Quantitation of Serum Urea as Microcapillary Columns of Dixanthylurea

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A simple technic is proposed for direct estimation of urea in terms of columns of dixanthylurea precipitated from serum. The procedure is reproducible and yields results comparable to those obtained colorimetrically using normal and uremic sera. An equation has been derived which permits conversion of levels of dixanthylurea columns, in millimeters, to urea nitrogen concentrations. The new method is useful in detecting as well as monitoring cases of uremia.

UREA readily combines with xanthydrol to form an insoluble precipitate, dixanthylea, which can be estimated gravimetrically (1–3) or colorimetrically (4–6). However, procedures which entail weighing precipitates are often too time-consuming for most routine purposes. Both xanthydrol and dixanthylurea, unfortunately, yield yellow color when treated with sulfuric acid. Therefore, xanthydrol must be removed by repeated washing of precipitates prior to colorimetric quantitation. To circumvent these disadvantages, Caraway (7) and Lawrie (8) have proposed turbidimetric determinations of dixanthylurea. In order to further facilitate estimation of dixanthylurea from serum, a technic has been developed whereby levels of centrifugally sedimented precipitate are measured in microcapillary tubes. Technical variables of the procedure have been investigated and the method applied to uniform drops of serum obtained from normal and uremic individuals.

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Materials and Methods

Serum Urea Technic

A drop of serum (28 μl.) delivered from a uniform-bore capillary tube* was mixed on a glass slide with 4 calibrated drops (160 μl.) of 50% acetic acid. A single drop (40 μl.) of 10% xanthydrol (British Drug Houses) in methanol was added to the mixture and uniformity obtained with an applicator stick. Homogeneous suspensions were drawn into capillary tubes to a precalibrated height of 60 mm. After flame-sealing, tubes were allowed to stand for 15 min. and centrifuged in a microcapillary centrifuge† for 15 min. Columns of sedimented precipitate were measured to the nearest 0.1 mm. by means of a stand magnifier.

Serum Urea Nitrogen Technic

A 0.02-ml. aliquot of serum was incubated at 37° with 0.2 ml. of buffered urease‡ for 15 min. Mixtures were then treated with 5.0 ml. of phenol color reagent and 5.0 ml. of an alkaline hypochlorite solution (9). Tubes were returned to the incubator for another 15 min. at 37° and colorimetric measurements performed in a spectrophotometer§ at 540 mμ. (10).

Results and Discussion

Effect of Time on Dixanthylurea Precipitation

Uniform drops of sera from 1 normal and 2 uremic subjects were treated with acetic acid and methanolic xanthydrol. Duplicate mixtures drawn into microcapillary tubes were centrifuged immediately while others were allowed to remain at room temperature for intervals ranging from 5 to 60 min. prior to centrifugation (Fig. 1). Formation of dixanthylurea was not instantaneous and, therefore, ample time was available for filling capillary tubes. Precipitation became evident in 5 min. and appeared complete within 15 min. No appreciable alteration in precipitate levels was observed in microcapillary tubes per-

*Available from Hyland Laboratories, Los Angeles, Calif. Tubes for delivering serum had an outside diameter of 0.069 ± 0.002 in., an inside diameter of 0.053 ± 0.002 in. and were 2.9 ± 0.03 in. in length. Reagents were dispensed from droppers calibrated to deliver 25 drops per milliliter. Dixanthylurea was sedimented in tubes with an inside diameter of 1.4 mm. and 75 mm. long.

†Model BB, International.
‡Type II, Sigma Chemical Company, St. Louis, Mo.
§Coleman Instruments, Inc., Maywood, Ill.
mitted to stand for as long as 1 hr. before centrifugation. Lawrie (8) found that turbidity of serum-xanthylrol solutions increased steadily 5 min. after mixing and reached a maximum within 15 min.

**Effect of Centrifugation Time on Sedimentation**

Dixanthylurea was precipitated from sera obtained from patients with moderate to marked uremia. After being left at room temperature for 15 min., microcapillary tubes were centrifuged for 5–25 min. Centrifugal sedimentation was rapid and packing was maximal after 15 min. (Fig. 2). Centrifugation for an additional 5–10 min. did not produce discernible diminution in columns of precipitate. A standard floor-model centrifuge, available in most laboratories, may be used for dixanthylurea sedimentation; however, more time is required to achieve a well-packed column of precipitate.

