THE DETERMINATION OF SODIUM IN BLOOD.*

BY EDWARD A. DOISY AND RICHARD D. BELL.

(From the Laboratories of Biological Chemistry of Washington University School of Medicine, St. Louis, and Harvard Medical School, Boston.)

(Received for publication, November 20, 1920.)

A knowledge of the amounts of sodium salts in tissues and biological fluids is of considerable importance in reaching an understanding of the physiological rôle of inorganic substances. However, an adequate study of the variations in the amount of sodium in blood, for instance, under different physiological and pathological conditions would be difficult if not impossible by methods now in use. The quantitative determination of sodium by existing methods requires the ashing of the material, the removal of sulfates, phosphates, iron, calcium, and magnesium, and the estimation of potassium in the weighed alkali chlorides. Such a long and tedious procedure seriously limits a study of the metabolism of sodium. Another limiting factor is the amount of material necessary for an analysis, at least 15 cc. of blood being required for the usual gravimetric method.

These considerations prompted the search for a shorter method for the determination of sodium in smaller amounts of material. It seemed essential that some insoluble compound of sodium be found which would permit its quantitative precipitation. The most promising of the very few sodium compounds having small solubility appeared to be the complex sodium cesium bismuth nitrite described by Ball (2) and used by him for sodium determinations. A method based upon the precipitation of this

* A preliminary report of this method was made at the meeting of the American Society of Biological Chemists at Cincinnati, December, 1919.

1 Kramer (1) has recently described a method for the determination of sodium based upon the insolubility of the pyroantimonate. We have had no experience with this method, but Kramer's data indicate that it is a satisfactory procedure for the estimation of sodium in small samples of tissue.
compound has proved to be readily applicable to blood and urine and with some modification should be equally serviceable for any tissue. The results are believed to be almost as accurate as those obtained by the older procedures while the amount of material and time required are much less. As small an amount of sodium as 0.01 mg. yields a precipitate in a final volume of 2 cc. None of the ions commonly occurring with sodium interferes with the precipitation.

Certainly this method has great promise. The chief objections to it as described by Ball are the formation of a scum of bismuth subnitrate during the precipitation of the complex sodium salt, and the solubility of the latter in all solvents of the precipitating reagent. After many experiments we feel that we have overcome these difficulties.

The formation of the scum is probably due to a loss of nitrous acid and a consequent decrease of acidity of the solution. We find that if the reagent is added to the cold solution of the sodium salt and the flask immediately put in a cold room (1°C.), a scum rarely forms within 24 hours. At this temperature the precipitation of the complex nitrite is more rapid and is generally complete in 18 hours.

All the solutions used by Ball for washing the precipitate in order to remove the mother liquor dissolve so much precipitate that serious errors are introduced. He attempted to apply a solubility correction which would amount to 10 or 15 per cent of the precipitate in some of our determinations. However, none of the solutions that we have tried was so efficient as the 50 per cent acetone in removal of the excess reagent. Consequently we have retained it but have reduced the solubility of the precipitate in it to almost zero. The 50 per cent acetone is saturated with solid sodium cesium bismuth nitrite at 1°C. The excess salt is filtered off at this temperature and the solution used for washing. Its temperature probably does not rise more than 2 or 3° before the washing is complete.

The method as published by Ball was gravimetric. We have been able to show that the nitrite is susceptible of both volumetric and colorimetric estimation. The nitrite may be oxidized

\[^2\text{Faber and Stoddard (3) have used the sodium method described by Ball for the analysis of potassium nitrate. They mention the titration of}\]

Downloaded from http://www.jbc.org by guest on September 1, 2017
to nitrate on titration with standard KMnO₄. 0.1 N permanganate is suitable since the precipitate from 1 mg. of sodium requires 4.35 cc. for oxidation of nitrite to nitrate.

\[
9 \text{CsNO}_2 \cdot 6 \text{NaNO}_2 \cdot 5 \text{Bi(NO}_3)\text{)}_3 = 30 \text{NO}_2
\]

\[
30 \text{NO}_2 + 15 \text{O}_2 = 30 \text{NO}_3
\]

1 gram-molecule (3,753.6 gm.) of the complex nitrite requires 15 gram-molecules or 480 gm. of oxygen for oxidation.

\[
\frac{9 \text{CsNO}_2 \cdot 6 \text{NaNO}_2 \cdot 5 \text{Bi(NO}_3)\text{)}_3}{30 \times 16} = \frac{3753.6}{480} = 7.82
\]

i.e., 1 mg. of O will be required for 7.82 mg. of the precipitate.

