Patients with Crohn’s disease (7). We saw no difference, so our suggestion of an alkaline phosphatase response to Crohn’s virus infection in man remains unsupported by animal experimentation. Nevertheless, our speculation may serve to focus further attention on children with transient hyperphosphatasemia and reinforce the importance of the recommendation (3) that such children should receive long-term follow-up.

References

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Creatine Kinase Isoenzyme BB Concentration in Serum as a Marker of Myocardial Infarction

To the Editor:

To characterize and compare the changes in several myocardial markers, including CK-BB (creatine kinase, EC 2.7.3.2, isoenzyme BB), after myocardial insult, we measured serum CK-BB concentration by a sensitive and specific radioimmunoassay (1), total CK activity by a method involving N-acetyl cysteine activation (Boehringer Mannheim GmbH), CK-MB isoenzyme activity by immunoinhibition (E. Merck, Darmstadt, E.F.G.), and serum myoglobin concentration by radioimmunoassay (Nuclear Medical Systems, Inc., Newport Beach, CA 92663) in serum samples obtained at frequent intervals from 56 patients with acute myocardial infarction (AMI) during the first 96 h after onset of chest pain. The average time of admission was 4.2 h after the onset of pain.

All four variables exceeded the normal limits in every patient, though not necessarily concurrently. While the 95th percentile for CK-BB in normal people is 6.2 µg/L (1), CK-BB was only considered abnormal when it exceeded 9 µg/L. Peak CK-BB results ranged from three- to 100-fold the mean for controls, and occurred between 7 and 22 h into the patient’s course. Among patients whose serum was sampled within 2 or 3 h after the onset of chest pain, CK-BB was elevated as early as 2 h in five of 13 cases, and as early as 3 h in 13 of 26.

Of 24 cases where all four analytes were normal at the time of admission, BB alone was the first abnormal test in five, myoglobin alone was first abnormal in 14, and total CK-activity in only one, while four cases had simultaneous abnormalities. CK-MB alone was never the first abnormal test. Myoglobin concentration seemed to be the most sensitive test, preceding BB at least 10 times, while BB was abnormal earlier than myoglobin in six cases. An elevated BB concentration occurred earlier than an elevated MB activity in at least 38 patients and preceded total CK activity in at least 17 patients.

In 33 patients admitted to the coronary care unit with chest pain and who were found not to have an acute myocardial infarction, all four tests reached abnormal values at least once in a third to a half of the cases. This reflects an appreciable degree of nonspecificity for all four myocardial markers.

It has been assumed that CK-BB is not present in serum after myocardial infarction (2–4). We and others reported increased serum CK-BB concentration after cardiac surgery (5–7). We have also observed CK-BB in fresh myocardial homogenates, by agarose electrophoresis and radioimmunoassay. All these findings together suggest that CK-BB is present in myocardium and may be released into the circulation after myocardial insult.

In summary: serum CK-BB is a sensitive and early marker of myocardial infarction, clearly more so than CK-MB by immunoinhibition and at least as sensitive as total CK catalytic activity.

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Effects of Heat and pH on Radioimmunoassay and Activity of Prostatic Acid Phosphatase Compared

To the Editor:

During studies of prostatic acid phosphatase (PAP; EC 3.1.3.2) in prostatic cancer patients as measured by radioimmunoassay (RIA) and from its enzyme activity, we noted a significantly higher ratio between these two values (amount/activity) for serum as compared with prostatic cancer tissue extracts, in specimens from the same patients. Our RIA of PAP was according to Choe et al. (1). The reagents, supplied by Choe, included highly purified PAP, rabbit antibody to PAP, 125I-labeled PAP, and second antibody.

