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BIOCHEMISTRY OF FLUOROSIS X - COMPARATIVE STUDY OF THE FLUORIDE LEVELS IN BIOLOGICAL FLUIDS

by

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SUMMARY: The fluoride content of drinking water is directly correlated with the serum and saliva fluoride. Serum fluoride levels are also directly related to those of saliva, but not to urinary fluoride.

These interrelations are so close that a mathematical formula can be devised for the calculation of fluoride in body fluids. For instance, a sample of saliva may serve for the quantification of serum fluoride. Whereas these interrelations are observed in general population groups they do not always apply to individuals.

Introduction

The object of our investigation was to determine up to what point the evaluation of fluoride levels in blood can be replaced by its quantification in other organ fluids. By this method venipuncture might be eliminated, the follow-up of patients and periodic studies on populations might be simplified We therefore analyzed the fluoride levels of serum, urine,

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and saliva in individuals residing in areas of low, medium and high fluoride concentrations in their drinking water and correlated the results.

Material and Methods

a) Methodology and instruments, for the sake of clarity, will be described for serum, urine, and saliva separately.

b) The biological samples were classified in accordance with the fluoride content of the drinking water of the 3 population groups, namely 10 - 13 healthy male and female adults, aged 20 to 60 years, residing in high fluoride zone 1: 2.6-8 ppm (area of Buena Esperanza in the South of the Province of San Luis, Argentina); medium fluoride zone 2: 1 ppm in the City of San Luis, Argentina and low fluoride zone 3: 0.1 ppm in the city of Buenos Aires, Argentina.

All subjects were well nourished and adhered to a balanced diet with respect to animal proteins, fats, and vitamins A and C. They had been residing for at least 6 years in their respective areas, the majority were born in area 1. Fasting blood samples of urine specimens and saliva were obtained simultaneously in every case. The serum was separated in polyethylene containers. Urine specimens were those first voided in the morning which we found equivalent to the 24-hour samples.

Saliva was also obtained in a fasting state, without stimulation, as follows: after thoroughly rinsing the mouth with deionized water, approximately 5 ml of total saliva was collected in a polyethylene container, during a period of approximately 10-15 min. All subjects were cautioned against the use of fluoridated toothpastes.

The difficulty of obtaining the samples (blood, saliva, and urine) simultaneously in each individual in order to correlate them with the fourth element, i.e.: the drinking water, necessitated a reduction in the number of subjects.

Experimental Data and Results

I. Serum Fluoride

Blood serum or plasma are adequate for fluoride determinations; they are more accurate than those obtained with whole blood. The level of fluoride in serum remains practically constant due to the action of regulating mechanisms (extracellular fluid, bone fixation, urinary excretion, etc.) (1-4). Most authors observed similar fluoride values in individuals drinking water, the fluoride content of which is low or medium (0.1 - 1 ppm)(5). However, the determination of the fluoride values in serum has generated much controversy among investigators (6-8).

We postulated that, depending upon the treatment to which the sample is submitted for the separation of fluoride, this element bound to proteins will be liberated to a greater or lesser degree and bring about an increase in the ionizable fraction (9). This, in our opinion, is the ex-

planation for the discordant results.

Methodology: Our modus operandi, was a reproduction of that proposed by different authors (10-16), adapted to our requirements. This enabled us to carry out determinations of ionized fluoride in blood by means of a selective electrode without prior blood deproteinization or microdiffusion.

Reagents and Apparatus: Standard F^- solution of 100 ppm F^-: dissolve 2.2105 g of NaF in distilled water and dilute to 1 liter.

Working F^- solutions: prepare solutions containing 0.1, 1, and 10ppm F^-. - Buffer solution to adjust ionic strength: TISAB (Orion), μ= 0.05; pH = 4.75 (20). - Sodium hydroxide 2.5 N. - Saturated solution of silicone fluid in hydrochloric acid 6N (Dow Corning 740 Fluid, 39 centistokes, Dow Corning, Midland, Michigan (14). - Magnesium oxide p.a. - Combination ion F^- electrode, model 96-09 (Orion). - Digital pH meter model 701 (Orion).- Lucite Conway cells (40 mm diameter) with covers of the same material.

Ionic F^- Determination: 1 ml serum is mixed with a similar value of TISAB in a small plastic tray. Evaluation is made with the electrode and the aid of calibration curves.

