Estimation of the Bromide Space With
a Modification of Conway's Method

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There is a constant transfer of water and electrolyte between extracellular
and intracellular fluids. These exchanges are not only responsible for alterations of
the volume of these respective fluid compartments, but also account for changes in
the acid-base balance of the blood (1). For this reason, methods are needed for the
following of changes in both extracellular and intracellular fluids. The estimation of
extracellular fluid permits one, under certain circumstances, to evaluate changes in
cell composition. All the proposed methods are based on essentially the same prin-
ciple. A measured amount of substance, which is thought to be largely confined to
extracellular fluids, is given. The apparent volume of distribution is calculated from
the serum concentration. All methods require critical evaluation of the errors of
assumption as well as of determination, since it is unlikely that any substance is
distributed uniformly throughout the heterogeneous fluids that lie outside of the cells.

Two groups of substances have been proposed. a) Certain disaccharides and
the large complex polysaccharides (2-4) have been employed. These substances
appear satisfactory in that there is little evidence of penetration into cells. However,
some are excreted so rapidly that equilibrium cannot be reached without a continu-
ous infusion which not only makes the determination of body content at one moment
difficult, but also makes maintenance of a constant level at equilibrium uncertain.
Moreover these substances penetrate ascitic fluid so slowly that they may not be
suitable for edematous patients (5). They penetrate connective tissue so slowly, if
at all, that the estimated volumes do not include the fairly large volume of connect-
tive tissues that contain considerable amounts of chloride. For technical reasons,
and because of the low volumes obtained, these substances are unsatisfactory. b) Cer-
tain inorganic ions have also been used. These include thiocyanate (6), thiosulphate
(7), radiosulphate (8), radiosodium (9), radiobromide (10) and bromide (11).
In general, these substances give apparent volumes of distribution that are too large
for extracellular fluids. This is due to penetration of the cells, perhaps to sequestra-
tion, and in some instances because of a participation in metabolic reactions.

The present paper reports a simple, accurate method of determining serum or
blood bromide. This is essentially the method of Conway (12) with some simple
modifications. The method was tested to determine its suitability for estimation of
the apparent volume of distribution of bromide. Both recent (13) and earlier work
(14-16) indicate that bromide and chloride are distributed in tissues in the same
ratio as that of serum. In other words the bromide space, multiplied by the concen-
tration of chloride in an ultrafiltrate of serum, measures approximately the total
body chloride.

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METHOD OF ESTIMATION OF SERUM BROMIDE

The solutions and apparatus required are set out by Conway (12). For the modifications described here a muffle furnace, capable of maintaining temperatures between 370° and 420° C, is necessary.

**Determination of Serum Water.** One cc of serum to be assayed is placed in a 15-cc centrifuge tube and weighed. About 1 cc of distilled water is added and the serum is dried in an oven at 95° C. The dry weight of the serum is noted so that the serum water may be calculated.

**Determination of Bromide.** Five cc of distilled water are added to the tube containing dried serum, and this is allowed to stand for about 30 min. The tube is gently agitated once or twice, and the mouth should be covered with paraffin paper. A 4-cc aliquot is taken and this is transferred to the outer chamber of the porcelain Conway unit (12). The unit is placed in the oven at 95° C and the aliquot is evaporated to dryness. The unit is then transferred to a muffle furnace at 370°-420° C for 18–30 min (20 min at 400° C was generally used). After cooling on an asbestos mat, the procedure is the same as that described by Conway (12), except that 1 ml distilled water is added to the outer chamber of the Conway unit. The whole procedure is done in duplicate or triplicate. With respect to the investigations presented below, the determinations were made in quadruplicate.

**Calculations.** \( V \times 0.01 \times 1.07 \times \left( \frac{5}{4} \right) \times 1000 = \text{mEq of bromide per liter,} \)

where \( V = \text{titration in milliliters of } 0.01 \text{ N thiosulphate;} \)

\( 1.07 = \text{the correction for back diffusion of the iodine liberated in the center chamber;} \)

\( \frac{5}{4} = \text{the correction for the aliquot taken. mEq/l} \times 79.9 \times \left( \frac{100}{1000} \right) = \text{mg of bromide/100 cc.} \)

The factor \( 1.07 \) is the back diffusion factor which arises from the slight back diffusion of the liberated iodine from the center to the outer chamber. This was determined by using standard iodine solutions together with the 20% potassium iodide in the center chamber, other reagents being the same as are usually employed. This factor is constant for the range of values determined here (1–2 mEq/l).