![Fig. 1 (left). Effect of time on dixanthylurea precipitation. Fig. 2 (right). Effect of centrifugation time on dixanthylurea sedimentation.](image-url)
Specificity of Xanthydril for Precipitation of Serum Urea

A 2.0-ml. aliquot of uremic serum mixed with 0.4 ml. of concentrated buffered urease was incubated at 37°. Small amounts of serum-urease were removed immediately and treated with acetic acid and xanthydril according to the proposed technic. Dixanthylurea was precipitated from additional quantities of the mixture at incubation intervals ranging from 5 to 25 min. Urea available for reaction with xanthydril disappeared quickly from serum-urease mixtures (Fig. 3). At the end of 25 min., the amount of precipitate was minute and difficult to measure. It is noteworthy that decreases in dixanthylurea isolated from serum-urease mixtures accompanied progressive rises in ammonia. Concentrations of ammonia attained a maximum after incubation for 25 min. These data suggest that a methanolic solution of xanthydril selectively precipitates urea from serum.

Standardization of the Proposed Technic

Since xanthydril is precipitated from methanol by water, an artificial standard urea solution could not be prepared (8). Therefore, serum collected from clinically healthy subjects as well as patients with moderate or severe azotemia was used to prepare three pools. Urea nitrogen determinations were performed on aliquots of the pools by di-
rect nesslerization (11) and the modified Berthelot reaction (9, 10). Duplicate dixanthylurea precipitates from uniform drops of the three pooled sera were also measured. A linear relationship obtained with increasing concentrations of urea nitrogen from 11 to 180 mg./100 ml. (Fig. 4). In most clinical laboratories a majority of sera exhibit concentrations of urea which fall within this range. Thus, a satisfactory standard curve can be prepared by using sera of high as well as low urea content.

Reproducibility of Dixanthylurea Measurements

A series of 6 sera containing normal and elevated amounts of urea were treated with xanthodrol according to the proposed technic. Duplicate levels of precipitate were in close agreement and exhibited a standard deviation of ± 0.15 mm. (Table 1). Replicate determinations of urea nitrogen performed by the modified Berthelot reaction yielded a standard deviation of ± 2.7 mg./100 ml. Therefore, the precipitation technic offers precision comparable to that obtained with colorimetric measurement of urea nitrogen.
**Table 1. Duplicate Estimations of DIXANTHYLUREA Precipitate Levels**

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Microcapillary columns of precipitate (mm.)</th>
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<tbody>
<tr>
<td>1</td>
<td>13.0</td>
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<tr>
<td>2</td>
<td>1.5</td>
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<tr>
<td>3</td>
<td>4.2</td>
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<tr>
<td>4</td>
<td>15.3</td>
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<td>5</td>
<td>1.3</td>
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<tr>
<td>6</td>
<td>3.6</td>
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**Fig. 5.** Comparison of dixanthylurea precipitate columns with serum urea nitrogen concentrations.
Comparison of Direct and Indirect Estimations of Serum Urea

Blood was collected from 100 hospital patients with normal as well as elevated circulating amounts of urea. Measurements of dixanthylurea precipitates were compared with colorimetric quantitation of serum urea nitrogen (Fig. 5). Precipitate and urea nitrogen levels ranged from 1.0 to 14.0 mm. and from 6 to 177 mg./100 ml., respectively. Values showed good correlation ($r = 0.965$) at serum urea concentrations studied. From these data an equation,

\[
\text{Mg. urea nitrogen per 100 ml. serum} = 13.5 \text{ (mm. dixanthylurea)} - 5.1
\]

was derived from a line of best fit. The expression permits conversion of capillary columns of dixanthylurea into urea nitrogen concentrations with a degree of accuracy satisfactory for most clinical purposes.

Application of the Proposed Technic

Microcapillary columns of dixanthylurea were isolated from serum of 100 clinically healthy blood donors. Precipitate levels averaged 1.7 mm. (Fig. 6). A majority of values fell between 1.0 and 2.0 mm. of precipitate which correspond to urea nitrogen concentrations of 8.4–21.9 mg./100 ml. Thus, estimation of urea in terms of dixanthylurea precipitate and application of the proposed conversion equation yield values within accepted normal limits.
References