A Rapid Method for the Determination of Urea in Blood and Urine

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A rapid method for the determination of urea in blood and urine based upon the urea-p-dimethylaminobenzaldehyde reaction is described. Results of determinations can be reported in 15 min. The method described is suitable for routine use in the clinical laboratory.

The purpose of this report is to present a rapid method for the determination of urea in blood and urine based upon the urea-p-dimethylaminobenzaldehyde reaction.

Ehrlich, Rhode, Brocher, Weltmann and Barrenscheen, and Werner (1-5) reported on numerous substances that form red, orange, violet, and yellow interaction complexes with p-dimethylaminobenzaldehyde. Activated carbon has the specific property of adsorbing strongly aromatic substances (6). By treating the blood and urine with activated carbon, these interfering substances are removed.

Thus, when deproteinized and carbon-treated filtrates of blood and urine are reacted with our modified Ehrlich reagent, an immediate, clear, stable yellow complex is formed. These same filtrates incubated with urease and then reacted with p-dimethylaminobenzaldehyde did not form colored complexes.

The method described here is based upon the above-mentioned principles.

Materials and Methods

Reagents

Trichloroacetic acid, 10%.

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**Darco activated carbon.**

**Modified Ehrlich reagent.**

Five grams of p-dimethylaminobenzaldehyde are dissolved in 20 ml. of concentrated hydrochloric acid. Then, 80 ml. of distilled water are added. This reagent stored in an amber glass-stoppered bottle is stable for 1 month.

**Standardization**

Aqueous solutions containing 100, 50, 25, and 12.5 mg./100 ml. of urea were prepared. One milliliter of each solution was diluted with 4.0 ml. of 10% trichloroacetic acid and then reacted with 1.0 ml. of modified Ehrlich’s reagent in 19 × 105-mm. cuvets. The resultant colored solutions were read in a Coleman Junior Spectrophotometer at 425 mμ against a blank of 1.0 ml. of distilled water to which had been added 4.0 ml. of 10% trichloroacetic acid and 1.0 ml. of modified Ehrlich’s reagent. The percentage transmittance was plotted against concentration on semilogarithmic paper (Fig. 1). It is noted that the curve obtained followed Beer’s law, in a straight line up to 100 mg./100 ml. urea.

![Fig. 1. Standard curve of percentage transmittance for urea.](image)

**Procedures**

**Blood Urea**

1. Three milliliters of whole oxalated blood are added to 12 ml. of 10% trichloroacetic acid, the proteins precipitated and the mixture filtered through Whatman No. 5 paper.

2. To this filtrate is added approximately 0.25 gm. of activated charcoal, the mixture shaken for 30 sec., and again filtered through Whatman No. 5 paper.

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*Atlas Powder Company, New York, N. Y.*
3. Five milliliters of this filtrate are reacted with 1.0 ml. of our modified Ehrlich reagent. The resultant color is read against a blank of 1.0 ml. of distilled water plus 4.0 ml. of 10% trichloroacetic acid and 1.0 ml. of the modified Ehrlich's reagent at 425 m\(\mu\) in 19 \times 105 mm. cuvets.

The observed per cent transmittance is interpolated on the standard curve (Fig. 1) and the concentration of urea is read directly.

**Urine Urea**

1. Four ml. of freshly voided urine are diluted with distilled water to 100 ml. in a volumetric flask.

2. Three ml. of the diluted urine are then treated exactly as in the blood-urea determination. The resultant color is read at the same wavelength and against the same blank as for blood.

3. The interpolated concentration on the standard curve (Fig. 1) is multiplied by 25 (the dilution factor). If the determined value exceeds 100 mg./100 ml. dilute the deproteinized and carbon treated filtrate of blood or urine with 8% trichloroacetic acid (prepared by adding 4.0 ml. of distilled water to 16.0 ml. of the trichloroacetic acid solution) and make the necessary dilution-factor correction.

**Discussion**

The yellow-green color produced by the interaction of Ehrlich's reagent with urea is immediate, intense, clear, and stable. The spectral analyses of the yellow-green interaction products of the deproteinized and carbon treated blood and urine filtrates and of urea-p-dimethylaminobenzaldehyde revealed identity with peak absorption in the near ultraviolet range, 420 m\(\mu\) (Fig. 2). We have not observed any variation in color over a 2-hour period.

Weltmann and Barrenscheen (4) in 1922, had studied the urea-p-dimethylaminobenzaldehyde reaction and had developed a technic whereby the appearance of the yellow-green complex indicated a blood level of nonprotein nitrogen greater than 36–40 mg./100 ml. They did not make any attempt at the quantitation of urea. It might very well be that the amounts of concentrated hydrochloric acid used did not permit sensitivity. We have found that reducing the concentration of acid gave increased sensitivity for a given amount of p-dimethylaminobenzaldehyde. The concentrations of reagents employed in our method were optimum for sensitivity and the resultant
curve permitted the quantitation of values from 11 mg./100 ml. upwards to be in the optimum range of the spectrophotometer—90–10%T (Fig. 1).

Rhode (2) in 1905, and Brocher (3) in 1932, listed many compounds giving yellow, red, and orange colors with Ehrlich's reagent. Of the substances recorded—urobilinogen, indican, phenol, allantoin, tryptophane, uric acid, sodium hydroxbutyrate, morphine, camphor, novocaine, antipyrine, phenacetin and acetone—only acetone was to be considered. Activated charcoal has the specific property of adsorbing strongly aromatic substances (6); acetone is not adsorbed by the charcoal. However, we found that 500 mg./100 ml. of acetone, which is higher than that usually found in the blood in diabetic

**Fig. 2.** Spectral analyses of urea-p-dimethylaminobenzaldehyde complex and blood-filtrate p-dimethylaminobenzaldehyde complex.
acidosis, did not give any significant color with our modified Ehrlich's reagent. Ammonia does not interfere.

Many patients on whom urine and blood urea determinations are made may be on sulfanilamide therapy. Sulfur derivatives when reacted with p-dimethylaminobenzaldehyde result in a yellow-green color (5). However, this interference was completely nullified by the treatment of the blood and urine with activated carbon prior to analysis of the deproteinized filtrate. The sulfur derivatives are completely adsorbed on charcoal and do not interfere in the final determination of urea. A method developed by us taking advantage of this selective carbon separation of urea from sulfur drugs for the determination of sulfur derivatives in blood and urine will be published shortly.

The method reported here is sensitive to 2 mg./100 ml. of urea in blood. The blood urea values obtained in 12 normal adults selected at random were between 21.2 and 40 mg./100 ml. with an average of 29.9. Table 1 shows the recoveries of urea added to 13 samples of whole oxalated blood. The mean recovery rate was 98.5 per cent. These losses are of about the same magnitude as those reported for the diacetyl monoxime method. Rosenthal (7) reported an average recovery rate of 94-103 per cent and Marsh et al. (8) 94.5%, obtained with an automatic dialyzing apparatus.

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