Early Diagnosis of Acute Myocardial Infarction by Enzymatic Urinary Creatine Determination

To the Editor:

Creatine, a major constituent of human skeletal and cardiac muscle (1) has a low relative molecular mass (M, 131). Thus the compound enters the plasma compartment very quickly after cellular lesions occur (2) and is partly excreted in urine (3). Therefore, we tested the possibility of using creatine determinations in serum, saliva, and urine as an early marker for the diagnosis of acute myocardial infarction (AMI).

For determining creatine, we used the enzymatic method of Suzuki and Yoshida (4). This method is not interfered with by cephalosporins and creatinine (5), so it is advantageous for determinations of creatine in urine. The method also offers the possibility of automated analysis. Creatinase, sarcosine oxidase, peroxidase, and 4-aminopyrine were purchased from Boehringer, Mannheim, F.R.G. We used an RA-1000 (Technicon Instruments Corp., Tarrytown, NY) for the analysis. Creatine kinase (CK) was assayed according to Rosalki (6).

When following 20 cases of AMI (ages 58.5 ± 8.4 yr), we observed an early, transient increase in serum creatine. A peak value of 12.8 (SD 3.6) mg/L was observed 8 h after onset of pain (normal ref. interval 5.4 ± 1.8 mg/L; p <0.01). Very similar curves were obtained for saliva, which is essentially an ultrafiltrate of plasma. The values then gradually decreased during the next 8 h.

Creatine in urine also dramatically increased, becoming maximal 30 min after the peak for serum. Maximum values ranged from 80 to 500 mg/L (normal ref. interval 1.5–10.0 mg/L; p <0.001). Values for urine returned to normal within 24 h. Figure 1 depicts the typical course of creatine values in serum and urine as compared with serum CK activity in an AMI patient.

Similar results were obtained experimentally for 10 dogs after ligation of the left main coronary artery. Evoked reversible cardiac ischemia in 10 other dogs did not result in any increase in creatine concentration in serum or urine; only necrosis of the myocardial cells resulted in a statistically significant (p <0.01) release of creatine.

Determining creatine in serum and urine of patients with tentatively diagnosed AMI offers a promising alternative for a quick and relatively simple biochemical diagnosis. The test becomes positive earlier than does any other currently used biochemical and electrocardiographic marker. Because the renal threshold for creatine excretion is 6.0 mg/L (7), which is slightly above the normal concentration in plasma, determinations of urinary creatine better distinguish between patients and controls than do serum creatine determinations. Muscular trauma may interfere with the interpretation of data on creatine in serum and urine (3), but this is also the case for serum CK and myoglobin determinations (8). However, muscular trauma is clinically detectable and a combination of both conditions remains an exception. In patients with coronary heart disease where the incidence of macro CK type I was shown to exceed that in the reference hospital population (9), serum and urinary creatine determination may offer an excellent tool for AMI diagnosis, because typical CK forms are known to interfere with most methods for CK-MB determinations.

References

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Protein Markers of Nutritional Status as Related to Age and Sex

To the Editor:

We read with interest the report of Sachs and Bernstein (1), in which they concluded that there is no need to segregate reference intervals for serum protein concentrations by age nor are there significant correlations between different serum proteins. In this connection, we would like to report results of a prospective survey of 157 sequential admissions to a general surgical ward in which protein markers were measured.

The mean albumin concentration in serum (measured in the smac 1; Technicon Ltd, Basingstoke, U.K.) was 36.8 (SD ± 7) g/L, with no significant sex-related difference. Patients younger than 40 years had a significantly higher (p <0.01) mean albumin concentration of 41 (SD ± 4) g/L than patients 40–69 years old—37 (SD ± 6.5) g/L—or patients over 70 years—35 (SD ± 6) g/L. The age-related difference that we observed may occur for pathological or nutritional reasons in the older age groups, because it was not observed in the healthy blood donors of Sachs and Bernstein. We also measured transferrin by nephelometry (Encore; Baker Instruments, Surrey, U.K.) and prealbumin (transhyretin) by laser nephelometry (Hyland; Travenol Laboratories Ltd, Norfolk, U.K.), finding significant correlations (p <0.001) between albumin, transferrin, and prealbumin concentrations. The coefficients of correlation (r) were as follows:

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for prealbumin/albumin, \( r = 0.596 \); for prealbumin/transferrin, \( r = 0.602 \); and for transferrin/albumin, \( r = 0.683 \). This indicates that these three proteins may be concurrently influenced by common factors affecting many serum protein concentrations such as hydration, capillary permeability, and fluid losses from or into the bowel and other body compartments.

