Background Levels of Bromide in Human Blood

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Abstract

A total of 183 random, whole-blood specimens was collected from healthy individuals within the State of Queensland (Australia), and the bromide concentration was determined by wavelength-dispersive X-ray fluorescence spectrometry (WDXRF). The intensity of the tube Compton scatter line was used to account for differences in matrices between the aqueous calibration standard and whole-blood specimens. Technical details of the WDXRF method are included in the experimental section of the paper. The overall mean for bromide in human blood was 5.3 ± 1.4 mg/L and ranged from 2.5 to 11.7 mg/L. Associations between bromide levels and variables including age, gender, weight, height, and postcode address were examined by ANOVA and Pearson’s correlation. Data indicate that aged persons (45-65+ years) are more likely to have higher bromide levels than younger persons (15-25 years). Our results also suggest differences in bromide levels between the sexes in similar age groups. Average levels were higher in females in most age groups. The reason for this difference requires further detailed investigation. No correlation was observed between bromide levels and height or weight of donors. No significant differences in bromide levels were found in persons living in the highly populated southeast region of Queensland compared with those living in the less urbanized northern parts of the state.

Introduction

Bromide intake in healthy individuals occurs through normal dietary means and, to a lesser extent, through environmental exposure. The daily intake of bromide from normal diets is in the range of 0.1 to 0.3 mg Br/kg body weight (approximately 8 mg/day for adults) (1,2), and estimates for the half-life of bromide in blood range from about 12 to 14 days (3-8). Bromide is mostly excreted via the kidney (3,9), and healthy adults eliminate approximately 4 mg/day via renal clearance (10-13). Bromide ion, like chloride ion, is mainly distributed in extracellular fluid (14).

Nondietary intake of bromide occurs from the use of bromine-based compounds such as methyl bromide (15-18), which is used as a fumigant and in fire extinguishers, an anesthetic such as halothane (5,15), pharmaceuticals (7,19,20), specialized chemicals (e.g., those used in photography [8] and swimming pools [11]), and the exhaust of motor vehicles fueled by leaded gasoline to which ethylene dibromide has been added as a supporting antiknock agent (21). Excessive uptake of these chemicals manifests itself in elevated bromide levels in body fluids, thus allowing assessments to be made of such things as drug abuse and occupational exposure. Occupational exposure to methyl bromide may also be assessed by measuring the levels of methyl cysteine produced in the blood. This adduct is produced from the reaction of sulphhydril groups in cysteine with methyl bromide (22,23). Health complications can arise from the misuse of bromine chemicals, especially in workplace environments where chemicals such as methyl bromide or ethylene dibromide are used. Recent accidental fatalities involving methyl bromide applications (24-26) highlight the toxicity of this chemical and its potential to adversely affect the health of occupationally exposed personnel.

For a number of years now, this laboratory has used the wavelength-dispersive X-ray fluorescence (WDXRF) technique for determining blood bromide in routine biologic monitoring and in assessing occupational exposure to methyl bromide. XRF is eminently suited for determining bromide in blood because of its simplicity, speed of analysis, high sensitivity, specificity, accuracy, and independence of chemical form. Our method involves direct examination of whole blood. Previous results from this laboratory on healthy (non-occupationally exposed) individuals have ranged from 2 to 12 mg Br/L with an average value of approximately 5-6 mg/L.

Much work has been carried out to establish a reliable reference concentration of bromide in the biological fluids of healthy humans, and some data are summarized in Table I. All data were converted into units of milligrams bromide per liter of body fluid (mg Br/L).

With the exception of the high cord serum (8) result shown in Table I, bromide concentrations in all examined body fluids (whole blood, serum, and urine) are similar with a range of

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3–12 mg/L and mean value around 5 mg/L, which is consistent with past routine clinical results of this laboratory. Recently, however, Rempel et al. (27) reported a blood bromide reference range of 0.5–2 mg/L, and technical details were unavailable. The reference range reported by Rempel et al. (27) is considerably lower than previously reported and has caused considerable concern in our community and prompted the present investigation. Our primary objectives were to establish a reference level for bromide in human blood, which was taken at random from healthy persons in the State of Queensland (Australia) and to examine any associations between bromide levels and age, gender, weight, height, and locality. In the present work, 183 random blood samples were collected throughout Queensland, and all analyses for bromide were conducted by WDXRF.

