SIMPLIFIED BROMIDE DETERMINATION IN BLOOD AND URINE*

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(Received for publication, May 15, 1942)

In 1938 Brodie and Friedman (1) published a method for the determination of bromide in tissues and biological fluids. Tissues, serum, urine, or spinal fluid was dried and then fused with sodium hydroxide on a sand bath and the melt containing the bromide was dissolved in water.

The use of bromide as a measure of the extracellular fluid was demonstrated by Brodie, Brand, and Leshin (2, 3). In the present paper, a method for determining bromide in the serum and the urine is reported. The fusion, with subsequent solution of the melt and transferring, is the most complicated and time-consuming step of the original method. A study was made of different protein precipitants to substitute for the fusion, and the use of trichloroacetic acid was found to give quantitative recoveries of added bromide to serum.

The filtrate from trichloroacetic acid is treated essentially as in the original method. The bromide is oxidized to bromate by sodium hypochlorite buffered with acid phosphate. The excess hypochlorite is reduced by sodium formate and the addition of iodide to the bromate in acid solution results in the liberation of 6 equivalents of iodine. The iodine is then titrated with standard thiosulfate solution.

A simplified and accurate method for the determination of bromide in urine is also included in this communication. The bromide and chloride are precipitated from urine as the silver salts in the presence of nitric acid (4). The supernatant fluid is removed, the halides are suspended in acid phosphate solution, and the bromides oxidized as for serum.

Reagents—

Trichloroacetic acid, 10 per cent.
Sodium dihydrogen phosphate (NaH₂PO₄·H₂O), 40 per cent.
Sodium hypochlorite, 1.0 N in about 0.1 N NaOH. Pass chlorine gas with constant stirring into a solution containing 44.8 gm. of NaOH in 1000 ml. of solution. The alkalinity is tested at intervals by destroying the hypochlorite in 1 ml. of solution with 2 ml. of 3 per cent hydrogen peroxide, diluted to 10 ml. and titrated with 0.1 N HCl. The titer should be between 0.8 and 1.2 ml. The reagent is stable for several weeks in the refrigerator.

* A preliminary report of this paper was presented at the meeting of the American Society of Biological Chemists at Boston, April, 1942.
Sodium formate, 50 per cent.
Potassium iodide, 20 per cent.
Ammonium molybdate, 10 per cent.
Silver nitrate, 7.5 per cent.
Nitric acid, 1 per cent.
Sodium thiosulfate, 0.005 N. This is made from a stock 0.1 N solution and standardized before use.
Starch, 1 per cent.

Procedure

Serum—Into a 125 ml. Erlenmeyer flask put 5 ml. of serum and to it add with constant shaking 25 ml. of 10 per cent trichloroacetic acid. Shake thoroughly with a clean rubber stopper, let stand 15 minutes, and filter through a dry 9 cm. Whatman No. 1 filter paper, refiltering the first portion. In this manner about 25 ml. of filtrate are obtained. 10 ml. of filtrate, equivalent to 1.67 ml. of serum, are transferred to a 125 ml. Erlenmeyer flask and the trichloroacetic acid is neutralized by the addition of 0.5 gm. of sodium bicarbonate. Now add 5 ml. of 40 per cent sodium dihydrogen phosphate and 8 ml. of 1.0 N sodium hypochlorite. The resulting solution is immersed in a boiling water bath for 10 minutes, at the end of which time the excess hypochlorite is reduced by the addition of 5 ml. of 50 per cent sodium formate. The sides of the flask are washed with water, and the flask shaken and replaced in the bath for 5 minutes. The solution is cooled to room temperature, 10 ml. of 6 N sulfuric acid added, and the flask further cooled in an ice bath to about 10°. Add 0.3 ml. of ammonium molybdate and 1 ml. of 20 per cent potassium iodide. After standing for 1 minute the liberated iodine is titrated with 0.005 N sodium thiosulfate. The starch-iodine titration end-point is difficult to recognize in natural light, but results can be duplicated to 0.01 ml. of 0.005 N thiosulfate by placing the burette in a box with the inside coated with white paint. The box is illuminated from an upper back corner with a 60 watt lamp and the end-point is observed by holding the flask against the side of the box opposite the lamp. A blank determination is run under identical conditions, with 8 ml. of 10 per cent trichloroacetic acid and 2 ml. of water for the 10 ml. of filtrate. The titration of this blank is subtracted from the volume of thiosulfate in the determination. 1 ml. of 0.005 N sodium thiosulfate is equivalent to 0.0667 mg. of Br. The thiosulfate is standardized by measuring accurately a 0.01 N bromate solution into a 125 ml. Erlenmeyer flask, made to 20 ml. with water, and 5 ml. of sodium dihydrogen phosphate, 5 ml. of 50 per cent sodium formate, and 10 ml. of 6 N sulfuric acid are added in the order named. Cool to 10°, add 1 ml. of 20 per cent KI, 0.3 ml. of 10 per cent ammonium molybdate, and titrate the liberated iodine with sodium thiosulfate.
Calculations: Since 1 ml. of 0.005 N thiosulfate is equivalent to 0.0667 mg. of Br, and an aliquot of 1.67 ml. of serum is used, the calculation may be made as follows:

