Microdetermination of Urea in Urine Using p-Dimethylaminobenzaldehyde (PDAB)

The solution:
Devise an improved microchemical method of analysis for urea in urine.

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Adapt the PDAB method for the determination of urea in urine to a micro scale.

How it's done:
Prepare a standard urea solution by dissolving 1.500 g of urea in distilled water in a 100 ml volumetric flask and diluting to the mark.

Dissolve 5 grams of analytical reagent grade p-dimethylaminobenzaldehyde in 100 ml of absolute ethanol in a 200 ml volumetric flask. Slowly add 5 ml of concentrated sulfuric acid with swirling and cool to room temperature. Add distilled water to the mark. Store this PDAB reagent at room temperature in an amber bottle in the dark when not in use. It is relatively stable for many months.

Prepare a blank by micropipetting 0.01 ml of distilled water into a tube, adding 5 ml of PDAB reagent, and mixing well two or three times on a vortex mixer. In separate tubes, repeat this procedure using the standard urea solution and the urine sample in place of distilled water. Allow the tubes to stand for 10 minutes. Put the blank in a one-cm stoppered cuvette and read absorbance in a spectrophotometer set at a wavelength of 435 nm and 0.02 mm slit width. Repeat the above step for the standard urea solution and the urine sample, respectively, and correct each for the blank.

A stable color develops when PDAB reagent is mixed with the standard urea solution or the urine sample. This color is proportional to the urea content. The concentration of urea in the urine sample is calculated by dividing the absorbance of the urine sample by the absorbance of the standard, and multiplying by the concentration of urea in the standard.

Beer's law is obeyed over the entire range of best photometric accuracy in the spectrometer. Readings greater than 0.9 or less than 0.1 absorbance would indicate that the colored solution must either be diluted by adding more PDAB reagent or the sample size increased to retain the best photometric accuracy.

This microchemical adaptation of the PDAB method avoids extra steps of deproteinizing or removing normal urinary chromogens, which are required by other adaptations of the method, except under unusual circumstances where interference from excess protein occurs or when a sulfa drug or p-aminosalicylic acid (PAS) is present. In the event of protein interference, a stable tungstic acid may be used for deproteinizing the urine sample. Interference from a sulfa drug or PAS can be detected or removed.

Note:
1. Accuracy and precision of the microchemical adaptation of the PDAB method for determining urea in urine are satisfactory. The use of 0.01 ml samples rather than 0.2 to 2.0 ml samples required by other adaptations of the method is an advantage. It should be adaptable with ease to automated analysis.
2. Clinical laboratories, drug companies, hospitals, doctors, veterinarians, pharmacologists, toxicologists, and biochemists may be interested in this information.

3. Documentation is available from:
   Clearinghouse for Federal Scientific and Technical Information
   Springfield, Virginia 22151
   Price $3.00
   Reference: TSP69-10317

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   No patent action is contemplated by NASA.
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