**Table I. Bromide Levels in Normal Healthy Subjects**

<table>
<thead>
<tr>
<th>Body fluid</th>
<th>Range found (mg Br/L)</th>
<th>Average (mg Br/L)</th>
<th>Method of quantitation*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>1.92–8.56</td>
<td>4.77</td>
<td>ICPMS</td>
<td>2</td>
</tr>
<tr>
<td>Serum</td>
<td>–</td>
<td>3.9 ± 0.7</td>
<td>WDXRF</td>
<td>5</td>
</tr>
<tr>
<td>Plasma</td>
<td>–</td>
<td>6.4 ± 0.8</td>
<td>Colorimetric I</td>
<td>6</td>
</tr>
<tr>
<td>Cord-Serum</td>
<td>3.1–28.5</td>
<td>8.6 ± 2.6</td>
<td>Ion chromatography</td>
<td>8</td>
</tr>
<tr>
<td>Serum</td>
<td>–</td>
<td>6.4</td>
<td></td>
<td>Calculated</td>
</tr>
<tr>
<td>Plasma (av)</td>
<td>–</td>
<td>4.1 ± 0.9</td>
<td>ICPMS</td>
<td>10</td>
</tr>
<tr>
<td>Plasma (male)</td>
<td>–</td>
<td>3.8 ± 0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma (female)</td>
<td>–</td>
<td>4.3 ± 0.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum</td>
<td>3.2–5.6</td>
<td>3.9</td>
<td>EDXRF</td>
<td>11</td>
</tr>
<tr>
<td>Urine</td>
<td>0.3–7.0</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urine</td>
<td>2.41–11.9</td>
<td>6.43</td>
<td>NAA</td>
<td>12</td>
</tr>
<tr>
<td>Serum</td>
<td>5.6–10.4</td>
<td>–</td>
<td>Ion chromatography</td>
<td>13</td>
</tr>
<tr>
<td>Blood</td>
<td>up to 10</td>
<td>3–4</td>
<td>Colorimetric I</td>
<td>15</td>
</tr>
<tr>
<td>Urine</td>
<td>–</td>
<td>6.3 ± 2.5</td>
<td>Colorimetric II</td>
<td>16</td>
</tr>
<tr>
<td>Blood</td>
<td>–</td>
<td>5.5</td>
<td>WDXRF</td>
<td>17</td>
</tr>
<tr>
<td>Plasma (male)</td>
<td>3.95–7.25</td>
<td>5.08 ± 0.94</td>
<td>EDXRF</td>
<td>32</td>
</tr>
<tr>
<td>Plasma (female)</td>
<td>3.42–7.45</td>
<td>5.14 ± 0.46</td>
<td></td>
<td></td>
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<tr>
<td>Blood (male)</td>
<td>2.64–5.37</td>
<td>3.46 ± 0.69</td>
<td></td>
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<tr>
<td>Blood (female)</td>
<td>2.63–5.90</td>
<td>4.04 ± 0.77</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood</td>
<td>–</td>
<td>5.30 ± 1.30</td>
<td>NAA</td>
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<tr>
<td>Serum</td>
<td>–</td>
<td>3.38 ± 0.75</td>
<td></td>
<td></td>
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<tr>
<td>Plasma</td>
<td>–</td>
<td>7.38 ± 0.44</td>
<td>NAA</td>
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<tr>
<td>Serum</td>
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<td>NAA</td>
<td>37</td>
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<tr>
<td>Plasma</td>
<td>1.28–7.48</td>
<td>4.87 ± 2.02</td>
<td>NAA</td>
<td>38</td>
</tr>
</tbody>
</table>

* Colorimetric I: bromate-rosaniline method; Colorimetric II: mercuric thiocyanate method; ICPMS: inductively coupled plasma mass spectrometry; WDXRF: wavelength-dispersive X-ray fluorescence spectrometry; EDXRF: energy-dispersive X-ray fluorescence spectrometry; and NAA: neutron activation analysis.

**Experimental**

**Collection of blood specimens**

Blood specimens were collected from healthy persons attending the volunteer blood banks at nine different sites in the State of Queensland. Samples were collected over a two-week period during March 1996, and each site collected 10–30 blood samples. Blood was drawn into 0.2 mL EDTA preserved vacutainer tubes of 10-mL capacity. Two samples were collected concurrently from each donor, which provided a total of approximately 18 mL blood (approximately 9-mL blood sample/tube). Blood samples were received within 24 h of collection and were chilled during transportation to the laboratory. Analyses were carried out upon receipt of the samples.

