Concentrations of Ionic, Total, and Bound Fluoride in Plasma

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We found no significant difference between the means for ionic, bound, and total fluoride concentrations in the plasma of male and female subjects of the same age, living in a community with fluoridated water. When results for the 264 fasting subjects were therefore combined according to age, they indicated that persons over 60 years of age have a significantly higher mean ionic (3.89 \( \mu \)mol/L) and total (6.58 \( \mu \)mol/L) fluoride concentration in plasma than do younger age groups. For younger age groups, means ranged from 2.74 to 3.05 \( \mu \)mol/L for ionic fluoride and from 4.74 to 5.58 \( \mu \)mol/L for total. The bound fluoride concentration was lower in individuals 21 to 30 years of age (1.89 \( \mu \)mol/L) than in older age groups (for whom means ranged from 2.42 to 2.68 \( \mu \)mol/L), but was not significantly different from that of individuals who were younger (2.21 \( \mu \)mol/L). A tendency for the mean ionic fluoride concentration to increase with age was noted, but the concentration was significantly higher than the preceding decade group only in those persons over 60 years of age.

Additional Keyphrases: normal values • ion-selective electrodes • age-related effects

The range and average concentration of ionic, bound, and total fluoride in plasma are difficult to delineate unless environmental and biological factors that may influence the fluoride content are specified. Results of an extensive study (1) suggest that there is a tendency for the ionic fluoride concentration in plasma to increase with age. Mean fluoride concentrations of 0.7 and 1.4 \( \mu \)mol/L were associated with populations consuming nonfluoridated and fluoridated water, respectively. Husdan et al. (2), in a similar study, reported "normal ranges" for the concentration of ionic fluoride in serum and found that the concentration increased with age; the fluoride-intake history of the subjects in the investigation was not well defined. No sex-related difference in ionic fluoride concentration was found in a very limited study by Fuchs et al. (3). Parkins et al. (4), examining the plasma of patients 17 to 82 years of age who resided in an area with fluoridated water (but for whom the period of residency was not reported), found a range of 1.0 to 5.89 \( \mu \)mol/L for ionic fluoride. In another study, the mean total fluoride concentrations in the plasma of individuals who had consumed water from the same supply (fluoride content within the range 0.15 to 2.5 mg/L) for at least five years were shown to be similar to one another; concentration in plasma was only slightly higher when the water supply contained 5.6 mg/L (5). The age and sex of the persons in the study were neither indicated nor considered.

Guy et al. (6) recently reported data on ionic and bound fluoride concentrations in 106 plasma samples from residents of five cities whose water supplies contain from 0.1 to 5.8 mg of fluoride per liter. The mean concentrations of ionic fluoride ranged from 0.38 to 4.3 \( \mu \)mol/L and were directly related to the fluoride concentration in the water supply. Bound fluoride in the plasma of these individuals averaged 1.35 \( \mu \)mol/L and was not related to the fluoride concentration in the drinking water.

We have made an extensive study of persons who have consumed fluoridated water (1 mg/L) for over a year, to determine the normal ranges for the fluoride fractions in plasma in males and females of different ages.

Materials and Methods

Apparatus

The Orion Model 94-09 ion-selective electrode for fluoride (Orion Research, Inc., Cambridge, MA 02139) and a conventional potassium chloride reference electrode (Corning Glass Works, Medfield, MA 02052) were used with an Orion Research Ionanalyzer, Model 801 digital pH meter, to measure fluoride concentration.

Polypropylene Conway cells in the Obrink form, 68 mm in diameter (Biological Research, Inc., St. Louis, MO 63155), were used for diffusion. During diffusion, the Conway cells were covered with 100 \( \times \) 15 mm polystyrene Petri dishes (Falcon Plastics, Los Angeles, CA 90045).

Reagents

Perchloric acid, 700 g/L, double vacuum-distilled (G. Frederick Smith Co., Columbus, OH 43223). Prepare 500 and 250 g/L solutions.

Redistilled water. De-ionized water from a central supply was passed through a column (36 \( \times \) 3.4 cm) of filter-ion mixed-bed resin (Illinois Water Treatment Co., Rockford, IL 61105). The water was then redistilled from an all-glass still and stored in polyethylene bottles. This water was used to prepare all reagents and sample dilutions.

Buffer A. Prepare a solution containing 100 mL of 0.5 mol/L NaOH, 12 mL of 2.25 mol/L HCl, and 10 mL of 1 mol/L acetic acid. Adjust the pH to 5.0 with 2.25 mol/L HCl and dilute to 1 L.

