The soluble isoenzyme from liver is known to be a dimer of ~200 kDa. The high-M₄ molecular ("slow") form is a tetramer with a mass of ~400 kDa (6); it is separated from the membranous complex comprising the high-M₄ ALP by the use of Triton X-100.

Both fractions showed the same behavior toward inhibitors. About 20% of the activity was inhibited by exposure to L-phenylalanine, 10 mmol/L, and 90% was inhibited with L-homoarginine, 10 mmol/L.

The biliary or high-M₄, ALP isoenzyme appearing in plasma of patients with cholestasis is an index of liver damage. It is particularly high in patients with invasive metastases in liver and other cell-disruptive processes. Some authors found a close association of this "particulate" ALP with other membrane-bound enzymes and the so-called lipoprotein X (7). Others (8, 9) proposed that the high-M₄, ALP in serum of cholestasis patients is generated by hepatocyte plasma membrane fragments shed into the circulation. In our preparations, from bile and plasma, the high-M₄ isoenzyme was always recovered in the 105 000 × g pellets. De Broe et al. reported that high-M₄ ALP can be pelleted by centrifugation (8). We found γ-glutamyltranspeptidase (EC 2.3.2.2), another membrane-bound enzyme, associated with the pellet material. The soluble form of ALP of hepatic origin is usually increased in serum in various hepatic conditions. In the method used, the soluble form was confined to the supernate and we could detect no contamination of the 105 000 × g pellets by this form. This was confirmed by determinations in plasma from 15 to 20 healthy nonurolithic renal detector, who have a strikingly high amount of soluble ALP.

Determination of high-M₄ ALP activity in plasma affords a sensitive way to assess hepatic damage. Methods so far proposed for the assay of the biliary ALP all present obvious difficulties when processing multiple samples, a fact that discourages application to the clinical laboratory. Separation of the biliary ALP by centrifugation as indicated here is comparatively simpler. It does not require previous dialysis or concentration of samples as other techniques do. There is no interference from other ALP isoenzymes generally present in serum. Our results indicate that the activity associated with the plasma 105 000 × g pellets represents high-M₄ ALP exclusively; no contamination with the soluble isoenzyme could be detected. Thus the method described offers a reliable technique for determination of biliary ALP isoenzyme.

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References

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Relationship between Circulating Platelets and Serum Concentrations of Creatine Kinase in Stroke

To the Editor:

Recently we described an inverse linear correlation between platelet and leukocyte counts during the first hours after ischemic stroke (1). We found also a positive correlation between leukocyte count and serum creatine kinase (CK; EC 2.7.3.2) concentrations, confirming data previously reported (2). Therefore, we looked for a correlation between platelet count and serum concentrations of creatine kinase.

Forty-four patients (26 women and 18 men, mean age 75 ± 9 years) were included in the study. Cerebral ischemic infarction was diagnosed by means of clinical evaluation and computerized axial tomography. None of the patients were treated with drugs interfering with the analytes studied.

A blood sample for the simultaneous evaluation of platelet count and CK enzymatic activity was taken in the morning, while fasting, from an arm vein with minimum stasis, within 47 (range, ± 25) h from the onset of symptoms. Whole blood was collected into Vacutainer Tubes (Becton Dickinson) containing tripotassium EDTA as anticoagulant for the platelet count, or without any additive for measuring serum CK enzymatic activity. The samples were stored at room temperature and analyzed within 4 h from collection. Platelet count was evaluated with a Coulter Counter Mod S Plus II in whole-blood mode (normal range in our laboratory: 140–440 × 10⁹/L) (1). Serum CK activity was measured with the ultraviolet kinetic method, optimized as recommended by the German Society for Clinical Chemistry (Sigma kit no. DG 147-K; normal range in our laboratory: women 24–170 U/L, men 24–196 U/L) (2).

As shown in Figure 1, the platelet count was inversely correlated with serum CK value (r = −0.48, P < 0.001).

The interpretation of this correlation is quite difficult, primarily because we did not determine the proportions of individual CK isoenzymes. If we accept the hypothesis that CK enzymatic activity may reflect the extent of brain tissue damage (3, 4), one...
could simply suggest that the decrease in platelet count, attributable to a platelet "in loco" consumption (1), is directly related to the size of the ischemic area. Nevertheless, many studies demonstrate that the concentration of serum CK activity (and of BB isoenzyme in particular) is a poor index of brain infarct size (5, 6). This lack of relationship may be attributed to an intact blood–brain barrier, the rapid elimination or inactivation of CK-BB, or both. Moreover, the different distributions of the enzyme in the various brain regions must be considered (7).

