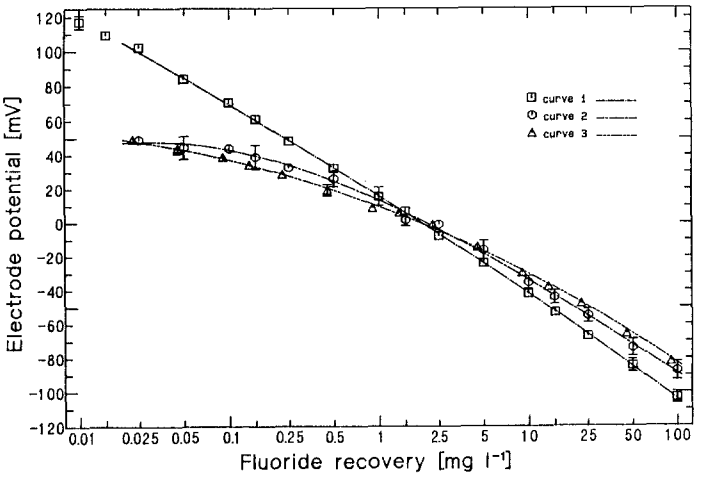

Fig. 4. Calibration of microdiffusion procedure (cf. Tables 1 and 2)

TISAB III to 5 ml of the solution used for curve 2. In each of the two cases the curve drawn through the experimental points represents a computed third order polynomial, fitted by the least squares method, employing BMDP statistical software [12]. Regression coefficients are given in Table 2. In both cases the contaminating influence of acids and alkaline solutions can be seen. The curves deviate considerably from linearity at lower fluoride concentration values, making an accurate evaluation of unknown samples impossible in this region. A blank concentration value of 0.27 mg/l was derived from these curves. This value was obtained by estimating the electrode potential at which the slopes of curves 2 and 3 tend to zero (approximately 50 mV) and by reading the corresponding fluoride concentration from curve 1. Only those solution concentrations above ca. 1 mg/l can thus be determined with any degree of confidence.

Figure 5 establishes the correlation between fluoride concentration in solution and the expected relative standard deviation (RSD) at any one concentration in the range examined and shows the decline in precision as lower concentration values are approached. Boundary lines were calculated with the TISAB calibration curve regression parameters (Table 2, dependent variable $\lg(c_F)$), assuming a mean RSD of ±2.24 mV. The pronounced effect of actual dis-

Table 3. Recovery of 25 μg fluoride from samples containing carbon dioxide

Sample	Fluoride recovered [μg]	Mean [μg]
Uric acid	28.3	27.9 ± 0.6
	28.1	
	27.2	
Ammonium	–	25.4 ± 0.2
Acid	25.5	
Urate	25.2	
Calcium	28.5	27.2 ± 1.1
Oxalate	26.5	
Monohydrate	26.7	

Table 2. Regression coefficients for diffused standard calibration curves (with (Fig. 4, curve 3) and without (Fig. 4, curve 2) TISAB III addition)

		Diffused standards[b]		Diffused standards[c]	
		without TISAB	with TISAB	without TISAB	with TISAB
Regression coefficients[a]	a_0	13.5735	9.7739	0.3885	0.2947
	a_1	−40.2272	−33.6749	$-0.2271 \cdot 10^{-1}$	$-0.2735 \cdot 10^{-1}$
	a_2	−8.9533	−6.2447	$-0.2034 \cdot 10^{-3}$	$-0.1681 \cdot 10^{-3}$
	a_3	1.7462	−0.3203	$-0.1824 \cdot 10^{-5}$	$-0.1021 \cdot 10^{-5}$
Residual mean square		5.2600	1.5483	0.0105	0.0014
Multiple R-square		0.9980	0.9993	0.9935	0.9991

[a] Computed by least squares fit of a third order polynomial in one independent variable to the dependent variable. The form of the regression equation is: $y = a_0 + a_1x + a_2x^2 + a_3x^3$

[b] Dependent variable y is E [mV], independent variable x is $\lg(c_F)$ [mg/l]

[c] Dependent variable y is $\lg(c_F)$ [mg/l], independent variable x is E [mV]

solved solids again shows the need for rather large aliquots of stone samples.