Table 1

Bueno Esperenza		San Luis		Bueno Aires	
Fluoride Content (ppm)					
Water	Serum	Water	Serum	Water	Serum
8.0	0.14	Home Water Supply 1 ppm	0.064	Home Water Supply 0.1 ppm	0.090
5.0	0.10		0.070		0.042
2.6	0.12		0.065		0.040
3.0	0.17		0.060		0.044
4.5	0.21		0.060		0.052
6.2	0.20		0.070		0.044
4.0	0.13		0.075		0.040
5.2	0.12		0.080		0.040
6.4	0.14		0.072		0.072
4.8	0.14		0.080		0.050
-	-	-	-	-	0.170
-	-	-	-	-	0.120
-	-	-	-	-	0.220
X=4.97	X=0.147	X-1	X=0.0696	X=0.1	X=0.078

Table 2

Bueno Esperenza		San Luis		Bueno Aires	
Fluoride Content (ppm)					
Water	Serum	Water	Serum	Water	Serum
8.0	0.25	Home Water Supply 1 ppm	0.13	Home Water Supply 0.1 ppm	0.18
5.0	0.20		0.12		0.10
2.6	0.24		0.12		0.12
3.0	0.30		0.10		0.10
4.5	0.38		0.12		0.10
6.2	0.38		0.13		0.12
4.0	0.23		0.15		0.10
5.2	0.24		0.16		0.12
6.4	0.25		0.13		0.18
4.8	0.26		0.15		0.12
-	-	-	-	-	0.30
-	-	-	-	-	0.29
-	-	-	-	-	0.40
$\bar{X}$=4.97	$\bar{X}$=0.273	$\bar{X}$=1	$\bar{X}$-0.131	$\bar{X}$=0.1	$\bar{X}$=0.171

In tables 1 - 5 all subjects had over 6 years residence in San Luis before the analysis and since birth in Buena Esperanza and Buenos Aires.

Total F^- Determination: Fundamentally the method consists of a) calcination, in order to destroy organic matter; b) microdiffusion, in order to separate fluoride; c) evaluation of separated fluoride by means of the selective electrode.

a) Calcination: 5 ml of serum and 75 mg magnesium oxide are placed in a platinum capsule. They are heated gently until volume is reduced by one-half, and placed in a 100°C. oven until dry, then heated in furnace for 30 minutes at 150°C., followed by 15 minutes at 300°C. and finally 90 minutes at 500°C.

b) Microdiffusion: Work is undertaken with lucite Conway cells prepared as follows:

Central chamber: 0.5 ml NaOH 2.5N. External chamber: add to all the ashes obtained during calcination 0.5 ml silicone solution in HCl 6N and 2 ml 50% perchloric acid. Diffusion time: 3 hrs. at room temperature.

c) Selective electrode readings: After microdiffusion, the contents of the central chamber are transferred quantitatively to a plastic tray, aided by 2-3 rinses with deionized water. Adjust pH to 7-7.2 with HCl (concentrated, IN and 0.IN, successively), arriving at final volume of 5ml with deionized water. Add 5 ml of TISAB, mix and proceed as in the case of ionic fluoride.

The values obtained for ionic and total F^- are shown in Tables 3 and 4.

Table 3

Buena Esperanza		San Luis		Bueno Aires	
Fluoride Content (ppm)					
Water	Saliva	Water	Saliva	Water	Saliva
8.0	0.12		0.055		0.079
5.0	0.10		0.062		0.058
2.6	0.10		0.058		0.040
3.0	0.14		0.050		0.050
4.5	0.15		0.060		0.100
6.2	0.16		0.060		0.060
4.0	0.10		0.065		0.060
5.2	0.15		0.065		0.032
6.4	0.15		0.075		0.050
4.8	0.15				0.080
-	-				0.150
-	-				0.078
$\bar{X}$=4.97	$\bar{X}$=0.132	$\bar{X}$=1	$\bar{X}$=0.060	$\bar{X}$=0.1	$\bar{X}$=0.068

Table 4

Sample N°	First Urine	24-hr. Urine
1	2.10	1.70
2	1.50	1.40
3	1.80	1.40
4	1.40	1.00
5	0.94	1.10
6	3.30	3.00
7	1.60	1.50
8	1.50	1.20
9	1.70	1.60
10	2.00	1.80
	$\bar{X}$=1.78	$\bar{X}$=1.57

II. Fluoride in Saliva

There is a wide discrepancy between the values given in the literature for salivary fluoride which range between 0.00 and 0.75 ppm (21-25). Some authors have used total saliva, others parotid saliva after stimulation. Table 3 presents the values obtained by us. Some investigators, however, have found no appreciable difference in fluoride excretion under normal conditions or after stimulation (26).

We worked with samples of spontaneously eliminated total saliva in order to facilitate extraction and in order to abide by the aim of our investigation i.e. to simplify the determinations and to render them useful for large scale population controls. For the serum we refer to ionic and total fluoride content, for saliva we refer solely to ionic fluoride. Because of the low protein content of saliva, equivalent values were obtained in the direct determinations with the selective electrode as compared with those established after calcination.