\[(\text{Titration} - \text{blank}) \text{ ml.} \times \frac{\text{thiosulfate N}}{0.005} \times 0.0667 \times 60 = \text{mg. \% Br}\]

The thiosulfate normality is known and the last three factors of the equation may be combined into one, giving the calculation directly from the titration. Thus, when the normality of the thiosulfate is exactly 0.005 N, 1 ml. is equivalent to 4.0 mg. per cent of Br.

Urine—Add 5 ml. of urine to a 50 ml. graduated conical centrifuge tube and bring the volume to 25 ml. with distilled water. Add 3 ml. of concentrated nitric acid and then with constant stirring add dropwise 7.5 per cent silver nitrate until no further precipitate forms. Overlay the solution with 95 per cent ethyl alcohol to lower the surface tension and prevent the formation of a silver halide film which forms during centrifugation. Let stand 20 minutes and then centrifuge at a moderate speed for 10 minutes. Siphon or decant the supernatant fluid and wash the precipitate with 20 ml. of 1 per cent nitric acid, and centrifuge again. The precipitate is next suspended with the aid of a stirring rod in 5 ml. of 40 per cent acid phosphate to which 10 ml. of sodium hypochlorite are added. The centrifuge tube is placed in a boiling water bath for 10 minutes and the suspension stirred at frequent intervals by vertical motion, after which time 5 ml. of sodium formate are added and the tube kept in the water bath 5 minutes longer. The solution is then filtered through a moistened 7 cm. Whatman No. 1 filter paper and the precipitate washed several times with water. To the filtrate are added 10 ml. of 6 N sulfuric acid, cooled in an ice bath, and after the addition of 1 ml. of 20 per cent KI and 0.3 ml. of 10 per cent ammonium molybdate the solution is titrated as in the serum determination. A blank is run with each series of determinations. The blank consists of 5 ml. of acid phosphate and 10 ml. of hypochlorite, and the solution is treated under identical conditions as for the urine determination. It is not necessary to filter the blank.

It is not possible to oxidize the bromide in urine directly, owing to the presence of interfering substances that react with hypochlorite. Isolating the silver halides makes the method applicable for urines of any composition. The normal excretion of bromide can be determined with a fair degree of accuracy by analyzing 10 ml. of urine. This amount of urine normally contains about 0.025 mg. of Br. When the chloride concentration in the urine is low, the addition of 2 ml. of 1 per cent NaCl should be made to 5 ml. of urine. The same amount of salt is also added to the blank.

Proteins in urine are removed by the addition of 15 ml. of 10 per cent trichloroacetic acid with constant stirring to 15 ml. of urine. The solution
is placed in a boiling water bath for 1 minute, cooled for 30 minutes, and filtered until clear. 10 ml. of the filtrate, equivalent to 5 ml. of urine, are determined as described.

**Calculations**—The blank is subtracted from the determination and the difference in titration is due to the amount of bromide in 5 ml. of urine.

\[
(Titration \ - \ blank) \text{ ml.} \times \frac{\text{thiosulfate N}}{0.005} \times 0.0667 \times 20 = \text{mg. % Br}
\]