Standard fluoride samples in the ng/mg concentration range are not readily available. The best is NBS SRM 1571 (orchard leaves) with 4 ng/mg fluoride, but this value has not been certified. Using about 1 g aliquots (with 7 ml acid mixture), the diffusion approach yielded (after correction for the estimated blank of ca. 0.27 mg/l) a value of 3.64 ± 0.23 ng/mg as mean of three standard additions.

Table 3 depicts the recovery of 25 μg fluoride from samples containing carbon dioxide. The fluoride content of the single test calculus is presented in Table 4 while the fluoride concentrations in each of the 20 calculi from India are given in Table 5.

Discussion

In evaluating the microdiffusion method, several aspects are worthy of comment. The slightly shorter recovery time required with HMDS (Fig. 2) is probably due to the immediate availability of more methylfluorosilane groups without the need of prior cracking of the long dimethylsilyl chains. As can be seen, complete diffusion is accomplished in less than 3 h which is about one tenth of the time needed for the noncatalytic reaction.

While it is generally accepted that diffusion times are strongly temperature dependent, Fig. 3 shows that in the presence of silicone fluids, the accelerating effect due to higher temperatures is minimal for total recovery, although

Table 4. Repeat analysis of struvite/apatite/calcium oxalate monohydrate calculus

Sample mass [mg]	Fluoride concentration [ng/mg]		
	without TISAB	with TISAB	std. add. mean
152.0	390	412	385
152.5	364	385	358
152.4	353	370	352
152.3	380	405	385
154.7	374	404	383
152.0	358	371	375
153.2	359	380	368
150.7	360	378	372
Mean	367 ± 13	388 ± 16	372 ± 13
% RSD	3.54	4.22	3.35

Table 5. Fluoride concentrations in the nuclei of 20 urinary calculi from India

No.	Main components	Fluoride concentration [ng/mg]
1	Calcium oxalate monohydrate	1,152
2	Uric acid	48
3	Ammonium acid urate	281
4	Calcium oxalate monohydrate, ammonium acid urate	60
5	Uric acid, calcium oxalate monohydrate	55
6	Ammonium acid urate	21
7	Ammonium acid urate, struvite	390
8	Uric acid	254
9	Ammonium acid urate	179
10	Ammonium acid urate	87
11	Ammonium acid urate	366
12	Calcium oxalate monohydrate, ammonium acid urate	485
13	Calcium oxalate monohydrate, calcium oxalate dihydrate	435
14	Ammonium acid urate	183
15	Calcium oxalate monohydrate	166
16	Calcium oxalate monohydrate	463
17	Calcium oxalate monohydrate	689
18	Calcium oxalate monohydrate	371
19	Ammonium acid urate	238
20	Calcium oxalate monohydrate, ammonium acid urate	389

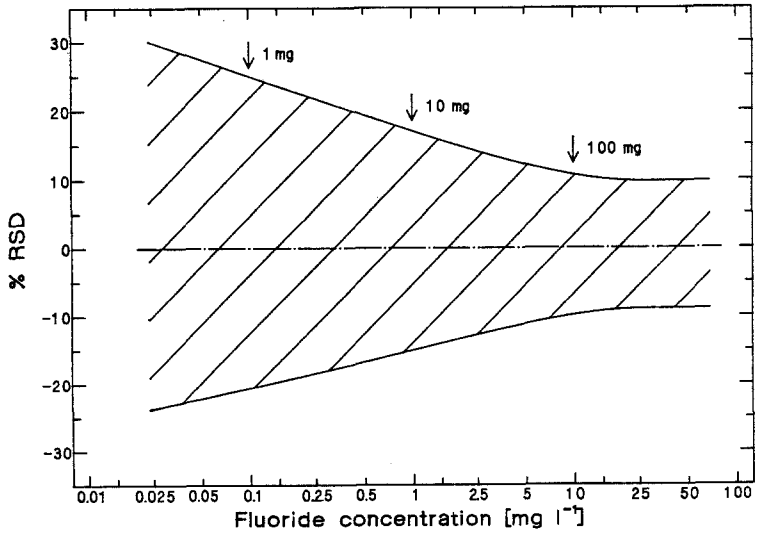

Fig. 5. Expected error as function of solution concentration

initially the percentage recovery is greater at elevated temperatures.

