THE ESTIMATION OF CHLORIDES IN BLOOD.

BY VICTOR C. MYERS AND JAMES J. SHORT.

(From the Laboratory of Pathological Chemistry, New York Post-Graduate Medical School and Hospital, New York.)

(Received for publication, July 31, 1920.)

Renewed interest in blood chlorides has been aroused by Allen,¹ who has endeavored to show that salt retention is the cause of pure hypertension, and also of the hypertension in many cases of kidney disease. The estimation of this constituent has been most often conducted on the plasma, rather than on whole blood, but there would now appear to be some question as to the reliability of plasma analysis in this connection unless the plasma is immediately separated from the corpuscles. In any case a significant change in the blood chlorides should definitely affect the chloride content of whole blood as well as that of the blood plasma. The practical use of various laboratory tests of diagnostic import has ordinarily kept pace with their reliability and simplicity. That the estimation of the blood chlorides has not received more clinical consideration must be ascribed either to lack of appreciation of the possible value of the test, or to the difficulties in technique. A method of chloride estimation is described below which we believe contributes to the simplicity and rapidity of the test, without impairing its accuracy.

Most American workers who have studied this question have employed the iodimetric method of McLean and Van Slyke,² or some modification of it. With their original method the blood proteins were removed with the aid of heat, acetic acid, magnesium sulfate, and blood-charcoal. The chlorides were then precipitated in acid solution with silver nitrate and the excess was titrated with iodine, using starch as an indicator. Owing to

the difficulty in securing suitable blood-charcoal, Harding and Mason\textsuperscript{3} modified the method by employing copper sulfate and alkali for the removal of the protein. Later in the same year Foster\textsuperscript{4} utilized \textit{m}-phosphoric acid to remedy the same difficulty. Almost simultaneously with the publication of the McLean and Van Slyke method, Myers and Fine\textsuperscript{5} described a technique of chloride estimation and later reported a few observations on the blood chlorides,\textsuperscript{6} although these were not then discussed. With this method the proteins were coagulated in the presence of five volumes of 0.01 N acetic acid with a total dilution of 1 to 10, the last trace of protein being removed with a few drops of colloidal iron. The chlorides were then titrated either according to the Volhard-Arnold method, using solutions one-tenth the strength of those employed for urine, or directly with the Mohr method in this practically neutral solution. The disadvantage of this method was in the difficulty of obtaining chloride-free colloidal iron. 3 years later Rappleye\textsuperscript{7} likewise made use of the Volhard titration for the estimation of chlorides in blood plasma. Still later Van Slyke and Donleavy\textsuperscript{8} greatly simplified the McLean-Van Slyke technique by introducing sufficient picric acid into the silver nitrate-nitric acid reagent to precipitate completely the proteins and chlorides in one operation. Although this method proved perfectly satisfactory for plasma, Austin and Van Slyke\textsuperscript{9} later noted that it gave too high results with whole blood, apparently as the result of the silver being bound by some constituent in the corpuscles. To overcome this difficulty for whole blood they now first precipitate the proteins with saturated picric acid solution. After filtration the chlorides are then estimated essentially as in the McLean-Van Slyke method.

\textsuperscript{3} Harding, V. J., and Mason, E. H., \textit{J. Biol. Chem.}, 1917, xxxi, 55.
\textsuperscript{4} Foster, G. L., \textit{J. Biol. Chem.}, 1917, xxxi, 483.
\textsuperscript{5} Myers, V. C., and Fine, M. S., Chemical composition of the blood in health and disease, New York, 1915, 34. also Myers, V. C., \textit{Post-Graduate}, 1915, xxx, 38.
\textsuperscript{6} Myers, V. C., and Fine, M. S., \textit{J. Biol. Chem.}, 1915, xx, 391.
\textsuperscript{7} Rappleye, W. C., \textit{J. Biol. Chem.}, 1918, xxxv, 509.
In connection with the present study we have tried a number of methods of protein precipitation, including the tungstic acid method of Folin and Wu. None of these has proved so satisfactory in our hands as the use of picric acid recommended by Van Slyke and Donleavy. Several years ago an attempt was made to utilize the picric acid filtrate employed in the estimation of sugar and creatinine for the chlorides, but at that time this method was not believed to be so satisfactory as the heat and acetic acid procedure. Our present observations, however, confirm the findings of Austin and Van Slyke as to the reliability of picric acid as a blood protein precipitant in the estimation of chlorides. Furthermore, they indicate that it makes little difference in the results obtained whether the blood is diluted 1 to 20 as in their technique, or 1 to 10 or 5. Since the 1 to 5 dilution is employed in the estimation of sugar and creatinine, the chloride estimation may be made on the same filtrate. We have preferred to adhere to the Volhard titration partly because this allows the use of the same solutions (diluted) employed in the Volhard-Harvey method for urine, and partly because of the rather rapid deterioration of the starch indicator used in the iodimetric method. When the starch solution is fresh the end-point is very sharp, sharper than in the thiocyanate titration, although with a little experience the latter is readily recognized. Many workers have complained of difficulties with the silver chloride filtration. We believe that the quickest and most satisfactory method of removing this rather troublesome precipitate is with the aid of the centrifuge as originally suggested.

