

Serum Ionic Fluoride: Normal Range and Relationship to Age and Sex

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We used the Orion fluoride electrode system to determine the normal range of serum ionic fluoride concentrations and to investigate its relationship to sex and age (A). 87 normal men, age 18-92 years (mean, 46 years), and 49 normal women, age 19-64 years (mean, 38 years), participated in the study. At the 95% confidence limits, males <45 years old had a normal range of 0.29 to 1.52 $\mu\text{mol/litre}$ and males ≥ 45 years old 0.29 + 0.0101 (A-45) to 1.52 + 0.0101 (A-45) $\mu\text{mol/litre}$. Females, however, had a normal range of 0.022A - 0.32 to 0.022A + 1.07 $\mu\text{mol/litre}$. A group of 51 men 18-44 years old was compared with a group of 36 men 46-92 years old. The mean serum F^- of the older group was shown to be significantly greater ($P < 0.01$) than that of the younger group. Factors related to serum ionic fluoride values are (a) tea as an important source of dietary F^- , (b) the lack of significant variation during daytime hours, and (c) the lack of significant difference in concentration between serum and plasma F^- .

Concentrations of serum constituents in normal people are in some cases affected by sex and age (1). Recently, in determining the normal range of serum ionic fluoride in groups of males and females of different ages, we also noted that age and sex exerted some influence. Although chemical methods (2-5) for measuring serum fluoride have been known for over 25 years, it is only since 1968 that it has been recognized that fluoride in human serum is partly ionized (6, 7)—chiefly because an instrument for conveniently measuring ionic fluoride (8-9) became commercially available in 1966. The importance of serum ionic fluoride measurements is further emphasized in view of the clinical use of fluoride as a treatment for osteoporosis, either alone (10, 11) or in combination with other substances such as calcium and vitamin D (12).

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Materials and Methods

Apparatus

We measured ionic fluoride with an ion-selective electrode system (Orion Research Inc., Cambridge, Mass. 02139) consisting of a Model 94-09 Fluoride Electrode, a Model 90-01 single-junction Ag/AgCl reference electrode, and a Model 701 digital millivolt meter.

Pipettes and flasks. The use of glass Pasteur pipettes (D. R. Taves, personal communication) leads to fluoride contamination and appreciably higher values for fluoride in serum. Standard fluoride solutions and specimens were measured with Falcon disposable plastic pipettes (Falcon, Division of Becton-Dickinson & Co., Oxnard, Calif.) but since results were the same with well acid-washed Pyrex volumetric class-A flasks as with plastic volumetric flasks, we used the former for preparing the series of standard solutions.

Reagents

Water used in preparing reagents and in the methodology had passed through a Barnstead Model D 0800 Bantam Cartridge holder containing a No. D0809 Ultrapure (mixed bed) cartridge. All reagents were stored in well-capped plastic containers.

Total ionic-strength adjustment buffer, Orion No. 94-09.

Concentrated stock standard fluoride solution (100 000 $\mu\text{mol/litre}$). Dissolve 4.1990 g of "Baker Analyzed" NaF (previously dried at 105 °C) in water, quantitatively transfer to a 1-litre volumetric flask, dilute to the mark, and mix well.

Diluted stock standard fluoride solutions (1000, 700, 500, 200, 100, 50, and 20 $\mu\text{mol/litre}$). Pipette 10, 7, 5, 2, and 1 ml of the concentrated stock standard into 1-litre volumetric flasks, dilute to the mark, and mix well. Pipette 2 ml of the concentrated stock standard into a 4-litre volumetric flask, dilute to the mark, and mix well. Pipette 10 ml of the 200 $\mu\text{mol/litre}$ standard into a 100-ml volumetric flask, dilute to the mark, and mix well.

Diluted working fluoride standard solutions (0.5, 0.7, 1, 2, 3, 5, 7, and 10 $\mu\text{mol/litre}$). Prepare according to the following protocol:

Concn of stock std, $\mu\text{mol/litre}$	Stock solution	Dilute to ml	Concn of resulting working stds, $\mu\text{mol/litre}$
1000	1.0	100	10
700	1.0	100	7
500	1.0	100	5
100	3.0	100	3
20	10.0	100	2
100	1.0	100	1
7	10.0	100	0.7
50	1.0	100	0.5

Prepare all diluted working standards freshly every three weeks.

