reference preparations to permit interconversion and thus valid comparisons of results. The absence of an international reference preparation for prealbumin, however, makes such comparisons problematic or at least much more difficult.

We hope that the efforts of the IFCC now underway to establish a new reference material for plasma proteins (including prealbumin) will meet with acceptance and will provide the needed comparability of prealbumin measurements.

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Anti-Asialoglycoprotein Receptor Antibody and Intestinal-Origin Alkaline Phosphatase in Plasma in Diabetes

To the Editor:

Intestinal-origin alkaline phosphatase (orthophosphoric-monoester phosphohydrolase (alkaline optimum), EC 3.1.3.1; ALP) in plasma is an asialoglycoprotein, thought to be cleared at the asialoglycoprotein receptor at the liver cell surface. Hepatocyte damage with impaired enzyme uptake by the receptor is considered to account for the increased intestinal-origin ALP observed in plasma in liver disease, particularly in cirrhosis (1). Increased intestinal-origin ALP activity also occurs in plasma in diabetes mellitus in the absence of obvious liver disorder, and no satisfactory explanation for the increase has been forthcoming. Circulating auto-antibodies to the hepatic asialoglycoprotein receptor (ASGP-R) have been observed in liver disease (2). We wondered whether anti-ASGP-R auto-antibodies might be present in diabetes also, might interfere with enzyme uptake, and might be relevant to the increased intestinal-origin ALP in plasma.

We, therefore, examined plasma samples from 17 diabetics, all of blood group O and mainly of maturity-onset type. Liver-function test results were available for 14 subjects and were normal, except for minor increases in aspartate aminotransferase (61 U/L, upper reference limit 40 U/L) in one subject and in γ-glutamyltransferase (68 U/L, upper reference limit 48 U/L) in a second. We divided the samples into two groups on the basis of the presence or absence of marked intestinal (β-mobility) ALP activity, visible by enzyme electrophoresis on cellulose acetate membrane. Nine plasma samples showed marked activity. For these, measurement of intestinal-origin (intestinal plus intestinal variant) ALP activity by immunocapture assay (3) showed a mean value of 52 U/L (range 32–115 U/L). Eight samples showed low or modest intestinal ALP activity by electrophoresis, and for these, intestinal-origin ALP activity averaged 7 U/L (range 1–24 U/L).

In the first group (high intestinal-origin ALP content), anti-ASGP-R auto-antibodies (measured by B.M. McF.) were present in three samples only, with titers of 1:300, 1:50, and 1:550 accompanying intestinal-origin ALP activities of 41, 115, and 32 U/L, respectively. In the second group, anti-ASGP-R antibody was also present in three samples, with titers of 1:1050, 1:550, and 1:50 at corresponding intestinal-origin ALP activities of 24, 6, and 4 U/L. Neither sample with increased liver enzyme activity contained anti-ASGP-R antibody, although both had high intestinal-origin ALP content.

We conclude that although anti-ASGP-R antibodies may be detectable in plasma in some patients with diabetes, they do not correlate with the concentration of intestinal-origin ALP activity and cannot account for the increased activity of this enzyme.

We thank Dr. P. Dandona for providing plasma samples from patients in his care.

References

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Iron Measurement in Patients with Monoclonal Immunoglobulin: a Further Caution

To the Editor:

We read with interest the observation of Bakker and Kothman-Tijkotte (1) concerning an artifactual high concentration of iron measured in a patient with monoclonal immunoglobulin. We recently experienced the same problem when measuring serum iron in three patients with a monoclonal immunoglobulin (identified by immunofixation electrophoresis as IgG-kappa in two cases and IgG-lambda in one case), which came serendipitously to our observation since last spring (2).

The concentrations of monoclonal immunoglobulin, measured by nephelometry (Behring, Marburg, F.R.G.), in the three patients were 44, 30, and 26 g/L. Serum iron concentration was measured by the following procedures: ICSH-proposed reference method provided by Boehringer Mannheim ("BMD," Mannheim, F.R.G.) with deproteinization by trichloroacetic acid, involving bathophenanthroline disulfonate (0.51 mmol/L) in acetate buffer (4 mol/L, pH 4.6) and sodium pyrosulfite and p-(N-methyl)aminophenol as reducing agents (method A); Synchroon CX5 analyzer and reagents provided by Beckman Instruments (Brea, CA), involving acetate buffer (500 mmol/L, pH 4.3), ferrozine (1 mmol/L), and hydroxyamine and thioglycolate as reducing agents (method B); Synchroon CX5 analyzer with reagents by BMD (prod. no. 759422; acetate buffer (170 mmol/L, pH 5.5), ferrozine (50 mmol/L), and ascorbic acid as reducing agent) (method C); and Beckman Instruments, Wilmington, DE), with pH 4.2 buffer and bathophenanthroline sulfonate and hydroxylamine as reducing agent (method D); RA-1000 (Technicon, Tarrytown, NY) and the previously indicated reagents from Beckman (method E); SMAC II (Technicon), with acetate buffer (1.65 mol/L, pH 12), ferrozine (0.69 mmol/L), and ascorbic acid and hydrochloric acid (pH 1.5) for liberating and reducing iron (method F).