The rather large volume was necessary to meet the critical-depth criteria required by the WDXRF method of analysis. We chose not to dilute smaller volumes of blood samples with water as a means of boosting volume to satisfy critical depth because this would raise the lower reporting limit by the dilution factor.

**Specimen preparation and standard**

The two blood specimens from each donor were mixed together, and approximately 16 mL was poured into a 32-mm diameter aluminum sample cup fitted with a fresh piece of mylar film on the base. The top was sealed with plastic, and the sample cup was then presented into the spectrometer for analysis. The calibration standard (100 mg Br/L aqueous solution) was similarly presented to the spectrometer.

The standard bromide 100 mg/L solution was prepared by diluting a 1000 mg/L stock bromide solution by 10 with deionized water. The 1000 mg/L bromide solution was prepared by dissolving 1.4893 g of previously dried KBr (105°C/3 h/air oven) in 1 L of deionized water.

**Spectrometer conditions**

Measurements were made with a Philips PW 1404 sequential wavelength-dispersive XRF spectrometer using a Rhodium target X-ray tube at a power setting of 60kV 40mA. A summary of operating conditions is as follows: crystal, LiF200; Br peak position/20, Kβ/29.93°; background offsets, -0.92°, +0.92°; Rh Kα, Compton 26°, 17.86°; counting times: peak 60 s, offsets, 30 s each, Compton, 20 s; collimator, fine (150 μm); PHD settings: lower, 25%; upper, 75%; detector, scintillation; X-ray path, air.

It should be noted that these operating conditions are not optimal. Some gain in sensitivity would be expected with a molybdenum target X-ray tube operating on both a higher kV and power setting (e.g., 80kV 35mA).

**Calibration and calculations**

Calibration was carried out on a freshly prepared 100 mg/L aqueous bromide solution. Differences in matrix between the aqueous calibration solution and whole blood samples were...
accounted for by calculating the ratio of the intensities of the respective $K_{\alpha}$ Rh tube Compton scatter lines. Calculations for bromide in the blood specimens were based on Equation 1:

$$C_{Br_{\text{sample}}} = C_{Br_{\text{std}}} \times \frac{I_{comp_{\text{std}}}}{I_{Br_{std}}^{\text{Comp}}} \times \frac{I_{Br_{\text{sample}}}}{I_{comp_{\text{sample}}}}$$  \hspace{1cm} \text{Eq 1}

where $C_{Br_{\text{sample}}}$ is the concentration of bromide in the sample; $C_{Br_{\text{std}}}$ is the concentration of bromide in the aqueous standard solution (in this work 100 mg/L); $I_{comp_{\text{std}}}$ is the intensity of the Compton line for the aqueous standard; $I_{comp_{\text{sample}}}$ is the intensity of the Compton line for the sample; $I_{Br_{std}}$ is the peak intensity of the $K_{\alpha}$ Br line (background corrected) for the aqueous standard; and $I_{Br_{\text{sample}}}$ is the peak intensity of the $K_{\alpha}$ Br line (background corrected) for the sample.

Critical depth

The critical depth of a liquid for any analyte line of wavelength $\lambda$ can be experimentally determined by collecting intensity measurements on the sample and progressively increasing the volume. The critical depth is taken at the point where a further increase in volume yields a negligible increase in intensity. In the present work, the shortest measured wavelength is the Rh $K_{\alpha}$ Compton scattered line with $\lambda = 0.6390\AA$, and all solutions (aqueous and whole blood) should be infinitely thick to this radiation. Critical thickness is dependent on a number of factors (28) including spectrometer configuration (incident and takeoff angles), and we found that a depth of 16 mm for both aqueous and blood media was required before critical depth was satisfied with respect to the Compton line under our operating conditions. For 32-mm diameter sample cups, this required a minimum of a 13-ml volume of whole blood or aqueous solution. Calibrations and all blood bromide determinations, however, were conducted on 16-ml volumes; this corresponds to a depth of approximately 20 mm.