The method used was that of Gron et al. (27), the reagents and instruments were the same as those used for the determination of F^- in blood. Five ml of saliva is mixed with an equal amount of TISAB in a plastic dish and shaken.

III. Urinary Fluoride

Urinary fluoride excretion is utilized to determine the degree of danger to which workmen are being exposed. Here again the values reported in the literature are highly variable since, in addition to the analytical methods employed, certain factors affect the results such as former and duration of exposure, age, pregnancy, kidney disease, etc. Fluoride concentrations in urine vary from hour to hour and from subject to subject. The influence of the factors responsible for the inconsistent values, has been confirmed once more in our investigation, and we were unable to find a mathematical formula to express the urine/blood/saliva relationship. The values obtained by us corresponded to the first specimen of the morning because we noted a close relationship between this sample and 24-hour specimens (Table 6). As in the case of saliva, it was impossible to distinguish between ionic and total fluoride due to the low protein content of normal urine.

The method used was an adaptation of that proposed by Neefus et al. (28), Cernik et al. (29), Tusl et al. (30), Singer et al. (31), and Mu-Wan Sun (32).

Reagents and Instruments: See Serum Fluoride.

Determination: In a polyethylene tray, mix 3-5 ml of urine with an equal amount of TISAB, and proceed as with the previous determinations. The values obtained are shown in Table 5. The fluoride concentration in water was established by direct measurement with the selective electrode.

Table 5

Buena Esperanza		San Luis		Buenos Aires	
Fluoride Content (ppm)					
Water	Urine	Water	Urine	Water	Urine
5.00	3.30	Home Water Supply 1 ppm	2.20	Home Water Supply 0.1 ppm	1.70
2.60	3.70		1.60		1.50
3.00	2.20		1.90		0.90
4.50	5.80		2.00		0.85
6.20	5.20		2.10		0.84
4.00	4.80		1.20		0.50
5.20	5.80		1.50		1.30
6.40	7.20		2.10		0.60
4.80	5.60		1.80		1.20
-	-		1.10		0.90
-	-		-		0.32
-	-		-		0.68
-	-		-		0.42
$\bar{X}$ = 4.63	$\bar{X}$ = 4.84	$\bar{X}$ = 1	$\bar{X}$ = 1.75	$\bar{X}$ = 0.1	$\bar{X}$ = 0.90

IV. Mathematic Interrelations

From the statistical study of the values given in the tables, based on the method of minimum squares, we were able to draw up the formula given for each particular case:

IONIC F^- /TOTAL F^- RELATION IN SERUM (TABLES 1 AND 2)

In areas of high (2.6-8 ppm) concentration in water:

Total F^- = 0.0232 + 1.70 x ionic F^- ± 0.0112 (S_{yx}); Correlation coefficient (r) = 0.98; Significant at: $p << 0.01$

In areas of medium (1 ppm) concentration:

Total F^- = 0.022 + 1.92 x ionic F^- ± 0.078 (S_{yx}); Correlation coefficient (r) = 0.89; Significant at: $p << 0.01$.

In areas of low (0.1 ppm) concentration:

Total F^- = 0.04 + 1.64 x ionic F^- ± 0.02 (S_{yx}); Correlation coefficient (r) = 0.98; Significant at: $p << 0.01$

Likewise a mathematic formula can be deduced from the extreme values (0.1-8 ppm) which makes an acceptable calculation possible ($p << 0.01$) of the concentration of total F^- from the ionic F^-.

Total F^- = 0.019 + 1.74 x ionic F^- ± 0.02 (S_{yx}); Correlation coefficient (r) = 0.99; Significant at: p <<0.01.

A study of the values obtained reveals a close relationship between ionic fluoride and total fluoride in serum, represented by a mathematical equation. Therefore, if the ionic fluoride values are known, it is possible to calculate the amount of total fluoride with a highly significant p value in each of the three groups included in our investigation. Depending upon the fluoride content in drinking water, one need only to apply the corresponding formula, although the following general one makes a close evaluation of the concentration of total fluoride in serum possible.

SERUM F^-/SALIVA F^- RELATION (TABLES 1,2,3)

IONIC SERUM F^-/SALIVA F^-

In areas with a high (2.6-8 ppm) concentration in water:
Ionic F^- = 0.022 + 0.944 x saliva F^- ± 0.026 (S_{yx}); Correlation coefficient (r) = 0.65; Significant at p = 0.05

In areas of medium (1 ppm) concentration in water:
Ionic F^- = 0.019 + 0.859 x saliva F^- ± 0.003 (S_{yx}); Correlation coefficient (r) = 0.91; Significant at p <<0.01

In areas of low (0.1 ppm) concentration:
Ionic F^- = 0.995 x saliva F^- ±0.023 (S_{yx}); Correlation coefficient (r) = 0.806; Significant at p <0.025.