**Table I**

<table>
<thead>
<tr>
<th>Bromide added (mg.)</th>
<th>No. of analyses</th>
<th>Average Br recovered (mg.)</th>
<th>Extreme range recovered (mg.)</th>
<th>Average recovery (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.049</td>
<td>6</td>
<td>0.0490</td>
<td>0.0480-0.0500</td>
<td>100.0</td>
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<td>0.079</td>
<td>8</td>
<td>0.0782</td>
<td>0.0075-0.0790</td>
<td>99.0</td>
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<tr>
<td>0.197</td>
<td>11</td>
<td>0.1961</td>
<td>0.1950-0.1990</td>
<td>99.5</td>
</tr>
<tr>
<td>0.493</td>
<td>10</td>
<td>0.4904</td>
<td>0.4870-0.4940</td>
<td>99.5</td>
</tr>
</tbody>
</table>

**Table II**

**Comparison of Recoveries by Fusion Method (1) and Trichloroacetic Acid Precipitation**

<table>
<thead>
<tr>
<th>Determination No.</th>
<th>Fusion method</th>
<th>Trichloroacetic acid method</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg. per cent</td>
<td>mg. per cent</td>
</tr>
<tr>
<td>1</td>
<td>13.1</td>
<td>13.3</td>
</tr>
<tr>
<td>2</td>
<td>11.6</td>
<td>11.9</td>
</tr>
<tr>
<td>3</td>
<td>18.6</td>
<td>18.8</td>
</tr>
<tr>
<td>4</td>
<td>16.5</td>
<td>16.5</td>
</tr>
</tbody>
</table>

**Results**

**Serum**—Amounts of bromide from 0.150 to 1.480 mg. were added to 5 ml. of normal serum, equivalent in amounts to 3 to 30 mg. per cent. The blank consisted of the same serum without added bromide. In Table I are shown the amounts added to the equivalent of 1.67 ml. of serum and the recoveries.

A series of determinations was made to find whether bromide in the circulating serum could be determined in the same manner as the bromide added to serum in vitro, as shown in Table I. A number of human sub-
jects were given from 3 to 5 gm. of sodium bromide and several hours later blood was withdrawn and aliquot determinations were made in duplicate by the trichloroacetic acid filtration and the original fusion method. The results of some of these are shown in Table II. Amounts of bromide added to serum in vitro were always recovered quantitatively by the trichloroacetic acid method, and the results checked favorably with those obtained by the fusion method in a large series of patients given bromide. However, in a few instances the fusion method yielded somewhat higher results. This discrepancy (less than 15 per cent) occurred in serum from diabetics taken when the patient was not in a fasting state. No explanation can be given at the present time as to why the bromide could not be filtered quantitatively in these exceptional cases. This problem is being studied further.

**Table III**

Recoveries of Bromide Added to Urine

<table>
<thead>
<tr>
<th>Bromide added</th>
<th>No. of analyses</th>
<th>Average Br recovered</th>
<th>Extreme range recovered</th>
<th>Average recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td></td>
<td>mg.</td>
<td>mg.</td>
<td>per cent</td>
</tr>
<tr>
<td>0.148</td>
<td>5</td>
<td>0.141</td>
<td>0.139–0.144</td>
<td>95.3</td>
</tr>
<tr>
<td>0.237</td>
<td>8</td>
<td>0.223</td>
<td>0.220–0.224</td>
<td>94.1</td>
</tr>
<tr>
<td>0.592</td>
<td>8</td>
<td>0.568</td>
<td>0.562–0.571</td>
<td>96.0</td>
</tr>
<tr>
<td>0.829</td>
<td>9</td>
<td>0.787</td>
<td>0.774–0.801</td>
<td>95.0</td>
</tr>
<tr>
<td>1.480</td>
<td>8</td>
<td>1.392</td>
<td>1.376–1.407</td>
<td>94.0</td>
</tr>
<tr>
<td>2.900</td>
<td>4</td>
<td>2.771</td>
<td>2.750–2.787</td>
<td>98.0</td>
</tr>
</tbody>
</table>

**Urine**—The recovery of bromide from urine is somewhat less than quantitative, though constant, as is seen from Table III. No further bromide could be recovered from the residue.

**SUMMARY**

A technique for the determination of bromide in serum is described. The proteins are precipitated with trichloroacetic acid, the bromide oxidized to bromate, and the latter determined iodometrically.

The bromide in urine is determined by precipitating the silver halides and oxidizing the bromide in the suspended salts.

I wish to express my appreciation to Dr. Louis R. Ferraro, Pathologist at Fordham Hospital, for his encouragement in making this work possible, and to Professor George B. Wallace and Dr. Bernard B. Brodie of New York University for their valuable suggestions.
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J. Biol. Chem. 1942, 144:519-524.

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