1 mg. of sodium produces 27.2 mg. of complex nitrite. Consequently

\[
\frac{27.2}{7.82} = 3.48 \text{mg. of O to oxidize precipitate from 1 mg. of sodium.}
\]

\[
\frac{3.48}{0.8} = 4.35 \text{ cc. 0.1 N KMnO}_4
\]

The colorimetric estimation is based on the coupling reaction of Griess (4) using napthylamine and sulfanilic acid. Advantage is taken of the fact that bismuth salts are soluble in alkaline tartrate solutions. The precipitate is dissolved in alkaline potassium tartrate, made up to a definite volume, and a suitable sample taken. The standard is 0.01 mg. of nitrite nitrogen. The colors are developed and read with a Duboscq colorimeter.

**Reagents.**

1. *Bismuth Cesium Nitrite Solution.*—Although we have attempted to improve Ball's reagent in several ways, we have been unsuccessful. It is much less stable at room temperature than at 1°C. If kept under an inert gas at 1°C. it is suitable for quantitative work for several weeks.

the precipitate with permanganate but give no data from which one may judge of its accuracy.

The authors are indebted to Mr. Faber and Mr. Stoddard for suggesting the possibilities of Ball's method for the determination of small amounts of sodium.
30 gm. of sodium-free potassium nitrite\(^3\) are dissolved in about 60 cc. of pure water. A solution containing 3 gm. of bismuth nitrate is added. (We keep on hand a 60 per cent solution of the crystallized salt in 2 N HNO\(_3\).) If a precipitate forms (due to excessive alkalinity of the KNO\(_2\)), dilute nitric acid is added carefully until it redissolves. A strong solution containing 1.6 gm. of CsNO\(_3\) and 1 cc. of 2 N HNO\(_3\) is added. The solution is diluted to 100 cc. and dilute nitric acid is used to remove any turbidity which may form. At this stage the reagent should be a clear orange-yellow. If sodium salts were present in any of the chemicals as impurity, the insoluble precipitate which has formed at the end of 24 hours is filtered off. The reagent is kept under illuminating gas in the cold room.

2. Acetone. Redistilled and kept ready for use at 1°C.

3. A 50 per cent solution of acetone saturated at 1°C. with sodium cesium bismuth nitrite.

*For Volumetric Procedure.*

1. Permanganate, 0.1 N or 0.05 N.
2. Oxalic acid, 0.1 N or 0.05 N.
3. H\(_2\)SO\(_4\), concentrated acid diluted with equal volume of water.

*For Colorimetric Procedure.*

1. Alkaline tartrate. Equal volumes of KOH (10 per cent) and tartaric acid (10 per cent) are mixed.
2. Sulfanilic acid, 0.8 per cent in 5 N acetic acid.
3. \(\alpha\)-Naphthylamine, 0.5 per cent in 5 N acetic acid.

\(^3\) Pure potassium nitrite has been a source of considerable difficulty which we have finally overcome. The potassium salts on the market generally contain large quantities of sodium. Since the nitrite cannot be purified by recrystallization, our only recourse was to make it. We have examined various samples of carbonate and have found that both Merck's Blue Label and Eastman's are substantially free from sodium. As an emergency procedure sodium-free potassium carbonate may be made by recrystallization of the oxalate. It is dried and ignited in a platinum dish.

The pure nitrite is made by passing nitrous fumes into a 25 per cent solution of sodium-free potassium carbonate. Nitric acid (sp. gr. 1.2) is dropped from a separatory funnel into a flask containing arsenious oxide. A delivery tube carries the fumes into the carbonate. The reaction is complete when the solution in the receiving flask gives off many fine bubbles of carbon dioxide on shaking. We generally run nitrite determinations at intervals to ascertain whether the reaction is running properly.
4. Nitrite standard. Made by recrystallizing AgNO₃ from hot water until free from nitrate. Add NaCl equivalent to the AgNO₃ and filter off silver chloride. Determine nitrite nitrogen by Devarda’s (5) method and dilute so that 5 cc. = 0.01 mg. N.

Preparation of the Material for Analysis.

When we started our analyses of blood and urine we thought that it would be necessary to remove the organic material by ashing. We have used both the wet and dry methods. In our hands the former has been the more successful. As many of our data were obtained on blood ashed with sulfuric and nitric acids, this procedure is given below in detail.