**Determination in Red Blood Cells.** Determinations on red cells are made as follows. A measured volume of heparinized blood is centrifuged. The plasma is removed and is used for the plasma bromide determination. From the volume of the packed red cells by hematocrit, the volume of red cells of the remaining mixture is estimated, and the correction for plasma bromide is made. This mixture is transferred to a beaker with washings of distilled water. The beaker is placed in an oven at 95° C. The fragments of blood are broken with a glass rod before they become hard. After drying is complete, the cells are extracted overnight with 10 ml of distilled water in a beaker covered with paraffin paper. A suitable aliquot is taken of the filtered extract and the determination of bromide is made in the Conway unit in the same manner as for serum.

**Accuracy.** One ml of a standard solution of potassium bromide (2 mEq of bromide/l., or 159.8 \( \mu \)g/ml) was added to 1 ml of normal serum. In 25 separate duplicate determinations the average and standard deviation were 159.7 \( \mu \)g/ml \( \pm 2.6 \). These results indicate that the error of duplicate determinations is unlikely to be greater than 3%. Serums of normal persons receiving no bromide revealed no bromide.

**Urinary Losses of Bromide.** Using the same technique as for serum, urinary excretion of bromide was determined in 6 normal adults. With oral administration of 2–3 gm, the average excretion after 6 hr was 1.95% of the dose (range, 0.92 and 2.90%). After 12 hr, urinary excretion averaged 3.8% of the administered amount (range 2.68–4.76%).
TABLE I. NORMAL MALES AND FEMALES

<table>
<thead>
<tr>
<th>Subj.</th>
<th>Age, Yr.</th>
<th>Ht, In.</th>
<th>Wt, Kg*</th>
<th>Serum Br, mEq/l.</th>
<th>Hr Following Dose</th>
<th>Br Space, Liters</th>
<th>Corrected Br Space, % Body Wt.</th>
</tr>
</thead>
</table>
| **Males**
| D. B. C. | 27 | 64 | 60 | 1.10 | 2 | 15.2 | 22.8 |
| | | | | 1.09 | 4 | 15.4 | 23.0 |
| | | | | 1.08 | 8 | 15.6 | 23.4 |
| | | | | 1.02 | 24 | 16.5 | 24.3 |
| W. E. S. | 28 | 71 | 81.8 | 1.34 | 1 | 18.8 | 22.1 |
| | | | | 1.13 | 2 | 18.1 | 22.1 |
| | | | | 1.09 | 4 | 23.0 | 25.2 |
| | | | | 1.15 | 7 | 22.0 | 24.2 |
| | | | | 1.03 | 25 | 24.0 | 26.7 |
| A. J. B | 29 | 77 | 74 | 1.41 | 2 | 18.1 | 22.1 |
| | | | | 1.41 | 4 | 18.1 | 22.1 |
| | | | | 1.37 | 8 | 18.5 | 22.5 |
| | | | | 1.23 | 24 | 20.5 | 24.9 |
| I. S. | 37 | 67 | 70 | 1.72 | 2 | 14.6 | 18.5 |
| | | | | 1.72 | 4 | 14.6 | 18.5 |
| | | | | 1.58 | 24 | 15.0 | 20.2 |
| P. F. | 35 | 64 | 74 | 1.70 | 2 | 14.8 | 17.3 |
| | | | | 1.69 | 4 | 14.9 | 17.4 |
| | | | | 1.57 | 24 | 16.1 | 18.7 |
| M. D. | 25 | 74 | 84 | 1.22 | 2 | 20.7 | 21.4 |
| | | | | 1.25 | 4 | 20.1 | 20.9 |
| | | | | 1.00 | 24 | 21.3 | 26.1 |
| R. E. C. | 32 | 71 | 79.6 | 1.45 | 2 | 17.3 | 19.7 |
| | | | | 1.45 | 5 | 17.3 | 19.7 |
| | | | | 1.21 | 26 | 20.8 | 23.5 |
| J. G. | 35 | 72 | 74 | 1.47 | 3 | 17.1 | 20.4 |
| | | | | 1.38 | 6 | 18.4 | 22.0 |
| | | | | 1.22 | 26 | 20.7 | 24.9 |
| **Females**
| F. C. | 29 | 62 | 50 | 1.67 | 2 | 10.0 | 18.0 |
| | | | | 1.63 | 4 | 10.4 | 18.7 |
| | | | | 1.69 | 8 | 10.2 | 18.4 |
| | | | | 1.58 | 24 | 10.6 | 19.1 |
| M. B. | 25 | 65 | 52.7 | 1.47 | 3 | 11.4 | 19.4 |
| | | | | 1.30 | 6 | 12.1 | 20.7 |
| | | | | 1.13 | 26 | 14.3 | 22.3 |
| E. T. | 20 | 65 | 55 | 1.34 | 3 | 12.5 | 20.6 |
| S. L. | 25 | 62 | 59.5 | 1.24 | 3 | 13.5 | 20.5 |
| J. S. | 27 | 65 | 59 | 1.27 | 3 | 13.2 | 20.2 |
| T. K. | 24 | 63 | 58 | 1.27 | 3 | 13.2 | 20.5 |
| G. C. | 22 | 63 | 52 | 1.41 | 3 | 11.9 | 20.6 |
| G. N. | 24 | 67 | 60 | 1.28 | 3 | 14.2 | 21.3 |