SERUM F^-/SALIVA F^-

Areas with high (2.6-8 ppm) concentration:
Total F^- = 0.05 + 1.67 x saliva F^- ± 0.04 (S_{yx}); Correlation coefficient (r) = 0.66; Significant at p <0.05.

Areas with medium (1 ppm) concentration:
Total F^- = 0.03 + 1.67 x saliva F^- ± 0.01 (S_{yx}); Correlation coefficient (r) = 0.70; Significant at p <0.025

Areas with low (0.1 ppm) concentration:
Total F^- = 0.06 + 1.41 x saliva F^- ± 0.05 (S_{yx}); Correlation coefficient (r) = 0.63; Significant at p <0.025

Evidently it is possible to calculate with a high degree of significance the value of ionic and/or total fluoride in blood serum if one knows the saliva fluoride concentration.

SERUM F^-/URINE F^- (TABLES 1,2,5)

The statistical study is not indicative of a relation between the levels of serum and urinary fluoride in any of the six possibilities under analysis. Consequently, it is not possible to calculate the amount of fluoride in serum from the amount in urine or vice versa.

SERUM F^-/DRINKING WATER F^- (TABLES 1,2)

We have calculated a general formula with respect to 0.1-8ppm fluoride in drinking water.

IONIC SERUM F^-/DRINKING WATER F^-

Ionic F^- = 0.072 + 0.0134 x water F^- $\pm$ 0.024 (S_{yx}); Correlation coefficient (r) = 0.58; Significant at $p < 0.001$

TOTAL SERUM F^-/DRINKING WATER F^-

Total serum F^- = 0.1509 + 0.0211 x water F^- $\pm$0.075 (S_{yx}); Correlation coefficient (r) = 0.54; Significant at $p < 0.01$

It is thus possible to calculate the concentration of ionic or total fluoride in serum when their values in drinking water are known, even in the presence of variations such as those ranging between 0.1 and 8 ppm in the drinking water. Higher concentrations were not investigated.

SALIVA F^-/URINE F^- (TABLES 3,5)

No mathematical relation was found for the values under study.

SALIVA F^-/DRINKING WATER F^- (TABLE 3)

The values of the three areas are likewise condensed in a single formula. Saliva F^- = 0.0607 + 0.0130 x water F^- $\pm$ 0.025 (S_{yx}); Correlation coefficient (r) = 0.76; Significant at $p << 0.001$

A close relation was found between the fluoride consumed with drinking water and that eliminated with saliva. Thus, saliva fluoride can be quantified with reasonable certainty when the fluoride content of the drinking water of a given individual is known.

URINE F^-/DRINKING WATER F^- (TABLE 5)

In this case as well, the three relations studied are considered in a single formula:

Urine F^- = 0.84 + 0.865 x water F^- $\pm$1.00 (S_{yx}); Correlation coefficient (r) = 0.94; Significant at $p << 0.01$

The above statistical study makes it possible to present a formula by means of which the amount of fluoride in the urine based on the fluoride content of drinking water can be determined with a $p << 0.01$ accuracy.

RELATION BETWEEN 24-HR. URINE AND THE FIRST MORNING SPECIMEN(FVM) (TABLE 4)

Urinary F^- (24-hr) = 0.026 + 0.8655 x FVM $\pm$ 0.173 (S_{yx}): Correlation coefficient (r) = 0.96; Significant at $p < 0.001$

A highly significant correlation exists between the 24-hr. urinary fluoride content and that of the first morning micturition. Therefore we conclude that the relationship is sufficient to determine the amount of fluoride in the latter.

Conclusions

Fluoride levels in drinking water are closely related to those in blood serum, urine and saliva. There is also a correlation between the fluoride content of the different biological fluids with the exception of urinary fluoride which is not related. In the serum a very close correlation between ionic and total fluoride was found.

The above correlations are so close that a mathematical formula can be worked out to enable the calculation of fluoride in urine, saliva and blood of a given individual provided the fluoride content of drinking water is known. Inversely, it is possible to calculate fluoride levels in drinking water or in the body fluids other than urine, when the levels of any of the others are known. As an alternative, samples of saliva may be used instead of blood for the quantification of serum fluoride. The first morning urinary specimen can be used instead of the total 24-hr. specimen.

Quantification of total fluoride in serum is possible with a sample of only 1 ml upon direct electrode evaluation of ionic fluoride, and the application of the corresponding formula , with an accuracy of $p << 0.01$.

Whereas, in general, the above-outlined interrelations exist because of individual differences from person to person, discretion in the application of these formulas should be used when dealing with individual patients.

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