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# COMMUNICATION

# Analysis of Serum Creatinine Using an Immobilized Enzyme Nylon Tube Reactor Incorporating a Four-Enzyme System

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Ginman et al. (*1*) recently described the use of a single nylon reactor with four immobilized enzymes for the analysis of creatinine in urine. The tube was incorporated in a continuous flow system. Good correlation with the Jaffé method has been obtained with a manual technique employing the same enzymes in solution ($r = 0.992$). The advantages of using enzymes for the quantitation of creatinine over Jaffé methods are precision, high specificity, simplicity, and speed of analysis.

However, when the method was applied to the analysis of serum, it was found that direct sample application of serum gave poor results, probably because of interference by serum proteins or by nonspecific NADH-consuming reactions. Pre-

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liminary treatment of the samples with 6.25% trichloracetic acid or sodium tungstate in order to remove problems was unsuccessful. Likewise, heat inactivation of the sample to destroy any ATP-converting enzymes proved unsatisfactory. When the method was modified to incorporate a dialysis system, it was found necessary to use serum standards, since a difference in dialysis rate was observed between aqueous and serum samples. A maximum of 24-in. dialyzer was used because any longer dialyzer caused significant carryover. Sensitivity was improved by increasing the ionic strength with 2% sodium chloride in the donor stream and dialyzing into the substrate solution at 37°C. Although it was possible to obtain a good standard curve, the results for serum samples did not correlate with the results obtained by a Jaffé method. No improvement was obtained by using glycylglycine buffer as the recipient stream and adding the NADH (ATP) PEP after the dialysis stage.

One substance that would interfere with the assay is pyruvate, the normal levels of which in blood are 34–80 μmol/L (*2*) by enzymatic methods of assays. The ratio plasma pyruvate: erythrocyte pyruvate is 2.3, so that plasma pyruvate is about 21% greater than whole blood pyruvate (*3*). Pyruvate is unstable in blood, but after an initial loss, levels rise to those above the original value (*4*) as a result of the following two sets of reactions (*5*).

(i) Pyruvate + triose phosphate ⇄ lactate + 3-phosphoglycerate

(ii) 3-Phosphoglycerate ⇄ 2-phosphoglycerate ⇄ phosphopyruvate ⇄ pyruvate

Oxalate inhibits reaction (ii), thus causing loss of pyruvate (*6*). Fluoride also causes increased loss, to lactate (4). Attempts made to remove pyruvate interference by using fluoride and oxalate as anticoagulants were unsuccessful, giving a marked depression in creatinine level compared with similar untreated sample. Another attempt to remove pyruvate was by preliminary treatment of the sample with iodine and potassium iodide, as described by Kirbrick and Mihorat (*7*). This was also unsuccessful, complete lack of correlation with the Jaffé method being observed.

The effect of interferences could be eliminated by the use of serum blanks. However, this would require an additional continuous flow channel, doubling the serum volume sampled and doubling the cost of reagents and other consumables such as pump tubes. Bonvinci et al. (*8*) have stated that serum blanks must be measured at the same time as the tests because of accessory reactions that proceed in parallel in both of them; this procedure creates problems when using endpoint methods of analysis. Bonvinci et al. have overcome these difficulties by using a discrete system of analysis, incorporating a modified kinetic method, originally proposed by Moss et al. (*9*).

In this work it was found that the successful way to adapt the method for urines to serum samples was to carry out a preliminary isolation of creatinine from serum. The technique used was based on that described by Polar and Metcoff (*10*), adsorbing the creatinine from acidified serum onto a Dowex 50W cation exchange resin. After elution from the resin with phosphate buffer, pH 12, the creatinine was determined by the continuous flow immobilized enzymatic method instead of by

the Jaffé method, as used by Polar and Metcoff. The sample (0.5 mL) was adsorbed onto 20 mg of resin. Complete adsorption was achieved by 30 min. After washing, complete elution from the resin into 0.5 mL of phosphate buffer took 10 min. These extractions were found to be stable for at least 1 week when stored at +4°C. The continuous flow system of analysis was that developed by Ginman et al. for urine creatinine determinations (*1*), although in order to gain maximum sensitivity, it was found necessary to use a 5-m length of enzyme tube. The calibration was performed using serum standards that were treated identically to the test. The precision of the method at high, intermediate, and low creatinine levels, involving 10 extractions on each serum sample, is shown in Table 1. The accuracy of the method, determined using quality control sera with stated assay ranges, is shown in Table 2, and the correlation and regression line, comparing the method with the Boehringer kit method, is shown in Fig. 1.