- Males above 60 kg were given 2013 mg of bromide, below 60 kg, 1343 mg. Females were given 1343 mg of bromide.
- Corrected for Donnan factor and serum water.
- Corrected for red cell bromide content (10–13%).
Estimation of Bromide Space. Three gm of potassium bromide were given by mouth to adults weighing over 60 kg and 2 gm to those weighing less than 60 kg. With these doses, the serum concentration stabilizes at 1-2 mEq/l. Assuming that average extracellular bromide concentration is that of an ultrafiltrate of serum, the following calculations are made:

\[
\text{Extracellular bromide concentration} = \frac{(\text{Br})_s}{(\text{H}_2\text{O})_s} \times 0.95
\]

Bromide space = bromide administered ÷ extracellular bromide concentration.

Where \((\text{H}_2\text{O})_s\) = proportion of water in serum; 0.95 = an average Donnan factor; \((\text{Br})_s\) = serum bromide concentration.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age,yr</th>
<th>Wt, Kg</th>
<th>Remarks</th>
<th>Br Given, Mg</th>
<th>Serum Br, mEq/l.</th>
<th>Br Following Dose</th>
<th>Br Space, Liters</th>
<th>Corrected Br Space, % Body Wt</th>
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<tbody>
<tr>
<td>D. A. (M)</td>
<td>31</td>
<td>61.6</td>
<td>Cirrhosis of liver, ascites (retaining chlorides), no edema obvious</td>
<td>2350</td>
<td>1.53</td>
<td>5</td>
<td>19.2</td>
<td>28.2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.51</td>
<td>9</td>
<td>19.4</td>
<td>28.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.51</td>
<td>22</td>
<td>19.6</td>
<td>28.6</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.48</td>
<td>30</td>
<td>19.8</td>
<td>28.9</td>
</tr>
<tr>
<td>M. H. (F)</td>
<td>50</td>
<td>72</td>
<td>Slight edema of legs (mild cardiac failure) and rheumatoid arthritis</td>
<td>2350</td>
<td>1.36</td>
<td>4</td>
<td>26.3</td>
<td>23.7</td>
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<td></td>
<td></td>
<td></td>
<td>1.43</td>
<td>9</td>
<td>28.6</td>
<td>25.7</td>
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<td></td>
<td></td>
<td>1.38</td>
<td>24</td>
<td>29.6</td>
<td>26.6</td>
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<td></td>
<td>1.38</td>
<td>98</td>
<td>29.6</td>
<td>26.6</td>
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<tr>
<td>O. S. (M)</td>
<td>60</td>
<td>58</td>
<td>Definite cardiac failure Carcinomatosis Generalized edema</td>
<td>2013</td>
<td>1.04</td>
<td>5</td>
<td>24.2</td>
<td>37.0</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.88</td>
<td>8</td>
<td>28.3</td>
<td>43.9</td>
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<td></td>
<td></td>
<td></td>
<td>0.90</td>
<td>33</td>
<td>28.0</td>
<td>43.9</td>
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<tr>
<td>E. V. (F)</td>
<td>50</td>
<td>132</td>
<td>Exceptional obesity Acromegaly?</td>
<td>3355</td>
<td>1.06</td>
<td>23</td>
<td>21.4</td>
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<td>1.96</td>
<td>10</td>
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<td>S. E. (M)</td>
<td>79</td>
<td></td>
<td>Cirrhosis of Liver, ascites</td>
<td>2013</td>
<td>0.96</td>
<td>5</td>
<td>26.4</td>
<td>29.8</td>
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<td>0.96</td>
<td>24</td>
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<td>29.8</td>
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<td></td>
<td></td>
<td></td>
<td>0.93</td>
<td>30</td>
<td>27.2</td>
<td>30.9</td>
</tr>
<tr>
<td>B. O. (M)</td>
<td>82</td>
<td></td>
<td>Edema and ascites Carcinomatosis?</td>
<td>2013</td>
<td>0.88</td>
<td>5</td>
<td>28.6</td>
<td>31.5</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.81</td>
<td>9</td>
<td>31.2</td>
<td>34.2</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>0.83</td>
<td>24</td>
<td>30.0</td>
<td>32.9</td>
</tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>0.83</td>
<td>30</td>
<td>30.0</td>
<td>32.9</td>
</tr>
</tbody>
</table>

