is rooted in minor variations in the use of DCC methodology from one laboratory to another. More important, our own consistent failure to confirm CAGE-detectable estrogen binding by the DCC method, suggests that this factor is not identical to the ER present in some breast tumors as suggested elsewhere (2).

The uniformity of results obtainable with CAGE, however, persuades us that this method will be most useful in further elucidation of EBF in blood. We are currently assessing possible cyclic fluctuations of this factor in blood from a group of 10 normal women and plan to extend observations to breast cancer patients.

References

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Albumin-bound Fluorescence: A Potential Source of Error In Fluorometric Assay of Creatine Kinase BB Isoenzyme

To the Editor:

Creatine kinase (EC 2.7.3.2) is separated by electrophoresis on cellulose acetate into three distinct isoenzyme fractions: MM, MB, and BB. On treatment of the electrophoretic strip with a fluorogenic-substrate reagent, these fractions become visible in ultraviolet light. Using such a procedure, several authors report the presence of the BB isoenzyme in serum various disorders, including chronic renal failure (1, 2).

We confirmed (3) the presence of a strongly fluorescing band anodic to CK-MB on electrophoresis, but showed this band to be completely unrelated to CK-BB isoenzyme. Because the native fluorescing material(s) in the serum of patients with chronic renal failure is a potential source of error in evaluating CK-BB isoenzyme, we have attempted to characterize it further.

Ultraviolet (365 nm) light was used. The procedure for the separation, identification, and fluorescence scanning of CK-isoenzymes was that previously described (0). Serum albumin was precipitated with rabbit anti-human albumin as follows: 10 μl of serum were incubated at room temperature overnight in the presence of two different volumes, 100 and 200 μl, of rabbit anti-human albumin (Bio-Rad Laboratories, Richmond, Calif. 94804). The mixture was diluted to 1 ml with phosphate buffer (1 mol/liter, pH 7.4). The samples were then centrifuged to sediment antibody-bound albumin. The supernatants were transferred to collodion bags (Sartorius Membrane-filter; BDH Chemicals, Ville St. Laurent, Quebec, H4T 1B3), concentrated under reduced pressure against Ringer’s solution to a final volume of 50 μl, and electrophoresed. Air-dried strips were scanned for their unique fluorescent band, which we found, after staining for serum proteins, to be associated with albumin.

For comparison, each test sample was accompanied by a “blank,” from which anti-human albumin was omitted.

Sera from patients with chronic renal failure were compared under the ultraviolet light with samples from patients with no evidence of kidney disease. All samples in the first group emitted a relatively strong fluorescence.

In another test, 5 μl of each specimen from each group was placed individually on Whatman no. 1 paper and air dried. The dried spots, under ultraviolet light, showed intense fluorescence in the case of all specimens from renal patients, but only faint fluorescence in the others.

Sera from hemodialysis patients were electrophoresed in parallel with a control sample (marker) containing all three CK isoenzymes. The support media were then incubated with a fluorogenic-substrate reagent. Figure 1 shows a superimposition of the patterns of fluorescence. Sera from renal patients exhibited two distinct fluorescent bands, one corresponding to CK-MM, a normal component of the serum, and the other occupying a position intermediate between the CK-MB and CK-BB isoenzymes.

To further characterize the nature of the fluorescent band of hemodialysis patients, sera were electrophoresed in duplicate (one application at each side of the median line) on the same cellulose acetate strip. After electrophoresis, the support medium was halved lengthwise. One half was treated with CK-isoenzyme substrate reagent; the other half was dried and scanned for its natural fluorescence pattern, and then stained to establish the pattern of protein electrophoresis. The results (Figure 2) showed that:

1) Serum electrophoresis of patients on long-term hemodialysis yielded a single fluorescent band (X), which was present before treatment with the CK-isoenzyme substrate reagent;

2) subsequent treatment of the electrophoretic strip with the substrate reagent resulted in the appearance of an MM band, without further increase in the fluorescent intensity of the intermediate (X) band; and

3) staining of the electrophoresed sample for protein indicated that the migration of the intermediate band was indistinguishable from that of serum albumin.

