On the other hand, and to our surprise, fructosamine concentrations in the survey specimens were higher in the ABA-VP (y') than in the Multistat (x') and least-squares regression of those data (n = 24) gave the equation: 
\[ y' = 0.992x + 0.389 \] 
\[ (r = 0.920, \text{S.D.}_x = 2.429, \text{S.D.}_y = 0.564, \bar{y} = 2.798, \text{S.D.}_y = 0.650) \]
for the slope of the regression line was 0.244 mmol/L. The intercept was significantly different from zero (0.025 < P < 0.01) but the slope was not significantly different from unity (0.95 < P < 0.99). Clearly, there was closer correlation between instruments, due in part to less apparent random error (smaller S_x, larger r), with survey specimens than with patients' specimens.

Although Baker et al. (1) state that "Program specimens exhibited similar behavior to patients' samples . . .", it was evident that, in our hands, they did not. This conclusion was reinforced by our observation that storage at -20°C and subsequent thawing was deleterious to the survey specimens but not to pooled patients' sera. We received the survey specimens by mail at ambient temperature and stored them at 4°C, as instructed, until assayed, following which they were stored at -20°C. At the completion of the first phase of the survey, the 12 survey specimens were thawed and assayed again in the Multistat. All speci mens were now noticeably more turbid and all had significantly lower fructo samine concentrations than before (mean_fresh = 2.479; mean_inann = 1.970 mmol/L; paired t-test, 0.05 > P > 0.001). In contrast, a pool of patients' sera stored similarly and assayed weekly, in duplicate, during three months showed no signs of deterioration (mean = 3.160, SD = 0.116 mmol/L, CV = 3.7%, n = 22).

The fructosamine assay has proved to be greatly influenced by slight variations in reaction conditions (2). In particular, assay pH, standardization, and the inherent reactivity of albumin have been troublesome and have undoubtedly led to disparate reference intervals being published (2, 3). Presumably, our observed behavioral differences between survey and patients' specimens are a manifestation of these difficulties. The poorer correlation seen with the latter specimens probably reflects the greater diversity of matrices in patients' sera. The failure of quality-control materials to simulate patients' specimens and hence to monitor the performance of assays is well recognized; examples abound in the literature (4-6).

Recently, a serum fructosamine assay that is said largely to nullify matrix and standardization problems has been proposed (7). It remains to be seen if this approach leads to greater commutability of results.

References

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Serum Protein Reagent for Use in the Parallel* Analytical System Not Susceptible to Interference by Dextrans

To the Editor:
A recent Technical Brief (1) listed the interference caused by dextran with reagents for serum total protein in several analyzers, including American Monitor's Parallel* analytical system. In February 1987, American Monitor released a new formulation of total-protein reagent recommended for use on their Parallel and Perspective* analyzers. This reagent is an optimized version of the type prepared by Flack and Woollen (2).

Dextran 40 was added to pooled sera at various concentrations (24 to 120 g/L), with care taken to keep the protein concentrations the same in each sample. The samples were then analyzed for protein, with each sample giving the same result, regardless of dextran concentration.

Evidently the reagent system is free from interference by dextran. It is also uninfluenced by hemolysis, bilirubin, glycerol, glucose, chloride, fatty acids, ascorbic acid, ammonia, epinephrine, and cysteine.

References

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How Early Can Ornithine Carbamoyltransferase Be Measured In Fetal Liver?

To the Editor:
Ornithine carbamoyltransferase (OCT; EC 2.1.3.3), a mitochondrial enzyme, has a major role in the urea cycle, facilitating the conversion of ornithine to citrulline. The enzymatic activity is limited to the liver. Deficiency of OCT is an X-linked disease manifested by severe hyperammonemia in neonates, leading to coma and eventually death. Management of an affected infant is very difficult and usually unsuccessful.

The OCT gene has been cloned and used for prenatal diagnosis (1). Hitherto, fetal liver biopsy was used for prenatal diagnosis (2, 3) and is still a useful method when examination of family members is noninformative for any of the known restriction fragment length polymorphisms. Recently we encountered such an uninformative family. Fetal liver biopsy was performed, the fetus was found to be affected, and the family chose to terminate the pregnancy. Further studies of
the liver specimen confirmed the antenatal diagnosis.

