a variable wavelength detector (Model 155-40), an "Ultrasphere-octyl" reversed-phase column (15 cm \times 4.6 (i.d.) mm, 5-\mu m particles), all from Beckman Instrument Co., Toronto, Ontario M5Z 2T2, Canada. Prepare the mobile phase by mixing 750 mL of acetonitrile, 250 mL of water, 0.5 mL of a 700 g/L solution of perchloric acid, and 0.5 mL of tetramethyl ammonium hydroxide solution (230 g/L of methanol). It is pumped at 1.2 mL/min, and the effluent is monitored at 240 nm.

A representative chromatogram of an extract of plasma from a patient on maintenance therapy on amiodarone is shown in Figure 1. The ratios of peak areas of amiodarone/internal standard and of peaks of desethylamiodarone/internal standard are linearly related to amiodarone and desethylamiodarone concentrations, respectively, over the range of 0.1 to 10 mg/L.

We have selected pentane rather than hexane for the extraction of plasma (6) because of its convenience: extracts can be evaporated rapidly without need for a current of nitrogen or reduced pressure. In single extraction with pentane 55 to 60% of the drug and its metabolite is removed; this is increased to 85–90% by the second extraction. In some procedures (3, 5, 6) a sulfur analog of amiodarone ("L 8040") is used as the internal standard. We find that its use in the present system prolongs the analysis from 10 min to 15 min for each injection. Prazepon, the internal standard we recommend, differs structurally from amiodarone but nevertheless behaves similarly in the extraction and chromatography. There is no change of peak-area ratio of amiodarone/internal standard when a mixture of the two compounds in acetate buffer is extracted with pentane.

Analysis of plasma supplemented with a 1 mg/L of each of amiodarone and desethylamiodarone showed both within-batch and between-batch CVs to be <5% for each compound. Blood is collected for this assay in green-capped (heparinized) Vacutainer Tubes (Becton Dickinson, Orangeburg, NY 10962). The use of separator and the like is avoided.

We thank Mr. C. Lafille (Sanofi Clinical Research Centre, Montpellier, France) for a gift of pure amiodarone, deethylamiodarone, and L 8040.

References


Ram N. Gupta

Dept. of Lab. Med.
St. Joseph's Hosp.
Hamilton, Ontario L8N 4A6 Canada

Stuart Connolly

Dept. of Med.
McMaster Clin. Unit
Hamilton General Hosp.
Hamilton, Ontario L8L 2X2 Canada

Isoenzyme 1 of Lactate Dehydrogenase Is Increased in Hepatic Diseases

To the Editor:

The value of isoenzyme 1 of lactate dehydrogenase (LD1), immunochemically determined (I) in serum as an aid to diagnosis of acute myocardial infarction (AMI), has been well examined (2–11) in recent years, with agreement that the diagnostic sensitivity and specificity of LD1 tests for detection of AMI are very high during the four days after onset of pain, with the upper normal reference limit, or some other close value, as the discriminant value. Many of these authors state that the LD1 test may produce falsely positive results in diseases other than AMI, but only Foo et al. (2) suggest hepatic disease as a possible cause of increased LD1.

We determined the catalytic concentration of LD1 in serum of 46 patients with various hepatic diseases. We used a reagent kit for this (no. 124893; Boehringer Mannheim, Mannheim, F.R.G.) at 30 °C, after immunoprecipitating the M-subunits with another reagent kit (no. 43145; Hoffmann-La Roche, Nutley, NJ). The patients' results ranged between 0.6 and 3.3 \mu kat/L; the reference interval previously obtained for healthy adults was 0.1 to 1.6 \mu kat/L.

Of these 46 patients, 18 had serum LD1 catalytic concentrations below the upper reference limit and 28 had LD1 values exceeding this limit; i.e., 61% of these patients were falsely positive for AMI when the upper normal reference limit was used as the discriminant value. This fact, surprisingly seldom described, shows the diagnostic specificity of immunochemically determined LD1 to be less than the literature indicates. We ascribe this to the lack of such study of patients with hepatic disease.

