### Table 1. Supine Patient's Response to 100 g of Oral Glucose

<table>
<thead>
<tr>
<th>Time, min</th>
<th>Glucose, mg/L</th>
<th>Norepinephrine* ng/L</th>
<th>Epinephrine* ng/L</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>970</td>
<td>186</td>
<td>86</td>
<td>None (glucose given)</td>
</tr>
<tr>
<td>30</td>
<td>1660</td>
<td>138</td>
<td>98</td>
<td>None</td>
</tr>
<tr>
<td>60</td>
<td>2710</td>
<td>170</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1940</td>
<td>160</td>
<td>60</td>
<td>Dizziness, abdominal cramps, diarrhea</td>
</tr>
<tr>
<td>120</td>
<td>1500</td>
<td>256</td>
<td>61</td>
<td>None</td>
</tr>
<tr>
<td>150</td>
<td>400</td>
<td>507</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>640</td>
<td>610</td>
<td>469</td>
<td>Feeling hot, flushed, tremulous, hungry</td>
</tr>
<tr>
<td>210</td>
<td>740</td>
<td>579</td>
<td>327</td>
<td>None</td>
</tr>
<tr>
<td>240</td>
<td>770</td>
<td>606</td>
<td>128</td>
<td>None</td>
</tr>
</tbody>
</table>

* Reference intervals (2) for healthy, supine adults (mean ± SEM): plasma norepinephrine 182 ± 21 ng/L, plasma epinephrine 44 ± 11 ng/L.

patients who had undergone vagotomy and pyloroplasty but did not have dumping syndrome (8). However, only baseline and maximum values were reported, and the authors did not comment regarding the temporal changes in these hormones as related to the presence or absence of symptoms.

The results we report indicate that in this patient, the early symptoms of the dumping syndrome did not correlate with changes in plasma catecholamines, but the late reactive hypoglycemia and associated symptoms were associated with a marked increase in these compounds. It appears, therefore, that the symptoms of early dumping syndrome are not primarily catecholamine mediated. Improvement in symptoms of the early and late phases in patients treated with 10 mg of oral propranolol 30 min before an OGTT has been reported (9); unfortunately, concentrations of catecholamine were not reported in that study. In view of the catecholamine results determined in the present study, propranolol may be exerting a beneficial effect by some means other than blockade of excess catecholamine action.


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### References


**An Abnormal Isoenzyme of Creatine Kinase in the Serum of a Patient with Metastatic Carcinoma: Identity with Mitochondrial Creatine Kinase**

**To the Editor:**

Recently, there have been increasing reports concerning an abnormal isoenzyme of creatine kinase (CK; EC 2.7.3.2) in human serum. In 1973, James and Harrison (1) reported 14 cases of abnormal CK isoenzyme migrating cathodal to the skeletal-muscle isoenzyme (CK-MM) in sera and considered them to be of mitochondrial origin. In 1980, Heinz et al. (2) found a case with an abnormal isoenzyme of CK that differed immunologically from CK-MM or CK of brain origin (CK-BB) and suggested that the abnormal isoenzyme was of mitochondrial origin.

Here we report a case with cancer metastatic to liver, bone, and lymph nodes, with an abnormal isoenzyme of CK in the serum.

A 63-year-old man who had undergone radiation therapy for cervical lymph node metastases in another hospital was admitted to our hospital on June 12, 1981. Scintiscans suggested liver and bone metastases causing lower back pain. He died of general weakness on June 28. The primary focus had not been determined.

On the day of admission, the activities of creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in the patient's serum were greatly above normal (Table 1). Mitochondrial AST was observed June 24, although we did not measure its activity on the other days. The isoenzyme pattern of LDH was not unusual throughout the study (LDH1 to LDH5: 17, 46, 29, 5, and 3%, respectively). Isoenzyme analysis of CK revealed the presence of an abnormal isoenzyme of CK, which migrated cathodal to CK-MM (Figure 1). On the 12th hospital day, a further band of CK-BB appeared. Thereafter, three isoenzymes (abnormal CK, CK-MM, and CK-BB) were present until death.

