obtained was due to the technique; four readings of absorbance are required, only a small change in absorbance (0.056 A per 0.1 mmol of creatinine per liter) is obtained, and the initial absorbance reading and the final absorbance reading, when the sample has a low creatinine concentration, are both made at an inherently imprecise part of the absorbance scale.

**Linearity.** Linearity was assessed by duplicate analysis of a series of seven samples generated by mixing aliquots of two sera of 0.90 and 0.08 mmol of creatinine per liter and by duplicate analysis of a series of serum dilutions of a solution of 10 mmol of creatinine per liter in 0.1 mol/L HCl. Readings and concentration were linearly related up to 1.50 mmol of creatinine per liter.

**Accuracy.** Regression analysis of results of assays done by the enzymatic and continuous-flow methods, encompassing 100 samples within and outside the reference range, gave the equation: enzymatic = 0.9991 continuous-flow = 0.0014. Statistical correlation was good (r = 0.9962), and the t-test (t = 0.1481) showed that results obtained by enzymatic analysis did not differ statistically from those obtained by the continuous-flow method. This finding contrasts with previous reports that creatinine values found by enzymatic techniques were significantly lower than those found by Jaffé methods (2, 5, 8, 9).

Analytical recovery was assessed by adding small volumes of a solution of creatinine in 0.1 mol/L HCl to pools of serum, yielding four samples of serum that were analyzed in duplicate. Mean recovery was 92.6% and the experimental results fulfilled the criteria of Logan (10).

Blind triplicate analyses of a series of quality-control materials and of materials utilized in the Wellcome Group Quality Control Programme were performed. The values obtained generally agreed well with the assigned or consensus values. These results confirm the findings of the patient-sample analyses that the enzymatic and continuous-flow methods give similar results, because the assigned or consensus values were obtained by, or much influenced by, continuous-flow alkaline picate methods.

Certain quality-control materials, however, gave results that were not consistent with their assigned values. This was shown to be due to exhaustion of all available NADH before the reaction was complete, owing to some secondary reaction. This implies that certain quality-control materials must be used with caution and these findings borne in mind when lyophilized materials are used in interlaboratory proficiency surveys.

**Specificity.** The effects of lipemia, icterus, and hemolysis on determination of creatinine were studied by triplicate analyses of a series of solutions prepared by mixing aliquots of each of a lipemic (triglycerides: 4.06 mmol/L), an icteric (bilirubin: 360 μM/L), and a hemolyzed (hemoglobin: 1.58 g/L) serum with aliquots of a normal serum. Interference was negligible.

**Instrumental Analysis.** We attempted to adapt the reagent set to kinetic and end-point analytical methods on a Centrifichem System 400 analyzer (Union Carbide Corp., Tarrytown, NY 10591). Kinetic approaches were not found to be feasible, because creatinine concentration was apparently unrelated to initial rate. Assays could be done in an end-point mode, but with unacceptable precision. The requirement of addition of creatininasise to either sample or reagent and the need to monitor both the reaction with creatininasise and the background secondary blank reaction without creatininasise caused problems with regard to practicability.

The enzymatic assay of creatinine was therefore shown to provide results of accuracy equivalent to the continuous-flow alkaline picate method, but of poorer precision and practicability. The method has the following inherent disadvantages: (a) expensive reagents are required, (b) the manual technique is time-consuming, and (c) certain quality-control materials do not react like samples from patients.

We therefore consider that this enzymatic technique cannot be recommended to replace suitable alkaline picate techniques for the determination of creatinine in the routine clinical chemistry laboratory.

### Table 1. Precision Studies on Three Sera

<table>
<thead>
<tr>
<th></th>
<th>Within-day</th>
<th></th>
<th>Day-to-day</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
<td>Medium</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Mean, mmol/L</td>
<td>0.06</td>
<td>0.22</td>
<td>0.39</td>
<td>0.05</td>
</tr>
<tr>
<td>SD, mmol/L</td>
<td>0.011</td>
<td>0.010</td>
<td>0.029</td>
<td>0.010</td>
</tr>
<tr>
<td>CV, %</td>
<td>19.3</td>
<td>4.5</td>
<td>7.5</td>
<td>20.9</td>
</tr>
</tbody>
</table>

References


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**Accuracy of a Fully Enzymatic Method for Creatinine, as Judged by Isotope Dilution–Mass Fragmentography**

To the Editor:

The simple enzymatic methods of analysis for cholesterol, glucose, urea, uric acid, and several other components in serum or blood are in general accurate, giving values near those obtained with highly accurate comparison methods (1). In contrast, many less-specific methods are in general use that are based on formation of more or less well-defined colored complexes. Of these the Jaffé reaction for determination of creatinine is probably most important quantitatively. It is well established that this method gives falsely high values, owing to the presence of "pseudocreatinines" in serum, which may increase the apparent creatinine concentration by 10 to 100%. On the average, methods based on the Jaffé reaction give a constant positive error of about 20 μmol/L (2).