To investigate the possibility that the native fluorescence is due to something
bound by albumin, we incubated serum samples with rabbit anti-human albumin, precipitated the albumin, and then electrophoresed the supernate, after concentrating it in a collodion bag. The amount of serum albumin and the intensity of the fluorescent band decreased in the same proportion. This observation was confirmed with two different concentrations of the antibody (Table 1).

It is well known that serum albumin binds a variety of exogenous and endogenous compounds, and serves as a carrier in the transportation of numerous chemical agents. It is therefore conceivable that in certain conditions, such as chronic renal failure, where there is a continuous impediment to the elimination of fluorescent drugs and metabolites, serum albumin would exhibit a high fluorescent activity.

While the recent introduction of fluorogenic-substrate reagents for identification and evaluation of CK-isoenzymes has been instrumental in increasing diagnostic accuracy of myocardial infarction, the mislabeling of serum albumin as CK-BB isoenzyme by several authors results in erroneous reports. Recently, the presence of CK-BB isoenzyme has been reported in various conditions, such as chronic renal failure (1, 2), prostatic carcinoma (4), pregnancy (5), and acute brain injury (6). However, our findings suggest that all previous electrophoretic reports in which an appropriate “blank” (without substrate reagent) was not included should be regarded with caution. Furthermore, a “blank” is essential, not only for differentiating CK-BB from albumin, but also when the pattern of LDH isoenzymes is evaluated, since albumin may give rise to an additional fluorescent band and interfere with measurement of the actual percentage of each LDH fraction (7).

References

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Erroneous Temperature Corrections for Blood pH and Gas Measurements

To the Editor:
This letter is to call attention to an arithmetic error and an incorrect implication that may be drawn from our previously published table of correction factors for blood pH and gas measurements (1).

The arithmetic error resulted in printed correction factors for pCO₂ between the temperatures of 40 °C and 43 °C that are lower than the actual values. Although the error in pCO₂ will almost never exceed 2–3 mmHg, the correct factor should, of course, be used.

A similar error is present in the reported pO₂ correction factors between 40 and 43 °C, but, more importantly, it should be noted, as stated in the text, that the pO₂ correction factor is a function of oxygen saturation. The factor changes rapidly between saturations of 90% and 100%, which includes a large percentage of arterial blood samples encountered.

Therefore it is recommended that neither our table nor other similar ones be used for correction of data, but that temperature-corrected values of pH, pCO₂, and pO₂ be calculated directly by use of the following equations (1), which are easily programmed and stored for use with a desk-top calculator.

\[
\text{pH (at } T\text{) = pH (at } 37\text{ °C) - 0.0147 (T - 37)}
\]

\[
\text{pCO₂ (at } T\text{) = pCO₂ (at } 37\text{ °C) - 10^{0.019(T-37)}}
\]

\[
\text{pO₂ (at } T\text{) = pO₂ (at } 37\text{ °C) - 10^{0.019(T-37)}}
\]

where

\[ f = 0.032 - 0.0282 e^{0.3 x - 30} \]

\[ x = \text{oxygen saturation in percent} \]

\[ T = \text{body temperature in degrees Celsius} \]

It is also worth re-emphasizing that calculated values of bicarbonate, CO₂ content, oxygen saturation, and oxygen content are not subject to temperature corrections. These quantities are either temperature-invariant by definition (CO₂ content and oxygen content) or only very slightly temperature-dependent (bicarbonate and oxygen saturation).

Reference

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A Case of Isolated Increase in Serum Alkaline Phosphatase Activity

To the Editor:
I give here a brief history of a patient with isolated elevated serum alkaline phosphatase and discuss its etiology.

H.J.P., a 56-year-old white man was admitted to this hospital in February 1975, for studies because of weight loss, weakness, and persistent cough, and for minor surgery of some skin tumors. A chronic alcoholic, he was known to drink six bottles of beer and four shots of whiskey after work every day since 1970. Contrary to his doctor’s advice, he continued drinking and at the time of this admission he was drinking a couple of shots of whisky and four cans of beer daily. He also had had a convulsive disorder for many years. Extensive work-