Enzyme activity was assayed with a
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(3)

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(2)

(1/2)

(1/4)

RIA, Activity, µg/L

S.U./mL

107

0.59

181

135

2.8

47.9

48

0.66

73.5

82.6

1.5

54.3

142

0.24

584

49

0.8

59.8

11

0.17

65

80

2.5

32.5

5.9

0.06

104

3.65

0.2

22.8

Mean I/E ratios

201.5

(p < 0.05)

43.46

kit (Sigma Chemical Co., St. Louis, MO 63178). The test substance (serum or prostate extract) was added to n-p-nitrophenyl phosphate in citrate buffer, with and without L-tartaric acid, according to Sigma Bulletin No. 104. The pH and readings in readings at 410 nm, after 30-min incubation at 37°C, between the specimen incubated in citrate alone and citrate plus L-tartaric acid represented the specific PAP activity, which was expressed as Sigma Units (S.U.) per mL (1 S.U. = 16.7 U; 1 U is defined as 1 nmol of substrate utilized per minute).

To serum to be assayed for PAP we added citrate tablet preservative, giving a final pH of 6.0 to 6.5. Specimens were stored at −85°C until assay. Normal values for PAP by this RIA are <24 μg/L and for the enzyme assay <0.20 S.U./mL.

We extracted tissue for use in both assays with tria(hydroxymethyl) methylamino buffer (pH 6.5, 50 mmol/L) containing 0.1 mol of KCl per liter, and either assayed the extract without delay or stored it at −85°C. We established that homogenization of tissue with a Tekmar homogenizer in pH 6.5 buffer, in a ratio of 1 g/100 mL and at a speed of 20,000 rpm for 30 s, repeated four times with 30-s cooling intervals, was optimal for analytical recovery of tissue PAP. The immunologic/enzymic (I/E) ratio of PAP in tissue and serum was expressed as nanograms of PAP by RIA per milligram of tissue or per milliliter of serum/S.U. per milligram of tissue or per milliliter of serum.

Both serum and prostatic cancer tissue were obtained from each of five patients with either Stage C or Stage D untreated cancer of the prostate, verified by biopsy or transurethral resection of the prostate. More than three-fourths of each tissue specimen was cancerous by histological evaluation.

Table 1 compares radioimmunoassay and enzyme assays for PAP in the serum and prostatic cancer tissue of these five patients. It can be seen that the PAP I/E ratio was significantly higher in serum than tissue, confirming the findings of Foti et al. (2) that RIA is more sensitive than enzyme assay of PAP in serum.

To explain this, one possibility was that a factor in serum was affecting PAP activity without affecting PAP as measured by RIA, resulting in an increased I/E ratio for PAP in serum. Another possible explanation was that the difference in pH between prostatic tissue and serum resulted in preservation of enzyme activity in tissue but loss of enzyme activity in serum and no effect on the RIA of PAP.

We therefore studied the effects of incubating standards of PAP in sera for 60 min at body temperature (37°C), at pH values ranging from 5.0 to 7.2. We found that 60 min of heating of purified Choe PAP standard added to these sera decreased the activity significantly at pH 7.2, but not at pH 6.5, and had little or no effect on results for aliquots of the same material measured by RIA. Similar results were noted when the purified PAP standard was put in buffer and heated at 37°C, thereby eliminating the possibility of serum factor being present that decreased enzyme activity. No effect of pH over the range 5.0 to 7.2 was noted on enzyme or RIA activity when the incubation was done at 0°C. It appeared, therefore, that PAP as measured from its activity was sensitive to the combination of pH 7.2 and heating, both of which are physiological processes, whereas the RIA was unperturbed by changes in pH over this range or by warming for 1 h at 37°C. Foti et al. (3) noted similar effects of heating serum at pH 8.4. The explanation for the much lower I/E ratio noted in prostatic tissue is probably that prostatic fluid has a pH of 6.5 (4–5) and because the PAP was extracted with pH 6.5 buffer, the enzyme is not exposed to higher pH values. As mentioned previously, the enzyme activity is stable at 37°C at pH 6.5.

The decrease is enzyme activity at pH 7.2 at 37°C and the lack of effect of these conditions on the results of RIA accounts for the greater sensitivity of RIA of PAP in serum as compared measurement of its activity. In prostatic tissue, which is extracted with pH 6.5 buffer and whose secretions have a pH of 6.5, there is no essential difference in relative RAP activity, whether measured by enzyme assay or RIA, and the enzyme assay is quite satisfactory for the tissue studies under the conditions described.

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