Enzymatically Active Component of Macro-Creatine Kinase Appears to Be BB Isoenzyme

To the Editor:

Electrophoretic analyses and studies of relative molecular mass (M_r) have provided evidence for the occurrence of several atypical forms of creatine kinase (EC 2.7.3.2) in serum (1). One of these forms, observed in about 1.6 per thousand samples, is characterized by an M_r of approximately 350,000 and by its migration between isoenzymes MM and MB during electrophoresis at pH 8.6 (2). This form, designated "macro-CK," has been described as a complex between creatine kinase and a gamma-globulin (1). Most of the evidence indicating that the enzymatically active component of macro-CK is a type BB isoenzyme is based on immunoinhibition studies (1). However, some inhibition of macro-CK by antibodies against the muscle-type isoenzyme has been reported (3, 4), and one could envision arrangements in the complex in which an M-subunit could be antigenically nonreactive. Furthermore, serum containing macro-CK often exhibits a normal total creatine kinase activity, although most of it is the atypical form (3, 4), which may indicate that the high concentration of macro-CK occurs at the expense of the muscle-type isoenzyme.

To characterize the enzymatically active component of macro-CK by a different criterion, we have isolated macro-CK and denatured and renatured it by a procedure we use frequently in our laboratory to study subunit interactions among the isoenzymes of creatine kinase (5). Serum samples (generously donated by Dr. A. Girgenti, St. Joseph’s Hospital, Tampa, FL) were subjected to gel filtration through Sephadex G-200, and two creatine kinase components were detected by electrophoresis in agarose (Corning ACPI apparatus and assay reagent). Elution of the high-M_r species corresponded to an M_r of 325 000 and electrophoretic migration was between isoenzymes MM and MB. The component of lower M_r, eluted and migrated electrophoretically like isoenzyme MM. The isolated macro-CK was totally inhibited by antibodies to human CK-BB (generously provided by Dr. R. Wicks, Hoffmann-LaRoche, Nutley, NJ). Ouchterlony double-diffusion analysis exhibited a single continuous line of precipitation between anti-CK-BB against serum containing macro-CK, isolated macro-CK, and human CK-BB. The isolated macro-CK was treated for 1 h with 8 mol/L urea in buffer containing 0.1 mol of potassium phosphate (pH 7.6) and 2 mmol of dithiothreitol per liter, then exhaustively dialyzed against the same buffer free of urea.

Electrophoresis of the products of this denaturation/renaturation cycle exhibited a single CK activity band, at the position identical to that of purified human CK-BB. The absence of other bands indicates that M-subunits were not a component of macro-CK. Furthermore, the finding that on dissociation/reassociation macro-CK did not reform suggests that either the immunoglobulin was irreversibly denatured by exposure to urea or that the affinity of a B-subunit for itself is considerably greater than its affinity for the immunoglobulin.

Further studies are in progress to ascertain if the CK-BB isolated from macro-CK is the same isoenzyme as CK-BB obtained from human brain.

Supported in part by a fellowship to J.J.L. from the American Cancer Society, Florida Division.

References

Jorge J. Lense
Steven H. Grossman
Dept. of Chem.
Univ. of South Florida
Tampa, FL 33620

Differences in Vitamin B_12 Results as Measured by Boll and No-Boll Kits

To the Editor:

Recently we evaluated seven kits offered for the determination of vitamin B_12 and folate in serum. Four of these kits are commercially available: Simultrac "True B_12" liquid phase (Becton Dickinson Immunodiagnostics, Orangeburg, NY 10962), No-boll Com- bostat II (RIA Products, Waltham, MA 02254), Quantaphase (Bio-Rad Laboratories, Richmond, CA 94804), and Vita-
min B₁₂/Folate Dual Radioassay kit (Amersham Corp., Arlington Heights, IL 60005). The other three kits are not yet commercially available: Simultrac boil solid phase and Simultrac no-boil solid phase (Becton Dickinson) and no-boil Dualcount (Diagnostic Products Corp., Los Angeles, CA 90045).

Patients' specimens that had vitamin B₁₂ values either <140 pmol/L (possible vitamin B₁₂ deficiency) or >1500 pmol/L, as analyzed by our present method (Simultrac "True B₁₂," liquid phase), were stored at −40 °C, and we obtained hematological information on these patients.

We assayed 27 specimens by all seven methods. Of these 27 patients, one was diagnosed as having chronic myelogenous leukemia, one had myeloproliferative disorder, one had hypochromic microcytic anemia, and the remaining 24 had a hematological profile typical of macrocytic anemia. Results obtained with all the kits agreed well with the hematological information in 26 of the 27 patients.