A critical-depth calculation based on pure water was performed (28,29) using the following parameters: PW1404 incident angle, $56^\circ$; PW1404 takeoff angle, $40^\circ$; Compton wavelength, 0.6390Å; incident wavelength, 0.6147Å (assumed to be monochromatic and taken as the Rh $K_{\alpha}$ characteristic line [30]); and density of water, 1 gm/cm$^3$. Mass absorption coefficients were taken from the tables of Champion et al. (31). The calculated critical depth for the Compton line was determined to be 28 mm. By definition, critical thickness is reached when the intensity of an analyte line (I$_{i}$) from a sample is at least 99.9% of the intensity of the same line (I$_{i}$) that would emerge from the same material at an infinite thickness (28) (i.e., I$_{i}$/I$_{i\infty}$ = 0.999). In the present study, the experimentally derived critical depth was found to be about 16 mm, which corresponds to an I$_{i}$/I$_{i\infty}$ ratio of 0.981 (based on pure H$_2$O). We found no discernible increase in intensity of the Rh $K_{\alpha}$ Compton line when blood or aqueous solutions were increased in depth past 16 mm, but we nevertheless used 20-mm depths throughout this study as a safety margin. It should be noted that a depth of 20 mm corresponds to a I$_{i}$/I$_{i\infty}$ ratio of 0.993, which is slightly short of the critical value of 0.999. Further increases in depth from 20 mm would result in negligible increases in intensities. Therefore, we were satisfied that our XRF measurements were conducted on liquids which fulfilled the critical depth criterion.

Validation of method

A plot of bromide concentration ($C_{Br}$) versus $I_{Br}/I_{comp}$ using a series of aqueous standards was linear to at least a concentration of 100 mg Br/L. A slight blank correction (using pure water) was necessary for all measurements. Standard addition experiments were carried out on a sample of pooled blood. Bromide-spike concentrations in the pooled blood ranged from 2 to 10 mg/L, and acceptable recoveries of 100 ± 7% were consistently obtained during the course of this study. The calculated lower reporting limit (LRL) for bromide in blood at the 99.7% confidence limit (3a) (30) using the conditions outlined here was 0.5 mg/L.

In principle, whole blood analyses for bromide by the method outlined here could be successfully carried out on smaller volumes of blood diluted with water to ensure that the critical depth requirement is satisfied. For example, analyses could be carried out with 5 mL blood diluted with 10 mL water in 32-mm diameter sample cups. The x3 dilution will, however, raise the LRL by the dilution factor (in the present case, the LRL would be 1.5 mg/L).

Statistical analysis

Bromide-spike concentrations in the pooled blood ranged from 2.5 to 11.7 mg/L. A slight blank correction (using pure H$_2$O) was made to account for blank contributions. The calculated lower reporting limit (LRL) for bromide in blood at the 99.7% confidence limit (3a) (30) using the conditions outlined here was 0.5 mg/L.

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Statistical analysis

Blood bromide results were log transformed to achieve normality, then statistically analyzed using SPSS (version 6.1) for Windows™. Analysis of variance models and Pearson's correlation were used to identify factors associated with significant variation in bromide level among the recorded variables, namely age, gender, weight, height, and postcode address of
the donor. Although weight and height were entered as continuous variables, age was grouped into six categories, 15–24, 25–34, 35–44, 45–54, 55–64, and 65+ years. Postcodes were categorized into groups, roughly corresponding to southernmost Queensland to Caboolture (a provincial town 35 km from Brisbane City) and northward from Caboolture. Multiway ANOVA models incorporated age, gender, and location as main effects and the continuous variables as covariates using unique sums of squares to allow all variables to be considered simultaneously.

Results

Bromide levels in the 183 blood specimens collected in Queensland ranged from 2.5 to 11.7 mg/L, with an overall mean of 5.3 ± 1.4 mg/L (Table II). A majority (89%) contained less than 7.0 mg/L, whereas the minority had higher levels (Figure 1).

Although mean age did not differ significantly by gender, males were significantly heavier and taller than females in the sample. Bromide levels were slightly higher among females than males, but this difference was not statistically significant ($F = 2.5; df 1, 181; p > 0.05$).

Bromide levels increased significantly with age group of the donor ($F = 3.1; df 5, 177; p = 0.01$; Figure 2). The highest levels among both males and females were found in the 65+ year age group (mean 6.0 ± 1.7 mg/L), which is considerably higher than among the 15–24-year-old group, where the mean was 4.2 ± 0.9 mg/L. Individuals with relatively high bromide levels (>7.5 mg/L) were found in all age groups except the 15–24-year-old group.