Previous reports (2, 3) stated that OCT activity was not detectable before 17 weeks after the last menstrual period, and that liver biopsy for prenatal diagnosis should not be attempted before 18 weeks of gestation. We have since expanded our data and would like to correct that statement. Fresh human fetal livers were obtained between eight and 24 weeks after the last menstrual period from patients who were undergoing dilatation and evacuation. Fetal age was determined by the last menstrual period and by fetal foot length, which was measured and compared with standard tabulated values. The samples were immediately frozen in liquid nitrogen and stored frozen at −70 °C.

The liver tissue was homogenized in 20 volumes of ice-cold sterile water in a mechanical grinder for 1 min. Protein concentration was measured with the Bradford assay (4) and adjusted to 2−4 mg/mL with sterile cold water. The reaction mixture was as follows: 150 μL of TES [N-tris(hydroxymethyl) methyl-2-aminoethane sulfonic acid] buffer (0.1 mol/L, pH 7.4), 20 μL of a 25 mmol/L L-ornithine solution, 40 μL of 11.7 mmol/L carbamyl phosphate reagent, 10 μL of [14C]carbamyl phosphate (1.26 mmol/L, specific activity 10.4 Ci/mol), and 10−30 μL of liver homogenate. The final volume was adjusted to 250 μL with sterile water.

We incubated the tubes at 37 °C for 15 min and stopped the reaction by adding 50 μL of trichloroacetic acid (TCA), 500 g/L. The unincorporated [14C]carbamyl phosphate was eliminated from the reaction mix as 14CO2 by boiling the samples in the hood for 10 min. For negative controls we used water and TCA-inactivated liver homogenates. The radioactivity was measured in a Model LS3801 scintillation counter (Beckman Instruments, Brea, CA).

The results, μmoles per milligram of protein per hour, are calculated as follows:

\[
\text{counts/min} \times \frac{2.5 \times 10^{-6}}{V \times C \times T}
\]

where \(2.5 \times 10^{-6}\) is a factor that combines all of the constant parameters in the formula (i.e., conversion of counts/min to disintegrations/min, to μCi, to μmol) to yield the total amount (μmol) of citrulline that was produced; V is the volume (μL) of liver homogenate; C is the protein concentration (g/L); and T is the time in hours.

The results (Table 1) indicate that OCT activity can be detected by eight weeks after the last menstrual period and that there is no substantial change in activity during the next 16 weeks. All the fetal livers tested as described above had measurable OCT activity. Therefore, we conclude that the prenatal diagnosis of OCT deficiency can be made by liver biopsy as early as gestation as fetal liver biopsy can be safely performed.

Identifying CK-MB and Macro-CK by Electrophoresis

To the Editor:

We have previously described a case of macro creatine kinase (CK) type 1 in which the electrophoretic mobility of the macro CK was identical to that of the MB isoenzyme of CK (1). Evidently macro CK may be an interference in the identification of CK isoenzymes by electrophoresis. We have since seen two additional cases of such macro CKs. In all three of these cases the amount of CK-MM was less than the amount of macro CK type 1. Furthermore, in all three cases the CK isoenzyme pattern remained stable over time. These observations form the basis of two criteria that can be used to raise suspicion that a band in the MB region might be a macro CK.

CK enters the bloodstream by leaking from damaged cells (2). All cells that contain CK-MM have even more CK-MM; myocardium is richest in MB of all tissue, with CK-MB constituting 20% to 40% of the total CK activity (2, 3). Therefore, as Galen observed (2), serum CK-MB should never exceed 40% of the total CK. This leads to the first criterion for suspecting that a band is not MB; activity greater than 40% of the total CK. Clearly, allowances must be made if CK-BB or another macro CK is present.

The most important diagnostic use of CK isoenzyme analysis is in acute myocardial infarction. Recently, more emphasis has been placed on the temporal pattern of CK; an appropriate increase and decline suggests an acute myocardial infarction, even if the total CK activity remains within the reference range (4). Conversely, a stable CK isoenzyme pattern is not consistent with an acute event. Hence, the second criterion for suspecting that a band is not MB is a CK isoenzyme pattern that is unchanged over several days, regardless of the total CK. It is most important to correctly identify CK-MM vs macro CK in the setting of a patient with coronary artery disease, because a persistent CK-MM band might be interpreted as evidence of ongoing ischemia, leading to increasingly invasive therapies.

If a suspicion of macro CK type 1 is raised, it can easily be confirmed by incubating the patient’s serum with anti-immunoglobulin antibodies (1).

References

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Table 1. OCT Activity in 20 Fetal and Postnatal Liver Samples

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* μmoles per hour per milligram of protein.