References

7. Clement GE, Havassey J, Gull J, Naccarato WF. Diagnostic performance of com-
bined isoenzyme analysis using the Du Pont ace analyzer to measure CKMB and LDH.


Javier Fuentes
Mariano Martínez
Margarita Fusté
Carme Biosca

Serv. de Bioquim.
Hosp. de Bellvitge “Princeps d’Espanya”
Feixa Larga s/n
L’Hospitalet de Llobregat
Barcelona, Spain

Rapid Quantification of Hb Hope in Heterozygotes

To the Editor:

Hemoglobin Hope [β136 (H 14)] Gly → Asp, although a rare hemoglobin (Hb) variant, is not exceptional in West Africa (1). It is most commonly observed in the heterozygous state, or in some instances in association with Hb S. Although its affinity for oxygen is low, no related pathology has been described. Diagnosis is relatively easy because it migrates slightly ahead of Hb A on cellulose acetate electrophoresis at pH 8.6 (2) (Figure 1). Identification is readily confirmed by electrophoresis on citrate agar (3), where it migrates slightly ahead of Hb F. Exceptional evaluation of Hb Hope in heterozygotes is not trivial in these populations, in whom the incidence of thalassemia syndromes and iron deficiency is high. Low expression of Hb Hope could indicate an associated α-thalasssemia or iron deficiency, whereas a high proportion of Hb Hope is the sure sign of an associated heterozygous β*-thalasssemia (4).

Except when Hb Hope is observed in association with Hb S, its quantification in heterozygotes is not possible from the direct densitometric scanning of the cellulose acetate strips, because it is so poorly separated from Hb A (Figure 1). Quantification is possible by scanning isoelectric focusing gels, because in such a system Hb Hope is clearly resolved from Hb A, but relatively few laboratories are adequately equipped to perform this technique.

We describe here the rapid, easy measurement of Hb Hope by ion-exchange chromatography on the Biorex 70 disposable columns commonly used in measurement of glycated hemoglobin.

Figure 2 shows the elution profile from an Hb A1 kit column (Bio-Rad Laboratories, Richmond, CA). The hemolysate was prepared as recommended by the manufacturer. Hb Hope is entirely eluted from the column by 25 mL of the kit’s phosphate buffer. In this particular experiment, Hb A was then eluted by increasing the ionic strength of the buffer. Thus we propose, for the measurement of Hb Hope, that the procedure recommended for Hb A determination be used except for an increase in the volume of phosphate buffer from 10 to 25 mL. By such a procedure, Hb Hope in a 46% proportion of total Hb was detected in the heterozygote patient corresponding to lanes 1 and 5 in Figure 1. To validate this result, we measured Hb Hope both by this chromatographic technique and by densitometric scanning of the cellulose acetate electrophoresis strip in the case of a double heterozygote for Hb S and Hb Hope (lane 4, Figure 1). Results were 52% and 51%, respectively. Although, by this procedure, the Hb Hope-containing fraction is obviously contaminated with the fast-moving Hb A1 fraction, this should not lead to its significant overestimation unless the patient is grossly unbalanced with respect to an eventual diabetic state.

We therefore consider this a rapid, easy test for Hb Hope measurement in heterozygotes, which should be helpful in the detection of associated iron deficiency or various thalassemic syndromes.

References


R. Ducroq
S. Berriche
M. T. Dalle
J. Elion

Lab. de Biochim. B
Hôpital X Bichat
46, rue Huchard
75018 Paris, France

1 Address correspondence to this author.

Comparison of Fluorescence Polarization Immunoassay and Enzyme Immunoassay for Free Carbamazepine in Serum or Plasma Ultrafiltrates from Epileptic Patients

To the Editor:

Carbamazepine (CBZ; Tegretol®) is indicated for treatment of complex partial seizures, generalized tonic–clonic seizures, and elementary partial seizures (1). Adjustment of CBZ dosage in patients with convulsive disorders is routinely guided by measurements of