1 cc. of whole blood, plasma, or urine is transferred to a pointed Pyrex tube. A few drops of H₂SO₄ (concentrated) and 5 cc. of HNO₃ (concentrated) are added. A low flame which keeps the liquid boiling gently is used. The digestion is continued in the usual manner until the liquid is colorless. Urine is completely oxidized in about 8 minutes but the blood generally takes 3/4 hour. As both iron salts and any appreciable amount of phosphates interfere with this method of determination of sodium, they must be removed from the blood digest. None of the samples of urine or plasma analyzed contained sufficient quantities to vitiate the analysis.

The digest of the whole blood is quantitatively transferred to a 25 cc. volumetric flask with about 20 cc. of water. 1 drop of methyl orange and 5 to 6 drops of 4 per cent bismuth nitrate are added. A strong solution of potassium carbonate (free from sodium) is added dropwise with shaking until the indicator changes color. The flask is made up to volume, mixed, and the solution transferred to a centrifuge tube. Centrifuging at moderate speed throws down the insoluble phosphates and iron salts.

These tubes are reclaimed from non-protein nitrogen determinations. After a tube has been rendered unserviceable by the phosphoric acid it is heated in an oxygen-gas flame and drawn out to a point. The tubes with small tips and thin walls stand heating best. The pointed tip provides a constant stream of bubbles which promote even boiling. No boiling stones are necessary.
20 cc. of the supernatant liquid are pipetted into a 50 cc. Erlenmeyer flask. This solution is evaporated on the hot plate to 2 to 3 cc. and rendered just acid with HNO₃. An excess of 0.5 cc. of 2 N HNO₃ is added and the precipitation carried out as described.

After carefully checking our method on the ashed blood, we found that a great deal of time and labor could be saved by deproteinization with trichloroacetic acid. Our data (Table I) indicate that a dilution of 1:5 or 1:10 is perfectly satisfactory.

The procedure is as follows. 5 cc. of whole blood or plasma are transferred to a 50 cc. flask containing 35 cc. of water, and 5 cc. of trichloroacetic acid (20 per cent) are added. The contents of the flask are diluted to the mark, mixed, allowed to stand about 30 minutes, and filtered through a dry paper. 10 cc. of filtrate (equivalent to 1 cc. of blood) are pipetted into a 50 cc. Erlenmeyer flask and 1 drop of concentrated nitric acid is added. The flask, closed with a trap, is heated on a piece of asbestos on a hot plate until brown fumes from the acid are evolved. It is removed, cooled, and the trap washed off with a few drops of water. Precipitation is then carried out as described below.

*Precipitation.*—The solution is cooled to 10–12°C. and 3 cc. of reagent are added for each milligram of sodium expected. The flask is stoppered with a two-hole rubber stopper bearing two short glass tubes bent at a right angle. One is fitted with a short rubber tube with a glass plug, the other with a Bunsen valve and plug. Illuminating gas freed from H₂S is passed into the flask for a few seconds and the plugs are replaced. The flask is put in the cold room at 1°C. A yellow crystalline precipitate begins to form in a few minutes. Precipitation is complete in 24 hours, whereas at room temperature 48 hours are required. A scum is much more likely to form before the precipitation is complete at the higher temperature.

The precipitate is rapidly filtered on a Gooch crucible which has previously been dried and weighed. Washing with the ice-cold 50 per cent acetone which is saturated with sodium

---

5 We advise the use of a trap in the mouth of the flask to prevent loss by bumping. Ours is made by blowing a bulb on the closed end of a small soft glass test-tube. A hole is then blown in the side of the bulb which is then cut off from the tube.
cesium bismuth nitrite is quickly carried out. Speed during the filtration and washing is essential for good results. 10 cc. of the 50 per cent acetone are used; 2 cc. are blown from a miniature wash bottle (made from a 10 cc. graduated cylinder) into the precipitation flask. The suction is stopped and the liquid poured onto the mat. This is repeated four times. 10 cc. of pure acetone are used to complete the transfer of the precipitate to the Gooch. If the volumetric or colorimetric procedure is used, complete transference of the precipitate is not necessary.

The Gooch is dried in an air bath at 100°C. until a constant weight is obtained.

Weight of precipitate × 0.03675 = Weight of sodium in solution

We prefer to avoid the weighing necessary for a gravimetric determination. Incidentally any scum which may have formed will cause the gravimetric result to be too high but will not affect the nitrite estimation. Very frequently potassium nitrate crystallizes at 1°C. This will also give erroneous gravimetric results. The precipitate is filtered as described on a Gooch crucible and estimated either volumetrically or colorimetrically.