Although TISAB also contributes to the fluoride blank (Fig. 4) it is nevertheless highly recommended as additive. Since each experimental point is the mean of three values obtained from different samples, it was possible to calculate the mean single RSD. For the two cases, i.e. with and without TISAB III, values of 2.24 mV ± 0.85 mV and 3.98 mV ± 2.04 mV, respectively, were obtained. Samples with TISAB therefore show a much smaller spread in their mV readings about the mean value, than do samples without TISAB. A mean RSD of ±2.24 mV might appear to be rather high, as it constitutes an error of ±0.16 mg/l at the 1 mg/l level, but this represents the overall reproducibility of the experimental procedure as a whole for different samples. This compares favourably with reported reproducible potential readings in the *same* sample of 1 [20, 25] to 1.5 mV [4] at any one fluoride concentration.

The fluoride concentration value of 3.64 ± 0.23 ng/mg obtained for the NBS SRM 1571 standard agrees well with the uncertified value of 4 ng/mg and is in good agreement with values reported by other workers (3.9 ± 0.6 ng/mg, 3.80 ± 0.32 ng/mg, 4.3 ng/mg [22]). This lends confidence to the reliability of the diffusion method.

The recovery of fluoride from samples containing carbon dioxide (Table 3) yielded higher values than were expected. This anomally is probably due to extraneous fluoride contributions. The results suggest that CO_2 does not interfere with accurate fluoride concentration determination in the procedure described. However, if the absorbing capacity of the trapping solution is exceeded, a situation might arise where some of the fluoride might remain uncollected. An erroneously low fluoride activity would thus be measured. In addition, high pressure build-up in the diffusion vessel might cause leakage of CO_2 and trimethylfluorosilane [21] again yielding low fluoride values.

Mean values obtained from the three different analyses of the test calculus are well within the RSD of the single mean values of each method all of which are in turn always within 4.5% RSD (Table 4). These results, together with the results obtained for the NBS standard evaluation show that both the precision and accuracy of the described method are highly satisfactory.

As far as the analysis of the 20 Indian calculi is concerned, the mean fluoride concentration is 316 ng/mg (range 21 to 1152 ng/mg). It is noted that in the present study the "pure" calcium oxalate calculi have higher fluoride concentrations (mean 546 ng/mg, range 166 to 1152 ng/mg) than the "pure" uric acid and urate stones (mean 184 ng/mg, range 21 to 366 ng/mg). This is in agreement with similar observations reported by other workers [2, 11, 26]. These observations are also in agreement with the results of Herman who reported a correlation between high calcium and high fluoride concentrations [8]. It is conceivable that with high concentrations of fluoride in urine, calcium fluoride may be precipitated, thereby acting as a potential initiator of crystal formation by aiding heterogeneous nucleation [13]. Calcium oxalate may grow on these crystals, in turn acting as a nucleus for uric acid precipitation [15]. As is evident from other investigations, stones containing calcium oxalate in particular show marked differences in their fluoride content when areas of fluoridated and natural drinking waters are compared [11].

The presence of fluoride in the Indian stones of the present study is likely to be related to the fact that certain areas in India have an average fluoride concentration of 16 mg/l in the drinking water [13] which far exceeds the level of 1–2 mg/l recommended by the WHO [27]. Detailed clinical, nutritional, compositional and biochemical data, will possibly shed some light on the role of fluoride in the pathogenesis of these calculi.