Indeed, the higher serum CK activity might also be partly or entirely related to muscle immobility, rigidity, and spasticity after an acute stroke—in which case, the MM isoenzyme would be most involved in this increment. In any case, our attempts to correlate the extent of muscle-skeletal processes with CK serum concentrations failed, leaving the question to be further investigated.

References

Effect of Bilirubin Covalently Attached to Albumin on Measurement of Serum Creatinine

To the Editor:

We read with interest the article by Franzini et al. (1), which described the use of ditaurobilirubin (DTB) as a surrogate for authentic conjugated bilirubin (Bc) in assessing interference from bilirubin in serum creatinine measurements. Although Franzini et al. compared the interference effect of unconjugated bilirubin (Bu), Bc, and DTB, they did not investigate the effect of bilirubin covalently bound to albumin (biliprotein or delta-bilirubin, Bd (2)) on creatinine measurement.

We have examined the effect of Bc on creatinine measurement, using serum-isolated natural Bd and chemically synthesized Bd adduct (3, 4), because Bc can be increased in the blood of adult icteric serum (5). Bu (Sigma grade; Sigma Chemical Co., St. Louis, MO) was dissolved in aliquots of sodium hydroxide, 0.1 mol/L, and diluted with distilled water. Commercial-grade DTB from Porphyris Products (Logan, UT) was dissolved in distilled water and used without further purification. Natural Bc was isolated from human bile according to the modified Lucassen procedure of Wu et al. (6). Bilirubin covalently bound to albumin (Bd) was isolated from icteric adult serum as described previously (3, 7), and was also synthesized from Bu and Woodward’s reagent K (N-ethylphénylisoxazolium-3’-sulfonate; Sigma) according to the method of Kuenzle et al. (4, 7). The synthetic or serum-isolated Bd was purified by ultrafiltration (Centrifree”; Amicon Div., W.R. Grace & Co., Beverly, MA) and by column chromatography (Sephadex G-25; Pharmacia Fine Chemicals AB, Upsala, Sweden). The Bd obtained by isolation or synthesis contained 99–100% of Bd as total bilirubin, as determined by HPLC (7). Furthermore, bile-isolated Bc was passed through a Sephadex G-25 column to remove the oxalate or sodium thiosulfate and undesirable materials. To 0.4 mL of each bilirubin sample with different bilirubin concentrations we added 0.1 mL of human serum albumin solution (Human Protein Standard; Dade, Miami, FL) reconstituted with 1.5 mL of 2.12 mmol/L creatinine (Kanto Chemical Co., Tokyo, Japan). The final concentration of albumin was 32 g/L, bilirubin ranged from 0 to 431 μmol/L, and creatinine was 424 μmol/L in each sample. Samples of isolated Bd and synthetic Bd were diluted with distilled water, and 0.4 mL aliquots of these were mixed with 0.1 mL of 2.12 mmol/L creatinine solution (final concentration of Bd, 0–451 μmol/L, with a constant bilirubin/albumin molar ratio of 0.49). No creatinine was observed in the samples of human serum albumin and isolated Bc and Bd.

Creatinine concentration was measured by three mechanized procedures, with commercially available reagents, as follows. Kinetic Jaiffé: kinetic picate reaction (Autosera CRE; Daiichi Pure Chemicals, Tokyo, Japan) performed with a Hitachi 705 analyzer (Hitachi, Ltd., Tokyo, Japan). Direct enzymatic procedure: enzymatic conversion of creatinine to creatinine (amidohydrolase, EC 3.5.2.10) and then to sarcosine (creatinine amidohydrolase, EC 3.5.3.3) followed by peroxidase-coupled (EC 1.11.1.7) colorimetric measurement of the hydrogen peroxide produced by the action of sarcosine oxidase (EC 1.5.3.1) with use of a commercial reagent (Determiner CRE 633; Kyowa Medex Products, Tokyo, Japan) with the Hitachi 705 analyzer. These two procedures had been confirmed by Franzini et al. (1) to display negative interference from bilirubin. Creatinine concentration was also measured with an Ektachem 700 analyzer (Eastman Kodak & Co., Rochester, NY) by a dry multilayer slide procedure, based on a sequence of reactions ending also in sarcosine production and oxidation, which reportedly shows no significant

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<th>Table 1. Effect of Bilirubin on Serum Creatinine Measurement</th>
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<td><strong>Creatinine</strong></td>
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*Values are means of three creatinine additions (424 μmol/L final concentration), and are expressed as the percentage recovered.*