Procedure

Collect 10–15 ml of blood with a disposable plastic syringe and transfer to a plastic tube (Falcon disposable plastic tube No. 2001 with snap cap, 17×100 mm). After the blood clots, dislodge the clot with a plastic rod and centrifuge the tube. Transfer the supernatant serum to another capped plastic tube with a 2-ml plastic pipette. Either analyze the specimen immediately or store it at -10°C in a freezer, where it will be stable for at least a month. Pipette 2-ml quantities of the dilute fluoride working standards (or specimens) into a "NALGENE" hollow No. 7 polyethylene stopper (No. S9066-7). Add an equal volume of the buffer, to buffer the sample to pH 5–6 and to maintain a total ionic strength that is relatively independent of individual specimen ionic variations (9). Place the plastic stopper, containing a small (13×3 mm) bar magnet on a heat-insulating cork mat set on a magnetic stirrer. Then carefully lower the electrodes into the solution so that they do not touch the rotating magnetic bar, the speed of which has been suitably adjusted. Equilibrium is attained and at 5 min the digital reading is noted. Rinse the electrodes with water from a wash bottle (washings collected in a beaker) and then blot dry with tissue paper before re-use. After measuring F^- in a specimen that has a high ionic fluoride concentration, run a 1 $\mu\text{mol/litre}$ standard solution before analyzing the next sample. In using the dilute working standards to calibrate the instrument, go from the most dilute one (0.5 $\mu\text{mol/litre}$) to the most concentrated. Prepare the

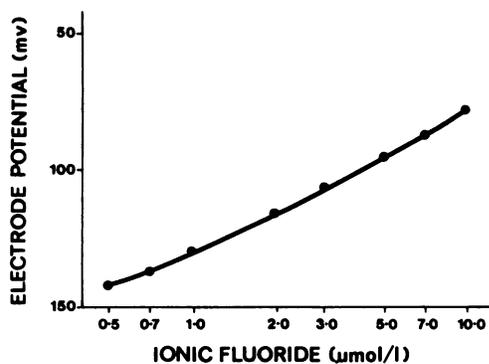


Fig. 1. Calibration curve for ionic fluoride concentration

working calibration curve (Figure 1) on two-cycle semilogarithmic paper. Read serum ionic fluoride values from this curve. Make measurements (both standards and specimens) in duplicate, average only those values not differing by more than 2 mV, and use the mean value. The graph is linear from 10 to 500 $\mu\text{mol/litre}$ with a nernstian gradient of 59 ± 1 mV per 10-fold change in concentration. Below 10 $\mu\text{mol/litre}$ (0.5–10 $\mu\text{mol/litre}$), we found a nonlinear response with an upward trend, attributable to a decrease in the sensitivity of the electrode. Temperature changes greater than 1°C during a series of measurements usually necessitate recalibration of the system.

Results

Precision. The within-day precision of the method involving duplicate measurements on 87 different sera (with values in the normal range) and 84 different sera (with values in the pathological range) is indicated by CV's of 6.9 and 4.5%, respectively. The between-day precision (CV) in the normal ($n = 14$) and pathological ranges ($n = 20$) were 6.8 and 4.8%, respectively. The mean analytical recovery of fluoride added to serum ($n = 24$ additions) was 99% (104–94%).

Subjects. The nonfasting normal male subjects consisted of 87 men, 18–92 years old (mean age, 46 years). Four were staff members, 65 were Red Cross blood donors, and 18 were residents of a home for the aged. The last were 69–92 years of age, ambulatory, and in apparent good health. They were selected by an internist specializing in geriatrics (C.G.). None had any clinical condition related to bone or kidney disease and all had normal values for serum creatinine (13, 14) and alkaline phosphatase (1, 15) (none exceeded 15 King-Armstrong units/100 ml) for their age. None was on medication known to influence serum ionic fluoride concentrations.

The 49 female subjects' ages ranged from 19–64 years (mean age, 38 years) and consisted of 25 staff members and 24 blood donors. None of the staff members had clinical histories of bone or renal disease.