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References

1. Anasuya A (1982) Role of fluoride in the formation of urinary calculi: studies in rats. J Nutr 112:1787–1795
2. Auermann E, Kühn H (1971) Fluoride content of kidney stones. Fluoride 4:150–151
3. Campbell AD (1987) Determination of fluoride in various matrices. Pure Appl Chem 59:695–702
4. Evans L, Hoyle RD, Macaskill JB (1970) An accurate and rapid method of analysis for fluoride in phosphate rocks. N Z J Sci 13:143–148
5. Frant MS, Ross Jr JW (1966) Electrode for sensing fluoride ion acitvity in solution. Science 154:1553–1555
6. Hering F, Briellmann T, Seiler H, Rutishauser G (1985) Fluoridation of drinking water: effects on kidney stone formation. Urol Res 13:175–178
7. Hering F, Briellmann T, Seiler H, Rutishauser G (1985) Role of fluoride in formation of calcium oxalate stones. In: Schwille P, Smith L, Robertson W, Vahlensieck W (eds) Urolithiasis and related clinical research. Proceedings of the 5th International Symposium. Plunum Press, New York, pp 383–386
8. Herman JR (1956) Fluorine in urinary tract calculi. Proc Soc Exp Biol Med 91:189–191
9. Herman JR, Papadakis L (1960) The relationship of sodium fluoride to nephrolithiasis in rats. J Urol 83:799–800
10. Hesse A, Dietze H-J, Berg W, Hienzsch E (1977) Mass spectrometric trace element analysis of calcium oxalate uroliths. Eur Urol 3:359–361
11. Hesse A, Müller R, Schneider H-J, Taubert F (1978) Analytische Untersuchungen zur Bedeutung des Fluorgehaltes von Harnsteinen. Urologe [A] 17:207–210
12. Jennrich R, Mündle P (1983) Polynomial regression, P5R. In: Dixon WJ (ed) BMDP statistical software. University of California Press, Berkeley, pp 283–288
13. Jolly SS, Sharma O, Garg G, Sharma R (1980) Kidney changes and kidney stones in endemic fluorosis. Fluoride 13:10–16
14. Juuti M, Heinonen OP (1980) Incidence of urolithiasis and composition of household water in southern Finland. Scand J Urol Nephrol 14:181–187
15. Lagergren C (1956) Biophysical investigations of urinary calculi. Acta Radiol [Suppl] 133:1–71
16. Ljunghall S (1978) Regional variations in the incidence of urinary stones. Br Med J 1:439

17. Luoma H, Nuuja T, Collan Y, Nummikoski P (1976) The effect of magnesium and fluoride on nephrocalcinosis and aortic calcification in rats given high sucrose diets with added phosphates. Calcif Tissue Res 20:291–302
18. Meyer JL, Nancollas GH (1972) Effect of stannous and fluoride ions on the rate of crystal growth of hydroxyapatite. J Dent Res 51:1443–1450
19. Orion Research (1977) Analytical methods guide, 9th edn. Orion, Cambridge, p 4
20. Raby BA, Sunderland WE (1967) Direct determination of fluoride in tungsten using the fluoride electrode. Anal Chem 39:1304–1305
21. Sara R, Wänninen E (1975) Separation and determination of fluoride by diffusion with hexamethyldisiloxane and use of a fluoride sensitive electrode. Talanta 22:1033–1036
22. Shroy RE, Kraner HW, Jones KW, Jacobson JS, Heller LI (1982) Proton activation analysis for the measurement of fluorine in food samples. Anal Chem 54:407–413
23. Summers JL, Keitzer WA (1975) Effect of water fluoridation on urinary tract calculi. Ohio State Med J 71:25–27
24. Taves DR (1968) Separation of fluoride by rapid diffusion using hexamethyldisiloxane. Talanta 15:969–974
25. Tušl J (1970) Direct determination of fluoride in human urine using the fluoride electrode. Clin Chim Acta 27:216–218
26. Wandt MAE, Rodgers AL (1987) Fluoride concentrations in a collection of urinary calculi. J Urol 138:644–647
27. World Health Organization (1965) Normes internationales pour l'eau de boisson. Geneve
28. Zipkin I, Lee WA, Leone NC (1958) Flurode content of urinary and biliary tract calculi. Proc Soc Exp Biol Med 97:650–653

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