ethylene glycol or glycerol permits storage at very low temperatures without ice crystals forming, which could cause enzyme denaturation.

No adequate control sera for creatine kinase (EC 2.7.3.2; CK) isoenzymes being currently commercially available, we have tested two different liquid controls during a six-month period.

**Glycerol-stabilized enzyme preparation.** Purified and lyophilized human creatine kinase isoenzymes MM, MB, and BB were generously donated by W. Gruber, Boehringer Mannheim, F.R.G. The enzymes were dissolved in the medium described by Rosalki (1). After 1 h of gentle mixing, the enzyme solution was dispensed in 2-mL glass vials with screw caps and stored at −80 °C.

**Ethylene glycol-stabilized controls.** The isoenzymes, prepared from human tissues, were separated by chromatography on diethylaminoethyl-Sephadex and the isolated isoenzymes were stored in a matrix containing albumin (4 g/L), ethylene glycol (333 mL/L), and di-thiothreitol (3 mg/L), and the pH was adjusted to 6.5. The enzyme solution was dispensed into dark-glass vials, flushed with nitrogen gas, then sealed and stored at −20 °C.

At the beginning of each working week, vials were taken out of the freezer and thawed at 4 °C. They were then analyzed daily with our routine serum CK and serum CK B determinations. The Scandinavian recommended method with and without the immunoinhibitor anti-M was used as previously described (2, 3) at 37 °C with a LKB 2096 enzyme analyzer (LKB, Bromma, Sweden). All three isoenzymes were run as single determinations each day. Between determinations the controls were stored at 4 °C. Table 1 shows the stability of the enzyme preparations during a five-day working week under the conditions described above. Table 2 shows the mean value and SD for each working month for the two different liquid enzyme controls. As shown in the Tables, the stability of the enzymes was quite adequate and the day-to-day imprecision (CV) was highly acceptable. Additional data for the ethylene glycol control show that this preparation is stable for at least 12 months.

Liquid control preparations for CK isoenzymes stabilized as described in this report yield very stable preparations for all three isoenzymes. Their main advantage over lyophilized controls is that, in our opinion, the liquid controls are simpler to handle in routine laboratory work.

**References**


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**Indirect Evidence for the Presence of Creatine Kinase Isoenzyme BB in Human Heart Tissue**

**To the Editor:**

In the process of evaluating several methods for quantifying CK-MB (isoenzyme of creatine kinase, EC 2.7.3.2), we generated data that strongly suggest the presence of CK-BB in the human heart. We evaluated two enzymic immunoinhibition kits: method A, Eka-chem CK-MB (Smith Kline Instruments, Inc., Sunnyvale, CA 94086), and method B, Isomune-CK (Roche Diagnostics, Nutley, NJ 07110). We also evaluated an immunoradiometric kit, method C (EMBRIA-CK) and a radioimmunoassay, method D (ABURIA-CK), both from International Immunoassay Laboratories, Inc., Santa Clara, CA 95050. All kits were used as directed by the manufacturers. We measured enzyme activity at 37 °C with a COBAS-BIO centrifugal analyzer (Roche Analytical Instruments, Nutley, NJ 07110); gamma counting was done with a PACE-1 gamma counter (Picker Instruments, Cleveland, OH 44143).

Methods A and B both include a blocking antibody directed towards...
CK-M. Method A measures CK-BB as well as CK-MB (possible interference from adenylate kinase and myokinase is compensated for in a separate blanking step), whereas method B measures only CK-MB, by blanking (tube 2) for CK-BB as well as for adenylate kinase and myokinase. Method C is a "sandwich"-type radioassay specific for CK-MB, in which CK-MB is sandwiched between anti-CK-M and anti-CK-B. In method D, detection of the antibody directed towards CK-B is used and both CK-BB and CK-MB are measured.

We correlated results from these procedures with those from our in-house (1) DEAE-Sephadex A-50 ion-exchange (method E) and electrophoretic (Corning ACl, Palo Alto, CA 94306) CK isoenzyme fractionation procedures. For 100 patients with suspected myocardial infarcts or who had had open-heart surgery, whose sera were positive for CK-MB by electrophoresis (≥4 U of CK-MB per liter), the smallest activity detectable with this electrophoresis system, results by methods A and B correlated well with those by method E (r = 0.98 and 0.95, respectively). For approximately 150 patients in this same category whose sera were negative for both CK-MB and CK-BB by electrophoresis, method A had approximately 10% positives (defined as ≥6 U of "CK-MB" per liter for both methods A and B), as compared with no positives for method B.

