THE DETERMINATION OF PROTEINS IN BLOOD—A MICRO METHOD.

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The use of sodium sulfate instead of ammonium sulfate in the precipitation of globulins makes it possible to determine the proteins of plasma or serum in small quantities of blood. The basis for the selection of sodium sulfate has been discussed in the preceding paper (1). Robertson (2) described a procedure for the determination of blood proteins in small quantities of blood with the aid of the refractometer. Cullen and Slyke (3) have proposed a procedure which gives consistent results and which does not require any special apparatus beyond that to be found in any laboratory. Their method, however, required 5 cc. of plasma for each constituent of the blood determined. The determinations described below can be performed with the usual laboratory apparatus and require but 0.5 cc. of plasma or serum for each determination.

The procedures involve the precipitation of fibrinogen with calcium chloride, the globulins with definite concentrations of sodium sulfate at 37° C., and non-protein nitrogen with trichloroacetic acid. In the case of fibrinogen and non-protein nitrogen the technique of Cullen and Van Slyke is followed. The globulins are precipitated by adding a concentration of sodium sulfate which is greater than the required percentage by the amount of sodium sulfate necessary to produce the desired percentage when added to the blood sample. The solutions are prepared by dissolving the required quantity of sodium sulfate in a little less than the final volume, which requires heat for the higher percentages, and then diluting to volume at 37° C. All precipitations and filtrations with sodium sulfate are carried out in the
incubator or hot room. The following concentrations of sodium sulfate are needed: 14, 18, and 22.2 per cent. When 15 cc. portions of these solutions are added to 0.5 cc. of blood the final concentrations are approximately 13.5, 17.4, and 21.5 per cent of sodium sulfate respectively. At 13.5 per cent of sodium sulfate euglobulin is precipitated, at 17.4 per cent euglobulin and pseudoglobulin I are precipitated, and at 21.5 per cent all globulins are thrown out of solution. In case blood plasma is used fibrinogen is present in each case and the nitrogen representing this protein must be deducted. Suitable blanks must be made for each determination.

The volume of solution used, 15.5 cc. (15 cc. of salt solution plus 0.5 cc. of blood), permits duplicate determinations to be made on each precipitation. This does not insure against errors in precipitation, but it has been our experience that simultaneous duplicate precipitations almost invariably agree. When it is desired to make duplicate precipitations it is advised that 13.5 and 14.5 per cent, 16.4 and 17.4 per cent, and 21 and 22 per cent of sodium sulfate, final concentrations, be used. With these concentrations the values obtained with each pair should agree within experimental error, except perhaps in the case of 16.4 and 17.4 per cent of sodium sulfate.

Precipitations are made in test-tubes or 50 cc. centrifuge tubes and then closed with a rubber stopper. The filtrations are conducted in the incubator using a dry 9 cm. filter paper. It is desirable to wet the filter paper with a small amount of the solution to be filtered before pouring on the bulk of the solution containing the precipitate. The funnels are covered with watchglasses. 1 inch test-tubes held in test-tube racks are convenient for filtration. With these tubes and the cover-glasses a reasonably tight filtration system is obtained.

For measuring, the accurately calibrated Ostwald pipettes and the 15 cc. graduated pipettes introduced by Folin are used.

The nitrogen determinations are conducted in large Pyrex test-tubes in general according to the original micro procedure of Folin and Farmer (4), and the distillations are carried out, according to the procedure of Folin and Wu (5) in their system of blood analysis, without cooling the distillate. In distilling, a Pyrex connecting tube is used which carries a distilling head and
has an enlargement on the tube which dips into the acid to guard against mechanical transfer of alkali and back suction. For titrations we use standard hydrochloric acid and sodium hydroxide which are approximately 0.05 and 0.025 N respectively. Our burettes deliver 25 cc. and are graduated to 0.05 cc. Methyl red is used as an indicator.

The determinations are as follows: Plasma is collected so that it contains 0.5 per cent of potassium oxalate. Both plasma and serum are centrifuged until clear.

**Total Nitrogen.**—0.5 cc. of plasma or serum is placed in a large Pyrex test-tube and the 2 cc. of concentrated sulfuric acid, 1 drop of 5 per cent copper sulfate, and a quartz pebble are added; the solution is digested over a free flame until clear, and then 7 to 10 minutes longer. Cool 3 to 5 minutes, add 25 to 30 cc. of ammonia-free distilled water, a small amount of talcum powder or powdered pumice stone, and concentrated sodium hydroxide solution sufficient to neutralize the acid, and distill into standard acid.

In place of using two 0.5 cc. portions of blood 15 cc. of 0.8 per cent sodium chloride solution may be added to one portion of 0.5 cc. and two 5 cc. portions of the diluted plasma taken for analysis.

