protocol. Two additional patients were subsequently hospitalized for coronary artery bypass graft surgery.

We constructed 2 × 2 contingency tables and used the chi-square test with Yates's correction factor to determine if patients with >5 U of CK-MB per liter of serum were at increased risk of subsequent hospitalization for acute myocardial infarction of angina pectoris. (The two patients who underwent coronary artery bypass graft surgery were excluded from this analysis.) Using CK-MB isoenzyme results as quantified with the Model 720 instrument, we found that patients with >5 U of CK-MB per liter of serum were not more likely to be subsequently hospitalized for cardiac disease (\(x^2 = 0.07; p \text{ not significant}\) than patients with <5 U/L. However, if we used CK-MB isoenzyme results as quantified with the Model 760 instrument, patients with >5 U/L were at a significantly increased risk of being subsequently hospitalized for cardiac disease (\(x^2 = 5.47; p < 0.05\)) than patients with <5 U/L.

We conclude that electrophoresis results for CK-MB, as quantified with the Model 760 fluorometer/densitometer, can be correlated with subsequent clinical course, but not those quantified with the Model 720 fluorometer/densitometer. Although the two instruments appear to be equally sensitive, many more renal-failure patients who were not subsequently hospitalized had CK-MB activity in serum >5 U/L, as quantified with the Model 720 instrument than with the Model 760. These "falsely positive" results obscured those patients with a similar increase in CK-MB in serum who were subsequently hospitalized for cardiac disease. We (5) have found that background fluorescence and non-CK-fluorescent artifacts, which are amplified by the Model 720 instrument, explain the difference in results with the two fluorometers.

Our results also suggest that increased CK-MB activity, quantified by electrophoresis and the Model 760 instrument in the patient population we studied, is a poor prognostic sign and may indicate low-grade myocardial necrosis. Perhaps more aggressive medical therapy is indicated in renal-failure patients with >5 U of CK-MB per liter of serum.

References

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Using Filtrates of Urine Centrifuged through a Microconcentrator Membrane to Distinguish False and True Positives for Protein by Sulfosalicylic Acid Precipitation

To the Editor:

Two procedures commonly used for measuring urinary protein are the "dip-stick" method and sulfosalicylic acid precipitation (1). In the dip-stick method of determining urinary protein, a reagent strip impregnated with bromphenol blue and pH 3.0 citrate buffer is used to exploit the so-called "protein error of indicators." Because of its convenience, the reagent-strip method is widely used in routine screening for proteinuria, but unfortunately it is essentially insensitive to proteins other than albumin.

Protein precipitation by sulfosalicylic acid is used as a confirmatory test when the reagent-strip result is positive, or when their presence is suspected, to detect proteins missed by the reagent strip (globulins, Bence Jones proteins, etc.). In practice, however, urine specimens for which the reagent-strip result is negative but the sulfosalicylic acid result positive usually contain extraneous acid-precipitable substances, such as roentgenographic contrast dyes, penicillins, tolbutamide, or other drugs; rarely is the acid-induced turbidity due to globulins or other proteins.

To help analysts determine whether the sulfosalicylic acid-precipitable material is protein or not, we have developed the following simple centrifugation–filtration procedure. When centrifuged within a microconcentrator membrane (10 000-Da cutoff), low-Mr substances (antibiotics, contrast dyes, etc. (2–4)) pass through the membrane and appear in the filtrate, whereas larger protein molecules are retained. Testing the filtrate enables one to distinguish true-positive from false-positive protein results obtained with the sulfosalicylic acid test.

We used microconcentrators (Centricon 10; 10 000-Da cutoff) purchased from Amicon Co., Danvers, MA. Clinitek Auto 2000 Reagent Cartridges (Ames, Elkhart, IN) were used with the Clinitek Auto 2000 automated urine chemistry analyzer to determine urinary protein by the reagent-strip method. Sulfosalicylic acid solution was prepared by dissolving 20 g of the acid in water and diluting to 100 mL. All samples were centrifuged in a Sorvall Superspeed RC2-B centrifuge with a fixed-angle head rotor (Du Pont Instruments, Wilmington, DE). Piperacillin was obtained from Lederle, Pearl River, NY, and oxacillin from Biocraft, Elmsford, PA, NJ.

We obtained from eight patients urine specimens that gave negative or trace results for protein with the reagent strips and positive results with the sulfosalicylic acid test. Six of the patients were being treated with a penicillin or cephalosporine and two had received radiographic dyes for intravenous pyelogram studies. We centrifuged (3000 × g, 30 min) 1 mL of each urine specimen in a Centricon 10 Microconcentrator and tested the filtrates with sulfosalicylic acid. In addition, we prepared centrifuged urine specimen to contain serum proteins or antibiotics by mixing 6 mL of urine (negative for protein by both the reagent-strip and sulfosalicylic acid methods) with 500 μL of human serum, or dissolved 20 mg of piperacillin or oxacillin in 10 mL of protein-negative urine, before centrifuging as above.

Filtrates from the eight patients' urine specimens were all positive by the sulfosalicylic acid test. One patient taking penicillin had 3+ protein results by sulfosalicylic acid in both the original urine specimen and the filtrate, and one patient who had received an intravenous radiographic dye had 2+ results for both the urine and the filtrate; for all others, protein results were 4+ for both types of samples.
When urine was mixed with either piperacillin or oxacillin, the results of sulfosalicylic acid tests were 3+ positive. The filtrates from both of these urine samples were also 3+ positive. Urine containing added serum was 4+ positive with the sulfosalicylic acid test but the filtrate was negative.

To test whether both protein and nonprotein acid-precipitable substances are present in urine, centrifuge the urine until all the liquid has passed through the membrane, wash the retentate by adding 1 mL of water, and again centrifuge until all the liquid has been filtered. These steps take about an hour. Redissolve the second retentate in 1 mL of water and test with sulfosalicylic acid. We observed both protein and nonprotein sulfosalicylic acid precipitates in urine samples to which serum and penicillin had been added.

Although we centrifuged urine specimens for 30 min at 3000 × g to obtain 1 mL of filtrate, one can perform the sulfosalicylic acid test with smaller volumes, which takes less centrifugation time. Moreover, the microconcentrator units can be cleaned for re-use at least once by inverting and filling the units with distilled water and centrifuging them for 15 min at 2000 × g. There is no evidence of any protein residue after this washing procedure.

References
4. Ibid., p 5622.

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