A Simple Turbidimetric Method of Estimating Blood Urea

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The classic estimation of urea in the blood, while not technically difficult, is time consuming, and a quicker method was sought for an emergency procedure. It has been known for many years that a solution of xanthodrol with urea forms an insoluble precipitate, dixanthylurea. Also that this precipitate dissolves in 50% sulfuric acid to give an intense yellow-colored solution which obeys Beer's law up to certain concentrations. Unfortunately xanthodrol itself gives the same yellow color in sulfuric acid, so the precipitate of dixanthylurea has to be washed free of the xanthodrol reagent before preparing it for the colorimeter.

Beattie (1), in 1928, described a method which involved the repeated washing of the precipitate and the final evaluation of the yellow solution in sulfuric acid. Later, in 1937, Lee and Widdowson (2) elaborated Beattie's work and devised a micro-method which, they claimed, was accurate. This method was tedious and unsuitable for our purpose. Ten years later, in 1947, Engel and Engel (3) reconsidered the whole matter and produced an elegant method, but again it involved repeated washing of a small precipitate and the colorimetric estimation of the sulfuric acid solution. Caraway (4), in 1955, proposed a turbimetric method using the same reaction of xanthodrol and urea but taking the turbidity of the mixture, after five minutes, as a measure of the urea present. A disadvantage of this method is the continuing development of the turbidity after the stipulated 5 minutes. Furthermore the estimation of turbidity by eye, without any standard for comparison, is very difficult and introduces the per-
Table 1. Turbidimetric Method for Estimation of Urea, Using 5% Xanthydrol in Methanol

<table>
<thead>
<tr>
<th>Original blood urea</th>
<th>Urea added</th>
<th>Turbidimetric estimation</th>
<th>Recovery percent</th>
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<tbody>
<tr>
<td>30</td>
<td>97</td>
<td>120</td>
<td>94</td>
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<td>45</td>
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</tr>
<tr>
<td>55</td>
<td>95</td>
<td>150</td>
<td>100</td>
</tr>
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</table>

*All values in milligrams of urea per 100 ml.

The urea was added as a solution of 10 mg. urea in 1 ml. of 50% acetic acid.

To 0.9 ml. of serum (or plasma) was added 0.1 ml. of the urea solution. The above are the means of duplicate determinations.

No satisfactory artificial urea standard solution could be devised so the curve was constructed from estimations made on serum (or plasma) whose urea content had been ascertained by the aeration method. It was found, also, that aqueous solutions of urea did not give good recovery figures; hence the use of the 50% acetic acid solution.

Reagents and Apparatus

50% Acetic acid. Mix equal volumes of glacial acetic acid and distilled water.

5% Xanthydrol. (British Drug Houses 10% xanthydrol in methanol diluted with an equal volume of methanol)

Thoroughly clean and dry 6" × ¾" test tubes.

Thoroughly clean and dry pipet for the xanthydrol solution.

Serum, plasma, or protein-free filtrate prepared from equal parts of whole blood and 20% trichloracetic acid.

E.E.L. Colorimeter with dry 8-mm (O.D.) tubes and the red filter No. 205.

The two reagents keep well. No change in the xanthydrol reagent has been found after four weeks if it is stored in a well-stoppered bottle in the dark.
METHOD

Pipet 0.2-ml of serum or plasma or 0.4 ml of the protein-free filtrate into a clean, dry, test tube. Add 4 ml of the 50% acetic acid, or 3.8 ml if protein-free filtrate has been used. Add 0.4 ml of 5% xanthydrol reagent, being careful that it does not touch the side of the tube as it creeps up glass.

Mix by gentle shaking and let stand, at room temperature, for fifteen minutes.

Transfer to a cuvet and read in the colorimeter, using the red filter No. 205.

If a high blood urea is suspected then dilute the specimen 1:1, 1:2, 1:4 etc. with normal saline and use 0.2 ml of the diluted fluid.

Test tubes and colorimeter tubes can be quickly cleaned with 25% to 50% sulfuric acid and water.

It is most important that all tubes and pipets are quite dry before use, since xanthydrol is precipitated from methanol by water so that high readings would result if moisture were present.

RESULTS

A series of trials was made with bloods of known urea content of from 44 to 406 mg per 100 ml, and it was found that the turbidity in-

Fig. 1. Time-turbidity curve.
creased steadily after 5 minutes but that the increase became so small at 15 minutes that the manipulation of the specimen and slight delay in reading introduced no significant error. The time-turbidity curve (Fig. 1) became almost flat at 15 minutes, no matter what the concentration of urea so long as it was below 250 mg. per 100 ml.

**Fig. 2.** Scattergram of 100 specimens showing range of urea concentrations examined (mg./100 ml.).

**Fig. 3.** Concentration-absorbance curve showing that mean values of more than 100 determinations of various concentration levels follow the Beer-Lambert law. Blocked areas show range of galvanometer readings with the mean value for that concentration area falling on the line.
The development of turbidity was not affected by temperature in the range 15° to 37°, or by excessive amounts of either glucose or cholesterol in the blood. Barbiturates or sulfa-compounds present in the specimen gave no interference. It was found, however, that badly hemolysed specimens gave unreliable readings and were best discarded.

One hundred bloods have been examined, with urea contents of from 25 to 760 mg. per 100 ml., and it has been found that up to 250 mg. per 100 ml. the method gives results reliable enough for clinical use. Figure 2 is the scattergram of these estimations. The urease digestion and aeration method of Van Slyke and Cullen (5) was always carried out as a check. In the majority of cases the turbidity reading was within 5% of the value as determined by aeration. Figure 3 shows the spread of urea values at points along the colorimeter scale. The test has proved particularly useful in cases of suspected uremia, for with levels of 100 mg. per 100 ml., and over, a definite turbidity develops within the first minute after the addition of the xanthydrol reagent.

SUMMARY

A rapid turbidimetric method has been described for estimating urea in serum, plasma, or protein-free filtrate. The method uses a xanthydrol reagent and 50% acetic acid, and measurement of the degree of turbidity produced in 15 minutes in a colorimeter using a red filter. Blood urea values of 100 mg. per 100 ml. and over are indicated, clearly, in less than 1 minute.

REFERENCES

5. Van Slyke and Cullen, J. Biol. Chem. 19, 211 (1914).