The frequency distribution of the 136 subjects' ages, in decades, were as follows: 18–27: (21♂, 15♀), 28–37: (23♂, 6♀), 38–47: (10♂, 11♀), 48–57: (9♂, 14♀), 58–67: (6♂, 3♀), 68–77: (4♂), 78–87: (9♂), and 88–97: (5♂).

Age-related effects. Figure 2A and B relates the serum ionic fluoride concentrations to the ages of 87 normal men (ages 18–92 years) and 49 normal women (ages 19–64 years), respectively. The ranges we found for serum ionic fluoride were for men, 0.5–2.0 $\mu\text{mol/litre}$, and for women, 0.5–2.3 $\mu\text{mol/litre}$. As Figure 2A shows, for men <45 years old, the serum ionic fluoride concentration is relatively constant—i.e., unrelated to age—with a mean value of 0.906 ± 0.306 (SE) $\mu\text{mol/litre}$. However, after age 45 years, the serum ionic fluoride concentration tends to be linearly correlated with age. For men ≥ 45 years old, the serum ionic fluoride concentration relationship is given by the equation $0.906 + (0.0101)(A-45) \pm 0.306$ (SE) $\mu\text{mol/litre}$, where A is the age in years and SE is the standard error of the mean.

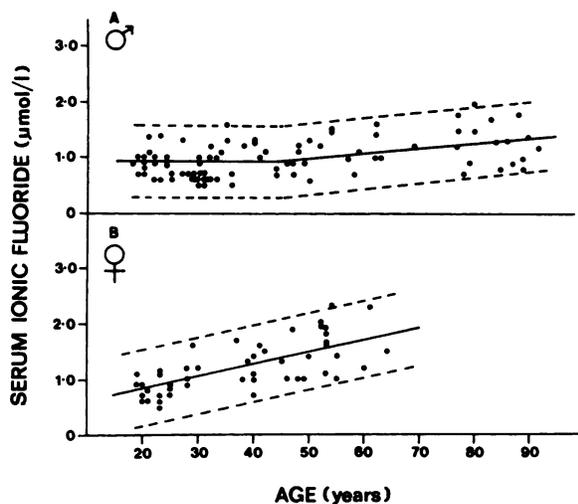


Fig. 2. Relationship between serum ionic fluoride ($\mu\text{mol/litre}$) and age (years), in 87 men (A) and 49 women (B)

At the 95% confidence limit, the normal range for men younger than 45 is therefore 0.294 to 1.518 $\mu\text{mol/litre}$ and for men ≥ 45 years of age, $0.294 + (0.0101)(A-45)$ to $1.518 + (0.0101)(A-45)$ $\mu\text{mol/litre}$.

We compared two groups of males. Because the lines relating serum ionic fluoride concentration with age intersected at age 45, these two groups were divided in the following manner. Group I consisted of 51 men, age 18–44 years, having a mean serum ionic fluoride of 0.876 ± 0.275 (SD) $\mu\text{mol/litre}$; Group II consisted of 36 men, 46–92 years of age, having a mean serum ionic fluoride of 1.183 ± 0.350 (SD) $\mu\text{mol/litre}$. The mean serum ionic fluoride value of the older group exceeded ($P < 0.01$) that of the younger group. In the case of women, however, Figure 2B indicates a quite different situation. A simple linear regression correlates serum ionic fluoride concentration [F] and age (A) by the equation $[F] = 0.375 + 0.022A$. The coefficient of correlation (r) is 0.66, with a SE of 0.345 and $P < 0.001$. The minimum and maximum values for serum ionic fluoride in normal women at different ages (95% confidence limit) are therefore $(0.022A - 0.315)$ and $(0.022A + 1.065)$ $\mu\text{mol/litre}$, respectively.

Effects of time of day and food intake. Experiments were designed to indicate (a) whether serum ionic flu-

oride concentrations varied during the laboratory working day, (b) whether values for serum and plasma differ, and (c) values for ionic fluoride concentrations in whole blood.

