agent set consisted of \( \alpha \)-naphthol AS-MX (3-hydroxy-2-naphthol-2,4-xylidide) and a diazo compound (Fast Blue RR), also from Helena Laboratories. A thin film of the stain was spread onto a second cellulose acetate strip and the electrophoresed strip was layered on top of this. This combination was incubated at 37 °C for 15 min, thus making the isoenzymes visible.

The fast band was identified by electrophoresing aliquots of the following solutions: (a) 200 mg of bilirubin per liter in a solution containing, per liter, 60 g of human albumin solution and 2 g of sodium carbonate; or (b) 200 mg of bilirubin per liter in a solution containing, per liter, 9 g of sodium chloride and 2 g of sodium carbonate; or (c) 60 g of albumin and 2 g of sodium carbonate per liter. The bilirubin used was Standard Reference Material No. 916 (National Bureau of Standards, Washington, D. C. 20234).

Alkaline phosphatase activities were measured by a kinetic modification of the method of Morgenstern et al. (4).

In reviewing about 1200 such alkaline phosphatase isoenzyme patterns run during the past two years, we found 27 patients whose sera showed the fast-migrating band. Twenty-six of the 27 also had abnormally high bilirubin concentrations (\( >10 \) mg/liter). To prove the association of this band with above-normal bilirubin, we electrophoresed and stained for alkaline phosphatase activity the following solutions: (a) bilirubin (200 mg/liter) plus human albumin (60 g/liter), (b) bilirubin (200 mg/liter), and (c) human albumin (60 g/liter). All three of these solutions, tested for alkaline phosphatase activity by a kinetic method, had insignificant (<3 U/liter) alkaline phosphatase activities but the albumin and bilirubin mixture had nonspecific staining in the albumin region (Figure 1). Figure 1 also shows the pattern from two patients whose serum showed the fast band in the same position as the nonspecific band for the albumin/bilirubin mixture.

We conclude that the alkaline phosphatase-like activity in the albumin region is merely an artifact due to a bilirubin/albumin complex that nonspecifically binds with this particular stain.

The separation by electrophoresis of albumin-bound bilirubin and free bilirubin (Figure 1, a and b) may be a useful method for measuring bound and free bilirubin in potential cases of bilirubin encephalopathy in the neonate. We are currently investigating this possibility.

Accuracy of Immunological Methods for Measurement of Creatine Kinase Isoenzyme Activities

To the Editor:

In his recent evaluation of methods for creatine kinase fractionation, Morin (1) indicates that “the immunological method [2] has unacceptable reliability” and that “very broad variations and poor reproducibility” were observed.

The data presented in his Table 2 obtained by the immunological assay “essentially performed as described” are indeed unacceptable. Since the accuracy of an immunological procedure depends on the quality of the antisera used, one may ask how good was the antigen-binding capacity of Morin’s antisera. For any quantitative approach a very careful evaluation of the inhibition and precipitation curves is fundamental. Surprisingly, Morin does not present these important data, thus rendering his study rather questionable.

References

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In our previous studies we have been able to demonstrate that isoenzyme activities in tissue extracts obtained by the immunological method are identical to data from electrophoretically separated isoenzymes after elution and photometric assay (2, 3). We have further reported the intraserial precision and sensitivity of the immunological method by investigating artificial mixtures of the three isoenzymes (3) and a serum from a patient with myocardial infarct (4). The following summarizes our results [mean ± SD (U/liter), CV (%)]:

(a) mixture of MM and MB: 50 ± 1.2, 4.2; MB = 11 ± 2.4, 21.8
(b) mixture of MM and BB: 60 ± 1.9, 3.2; BB = 59 ± 1.5, 2.5
(c) mixture of the three isoenzymes: MM = 112 ± 4.3, 3.8; MB = 31 ± 8.5, 27.4; BB = 154 ± 5.0, 3.2
(d) myocardial infarct serum: MM = 357 ± 12.5, 3.5; MB = 49 ± 13.4, 27.3

The minimal MB-activity still detectable is 4% of the total CK activity. Measurement of the MM and BB isoenzyme activities, which are determined by a single photometric test, is nearly as precise as the total CK-activity assay itself; the large standard deviation of the MB value stems from the fact that it has been determined by differential calculation. Recently a modified immunological assay has been developed that permits direct measurement of MB-activity in the absence of BB (E. Mercck).

However, a comparison of these two methods by investigation of 73 sera indicated that the MB activities determined by our immunoprecipitation assay correlated with those directly measured by immunoinhibition (5). Moreover, the immunological test in general was found to be superior to other commercial kits with respect to practicability and sensitivity (6). The application of immunological methods thus provides a sensitive and accurate measure of the three creatine kinase isoenzymes, fulfilling all clinical requirements for a diagnostic tool.

References

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Dr. Morin responds:

To the Editor:

I did not intend my paper to cast doubts on the integrity or value of Dr. Jockers-Wretou’s and Dr. Pfleiderer’s original work. I agree with them that the difference between their results and mine are probably due to differences in the properties of the antisera used. Their protocol was followed closely with only minor modifications. The titers were approximately 1.3 and 2.2 of the values they reported for MM and BB, respectively. I hope to publish data on these continuing studies in the near future, but, for the present, I can report that there were three equivalence points: activating, precipitating, and inhibiting. This was not evident to me originally and undoubtedly was the source of the reported broad variations and poor reproducibility.

I am confident that immunological methods can be made to work quite well and that Drs. Jockers-Wretou and Pfleiderer do this routinely. Nevertheless, most clinical laboratories are not prepared to adequately purify human CK, raise and characterize antibodies, and quality-control that uncertain supply. To my knowledge, no commercial source of CK antisera is available in the USA.

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