Although Polar and Metcoff (*10*) obtained good results using a Jaffé method following isolation of the creatinine, with recoveries ranging from 98–101%, the advantages of the enzymatic method are its complete specificity, speed of analysis (5 min throughput time; 60 samples/h), simplicity (a direct method using a single stream), and avoidance of the use of explosive picric acid. Stability of the enzyme tubes is, however, a factor to be determined, and this in turn will affect the cost of the assay.

TABLE 1
Precision of the Serum Creatinine Method at Low, Intermediate, and High Creatinine Levels

| | Low | Intermediate | High |
|---|---|---|---|
| $\bar{x}$ μmol/L | 112.5 | 275.2 | 523.9 |
| $s$ | 7.56 | 3.85 | 17.46 |
| cv, % | 6.72 | 1.40 | 3.33 |

TABLE 2
Accuracy of the Serum Creatinine Method

| Assayed mean of quality control material, μmol/L | Creatinine value obtained, μmol/L |
|---|---|
| 153 | 150, 145 |
| 291 | 295, 300 |
| 548 | $\bar{x}$ = 522 ($n$ = 12, $s$ = 19.6, cv = 3.75) |

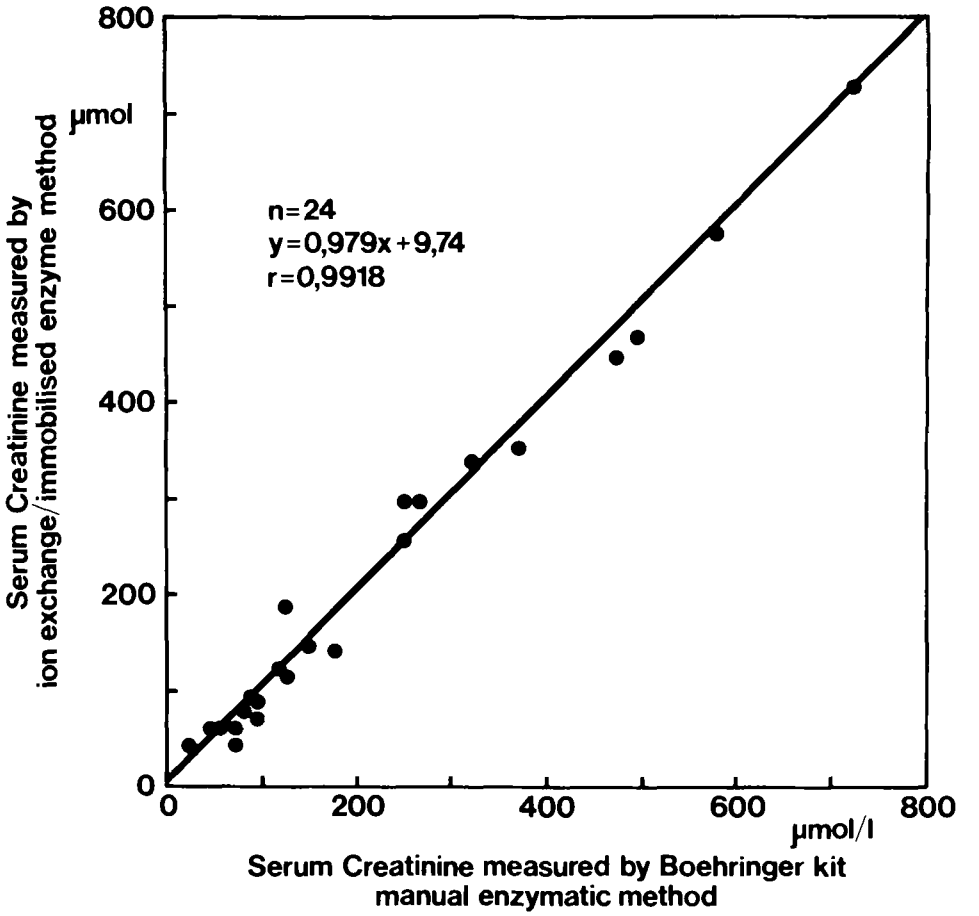


Fig. 1. Correlation and Regression line for serum creatinine measured by the ion exchange/immobilized enzyme method against serum creatinine measured by the Boehringer manual enzymatic kit method, by the method of linear least squares.

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