Sachs and Bernstein may have failed to detect the variation of serum albumin concentration with age and the correlations between serum proteins because of their small patient numbers (48) or because of their patient selection, because all but one had a serum albumin concentration of <32 g/L. The use of prealbumin as a marker for instant nutritional assessment in preference to albumin or transferrin because of its shorter half-life is not justified from the available information (2), and there certainly is a need to be aware of the potential variation of serum albumin concentrations with age when one is using this as a factor in assessing nutrition.

References

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Rapid Determination of the p-Aminobenzoic Acid Excretion Index in Urine without Use of Radioactivity

To the Editor:

We read Dr. Van den Bergh and his colleagues' description (1) of their technique for the rapid determination of the p-aminobenzoic acid (PABA) Excretion Index (PEI) with considerable interest. We have developed a similar technique, which depends on the coadministration of a marker along with bentomidine (N-benzoyl-L-tyrosyl-PABA), to correct for any malabsorption of PABA by the gut. Rather than p-aminosalicylic acid as used by Van den Bergh et al., we use anthranilic acid (p-aminobenzoic acid).

As we have reported elsewhere (2), we initially obtained excellent clinical results with our test. However, we recently have found that the technique tends to produce low PEI’s (false-positive results) in patients with liver disease but without pancreatic disease. We are actively investigating this phenomenon and our initial results suggest that, in at least some patients with liver disease, PABA may be less rapidly conjugated than anthranilic acid.

We thus suggest that any workers who are assessing the utility of non-isotopic markers of PABA absorption and excretion in determining the PEI should examine the metabolic kinetics of the putative marker and PABA in patients as well as in normal subjects.

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References

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Unreliability of Electrophoretic Determination of Neuron-Specific Enolase in Plasma

To the Editor:

We recently described (1) an electrophoretic technique for separating enolase (EC 4.2.1.11) isoenzymes. Combining this with a bioluminescence assay of total enolase activity (2) permits rapid determination of neuron-specific enolase in human serum as a marker for small-cell lung cancer and neuroblastoma. We report here our recent findings that plasma cannot be substituted for serum in these determinations, owing to the interference of platelets.

Figure 1 shows results of enolase separation done on serum and plasma obtained from whole blood by centrifugation at 1600 × g for 8 min. The samples are from the same normal subject. The serum produced a major fluorescent band for \( \alpha \) isoenzyme and a faint minor band (not visible in the figure) for the \( \gamma \) isoenzyme (lane 2). In the plasma an additional band was observed (band \( P \), lane 3), slightly anodic to the \( \alpha \) band, at the point of application on the electrophoretic plate. Experiments with 2-phosphoglycerate omitted from the detection medium showed that all the three bands corresponded to enolase activity.

The serum and plasma spotted on the plates contain equal amounts of total enolase activities, as determined by the bioluminescence method (2). After electrophoresis, however, the plasma enolase bands showed much higher fluorescence than the serum enolase bands, indicating that some enolase activity in plasma that was not measured by the bioluminescence assay was being unmasked by some procedure in the electrophoretic step.

Platelets, which often contaminate plasma preparations, have been known to affect the determination of lactate dehydrogenase activity in certain methods (3, 4). Further, because enolase is known to be present in platelets (5, 6), we examined their possible role as an additional source of enolase activity.

Total enolase activity in serum and plasma samples, as determined by bioluminescence, did not differ substantially. However, integration of the isoenzyme bands after electrophoresis (Auto Scanner Plus Vis; Helena, Beaumont, TX) showed a 12-fold greater intensity of total fluorescence in plasma than in serum. The \( \alpha \) and the \( \gamma \) isoenzymes made up 97% and 3% of total activity in serum, whereas in plasma the proportions were 79% \( \alpha \), 9% \( \gamma \), and 12% isoenzyme \( \gamma P \). When plasma was centrifuged (3000 × g, 30 min), all of band \( P \) was in the sediment (the platelets), which had an isoenzyme distribution similar to that of

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**Fig. 1. Electrophoretic separation of enolase isoenzymes from human serum and plasma (and total activity, U/L, as determined by the bioluminescence assay)**

Lane 1: human brain extract (reference); Lane 2: human serum (1.60); Lane 3: human plasma (1.30); Lane 4: supernate from human plasma after centrifugation (1.66); Lane 5: sediment from human plasma after centrifugation (0.85)