**Volumetric Procedure.**—The Gooch crucible and contents are placed in a tall 200 cc. beaker. A large excess of standard permanganate (at least twice the amount necessary for oxidation) and enough water to cover the crucible are added. The precipitate is stirred loose from the crucible and asbestos. 10 cc. of 1:1 sulfuric acid are poured in while the liquid is being stirred. After a few minutes the solution is heated to 75°C., an excess of standard oxalic acid added, and the titration finished with permanganate. A blank must be run on the reagents under similar conditions.

\[
\text{Ce. KMnO}_4 \times \text{normal factor} \times 8 = \text{Mg. O used}
\]
\[
\text{Mg. O} \times 7.82 = \text{Mg. precipitate}
\]
\[
\text{Mg. precipitate} \times 0.03675 = \text{Mg. sodium}
\]

**Colorimetric Procedure.**—For those who prefer a colorimetric method we have established a suitable technique. The precipitate is completely transferred to a beaker and 10 cc. of the alkaline tartrate are added. Upon warming, the salt rapidly dissolves. The solution is quantitatively transferred to a 100 cc. volumetric
flask, cooled, made up to volume, and mixed. A further dilution is made so that a volume containing approximately 0.01 mg. of N can be taken for colorimetric comparison.

The standard and unknown in 100 cc. volumetric flasks are diluted to about 90 cc. 2 cc. of the sulfanilic acid and napthalamine solutions are added to each. The flasks are made up to volume, mixed, and allowed to stand 20 minutes for the full color development. There is a very wide range of proportionality of color intensity to the amount of nitrite present. The colors are very stable.

The calculation is simple.

$$\frac{20 \times 0.01}{\text{Unknown reading}} = \text{Mg. N in sample used}$$

Suppose the sample was 1 cc. of a dilution of 1:1,000 then

$$\frac{9 \text{CsNO}_2 \cdot 6 \text{NaNO}_2 \cdot 5 \text{Bi(NO}_2\text{)}_3}{30 \text{N}} = \frac{373.6}{420.3} = 8.93$$

$$\text{Mg. N} \times 8.93 = \text{Mg. precipitate}$$

$$\text{Mg. precipitate} \times 0.0367 = \text{Mg. sodium in sample}$$

We prefer the volumetric method to either the gravimetric or colorimetric on account of its greater speed and accuracy. Possible contamination of the precipitate with either bismuth subnitrate or potassium nitrate renders the gravimetric values doubtful. The colorimetric procedure is open to the usual errors of such methods. The red color is very bright and comparison is rather difficult.

In Table I the figures given under "Indirect" were obtained by ashing the material in a platinum dish. The ash was taken up in water, acidified with hydrochloric acid, and the sulfates were precipitated. The barium sulfate was filtered off and the filtrate made alkaline with ammonia. After the barium phosphate had precipitated, an excess of ammonium carbonate was added. The precipitate was filtered off and the filtrate evaporated in a platinum dish. The salts were carefully dried and gently ignited. The salts were dissolved in water and tested for complete removal of barium and calcium. Generally a small amount of insoluble
material was present. It was filtered off and the filtrate caught in a small weighed platinum dish. Evaporation to dryness, and ignition were carried out as previously described.

TABLE I.
Sodium in Urine and Blood.

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Source of sample</th>
<th>Sodium per 100 cc.</th>
<th>Method.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Indirect.</td>
<td>Direct ash.</td>
</tr>
<tr>
<td>1</td>
<td>Urine, normal.</td>
<td>414</td>
<td>403</td>
</tr>
<tr>
<td>2</td>
<td>&quot;</td>
<td>351</td>
<td>349</td>
</tr>
<tr>
<td>3</td>
<td>&quot;</td>
<td>351</td>
<td>348</td>
</tr>
<tr>
<td>4</td>
<td>Blood, beef.</td>
<td>284</td>
<td>285</td>
</tr>
<tr>
<td>5</td>
<td>&quot;</td>
<td>278</td>
<td>279</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>272</td>
<td>263</td>
</tr>
<tr>
<td>7</td>
<td>&quot;</td>
<td>266</td>
<td>&quot;</td>
</tr>
<tr>
<td>8</td>
<td>&quot;</td>
<td>281</td>
<td>272</td>
</tr>
<tr>
<td>9</td>
<td>Plasma, &quot;</td>
<td>336</td>
<td>339</td>
</tr>
<tr>
<td>10</td>
<td>Blood, swine.</td>
<td>221</td>
<td>219</td>
</tr>
<tr>
<td>11</td>
<td>&quot;</td>
<td>206</td>
<td>204</td>
</tr>
<tr>
<td>12</td>
<td>&quot;</td>
<td>217</td>
<td>216</td>
</tr>
<tr>
<td>13</td>
<td>&quot;</td>
<td>209</td>
<td>212</td>
</tr>
<tr>
<td>14</td>
<td>Plasma, &quot;</td>
<td>360</td>
<td>358</td>
</tr>
</tbody>
</table>

* Determination on 0.4 cc. of blood.