Recently, fully enzymatic methods for determining creatinine were described (3, 4). These methods are not yet widely used, and there is still some uncertainty with respect to their accuracy. We have now compared results by a fully enzymatic method with those by a highly

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Table 1. Constants Obtained in the Regression Analysis between the Comparison Method and the Two Routine Methods for Creatinine

<table>
<thead>
<tr>
<th>Constant</th>
<th>Enzymatic method (µmol/L)</th>
<th>Kinetic Jaffé method (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coefficient of correlation (r)</td>
<td>0.94 ± 0.06²</td>
<td>1.07 ± 0.06²</td>
</tr>
<tr>
<td>Slope</td>
<td>9 ± 7³</td>
<td>22 ± 6³</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.01</td>
<td>0.939 + 8.9</td>
</tr>
</tbody>
</table>

² Mean ± SD, not significantly different from 0.01 (p > 0.01, Student’s t-test).
³ Mean ± SD, not significantly different from 1.0 (p > 0.01, Student’s t-test).

We compared results obtained with each of the two routine methods with results obtained with the mass-fragmentographic method (x-axis); (Table 1). For the enzymatic method, comparison, the regression equation was y = 0.939 + 8.9. Both routine methods correlated very well with the comparison method. Both routine methods gave a slope in the regression analysis that was near 1.0; the difference from 1.0 was not statistically significant (Student’s t-test). The intercept obtained in the comparison between the enzymatic method and the comparison method (9 µmol/L) was considerably smaller than the intercept obtained in the comparison between the Jaffé method and the comparison method (22 µmol/L). Only the intercept obtained in the latter analysis differed statistically from 0 (Table 1).

From a clinical point of view, the most interesting concentration of creatinine corresponds to the upper limit of the normal range, about 100 µmol/L. The ideal method for creatinine should thus be most accurate at this concentration. From the data given (Table 1), it can be seen that the slope of the enzymatic regression line, being somewhat smaller than 1.00, compensates for the intercept in that range. Thus a concentration of 100 µmol/L according to the comparison method corresponds to a concentration of 105 µmol/L according to the enzymatic method. At a concentration of 146 µmol/L both methods give the same value.

We conclude that the enzymatic method and mass fragmentography give almost identical values in the upper normal range of creatinine concentrations. Evidently the upper-normal limit for creatinine concentration should be lower with the present enzymatic method than with most modifications of the Jaffé methods. The enzymatic method is considerably more laborious than most Jaffé methods. On the other hand, at present, the enzymatic method is probably the best routine method available for discriminating between normal and pathological creatinine concentrations.

References

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Rate of Achieving Thermal Equilibrium in Two Models of Centrifugal Analyzers

To the Editor:

The importance of temperature stability in kinetic analyses cannot be overemphasized. The time needed to establish it is equally important, but not always monitored as carefully.

When enzyme activities are measured at 37 °C, as they are in our laboratories, the time required to reach a stable temperature may be significant, and may vary from instrument to instrument. These variations became apparent when results for serum lactate dehydrogenase (EC 1.1.1.27) as measured in a 36-cuvette Rotochem IIa were compared to those with a 15-cuvette Rotochem II (American Instrument Co., Silver Spring, MD 20910). The short lag time (30 s) that we use for lactate dehydrogenase showed a significant nonlinearity of absorbance vs. time in the initial intervals of the reaction in the Rotochem IIa.

An “optical thermometer” was designed similar to one described by Bowie et al. (1). A 0.1 mol/L solution of tris(hydroxymethyl)methylamine (Tris) was adjusted to pH 8.0. Phenol red (phenosulfophthalein) was added to give an absorbance of approximately 0.3 at 550 nm in a 1-cm cuvette. At this absorbance, which corresponds to 50% transmittance, optical systems of centrifugal analyzers most sensitively detect changes in the intensity of transmitted light.

A change in temperature causes a shift in the pK of the Tris and therefore a change in pH. This change in pH can be monitored by an indicator that responds in the correct pH range (near the pK of the indicator). Therefore, a change in temperature causes a change in color.

I used a Model 34 spectrophotometer with a thermostated flow cell (Beckman