acid, centrifuge the mixture, mix 2 mL of the supernate with 0.5 mL thiobarbituric acid (50 mmol/L), and incubate at 45 °C for 30 min. Measure the absorbance. A 10 g/L concentration of Hb A₁ cor "... correspondence to an absorbance of 0.060 at 458 nm.

With fresh hemolysate there is an excellent correlation between the isolated Hb A₁ fraction from the column method and data from the colorimetric assay (r = 0.96, n = 50, intra-assay CVs for the colorimetric assay and the column method are respectively 1.5 and 1.7%).

The quality control of the colorimetric assay cannot be improved by using 5-hydroxymethylfurfural, as has been suggested (7), because the internal standard cannot be correlated to the amount of 5-hydroxymethylfurfural formed; the main problem is a breakdown of 5-hydroxymethylfurfural. In standard, it makes sense to use a reference standard such as human Hb (Sigma Chemical Co., St. Louis, MO 63178; Human no. H, Type IV, 2X crystallized) or a simple hemolysate from pooled blood.

In our comparative study with four hemolysate samples stored at 5 °C during 80 days, the column-chromatographic method shows an apparent increase in the Hb A₁ fraction after the sample has been stored for 10 days, increasing linearly by fourfold in 80 days.

In contrast, no sample instability was found with the colorimetric assay. Again, in experimental animal studies with rabbits we obtained a normal value for Hb A₁ with the colorimetric assay (5%), in contrast to the column-chromatographic assay, in which the "fresh" blood samples showed an Hb A₁ that was too low (1.5%).

Because of its reliability and relative simplicity, the colorimetric assay evidently is to be preferred for routine work.

References

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Bence Jones Proteinuria Revisited

To the Editor:

The classical heat-precipitation test for detection of Bence Jones proteinuria (1) is now replaced by more specific and sensitive methods such as electrophoresis and various immunodiffusion procedures, which may be used to detect the presence of monoclonal light chains (FMULC). FMULC in low quantities are not always associated with the diagnosis of malignant monoclonal gammopathy (2).

We have investigated the clinical significance of FMULC in 3850 hospitalized patients. The protein content of urines was measured, and electrophoresis was performed whenever proteinuria was found to exceed 0.5 g/L. Immunoelectrophoresis was performed when an abnormal electrophoretic band was detected in either urine or in serum. All patients' sera were studied by electrophoresis on cellulose acetate. Fifty-six myeloma cases (including eight cases of light-chain disease), 72 benign monoclonal gammopathies (BMG), and 10 cases of Waldenström disease were identified. FMULC was observed in 40% of the myeloma cases, 20% of the Waldenström diseases, and 23% of BMG. If only proteinuria >0.6 g/L were considered (3), the percentage of FMULC-positive myelomas was 45% vs 6% only for BMG.

This work confirms the classical conclusion of the occurrence of FMULC in one myeloma case out of two and one BMG case out of four. It re-emphasizes the importance of the severity of the proteinuria. The discriminative value is 0.6 g/L according to Dammaco and Waldenström (2).

However, the 95th percentile for BMG is 0.75 g/L, but discriminant analysis shows that the probability of BMG is only 0.001 for a concentration of 1.2 g/L.

Perhaps an even better discrimination may be to use the actual value for Bence Jones protein. All the values described here refer to total proteinuria, which in many cases may significantly exceed Bence Jones protein. However, a simple, reliable, and selective assay for Bence Jones protein is still lacking.

In some cases, FMULC may be present without any detectable abnormal electrophoretic peak in the serum. In our series of 10 cases, all these patients had malignant diseases (light-chain disease: eight cases; chronic lymphocytic leukemia: one case; non-Hodgkin lymphoma: one case). A similar observation of one case of myeloblastic leukemia associated with FMULC was elsewhere reported (4).

References

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A Jendrassik–Grof Method Modified to Eliminate Hemoglobin Interference with Assay of Total Serum Bilirubin

To the Editor:

Shull et al. (1) discuss a proposed mechanism to account for interference by hemoglobin in determination of total bilirubin by the Jendrassik–Grof method. I agree, because results of my experiments are consistent with their proposal.

A sample blank is used to correct the spectral interference due to alkaline