To identify the abnormal CK, we filtered a sample of the patient's serum through a Sephacryl S-300 column. A single peak of CK activity was observed at almost the same elution volume as that of albumin (Figure 2). After gel filtration, the abnormal CK isoenzyme was still observed on electrophoresis (Figure 1), indicating that it was different from high-molecular-mass CK such...
as macro- or immunoglobulin-bound CK, but had a molecular mass similar to that of normal CK.

Immunological difference of the abnormal CK from CK-MM was demonstrated by using antibody against human CK-M monomer. Incubation of this antibody with the sample obtained after gel filtration (Figure 2) eliminated the isoenzyme corresponding to CK-MM but not the abnormal CK isoenzyme (Figure 3).

Table 2 shows $K_m$ values for ADP and phosphocreatine. $K_m$ values for the abnormal isoenzyme, calculated from the kinetics of CK and the patient's CK, were about a tenth those of CK-MM. Both $K_m$ values for CK of patient's sample were between those of CK-MM and the abnormal CK isoenzyme. Both $K_m$ values for the abnormal CK were quite similar to those of mitochondrial CK (4, 5) and were different from those obtained for CK-MM (6–9).

These results suggest that the abnormal CK found in the serum of the patient was of mitochondrial origin.

James and Harrison (1) found that 11 of 14 cases with mitochondrial CK in their serum were diagnosed as having cardiac pathology, including five cases with myocardial infarction; the remaining three cases had no demonstrable cardiac pathology. In our case, CK-MB was not found throughout the study and this patient had no signs of myocardial infarction, the same as the case described by Heinz et al. (2).

Accordingly, the diagnostic significance remains uncertain because of the few cases. The origin of mitochondrial CK in the absence of cardiac disease is unknown, but it may be suggestive that both the case of Heinz et al. (2) and our case had a metastatic carcinoma to the liver (or other tissues). As described by Heinz et al. (2), the abnormal CK may have been released from the metastatic carcinoma. At least, in our case, the presence of mitochondrial AST may indicate that mitochondrial membrane damage had occurred in a certain tissue.


References

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**Table 1. Laboratory Data on Serum Enzymes of the Patient**

<table>
<thead>
<tr>
<th>Date</th>
<th>CK, U/L</th>
<th>CK-abnormal %</th>
<th>CK-MM</th>
<th>CK-BB</th>
<th>LDH, U/L</th>
<th>AST, %</th>
<th>M-AST, %</th>
<th>ALT, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/12</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td>10 480</td>
<td>359</td>
<td>ND</td>
<td>27</td>
</tr>
<tr>
<td>(admission)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/15</td>
<td>915</td>
<td></td>
<td></td>
<td></td>
<td>10 125</td>
<td>411</td>
<td>ND</td>
<td>27</td>
</tr>
<tr>
<td>6/17</td>
<td>437</td>
<td>52</td>
<td>43</td>
<td>0</td>
<td>14 175</td>
<td>252</td>
<td>ND</td>
<td>39</td>
</tr>
<tr>
<td>6/24</td>
<td>528</td>
<td>56</td>
<td>40</td>
<td>3</td>
<td>15 000</td>
<td>638</td>
<td>118</td>
<td>132</td>
</tr>
<tr>
<td>6/26</td>
<td>549</td>
<td></td>
<td></td>
<td></td>
<td>18 450</td>
<td>1359</td>
<td>ND</td>
<td>290</td>
</tr>
<tr>
<td>6/27</td>
<td>655</td>
<td>63</td>
<td>33</td>
<td>4</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6/28</td>
<td>ND</td>
<td></td>
<td></td>
<td></td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>(death)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Normal ranges: CK, 5–115 U/L; LDH, 150–380 U/L; AST, 5–38 U/L; ALT, 5–30 U/L.

* Enzyme activity was estimated with the following kits of Boehringer Mannheim: CK-NAC for CK, GPT-opt for AST, GPT-opt for ALT, and LDH reagent for LDH. * M-AST (mitochondrial AST) measured by the method of Schmidt et al. (3). * Percentage distribution of CK isoenzymes was calculated based on the densitometric analysis after electrophoresis as described in Fig. 1. * ND, not determined.