Buffer B. Dissolve 6.4 g of reagent-grade sodium chloride in 500 mL of 0.05 mol/L acetate buffer (pH 5.0), check and adjust the pH, and dilute to 1 L with acetate buffer. Final concentrations: 0.11 mol of sodium chloride and 0.05 mol of acetate buffer per liter.

Standard fluoride solutions. Prepare stock solutions in Buffer A and in Buffer B to contain 100 mg of fluoride per liter by dissolving 221.0 mg of reagent-grade NaF per liter. Also

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prepare a more dilute stock solution containing 1 mg of fluoride per liter by diluting with the appropriate buffer. Prepare working standards to contain 10 to 200 μg/L in the appropriate buffer. Store all solutions in polyethylene bottles.

*Calcium phosphate (low in fluoride).* Prepare calcium phosphate by the method described by Singer and Ophaug (7).

**Collection of Specimens**

From each of 264 donors we collected 12–15 mL of blood in evacuated tubes that contained powdered ethylenediaminetetraacetic acid as anticoagulant. The donors, who had fasted for 12 h, lived in Richfield, MN, a community with a municipal water supply containing 1 mg of fluoride per liter. Blood was drawn while each donor was seated, after a tourniquet had been removed. The plasma obtained by centrifugation was stored frozen until analysis. Together, the anticoagulant and collection tube gave a blank value of 0.032 μg ± 0.0064 μg (12 trials) of fluoride.

**Procedures**

Ionic fluoride was determined at pH 5.0 in 1–2 mL of plasma diluted with 1–3 mL of Buffer B. Standard fluoride solutions were prepared in Buffer B, and the fluoride concentration of the unknowns and standards was measured at equilibrium.

The total fluoride content of the plasma was determined in samples (3–4 mL) that were ashed at 500 °C (8) after addition of 10 mg of low-fluoride Ca₃(PO₄)₂ as a fluoride fixative. The fluoride in the ash was isolated by diffusion (9) and determined colorimetrically (8) or with the ion-selective electrode for fluoride. When the fluoride electrode method was used, as was the case for most determinations, the diffusates (which were in 500 μL of 0.5 mol/L NaOH) were removed and neutralized with 2.25 mol/L HCl (70–80 μL). The solutions were then buffered to pH 5.0 with 1 mol/L acetic acid (90–110 μL) and diluted to a total volume of 5 mL. Blanks and analytical recovery-assessment (0.25 to 0.30 μg of fluoride) samples were taken through the entire procedure. Fluoride standard solutions covering the range of fluoride found in the plasma (0.01 to 0.20 mg/L) and recovery specimens were prepared in Buffer A. The fluoride ion concentration of all samples was recorded after a 30-min equilibrium period. Bound fluoride was calculated as the difference between the ionic and total fluoride concentration of plasma.

**Statistics**

Means were compared statistically by calculating the Student’s t value (10). A p value of <0.025 was considered statistically significant.

**Results**

The blanks contained an average of 0.14 μg of fluoride, and recoveries averaged 97.1%. The CV was 5.62%.

Mean values for the concentration of ionic, bound, and total fluoride were not statistically different for males and females grouped according to age decade. Therefore, results for male and female donors were combined and are summarized in Table 1.

The mean concentrations of the various fractions of fluoride in plasma were not different for individuals in the age decades of 10 to 60 years. Ionic, bound, and total fluoride concentrations ranged from 0.53 to 5.79, 0.00 to 5.79, and 1.05 to 9.47 μmol/L, respectively. Those persons over 60 years of age had significantly higher mean values for ionic and total fluoride than any of the younger age groups, but the bound fluoride concentration was not significantly different at any decade. Ionic, bound, and total fluoride concentrations in the plasma of individuals older than 60 years ranged between 1.05 to 6.84, 0.00 to 5.26, and 2.11 to 11.1 μmol/L, respectively.

**Discussion**

The trend towards a higher mean ionic fluoride concentration in the plasma with increasing age was somewhat similar to that found by Kuo and Stamm (11), who reported that the mean serum inorganic fluoride concentrations among males who consumed water containing 0.1 mg of fluoride per liter increased from the second (1.00 μmol/L) to the seventh (2.16 μmol/L) decade of life. Our results also support the suggestion of Fuchs et al. (3), from a limited study, that there is a tendency for ionic fluoride concentrations to increase with age. The mean concentrations of ionic fluoride in our study are similar to those found in a fluoridated area by Jardillier and Desmet (12), who reported a value of 3.3 μmol/L. These values are only slightly higher than those reported by Parkins et al. (4), who examined the plasma of 41 inpatients from 17 to 82 years of age (of whom only 36 were currently living in an area with fluoridated water) and found a mean ionic fluoride concentration of 2.46 μmol/L and a range of ionic fluoride from 1.0 to 5.9 μmol/L. Other investigators have reported lower concentrations of ionic fluoride in plasma. Hall et al. (13) reported a mean of 1.96 μmol/L with a range of 0.84 to 2.90 μmol/L for a series obtained in an area with fluoridated water, whereas Guy et al. (6) reported values of approximately 1 μmol/L in two communities with fluoridated water. It is interesting that Cowell (14) found that the mean ionic fluoride concentration in plasma was 1.26 μmol/L for a series of 51 apparently healthy individuals who drank water with a fluoride concentration of 0.05 mg/L.