The mixed sodium and potassium chlorides were dried to constant weight and the potassium was determined in the usual manner as perchlorate. The sodium perchlorate was dissolved in
97 per cent alcohol containing 0.2 per cent perchloric acid. Consequently the results for potassium are possibly a little low and sodium a little high (6).

Table II illustrates the accuracy to be expected when weighed quantities of sodium cesium bismuth nitrite are titrated with permanganate. The volumetric estimation of known amounts of sodium salts also throws light on the reliability of a titration procedure.

Table III gives some values obtained by the gravimetric, volumetric, and colorimetric procedures on pure sodium nitrate.

### TABLE II.

**Titration of Sodium Cesium Bismuth Nitrite with Permanganate.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>62.6</td>
<td>62.0</td>
<td>-0.6</td>
</tr>
<tr>
<td>2</td>
<td>55.3</td>
<td>55.8</td>
<td>+0.5</td>
</tr>
<tr>
<td>3</td>
<td>89.4</td>
<td>88.4</td>
<td>-1.0</td>
</tr>
<tr>
<td>4</td>
<td>51.5</td>
<td>52.1</td>
<td>+0.6</td>
</tr>
<tr>
<td>5</td>
<td>54.1</td>
<td>55.1</td>
<td>+1.0</td>
</tr>
<tr>
<td>6</td>
<td>118.6</td>
<td>118.0</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

### TABLE III.

**Determination of Sodium in Pure Sodium Nitrate.**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mg.</td>
<td>mg.</td>
<td>hrs.</td>
<td>°C.</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>0.96</td>
<td>44</td>
<td>22</td>
<td>Gravimetric.</td>
</tr>
<tr>
<td>1.0</td>
<td>1.01</td>
<td>65</td>
<td>22</td>
<td>Volumetric.</td>
</tr>
<tr>
<td>1.0</td>
<td>0.99</td>
<td>69</td>
<td>22</td>
<td>&quot;</td>
</tr>
<tr>
<td>2.0</td>
<td>1.62</td>
<td>20</td>
<td>22</td>
<td>Gravimetric.</td>
</tr>
<tr>
<td>2.0</td>
<td>2.02</td>
<td>44</td>
<td>22</td>
<td>Volumetric.</td>
</tr>
<tr>
<td>2.0</td>
<td>1.97</td>
<td>44</td>
<td>22</td>
<td>&quot;</td>
</tr>
<tr>
<td>2.0</td>
<td>2.03</td>
<td>44</td>
<td>22</td>
<td>&quot;</td>
</tr>
<tr>
<td>2.0</td>
<td>2.04</td>
<td>44</td>
<td>22</td>
<td>Gravimetric.</td>
</tr>
<tr>
<td>2.0</td>
<td>1.85</td>
<td>7</td>
<td>1</td>
<td>Colorimetric.</td>
</tr>
<tr>
<td>2.0</td>
<td>1.92</td>
<td>15</td>
<td>1</td>
<td>&quot;</td>
</tr>
<tr>
<td>2.0</td>
<td>2.04</td>
<td>24</td>
<td>1</td>
<td>&quot;</td>
</tr>
<tr>
<td>3.0</td>
<td>3.06</td>
<td>44</td>
<td>22</td>
<td>Gravimetric.</td>
</tr>
<tr>
<td>3.0</td>
<td>2.98</td>
<td>24</td>
<td>1</td>
<td>Volumetric.</td>
</tr>
</tbody>
</table>
Our early work in agreement with Ball’s demonstrated that at least 44 hours were necessary for the complete precipitation at room temperature. We have found 24 hours to be sufficient at 1°C. 15 hours at this temperature gave low results.

Table I shows a comparison of values obtained by the usual procedure and by our different modifications of the sodium cesium bismuth nitrite method. We think that the results are sufficiently accurate for most work but hope to increase their accuracy by a few refinements on which we are now working.

Attention should be called to the constancy of the amount of sodium in the blood of the same species. In the case of both swine and beef the maximum variation is only about 7 per cent.

BIBLIOGRAPHY.

THE DETERMINATION OF SODIUM IN BLOOD
Edward A. Doisy and Richard D. Bell

J. Biol. Chem. 1921, 45:313-323.

Access the most updated version of this article at http://www.jbc.org/content/45/2/313.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/45/2/313.citation.full.html#ref-list-1