* Corrected for Donnan factor and for serum water. † Corrected for red cell bromide content (10-13%).

RESULTS

Tables 1 and 2 give the results on 16 normal adults and a few edematous and abnormally fat individuals. The time following the administration of potassium bromide is given for each determination. Bromide concentration is expressed in mEq/l. of ultrafiltrate of serum. The apparent volume of distribution (bromide space) is shown. The last column shows the bromide space corrected for bromide in
red cells. These values are expressed as percentage of body weight. No values include corrections for urinary losses. It is assumed in all cases that the body contains 40 ml of red cells/kg body weight. Hence 40 × bromide concentration/ml of red cells was subtracted from the administered bromide to obtain the total bromide excluding that of red cells. In the patients studied, this assumption indicated that 10–13% of the administered bromide is in the cells. When red cell bromide was not determined, an arbitrary 10% was assumed to penetrate the red cells. In calculating the values in the last column of the tables this factor has been taken into account.

In normal individuals, the bromide concentration remains constant within 2.5% from 2–7 hr after the administration of potassium bromide. This constancy is within the error of the determination. The concentration of bromide after 2.5–3 hr is satisfactory to measure the apparent volume of rapid distribution of bromide. After 24 hr the concentration decreases significantly and urinary losses undoubtedly account for this decrease. Since the edematous patients were excreting practically no chloride in the urine, bromide excretion was also negligible. It is not surprising, therefore, that the serum levels remained constant within the error of the determination for 24–30 hr.

The average apparent volume of distribution of bromide (excluding the red cells) was 21.0% of the body weight of normal, young males and 20.4% of normal, young females. The variations seemed to follow the differences in body fat. Patient E.B., who was exceptionally fat, showed a corrected bromide space of 14.5% of the body weight. The patients with generalized edema and ascites showed corrected bromide spaces of 26–43% of the body weight.

**DISCUSSION**

Conway precipitated the proteins and extracted the bromide with methyl alcohol. Ashing was carried out over a Bunsen burner. The present method avoids the difficulty of a quantitative transfer of small amounts of ash to the Conway dishes and the uncertainty of ashing over a Bunsen burner. Indeed the original Conway method was unsuccessful in these hands and complete recovery of bromide could not be obtained. Heating the serum to dryness and extraction with water remove most of the proteins and in the muffle furnace satisfactory ashes were obtained. The ash contains some carbon but large amounts led to incomplete recovery. Temperatures, which produced an ash free of carbon, volatilized some of the bromide. The addition of NaHCO₃ to the solution to be ashed made it necessary to raise the temperature in order to oxidize the carbon. Probably each oven should be tested to determine satisfactory conditions for recovery. The time and temperature must be controlled but the margin in these two variables is sufficient to give reliable results.

The amount of potassium bromide given for the fluid space measurement is about the same as the daily dose recommended for epilepsy. The therapeutic level is 10 mM/l. and the toxic level is 20 mM/l. (17). The level of 2 mM/l. is, therefore, unlikely to cause disturbances. This concentration can be determined within 2% which is as accurate as is likely to be significant for fluid space measurements. A small dose is also advantageous because it introduces as little osmotically active material as is desirable when measuring fluid volumes.

Previous work (14, 16) has shown that the concentration of bromide in cerebrospinal fluid is, after 5 hr, about 25% of that of serum. Since the ratio of cerebrospinal fluid chloride to bromide is the same as that of the brain, the bromide of the brain follows the cerebrospinal fluid level rather than that of the serum. It follows that the
measurement of extracellular space as determined from serum bromide does not include about three-fourths of the extracellular space of the brain cavity. In the adult this error is negligible because it is no greater than some fraction of 1200 gm, the brain weight. In infants the error is proportionately greater.