The subjects, three men and three women, fasted from midnight of the previous day to 1530 hours of the day of the experiment. During the study, no fluids other than de-ionized water were consumed. At 0800, 1200, and 1530 hours, we collected 12-ml specimens of clotted blood in plastic tubes. Additionally, at 0800 hours, 15 ml of heparinized blood was collected into each of two plastic tubes. The mean serum ionic fluoride concentration showed (by analysis of variance) no statistically significant variation during the period 0800 to 1530 hours ($P > 0.05$) when the subjects were fasting (Table 1).

Plasma vs. serum. No significant difference was noted in the mean concentrations of fasting serum and plasma ionic fluorides ($P > 0.05$) derived from the same blood specimen. Ionic fluoride in whole blood (heparinized) showed a mean of 1.50 $\mu\text{mol/litre}$ (range, 0.9–2.2 $\mu\text{mol/litre}$) in five fasting subjects.

Discussion

The literature contains many references to normal values for serum (or plasma) fluoride in humans, but they differ widely. Some authors (6, 7, 16) indicate that only 10–20% of the total fluoride in human serum is inorganic fluoride ion, and not bound to serum protein. The values quoted in the earlier literature were based on chemical methods (diffusion, distillation, or ashing, followed by spectrophotometry) that measured total (i.e., both nonionic and ionic) fluoride. Consequently, these values are appreciably greater (17) than values for F^- in plasma (or serum). Furthermore, if an arm tourniquet is used or if the subject is in an upright posture during blood collection, these factors probably contribute to an artificially high concentration for total (but not ionic) serum fluoride. Modern ion-selective electrode methods (18) [and the diffusion-fluorometric chemical method of Taves (5)] specifically measure ionic fluoride. Table 2 shows some representative published values for serum ionic fluoride (17, 19–23). Of these studies, only one (23) was based on fasting specimens and only one (22) indicated the sex of the

Table 1. Ionic Fluoride Concentrations in Six Fasting Subjects: Variation during the Day, and Concentrations in Serum, Plasma, and Whole Blood

Sex	Age, y	Daytime variation			Serum F^- , $\mu\text{mol/litre}$	Plasma	Whole blood
		0800 h	1200 h	1530 h			
♂	63	1.0	1.2	1.3	1.0	1.4	0.9
♀	41	1.1	0.7	0.9	1.1	1.1	1.0
♂	58	1.9	2.3	—	1.9	2.0	1.6
♀	57	1.9	1.9	2.6	1.9	2.2	1.8
♀	55	—	2.2	2.3	—	2.4	2.2
♂	72	2.9	2.7	2.7	2.9	3.1	—
	Av	1.75	1.83	1.96	1.76	2.03	1.50

Table 2. Reported Normal Serum Fluoride Ranges in Relation to Sex, Age, and Water Fluoridation

Ref. ^{a,b}	n	Sex	Age range (mean), ^a	Water fluoridation, mg/litre	Range, $\mu\text{mol/litre}$
17	16	—	—	—	0–1.5 ^c
19	14	—	—	0.15	1.4–5.2
	28	—	—	3.80	3.0–14.6
20	26	—	—	1.0	0.84–2.90
21	12	—	—	0.1	0.68–1.74 ^c
22	20	11♂ 9♀	23–71 (34)	0.18	0.31–0.99
23	41	—	17–82 (52)	optimal fluoridated	1–5.9
This paper	136	87♂ 49♀	18–92 (46) 19–64 (38)	1.0	0.5–2.0 0.5–2.3

^a Serum specimens: ref. 19, 20, this paper. Plasma specimens: ref. 17, 21–23.

^b Chemical method (17). Fluoride electrode method (19–23, this paper).

^c Calculated statistically and based on 95% confidence limits.

subjects. The data in Table 2 are mainly actual ranges, not statistically summarized values. They show some variations in values that may be due to (e.g.) fluoride content of the drinking water, fluoride content of the diet, and specimen contamination.

Several investigators (10, 19, 24) have shown that the serum ionic fluoride concentration increases with increasing concentration of fluoride in the drinking water, which has been shown to vary from 0–5.44 mg/litre (25).

