metastasis, as confirmed by either laparotomy or necropsy. Our control group consisted of 53 ostensibly healthy volunteer blood donors.

When the patients with benign biliary obstruction were compared with the control group, the mean values for GR activity were significantly higher (p < 0.01), but we saw no important differences in GPI. However, in those patients with neoplastic obstruction of the biliary tract without hepatic metastasis, the mean values for GR and GPI were higher than those of the control group (p < 0.001 and p < 0.02, respectively). The difference between this group and those with benign biliary obstruction was significant only for GR (p < 0.02). Table 1 gives the mean values found for all these analyses in the three groups.

While in 13 (81.2%) of the 16 patients with obstructive neoplastic icterus GR values were high, this was the case in only seven (22.5%) of the 31 patients with benign biliary obstruction (p < 0.001). In the cases with benign obstructive icterus the GR was above the normal range in 16.1%, and in 43.7% of those with neoplastic obstruction (p < 0.05). Simultaneously measured GR and GPI values were normal in 22 (71.0%) of 31 patients with benign obstruction and in one case with neoplastic obstruction (p < 0.001). Activities of the remaining enzymes studied were increased significantly in both types of icterus (Figure 1).

From our results we conclude that determination of GR is useful in the differential diagnosis of benign and malignant obstructive icterus.

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Colorimetry vs Column Chromatography in Hb A1 Assay

To the Editor:

Hemoglobin A1 is a glycosylated form of hemoglobin A, the concentration of which is an integrated index to a person’s blood sugar concentration during the previous several weeks. Thus, measurement of Hb A1 concentration may be used in assessing diabetic control. A column-chromatographic method (1) is widely used because it is simple and can be performed in any laboratory. However, separations of glycosylated hemoglobins are affected by pH (2), osmolality (3), and temperature (2). Moreover, whole blood or hemolysate cannot be stored for more than one week. The Hb A1c fraction, usually 20% of Hb A1, may increase by 179% when hemolysate is stored for 10 days at −20 °C or even by 314% when stored at room temperature, in contrast to Hb A1c, which remains constant at these temperatures for two weeks (4). Finally, Hb F is known to interfere (5).

We compared the column-chromatographic method (1) with a modified colorimetric method (6) that is done as follows:

To 2 mL of hemolysate, containing 60 mg of Hb, add 1 mL of 1 mol/L oxalic acid and digest the mixture at 100 °C for 90 min (into each digestion tube insert a rubber stopper, punctured with a 38 × 0.8 mm hypodermic needle; this minimizes evaporation). After adding 1 mL of a 400 g/L solution of trichloroacetic acid.
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 corresponds 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-hydroxymethylfurural, as has been suggested (7), because the internal standard cannot be correlated to the amount of 5-hydroxymethylfurural formed; the main problem is a break-down of 5-hydroxymethylfurural. In standardization it is sensible to use a reference standard such as human Hb (Sigma Chemical Co., St. Louis, MO 63178; Human no. H, Type IV, 2X crystalized) 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.

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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 urinary 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 54% 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