The various concentrations that have been reported may in part reflect the variation in daily fluoride intake reported for the various dietary regions of the United States (15). For example, the daily intake from all sources, based on Food and Drug Administration market basket food collections (representing the relative consumption of 117 food items by a 16- to 19-year-old male over a four-week period) from Atlanta, GA, and Buffalo, NY — both communities with fluoridated water — was found to be 1.72 and 0.99 mg, respectively. The methodology for ionic fluoride determination has been extensively examined by ourselves and others and does not appear to be the cause of the differences in values reported. The

### Table 1. Concentration of Fluoride in Plasma of Donors Living in a Community with Fluoridated Water

<table>
<thead>
<tr>
<th>Age range, years</th>
<th>No. donors</th>
<th>Ionic Fluoride form</th>
<th>Bound Fluoride form</th>
<th>Total Fluoride form</th>
</tr>
</thead>
<tbody>
<tr>
<td>10–20</td>
<td>52</td>
<td>2.74 ± 0.194</td>
<td>2.21 ± 0.232</td>
<td>5.00 ± 0.274</td>
</tr>
<tr>
<td>21–30</td>
<td>66</td>
<td>2.84 ± 0.168</td>
<td>1.89 ± 0.159</td>
<td>4.74 ± 0.216</td>
</tr>
<tr>
<td>31–40</td>
<td>30</td>
<td>3.00 ± 0.258</td>
<td>2.68 ± 0.284</td>
<td>5.68 ± 0.316</td>
</tr>
<tr>
<td>41–50</td>
<td>37</td>
<td>3.05 ± 0.189</td>
<td>2.42 ± 0.253</td>
<td>5.46 ± 0.295</td>
</tr>
<tr>
<td>51–60</td>
<td>40</td>
<td>3.05 ± 0.226</td>
<td>2.42 ± 0.258</td>
<td>5.47 ± 0.326</td>
</tr>
<tr>
<td>Over 60</td>
<td>39</td>
<td>3.89 ± 0.258</td>
<td>2.63 ± 0.216</td>
<td>6.58 ± 0.342</td>
</tr>
</tbody>
</table>
limited number of volunteers in some studies may explain some of the reported variations in concentration.

In our study, bound fluoride concentrations were similar for individuals between the ages of 10 to 30 years and reached a higher plateau after 30 years of age. The total fluoride remained relatively constant between 10 and 60 years of age, reaching its highest mean concentration in those over 60. The mean concentration for the 21- to 30-year-old group is significantly lower than for the older groups but does not differ significantly from that for the 10- to 20-year-old group. The residents of this community, according to our survey, are generally not a transient population and do not represent (e.g.) an influx of college students in the 21- to 30-year-old group.

The significantly higher concentrations of total fluoride observed in this study in the plasma of persons over 60 years of age as compared to those of the younger groups are largely the result of significantly higher ionic fluoride concentrations. This may be because of alterations in food-intake patterns by the elderly that might have resulted in a higher fluoride intake than was true for the younger persons in the study. Moreover, with increased age and fluoride exposure, a decreased ability of the skeleton to remove fluoride from the blood would also result in a higher fluoride equilibrium between body fluids and bone. The higher ionic fluoride value could also result from an increased bone resorption rate and a greater release of fluoride from the skeletal tissues, or from depressed renal function, particularly a change in glomerular filtration rate, which is associated with advanced age. There is no supporting evidence to suggest which, if any, of these speculations is most responsible for the results we observed.

In 1960, Singer and Armstrong (5) reported a mean ± SD of 7.89 ± 2.32 μmol/L for total fluoride in the plasma of 18 individuals who consumed fluoridated water (1 mg/L) and who resided in the metropolitan area from which the donors in the present study were obtained. The mean values for total fluoride in the 1960 populations examined by Singer and Armstrong are significantly higher than those found in the current study (4.74 to 6.58 μmol/L). There is no indication from the results of this investigation that there is an environmental contamination with fluoride, as suggested by Marrier (16). The range for total fluoride in plasma in the present study (1.05 to 11.1 μmol/L) is similar to that in the 1960 study.

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References