Upon further investigation, of 14 of these patients who were positive for CK-MB by method A but negative by method B, all were found to be negative (<4 U/L) by method C and to have unusually high blanks (>8 U/L) by method B. After the sera from these 14 patients were heated at 40 °C for 1.5 h, all these methods gave negative results for CK-MB and the blank values for method B were <1 U/L. This suggests the presence of a very heat-labile variant of CK-BB that is destroyed by the heat generated during electrophoresis (contrary to CK-BB from brain and bone as well as that from control materials, which is not destroyed by our electrophoresis system) but is detected by method A and the blank of method B.

Table 1. Results for CK isoenzymes

<table>
<thead>
<tr>
<th>Source of specimen</th>
<th>Total CK, U/L</th>
<th>CK-MB or CK-BB, U/L, by method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>264</td>
<td>15</td>
</tr>
<tr>
<td>Patient 2</td>
<td>45</td>
<td>9</td>
</tr>
<tr>
<td>Patient 3</td>
<td>72</td>
<td>9</td>
</tr>
<tr>
<td>Control*</td>
<td>135</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Method</th>
<th>A</th>
<th>B (blank)</th>
<th>C</th>
<th>D</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14</td>
<td>6</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>9</td>
<td>2</td>
<td>2.5</td>
<td>9</td>
</tr>
</tbody>
</table>

* Beckman I.D.-Zone CK isoenzyme control, lot no. CO11269. Positive for CK-MB and CK-BB by electrophoresis; all three patients' specimens negative for both by electrophoresis.

We conclude that the CK-BB found in these patients came from the heart and not bone or brain, because (a) none of these patients had had a cardiac arrest, cerebrovascular accident, or prostatic cancer; (b) all had had either a myocardial infarct or open-heart surgery (in each case these results were from their first specimens after the infarction or cardiac surgery; subsequent specimens were positive for CK-MB by all methods); and (c) this combination of results (methods A and D positive, method B negative with a high blank, and electrophoresis and method C negative for CK-MB) was not found in the sera from more than 130 patients who had no heart damage.

Apparently, CK-BB is being released transiently before CK-MB during necrosis of the heart muscle. A more extensive study is now underway to document these initial findings and establish their clinical significance.

Reference

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Carpal Tunnel Syndrome and Vitamin B6 Deficiency

To the Editor:

A recent report (1) indicates a connection between the "carpal tunnel syndrome" (sensibility changes and paresis in the area innervated by n. medianus, believed to depend on compression of the nerve in the narrow canal carpi) and indirect signs of vitamin B6 deficiency.

For this reason I determined the concentration in plasma of the coenzymically active form of vitamin B6, pyridoxal phosphate, by a modification of my direct method (2). The subject was a 25-year-old, otherwise healthy woman with no history of trauma, but with typical signs of carpal tunnel syndrome, for which she was operated on.

I found a low value, 12 μmol/L (reference interval: 20–60 μmol/L), which earlier has been shown (3) to indicate decreased intracellular activity of pyridoxal-phosphate-dependent enzymes.

Also in about 25 other cases of carpal tunnel syndrome, sometimes combined with tarsal tunnel syndrome, I have found values for pyridoxal phosphate in plasma that were in the lower range, combined with electromyographically verified signs of recovery on treatment with pyridoxine.

I conclude that a metabolic disorder related to pyridoxine deficiency contributes to the carpal and tarsal syndromes, and that pyridoxine therapy should be considered in the diagnostic and therapeutic handling of these syndromes.

References

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Serum Taurine Assay: A Caution

To the Editor:

An excellent technique for the rapid determination of taurine in biological fluids was presented by Stabler and Siegel (1), similar to an earlier technique developed by Larsen et al. (2), but even simpler. Both involve passage of specimens through cation–anion exchange resin beds, taurine being the only amine removable from both resins with water alone, followed by quantitation via "high-pressure" liquid chromatography, with fluorescamine (1) or o-phthalaldehyde (2) adducts for detection. However, both mention serum among biological fluids analyzable with these methods. Analysis of serum yields spuriously higher (three- to fourfold) taurine values than plasma, presumably due to release of taurine from taurine-rich platelets and (or) leukocytes during clotting (3).