To summarize the advantages of the technique described for blood chlorides, the estimation may be carried out: (1) on the same filtrate as used in the estimation of creatinine and sugar; (2) with the same reagents (diluted) as commonly employed for chloride estimation in urine (Volhard-Harvey method); and (3) considerable time may be saved by removing the troublesome silver chloride precipitate by centrifuging rather than by filtration.

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Method for Chloride Estimation in Blood.

When blood chlorides only are to be estimated there are some advantages in employing the 1 to 10 dilution and this technique will be described first.

Solutions Required.

1. Silver nitrate of such strength that 1 cc. is equivalent to 1 mg. of sodium chloride, 2.904 gm. to 1,000 cc. It may be prepared by a 1 to 10 dilution of the silver nitrate employed in the Volhard-Harvey method for urine.

2. Acid ferric alum indicator prepared by dissolving 100 gm. of crystalline ferric ammonium sulfate in 100 cc. of 25 per cent nitric acid and adding four parts of distilled water. This is one-fifth the strength employed for urine.

3. Ammonium thiocyanate of such strength that 2 cc. are the equivalent of 1 cc. of the above silver solution, or one-twentieth of the strength employed for urine. It contains approximately 0.65 gm. of the thiocyanate to 1,000 cc.

To simplify the test further it is possible to combine Solutions 1 and 2, two parts of No. 1 and one part of No. 2 being used with the 1 to 10 dilution of the blood, this combined solution being further diluted with one part of distilled water when the 1 to 5 blood dilution is employed.

3 cc. of whole blood (or plasma) are added to 27 cc. of distilled water in a 50 cc. centrifuge tube. About 0.5 gm. of dry picric acid is then added and the mixture stirred until protein precipitation is complete and the whole mixture turns a bright yellow color. The precipitate is now thrown down by centrifuging for a few minutes at moderate speed and the clear supernatant fluid decanted into a clean dry beaker. (If any particles remain suspended the fluid should be filtered.)

20 cc. are then pipetted into a clean dry 50 cc. centrifuge tube and 20 cc. of standard AgNO₃ solution, of such strength that 1 cc. is equivalent to 1 mg. of NaCl (Solution 1), and 10 cc. of the dilute acidified ferric alum indicator added (Solution 2). The contents are stirred to insure thorough mixing and the AgCl pre-precipitate is thrown down in the centrifuge. The clear supernatant fluid is decanted into a clean, dry beaker and 20 cc. portions are pipetted into each of two small porcelain evaporating dishes for duplicate titrations.
The titration is made with ammonium thiocyanate solution of such strength that 2 cc. are equivalent to 1 cc. of the AgNO$_3$ solution (Solution 3). The end-point is definite and consists of the first permanent tinge of reddish brown which extends throughout the mixture. Some experience may be necessary before the end-point is always recognized, but thereafter there need be no difficulty in obtaining exact duplicate titrations. Passing the end-point by one drop will introduce an error ordinarily of about 0.5 per cent in estimating chlorides in 100 cc. of blood or plasma.

When it is desired to utilize the 1 to 5 picric acid filtrate employed for sugar and creatinine, 5 cc. (equivalent to 1 cc. of blood) are employed, instead of 10 cc., for a single determination. If this method is to be followed regularly it is desirable to dilute the indicator solution further by one-half. In this case 5 cc. of the filtrate are pipetted into a 25 or 50 cc. centrifuge tube and 10 cc. of both the standard silver nitrate and ferric alum indicator added (or 20 cc. of the mixture referred to under “Solutions required”). After centrifuging titration is carried out on 20 cc. of the supernatant fluid as described above.