**Fibrinogen.**—0.5 cc. of plasma is measured into a tube, 14 cc. of 0.8 per cent sodium chloride solution at room temperature are added, then 1 cc. of 2.5 per cent calcium chloride, a small crystal of thymol, and the tube is stoppered. The tube and contents are allowed to stand until the fibrin is formed and then filtered on a dry filter. Two 5 cc. portions of the filtrate are taken for analysis.

**Euglobulin.**—0.5 cc. of plasma or serum is measured into a tube, 15 cc. of 14 per cent anhydrous sodium sulfate at 37°C. and a little thymol are added, and the tube is stoppered, shaken, and allowed to stand for at least 3 hours, or until the precipitate has settled. The solution is then filtered through a dry filter and two 5 cc. portions are taken for analysis. The results represent euglobulin in the case of serum and fibrinogen plus euglobulin in the case of plasma.

**Euglobulin Plus Pseudoglobulin I.**—The procedure is the same as for euglobulin except that 18 per cent sodium sulfate is used.
Total Globulins. The procedure is the same as in euglobulin except that 22.2 per cent of sodium sulfate is used.

Non-Protein Nitrogen.—0.5 cc. of plasma or serum is measured into a tube and 15 cc. of 5 per cent trichloroacetic acid at room temperature are added. The remainder of the procedure is the same as in euglobulin.

The calculations of nitrogen are those ordinarily associated with Kjeldahl determinations. The volume of solution from which the aliquot portions for analysis are taken is 15.5 cc. We have expressed our results in terms of grams of nitrogen in 100 cc. of blood. As the result of the analytical procedures the following results can be calculated for serum:

- Total nitrogen.
- Euglobulin nitrogen = Total nitrogen — nitrogen in filtrate from 13.5 per cent sodium sulfate precipitation.
- Pseudoglobulin I nitrogen = Nitrogen in filtrate from 13.5 per cent sodium sulfate precipitation — nitrogen from 17.4 per cent sodium sulfate precipitation.
- Pseudoglobulin II nitrogen = Nitrogen in filtrate from 17.4 per cent sodium sulfate precipitation — nitrogen in filtrate from 21.5 per cent sodium sulfate precipitation.
- Total globulin nitrogen = Total nitrogen — nitrogen in filtrate from 21.5 per cent sodium sulfate precipitation.
- Albumin nitrogen = Nitrogen in filtrate from 21.5 per cent precipitation — non-protein nitrogen.
- Non-protein nitrogen = Nitrogen in filtrate from trichloroacetic acid precipitation.

For plasma the euglobulin is estimated by subtracting the filtrate nitrogen from the nitrogen in the filtrate after the precipitation of fibrinogen.

Table I contains some comparative data on the Cullen-Van Slyke procedure and the micro method. Determinations using sodium sulfate and magnesium sulfate in the Cullen-Van Slyke method are included. Determinations for pseudoglobulin I are not included in the table since they were not made at the time the plasma was analyzed. The results are expressed in terms of the nitrogen remaining in the filtrate after precipitation without calculating the various fractions.
**TABLE I.**
Comparative results obtained with the method of Cullen and Van Slyke "macro" and the micro method. Results are expressed as grams of nitrogen per 100 cc. of blood remaining in the filtrate after precipitation.

<table>
<thead>
<tr>
<th></th>
<th>Cow.</th>
<th>Pig.</th>
<th>Goat.</th>
<th>Horse.</th>
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<tbody>
<tr>
<td></td>
<td>Macro</td>
<td>Micro</td>
<td>Macro</td>
<td>Micro</td>
</tr>
<tr>
<td>Total nitrogen...</td>
<td>1.67</td>
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<td>1.05</td>
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<td>CaCl₂.............</td>
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<td>1.39</td>
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<td>0.92</td>
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<td>0.81</td>
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<td>Sodium sulfate.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13.5 per cent...</td>
<td>1.01</td>
<td>0.41</td>
<td>0.74</td>
<td>0.39</td>
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<tr>
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<td>0.29</td>
<td>0.41</td>
<td>0.39</td>
</tr>
<tr>
<td>21.3 &quot; &quot; .......</td>
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<td>0.29</td>
<td>0.40</td>
<td>0.39</td>
</tr>
<tr>
<td>23.2 &quot; &quot; .......</td>
<td>0.27</td>
<td>0.27</td>
<td>0.31</td>
<td>0.37</td>
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<td>0.02</td>
<td>0.05</td>
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<td>0.53</td>
<td>0.44</td>
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<td>Magnesium sulfate.</td>
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<td></td>
<td>0.32</td>
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**BIBLIOGRAPHY.**

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