There was little variation in bromide levels among the intervening age groups ranging from 25 to 64 years. Some sex-related difference in the blood bromide levels in these intermediate age groups is suggested in Figure 2. Females showed a consistent increase through all age groups, whereas bromide levels in males were slightly lower among the 45–64-year-old group.

No correlation was observed between bromide levels and the height and weight of the donor (Pearson’s $r = -0.10, -0.07$, respectively; $p > 0.05$). Blood bromide levels among donors living in southeast Queensland, which is largely metropolitan, did not differ significantly from those living in northern parts of the state ($F = 1.5; p > 0.05$).

A multivariate analysis of variance model incorporating all of the variables revealed that age ($F = 3.1; df 5, 171; p = 0.01$) and sex ($F = 3.7; df 1, 171; p = 0.055$), but not height, weight, or home location, explained significant variation in bromide levels. The interaction between age and sex suggested in Figure 2 was not found to be statistically significant ($F = 0.9; p > 0.05$).

Age group and gender explained approximately 12% of the variation in blood bromide levels among the sample group ($F = 2.3; df 11, 171; p = 0.013$).

Discussion

The average and range of bromide levels in the blood specimens examined in this study are in good agreement with those of other studies (Table I). Other authors have reported higher bromide levels among healthy females compared with males (10,32). Furthermore, a recent study involving controlled bromide administration found that females retained more bromide than males given equal exposures (6). Our data support a
These trends were not statistically significant, and follow up studies using larger sample groups are needed to confirm these observations. Studies are also needed to determine if this difference is related to higher exposure or higher retention of bromide among females compared to males.

The increasing trend in bromide level with age is unlikely to reflect accumulation because the half-life of bromine in the body is relatively short. It is also unlikely to reflect occupational exposure because the highest group was retirement age (65+ years). The elevated bromide levels in aged persons may result from a higher frequency of minor ailments leading to the use of common bromine-based over-the-counter medications (20), such as bromopheniramine maleate and hyoscine hydrobromide. Although lithium therapy is also known to elevate blood bromide levels (33,34), the sample group was made up of healthy blood donors who were unlikely to have been taking lithium-based medications.

Comparisons of blood bromide levels among donors living in the southernmost part of Queensland and those living north of metropolitan Brisbane in the state did not suggest that bromide exposure increased in the most heavily populated and industrialized area. The data did not show a strong environmental association between bromide level and location; however, more precise assessments of potential environmental exposure associated with individual donors would be a more powerful way of examining this question.

Multivariate analyses incorporating age and gender were able to explain just 12% of the overall variation in bromide levels among the sample group, indicating that a greater deal of variation remains unexplained (21,35). The absence of occupational data prevents the examination of potential work-related exposures to the element. Recent studies have also suggested that other factors, such as low-salt diets (1,3,14,20), may also influence bromide levels by increasing its retention in the body through altered renal function (3). The molar sum of extracellular chloride and bromide remains constant in healthy individuals (approximately 110 mmol/L) (14). When the concentration of chloride is lowered through reduced dietary intake, bromide appears to replace the depleted chloride (14,20). This results in an increase in the half-life of bromide, and, consequently, its concentration rises above the normal level (1). It is possible that this factor is responsible for some of the age-related variation because older individuals may be more likely than younger people to be limiting their salt intake for health reasons and the remaining unexplained variation in bromide levels between individuals.

Further studies with larger samples linked with information on potential occupational and environmental exposure as well as dietary habits (including salt intake) of the donors are needed to confirm and explain the observed trends in blood levels of human populations. Quantitating the relative importance of medication-related versus environmental and occupationally related sources of exposure in determining blood bromide levels would be useful in the management of human exposure to the element.

Conclusion

The present WDXRF study obtained a mean blood bromide concentration of 5.3 ± 1.4 mg/L in healthy individuals from Queensland. Rempel et al. (27) reported a substantially lower reference range of 0.5–2 mg/L. The results of this study are supported by numerous other workers who obtained values similar to those presented here. The present study suggests that older persons are more likely to have higher blood bromide levels than younger persons, with levels in females higher than males within an age group. The reasons for these patterns are not clear but may be related to differences in diet or ingestion of bromine-based medication.

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References