Measurement of the bromide space presents considerable advantages. The small amount of administered bromide is unlikely to alter extracellular volumes significantly by osmotic effects. Furthermore continuous infusion into the volume to be measured is avoided, since the bromide reaches equilibrium rapidly. The amount in the body remains relatively constant over several hours owing to the slow rate of excretion. A single dose of bromide should enable one to follow the changes in the bromide space for several hours, as urinary excretion involves only small losses, and these are unlikely to interfere significantly with equilibrium. Discrepancies in the distribution of bromide, chloride and sodium might be followed in acute cases over several hours, during which loss or gain of body sodium and chloride occurs. In edematous patients the period of observation might be extended with a single dose of KBr.

Obviously the bromide space cannot be directly equated with extracellular volume. The correction for red cell bromide should be fairly accurate if blood volume is determined. However the actual concentration of bromide or chloride in most tissue cells is not known. Since the muscle contributes about 70% of the intracellular water of the body and apparently contains chloride at a concentration of about 4 mM/l., it is unlikely that the remaining 30% of intracellular fluid contains large amounts of chloride. Values for extracellular fluid of 16% predict that as much as one-third of the total chloride is intracellular and occurs at average concentrations as high as 40 or more mM/l. Extracellular volume is probably somewhat larger than 16% but probably a little less than 20%.

**SUMMARY**

A rapid and accurate method for determining serum bromide on 1 ml of serum containing 1 to 2 mEq of bromide/l. is described. The method is a modification of the microdiffusion technique of Conway, and it can be adapted to the estimation of bromide within the red cells. The bromide space corrected for erythrocyte bromide is about 20% of body weight in young adults. High values were obtained in edematous patients and low values in one excessively fat adult. Because of the slow urinary excretion, and the rapid equilibrium with extracellular fluids, the bromide space should prove a useful research method. The difficulties of measurement of extracellular space are briefly discussed. At present, no method is satisfactory and the bromide space cannot be equated with the extracellular space without further studies to define the intracellular bromide penetration of cells other than erythrocytes.

**ADDENDUM**

Since the completion of this paper, this method has been applied by Drs. S. Chaudhuri and G. Odell to 4 children suffering from nephrosis. It was found that the bromide in the ascitic fluid was in equilibrium with the serum bromide. The space measurement in terms of the body weights were 38, 42, 41 and 28% (for one early case). It must be pointed out that in the process of extracting bromide from the dried serum in nephrotic patients, ether should be used instead of water; otherwise a loss of bromide will occur. This is due to the extremely rich lipid content of the serum and the ascitic fluid. The bromide space of one nephrotic patient (weighing
20 kg) was repeatedly measured over a 48-hr period, and at the beginning of ACTH therapy. The values fluctuated from 42–44%. Estimations of the urinary bromide loss were made throughout. When diuresis commenced, the body weight had increased to 22 kg and this excess weight (2 kg) was quickly lost. The bromide space then measured 52%. At the end of a further 48 hr the weight was 17.5 kg and the bromide space was 50%. Calculations of the change in total body chloride content over this 48-hr period revealed a deficit of 290 mEq of chloride. (Total chloride = bromide space × serum chloride.) Actual measurement of the chloride loss in the urine revealed a value of 300 mEq.

These workers also studied a 4-wk-old infant (over a 17-hr period) suffering from pyloric stenosis. The increase in the bromide space, which followed electrolyte and fluid therapy, was measured. The total retention of ions was obtained from a balance study. At the beginning of this investigation the concentrations for Na, K and Cl in the extracellular fluid were 133, 4.9 and 93 mEq, respectively (serum values corrected for Donnan factor and serum water). At the end of the 17 hr, these values had increased to 142, 5.0 and 120 mEq, respectively. The initial bromide space was 506 ml and the final (after 17 hr) was 706 ml. The gain in extracellular electrolyte was therefore 25.5 mEq of Na, 0.80 mEq of K and 32 mEq of Cl. The total ionic retentions (by balance study) were found to be 21 mEq for Na, 4.2 mEq for K and 42.8 mEq for Cl. One can therefore deduce that the extracellular electrolyte retention was −4.5 mEq for Na, + 3.4 mEq for K and + 10.8 mEq for Cl, which is compatible with the known electrolyte change which occurs in the state of hypochloremic alkalosis due to pyloric obstruction (1).

This method has also recently been applied by this author to the investigation of a case of infantile acrodynia, where a finding of a diminished extracellular space and a significant reduction of total body chloride were revealed (18).

My thanks are due to Dr. Daniel C. Darrow for assistance in the presentation of this paper and for valuable guidance and instruction.

REFERENCES