The diet, *exclusive of drinking water*, also contributes to the total fluoride input of the individual, particularly if it contains fish, tea, and certain wines. Consequently the diet can also influence serum ionic fluoride values. Several reports (26–28) deal with the fluoride content of foodstuffs. Kramer et al. (26) showed that fluoride present in foodstuffs varied two- to threefold in different geographical areas of the U.S.A. Other reports indicate appreciable dietary variations, from about 10–25 $\mu\text{mol/day}$ (27, 28) to 85–170 $\mu\text{mol/day}$ (26). However, these data represent total, not ionic, fluoride. A British report (29) indicates an appreciable ionic fluoride contribution from tea drinking (27 μmol of F^- per cup), a finding consistent with our own determination of 25.6 μmol of F^- in a cup of “strong tea” with no added milk or sugar.

Finally, high serum ionic fluoride values could also arise in measurements where contamination might originate from the use of glassware, e.g., disposable glass Pasteur pipettes.

Several studies relate plasma fluoride with age and sex. Hanhijarvi et al. (24) studied 2000 people, age 1–80 years, sex unspecified, and found increased plasma ionic fluoride values with increases in both the fluoride concentration in drinking water and with age; mean plasma ionic fluoride values 0.7 and 1.4 $\mu\text{mol/litre}$ were associated with nonfluoridated and artificially fluoridated drinking water, respectively. They also found that plasma ionic fluoride increased with renal impairment (as reflected by increased serum creatinine).

Fuchs et al. (22), using ion-selective electrodes and the known-addition technique, found a range for plasma ionic fluoride of 0.31–0.99 $\mu\text{mol/litre}$ in a group of 11 men and nine women whose ages ranged from 23–71 years (mean, 34 years). They found no sex-related difference statistically. However, the fluoride content of the drinking water was quite low (0.18 mg/litre) and the numbers of male and female subjects studied were insufficient for the results to be conclusive.

Parkins et al. (23) used the ion-selective electrode to study the fluoride content in plasma from fasting subjects and in biopsies of the iliac crest, obtained at autopsy. They showed correlations among plasma fluoride, bone fluoride, and age. They concluded that their data indicated an interrelationship between bone and plasma fluoride concentrations and that plasma fluoride, measured in fasting individuals, increases with increasing bone fluoride. However, they also did not indicate the numbers of males and females studied.

A re-examination of Figure 2, A and B, indicates that the essential difference between males and females in the age-related change in serum ionic fluoride concentration is the apparent constant rate of increase in the serum ionic fluoride concentration of women during the ages 19 to 64 years. Men, on the other hand, appear to have a serum ionic fluoride concentration that remains constant from 18 years to 45 years of age. From 45 to 92 years, however, it increases with age at a constant rate. The rate of change of serum ionic fluoride concentration with age for women (22 nmol/litre per year) is about twice that for men over 45 years of age (10 nmol/litre per year). The intersection of the mean lines relating serum ionic fluoride concentration with age at age 45 is not entirely surprising. Renal function decreases with age (30) and, although the actual age at which the first marked decrease in glomerular filtration rate (inulin clearance) is observed is uncertain, according to the literature, the data of Davies and Shock (31) and Figure 1A suggest that it lies between the 4th and 5th decade.

Three factors (or some combination of them) could be responsible for the observed increase with age of serum ionic fluoride: increased intake, decreased excretion, and increased release of previously deposited bone fluoride. Perhaps older people take more fluoride in their diet (e.g., drink more tea) than do younger subjects. Despite the relative constancy of serum creatinine with age (13, 14) a decrease in glomerular filtration rate nonetheless occurs (30). This decrease in kidney function may be responsible for the increase in serum fluoride after the age of 45 years observed in both men and women. Although fluoride clearance data were not obtained in our study, the experiments of both Carlson et al. (32) with humans and Chen et al. (33) with dogs yielded fluoride clearances smaller than their creatinine clearances. The greater rate of increase of serum ionic fluoride with age, noted in females and referred to above, is probably due to the enhanced release of F^- from bone observed after the menopause (34). The difference between men and women in the age group below 45 years is somewhat difficult to explain. The smaller creatinine clearance observed in females (35) or diminished deposition of fluoride in bone, or both, may be responsible.

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