**Table 2. $K_m$ Values for ADP and Phosphocreatine**

<table>
<thead>
<tr>
<th>Michaelis constant, $K_m$ (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADP</td>
</tr>
<tr>
<td>0.55</td>
</tr>
</tbody>
</table>

* Both control and patient's sera (June 17) were filtered through Sephacryl S-300 column as described in Fig. 2. After gel filtration, the abnormal CK and CK-MM in patient's sample was 31% and 69%, respectively, as evaluated by electrophoresis. The control serum contained only CK-M isoenzyme.

For the kinetic experiments, CK activity of the samples was adjusted to the same value (200 U/L). Since the CK activity of the patient's sample contained 69% of CK-MM activity, the initial velocity ($v$) of the abnormal CK at each concentration of the two substrates in the sample was calculated from the following equation: $v = v_o - 0.99 \times v_o$, where $v_o$ is the velocity of total CK of patient's sample and $v_o$ is that of CK-MM of control sample. $K_m$ values were calculated for the least-squares best-fit line. The correlation coefficients for double-reciprocal plots were $>0.99$ in all experiments.

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**Fig. 2.** Gel filtration of the patient's serum through Sephacryl S-300 column. The column (1.9 × 90 cm) of Sephacryl S-300 was equilibrated with 50 mmol/L phosphate buffer, pH 7.4, containing 100 mmol/L KCl. ---, absorbance at 280 nm; ----, creatine kinase activity (U/L).

**Fig. 3.** Inhibition by anti CK-M

1. control human serum without treatment by the antibody; 2. patient's serum (June 24); 3. patient's sample after gel filtration of 2; 4. control human serum. After electrophoresis, no 2 to 4 were treated with the antibody against human CK-M (CK-M is antigen of Boehringer Mannheim), before staining for CK activity.


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Toluene Extraction for the Fluorometric Determination of Quinidine

To the Editor:

The recent publication of a Proposed Selected Method for the fluorometric determination of quinidine (1) was of some interest to me in that I have also had experience with the satisfactory performance of toluene as a replacement for benzene in extraction procedures for this drug.

Specimens for quinidine determination were assayed by a protein precipitation method (2) and by extraction with benzene and toluene; 0.1 mL of serum was added to 0.4 mL of 100 mmol/L NaOH and extracted with 5 mL of benzene or toluene. After centrifugation, 4 mL of the organic phase was transferred to a second tube, and reextracted with 3 mL of 100 mmol/L H$_2$SO$_4$. The fluorescence of the acid phase was measured with an Aminco-Bowman spectrofluorometer (excitation 342 nm, emission 453 nm). Standards prepared by adding known amounts of quinidine to drug-free plasma were carried through each procedure.

Table 1 presents results of the three procedures. Both extraction procedures gave substantially lower values than did the protein precipitation method. The superior specificity of extraction procedures has long been established, and the difference between precipitation and extraction results is similar to that previously reported (3). Benzene and toluene extractions yielded virtually identical results. Analytical recovery of toluene extraction, as compared with unextracted aqueous standards, averaged 87% vs 95% for benzene: fluorescence readings for samples extracted with toluene were slightly lower than those extracted with benzene, but this is compensated for by the use of extracted standards in both procedures.

Benzene is now clearly recognized as a carcinogen by OSHA and CAP Laboratorv Accreditation Program standards (4), and is a significant fire hazard as well. It seems inconceivable at this time that the Editors could recommend a procedure involving this hazardous and restricted solvent as a Selected Method when a satisfactory alternative is readily available. It seems equally inconceivable that, given the clearly unsatisfactory specificity of protein precipitation methods for quinidine, a substantial number of laboratories still use procedures of this type. In a recent CAP proficiency survey (5), 87 of 152 laboratories determining quinidine by fluorometry used protein precipitation procedures.

Toluene is an acceptable substitute for benzene in extraction procedures for quinidine. I have also found toluene satisfactory for fluorometric procedures for procainamide (6) and N-acetylprocainamide (7), and acceptable substitutes should be available for benzene or other hazardous solvents in other extraction procedures as well. Fluorometric procedures still have their place in laboratories where methods requiring sophisticated instrumentation or reagents are not feasible. An extraction procedure such as the Proposed Selected Method would be a significant improvement for many laboratories, but the recommendation of a hazardous solvent such as benzene is inappropriate.

References


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