The results are summarized in Table 1. We were not able to carry out all
the assays of the panel with samples from all three patients, but we think that the available data support the closing remarks of Bakker and Kothman-Tijkotte, who hypothesized that the interferences they described do occur in other procedures in which these kinds of reagents are used.

References

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Rare Error in Glucose Determination in Hemolysate

To the Editor:

Blood glucose is usually measured in hemolysates. Only in very rare cases, e.g., samples with highly increased leukocyte counts, do problems arise (1, 2). Here we report the occasional erroneous determination of blood glucose in patients with high leukocyte counts, presumably from the contents of lysed leukocytes in the sample, due to high sample viscosity. Because the changes in viscosity are not readily noticed by the technicians, and because duplicate measurements given identical results, the erroneous blood sugar measurement is not detected. This phenomenon appears only with dodecylsulfate-based hemolyzing reagents but not with digoxigenin-based reagents. However, because dodecylsulfate-based reagents have a longer shelf life, most factory-prefilled sample cups are prepared with these reagents, rather than with digoxigenin-based reagents.

Case report. A 67-year-old man who had had chronic myeloid leukemia (CML) for one year and diabetes for 20 years was admitted for treatment of CML. Under steroid medication his diabetes became unstable. Blood sugar determinations performed with Boehringer (Mannheim, F.R.G.) hemolyzing reagent fluid (no. 750689, which contains dodecylsulfate, 1.8 mL/L) and an EPOS analyzer (Eppendorf, Hamburg, F.R.G.) were as follows: on admission, measured blood glucose was 33.3 mmol/L; the following night, after the patient was treated by insulin infusion, he showed symptoms of hypoglycemia, although his measured blood glucose was 20 mmol/L. However, a test strip (HGT 20-800; Boehringer) showed a glucose value <2.8 mmol/L. A simultaneous assay of glucose in his serum showed a value of 1.5 mmol/L. Other laboratory findings included: hypergamma globulinemia with oligoclonal IgG-kappa; total protein was 70 g/L. No cold-agglutination proteins were present, but the leukocyte count was 74 x 10^9/L.

We examined the whole-blood hemolysate and found it quite viscous. Shaking the sample, as recommended for complete lysis, trapped air bubbles, so the sample was not homogeneous. Because treating plasma and serum from the patient with the hemolyzing solution did not change their viscosity, we conclude this phenomenon is linked to the formed elements of the blood. We were able to reproduce the same phenomenon in samples from patients with high leukocyte counts (>50 x 10^9/L).

Using the digoxigenin-based hemolyzing reagent (Boehringer, no. 262838) to lyse the same sample did not lead to a change in viscosity. After centrifugation of the latter sample, we found leukocytes in the centrifugation pellet, whereas no leukocytes were in the pellet obtained from samples lysed with dodecylsulfate.

Electrophoresis of the original sample showed the presence of large amounts of DNA (1 µg/L for a sample of 160 x 10^9 leukocytes per liter). We conclude that the changes of viscosity were caused by coagulation of the sample DNA by dodecylsulfate, a procedure well known and used to isolate DNA from leukocytes. The resulting high viscosity led to an inhomogeneous sample with trapped air bubbles. At high leukocyte counts, proper pipetting and mixing was impossible. Especially with methods based on a two-point measurement such samples may be misanalyzed. Use of six-point measurement would show nonlinear results and samples would be re-assayed.

We thus recommend the use of digoxigenin-based hemolyzing reagents and a multiple-point measurement when determining blood glucose in hemolysates.

We thank Dr. S. J. M. Conrad for carrying out electrophoresis and Mrs. J. Wolf for carrying out the viscosimetric measurements.

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Measurement of Glucose in Cerebrospinal Fluid with Reagent Strips and a Reflectance Photometer

To the Editor:

Measurement of glucose in blood and plasma by using reagent strips and reflectance photometry has become well established, and is widely available in both rural and general practice. However, little has been published on using these reagent strips and reflectance meters to measure glucose in cerebrospinal fluid (CSF)