blank measurements + 2 SD) and the upper limit of linearity was 50 mg/L. Samples of urine containing protein concentrations >200 mg/L, as detected by dipstick assay were diluted 10-fold with saline before assay. Evaluation of 140 diabetic urines (albumin concentration 1 to 200 mg/L), performed in parallel by this test and by radioimmunoassay (Pharmacia, Uppsala, Sweden), gave a correlation coefficient of 0.975, slope 0.963, and intercept 0.31. The upper physiological limits of the excretion rate of urinary albumin (in 11 healthy subjects) were 16.5 μg/min when untimed urine specimens were collected during daytime or overnight, and 8.0 μg/min when they were collected for 10 h at night under strict bedrest conditions.

References


The indophenol colorimetric method of Simpson and Stewart (1), commonly used for detection of acetaminophen in urine of patients suspected of drug overdose, can give false-negative results. A simple modification, the addition of copper sulfate, overcomes this problem.

We hydrolyze urine samples (five drops of urine and two drops of conc. HCl) in sealed, screw-capped 10.0-mL test tubes at 100 °C for 7 min rather than heating them briefly over a flame. This prevents splashing and overheating of the sample, and results are more reproducible. After cooling the tubes we add 1.0 mL of aqueous o-cresol (10 g/L), 2.0 mL of ammonium hydroxide (4.0 mol/L), and 1.0 mL of 4.0 mmol/L copper sulfate. A positive reaction is indicated by development of a blue color within 1–2 min.

This modification is based on the report of Hammond and Scawen (2), who showed that the time required for maximum color development for the indophenol reaction in plasma was shortened by the addition of ammoniac copper reagent. Adapting this simple modification to the Simpson and Steward method overcame the false-negative results (Figure 1). The color developed was maximum within 1 min for three urine samples that had given false-negative results in the unmodified method, and the color was stable during the 10-min period of observation. A comparison of absorption spectra of the blue product formed with and without added copper sulfate verified the presence of the same indophenol complex in the modified procedure.

Just why results are false-negative with some samples is unclear. All had high ketone concentrations (>16 mmol/L), as determined with "Keto-Diastix" (Ames Div., Miles Labs, Elkhart, IN), suggesting competition between ketones and o-cresol for condensation with p-aminophenol, the hydrolys product of acetaminophen. The fact that ketone concentrations decreased in urine specimens left standing and the false-negative results were reversed supports this hypothesis. However, attempts to block indophenol formation in urine samples by adding ketones or aldehydes were unsuccessful.

References