The case in which these did not agree was that of a 60-year-old native Indian woman, who was admitted to hospital with a history of feeling weak and tired, and with numbness and "pins and needles" sensations in both arms and legs. Her hemoglobin concentration was 75 g/L (normal: 120–160 g/L). The mean corpuscular volume was markedly increased at 145 fL (normal: 79–97 fL). The peripheral blood smear showed marked oval macrocytosis and poikilocytosis of erythrocytes, and a large proportion of the polymorphonuclear leukocytes showed hyposegmented nuclei. Serum lactate dehydrogenase (LDH) activity was increased at 418 U/L (normal: 100–225 U/L); LDH isoenzyme electrophoresis showed increased LDH1 and LDH2. All other routine biochemical values, including iron and total iron-binding capacity, were within the normal reference interval. The serum vitamin B₁₂ value by our current method was <75 pmol/L and the serum folate concentration was 11 nmol/L. A Shillings test was performed. For ⁵⁷Co with intrinsically factor, the excretion was 0.03 (normal: 0.10 to 0.42); for ⁵⁸Co without intrinsically factor, the excretion was 0.01 (normal: 0.10 to 0.40). The ⁵⁸Co/⁵⁷Co ratio was 2.97, which is characteristic of pernicious anemia. Tests for serum intrinsically factor antibody and parietal cell antibody were positive. After an intramuscular injection of 1000 units of vitamin B₁₂ the patient was discharged.

In a serum specimen obtained from this patient three weeks after discharge, the vitamin B₁₂ concentration was 93 pmol/L by our current method; the serum folate remained unchanged at 11 nmol/L. The serum ferritin concentration was 307 μg/L. No trace of ⁵⁷Co was detected in this specimen.

We also measured vitamin B₁₂ and folate concentrations in duplicate in this specimen with the kits mentioned earlier. Table 1 summarizes the results. We followed the manufacturer's instructions for each kit. In addition, the specimen was analyzed in duplicate by all methods on another occasion and the paired results by each method were within allowable limits of analytical error.

All values for vitamin B₁₂ obtained on this specimen with kits involving boiling as a denaturation step were below or near the lower limit of normal, whereas those obtained from kits involving alkali denaturation gave vitamin B₁₂ values that were at least twice as high and were within or above the reference interval.

Eight weeks after beginning the vitamin B₁₂ treatment, another specimen was taken. Table 1 also summarizes these results.

Evidently, for certain patients the results obtained with kits involving alkali denaturation differ considerably from those obtained with kits involving boiling denaturation. We recommend caution in evaluating results obtained with different kits for vitamin B₁₂ determinations.

On the basis of this and in-house data, Becton Dickinson is modifying their no-boil solid-phase kit.

Trefor Higgins
A. Wu

Concept of "Robustness" for Emergency Test Selection

To the Editor:

A statistical test is defined as "robust" if the α risk (the probability of rejecting the null hypothesis—the hypothesis of no difference or effect—when it is true) has little variation when the conditions for applying the test are not fully met. This definition is also applicable to biochemical tests and may be of value in test selection.

Results of biochemical tests are usually compared with the normal reference interval. In this comparison the α risk is fixed a priori (conventionally equal to 0.05) in such a way that there is an α probability to reject the null hypothesis (the test result is "normal") when it is in fact true. The reference limits used for this comparison must be produced according to defined criteria for specimens collection from reference (well-characterized) individuals (1). These criteria must also be followed for specimen collection from patients (application conditions for the test); if they are not, the α risk varies, usually becoming larger. Consider, for example, the analysis for a constituent that must be performed with serum obtained after a defined interval of fasting. If this time is shorter than defined, the probability of obtaining a result that is out of the reference limits increases and therefore the probability of obtaining a falsely positive result also increases.

A biochemical test may be defined as "robust" if no error of interpretation of results is induced when the prescribed standards for specimen collection are not met. This concept of robustness of a biochemical test should be especially applicable in the selection of tests to be used in the emergency laboratory, because such tests frequently must be

Table 1. Comparison of Results Obtained with Different Kits for Vitamin B₁₂ and Folate Concentrations Three Weeks and Eight Weeks after Commencing Vitamin B₁₂ Treatment

<table>
<thead>
<tr>
<th>Vitamin B₁₂, pmol/L</th>
<th>Mean At 3 wks.</th>
<th>Lower limit of normal*</th>
<th>Folate, nmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boil kits</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simultrac liquid phase</td>
<td>93</td>
<td>77</td>
<td>140</td>
</tr>
<tr>
<td>Simultrac boil solid phase</td>
<td>142</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>Quantaphase</td>
<td>152</td>
<td>184</td>
<td>11</td>
</tr>
<tr>
<td>No-boil kits (alkali denaturation)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combotest II</td>
<td>350</td>
<td>169</td>
<td>160</td>
</tr>
<tr>
<td>Simultrac no-boil solid phase</td>
<td>1798</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>No-boil Dualcount</td>
<td>302</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Dual radioassay (Amersham)</td>
<td>805</td>
<td>133</td>
<td>11</td>
</tr>
</tbody>
</table>

* As stated by suppliers.