THE QUANTITATIVE DETERMINATION OF ALBUMIN IN URINE.

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No abnormal urinary constituent is so frequently tested for qualitatively as albumin, yet there is curiously enough no reasonably accurate and convenient method available for its quantitative determination. The clinical Esbach method is rapid, but, like all "sedimentation" methods, is wholly untrustworthy. And the coagulation methods with accompanying filtrations, washings and weighings, or nitrogen determinations, are so tedious and laborious that they are very seldom used. Whether or no quantitative determinations of albumin in urine are capable of yielding much valuable information suitable methods for the determination of this abnormal urinary constituent are manifestly needed. In this paper we shall describe two fairly convenient methods neither of which is particularly original but both of which appear to give satisfactory results.

Turbidity method. Kober\(^1\) has described a nephelometric method for the determination of proteins in milk and in digestion mixtures, but so far as we know the principle has not yet been applied to the determination of albumin in urine.

As applied to urine the turbidity method for the determination of albumin is as follows:

To about 75 cc. of water in each of two 100-cc. volumetric flasks is added 5 cc. of a 25 per cent solution of sulphosalicylic acid.\(^2\) To one flask is then added 5 cc. of the standard protein


\(^2\) After having tried out a number of different precipitants for albumin we came to the conclusion previously reached by Kober, namely that sulphosalicylic acid is the best reagent for the quantitative precipitation of albumin.
solution containing 10 mgms. of albumin, and to the other is added the albuminous urine 1 cc. at a time (by means of an Ostwald pipette) until the turbidity obtained seems to be reasonably near that of the standard. The two flasks are then filled up to the mark with water, cautiously inverted a few times to secure mixing, and are then ready for the quantitative comparison in the colorimeter tubes. The standard must invariably first be read against itself to secure the adjustment of the colorimeter (and of the eye). The contents of one of the Duboscq colorimeter cups is then replaced by the suspension of the unknown, and the turbidity comparison is made exactly as in colorimetric work.

The standard containing 10 mgms. of protein is set at 20 mm. The unknown must not read less than 10 nor more than 30 mm.

Dividing 200 by the product of the reading of the unknown and the number of cubic centimeters of urine taken gives the albumin in milligrams per cubic centimeter of urine. It is very important not to shake the albuminous suspensions in the volumetric flasks because of the tendency of the precipitate to agglutinate. The preliminary mixing must therefore be accomplished by means of a few gentle inversions.

A quantitative albumin determination can be made with a very satisfactory degree of accuracy in the course of a few minutes in the manner described provided only that a standard albumin solution is available.

The standard protein solution is prepared from fresh blood serum free from haemoglobin. For the preparation of this serum either slaughter house or normal human blood may be used. The so-called blood serum sold for the preparation of bacteriological culture media should be avoided as it is usually several days old and is frequently partially decomposed. The dried preparations of “blood albumin” listed by chemical dealers are also not satisfactory for the preparation of standard solutions.

To prepare the standard 25-35 cc. of serum are diluted with a 15 per cent solution of chemically pure sodium chloride to about 1500 cc. The solution is mixed and filtered. By means of nitrogen determinations the protein content of the filtrate is determined (protein = N × 6.25) and on the basis of the figure obtained the solution is diluted with 15 per cent sodium chloride solution so that it contains 2 mgms. of protein per cubic centimeter.
Sodium chloride in the concentration mentioned is fairly effective as a preservative. Nevertheless it is best to saturate the standard albumin solution with chloroform (20 cc.). We have had several different protein standards in the laboratory for some months and have been unable to find any change. As a matter of further precaution we have made it a practice, however, to keep the stock solutions in a refrigerator.

The above method is not applicable to urines which are very deeply colored with blood or bile pigments. The method is of course applicable to other albuminous fluids than urine as for example exudates, transudates and the cerebrospinal fluid.

**Gravimetric method.** The gravimetric determination of albumin in urine here described was devised for the purpose of checking up the values obtained by the preceding turbidity method and differs only in minor details from the gravimetric determinations long since described by others. It is as follows:

Ten cubic centimeters of urine are pipetted into an ordinary conical centrifuge tube which has been previously weighed. To this urine is then added 1 cc. of 5 per cent acetic acid and the tube allowed to stand for fifteen minutes in a beaker of boiling water. At the end of this time the tube is removed from the water bath and centrifuged for a few minutes. The supernatant liquid is then poured off, the precipitate in the tube is stirred up with about 10 cc. of boiling 0.5 per cent acetic acid and again centrifuged. The supernatant liquid is then again poured off and the precipitate in the tube again washed, this time with 50 per cent alcohol. After centrifuging and pouring off the supernatant liquid for a third time the tube is placed for two hours in an air bath at 100–110°, then cooled in a desiccator and weighed.

The following results were obtained by means of the gravimetric method on sodium chloride solutions to which standardized solutions of blood serum had been added:

<table>
<thead>
<tr>
<th>Grams per liter of protein added</th>
<th>Grams per liter of protein found</th>
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<tbody>
<tr>
<td>1.30</td>
<td>1.22</td>
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<tr>
<td>1.05</td>
<td>1.08</td>
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</tr>
<tr>
<td>1.05</td>
<td>1.02</td>
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</table>
The following results were obtained on a number of albuminous urines taken at random from material sent in to the hospital laboratory.

<table>
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<tr>
<th>URINE NO.</th>
<th>GRAMS PROTEIN PER LITER BY THE GRAVIMETRIC METHOD</th>
<th>GRAMS PROTEIN PER LITER BY THE TURBIDITY METHOD</th>
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</table>
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