The calculation is $10 - \left(\frac{\text{Titer}}{2} \times \frac{5}{4}\right) \times 100 = \text{mg. of NaCl}$ in 100 cc. of whole blood or plasma.

With the above method it has been found possible to determine the sodium chloride content of pure solutions with an error of less than 1 per cent, and sodium chloride added to blood may be recovered with a similar degree of accuracy. A parallel series of results obtained with the Austin-Van Slyke method and the method described is given in Table I. As will be noted there is very close agreement between the two methods. In the case of the method described it apparently makes little difference whether a dilution of 1 to 5 or 1 to 10 is employed. All the observations reported in the table were obtained on whole blood.

In his first paper dealing with the chlorides of the blood plasma McLean$^{14}$ considered the influence of the plasma being allowed to stand in contact with the cells. He concluded that the change took place very slowly and that it was necessary only to centri-

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fuge within 2 to 3 hours to avoid the danger. In their first paper on the plasma bicarbonate Van Slyke and Cullen\textsuperscript{15} called attention to the effect which carbonic acid changes in whole blood might have on the chloride content of the plasma, a loss in bicarbonate resulting in an increase in the plasma chloride. This observation has recently been confirmed and extended by Fri-dericia,\textsuperscript{16} who states that estimation of the chlorides in the plasma or serum from blood which has been kept in open receivers must give too high results, because chlorides have passed into the plasma (or serum) on account of the decreasing CO\textsubscript{2} tension. McLean, Murray, and Henderson\textsuperscript{17} have likewise considered this question in a preliminary way. Observations bearing on this question are given in Table II. In taking the control

\textbf{Table I.}
Comparison of Results Obtained with Austin-Van Slyke Method and Method Described.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>NaCl per 100 cc.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Austin-Van Slyke method.</td>
<td>Method described.</td>
</tr>
<tr>
<td></td>
<td>mg.</td>
<td>mg.</td>
</tr>
<tr>
<td>2. M. B...</td>
<td>487</td>
<td>481</td>
</tr>
<tr>
<td>3. S. D...</td>
<td>487</td>
<td>481</td>
</tr>
<tr>
<td>4. I. S...</td>
<td>475</td>
<td>462</td>
</tr>
<tr>
<td>5. W. S...</td>
<td>447</td>
<td>444</td>
</tr>
<tr>
<td>6. R. I...</td>
<td>462</td>
<td>462</td>
</tr>
<tr>
<td>7. D...</td>
<td>475</td>
<td>475</td>
</tr>
<tr>
<td>9. F. M...</td>
<td>587</td>
<td>584</td>
</tr>
<tr>
<td>10. J. C...</td>
<td>531</td>
<td>537</td>
</tr>
<tr>
<td>11. M. D...</td>
<td>475</td>
<td>460</td>
</tr>
<tr>
<td>Average...</td>
<td>485</td>
<td>487</td>
</tr>
</tbody>
</table>

\textsuperscript{16} Fridericia, L. S., \textit{J. Biol. Chem.}, 1920, xliii, 245.
specimens of blood under oil the technique of Van Slyke and Cullen was followed. The subjects were normal, two being men and two women. In general it will be noted that standing increased the chloride content of the plasma. This being the case, results obtained on whole blood would appear to be more trustworthy than those obtained on plasma. Whole blood ordinarily contains about 100 mg. less NaCl per 100 cc. than plasma.

TABLE II.

Influence of Standing on Plasma Chloride.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Chloride content of whole blood (NaCl per 100 cc.)</th>
<th>Chloride content of blood plasma (NaCl per 100 cc.).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Taken under oil and separated immediately.</td>
<td>Taken in open container and separated immediately.</td>
</tr>
<tr>
<td>1. J. S.</td>
<td>506</td>
<td>600</td>
</tr>
<tr>
<td>2. L. J.</td>
<td>475</td>
<td>587</td>
</tr>
<tr>
<td>3. H. C.</td>
<td>538</td>
<td>644</td>
</tr>
<tr>
<td>4. F. T.</td>
<td>538</td>
<td>612*</td>
</tr>
</tbody>
</table>

* After slow aeration for 5 min. the figure was 616 mg.

SUMMARY.

A method of chloride estimation in blood is described, in which the same picric acid filtrate employed for the estimation of sugar and creatinine, and the same solutions (diluted) ordinarily employed for the estimation of chlorides in urine are utilized.
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