A series of 25 sera were assayed with the proposed system, in duplicate, each day for five days; the standard deviation of the results was ±2.5 mg/dl.

Correlation results for 33 sera assayed with the proposed system and with an automated o-toluidine one with dialysis (13) was: $Y_{(hexokinase-INT)} = 5.1 + 0.942X$; $S_{y.x} = 3.27; r = 0.992; n = 33$. A comparison of urine specimens was made by using an o-toluidine-glucose oxidase manual method, which is done as follows: Two 0.2-ml urine aliquots were respectively diluted with 0.2 ml of water and 0.2 ml of glucose oxidase (Worthington Biochemical Corp., Freehold, N.J., 07728; “4×” vial diluted to 25 ml with water). After incubation at 37°C for 45 min, 4 ml of o-toluidine reagent (19) was added to each aliquot, and the mixtures were heated at 100°C for 10 min. Absorbances were measured at 635 nm; the absorbance difference between the untreated and the glucose oxidase-treated specimens was used to calculate the glucose concentration. Correlation was $Y_{(hexokinase-INT)} = 2.22 + 1.05X; r = 0.998; S_{y.x} = 2.32; n = 24$.

The approximate cost is 20¢/test, based on a retail price of $28.00 per “GlucoStrate” kit. Enough reagents are supplied for 200 min of operation of the AutoAnalyzer (150 samples; 15 standards). This cost is competitive with the spectrophotometric hexokinase method with measurements at 340 nm (25¢/test).

References


Simple, Rapid, Kinetic Method for Serum Creatinine Measurement

Jack A. Lustgarten and Robert E. Wenk

A study of the reaction between creatinine and alkaline picrate confirmed that there is a linear relation between absorbance change and creatinine concentration over the entire clinical range of values. The specificity and accuracy of the method are comparable to that of the end-point Jaffé reaction with deproteinization. The test is somewhat imprecise in the low range, but the simple, rapid procedure is appropriate for the monitoring of patients with glomerular disease and those undergoing therapeutic dialysis.

Additional Keyphrases hemodialysis • emergency procedure • Jaffé reaction • urinary creatinine • renal disease • creatinine clearance • alkaline picrate reagent • uremia

Increasing medical emphasis on renal disease and therapeutic dialysis has caused more demand for determinations of serum creatinine concentrations as an aid in monitoring patients. Diagnostic unreliability in interpreting urea concentrations (1) and the unavailability of commercial kits for creatinine (2) prompted a search for a simple, rapid creatinine procedure.

Recently, Cook (3) described a specific assay that was based on a direct relationship between serum
creatinine and the change in absorbance over a fixed time interval, when creatinine is reacted with alkaline picrate. Fabiny and Ertingshausen (4) adapted a reaction-rate method to the automated "CentrifilChem" Fast Analyzer (Union Carbide Corp., Tarrytown, N.Y. 10591).

We have adapted the kinetic method to more readily available instruments and investigated the suitability and limitations of the procedure.

Materials and Methods

Apparatus: (1) Microsample Spectrophotometer 300N, Gilford Instrument Laboratories Inc., Oberlin, Ohio, 44074 (with "Thermo-Cuvette" Model 3017 and "Data Lister" Model 4008).

(2) Automatic Pipette, Micromedic Systems Inc., Philadelphia, Pa. 19105. (The calibration of the instrument was verified by comparison with mercury-calibrated, "to contain" micropipets.)

Working reagent: NaOH, 0.5 mol/liter, and saturated aqueous picric acid (ACS, prepared at room temperature) were mixed in equal volumes.

Standards: Creatinine standards were prepared by dissolving National Bureau of Standards creatinine (SRM No. 914) in dilute HCl (20 mmol/liter).

Reference method: Creatinine was determined in standards, sera from patients, and serum pools by both the experimental method and with the AutoAnalyzer (Technicon Instruments, Tarrytown, N.Y. 10591) N-11b creatinine-alkaline picrate reaction (which includes protein removal by dialysis). Calibration curves for the reference method were prepared with standards made from NBS creatinine.

Procedure: Samples of 100 µl (standards, pools, patient material) were added to 2.0 ml of working reagent, mixed immediately (zero time), and a stopwatch was started. At 20 s the absorbance (A20) of the clear solution was read and recorded by the Data Lister. At 80 s a second reading (A80) was recorded. The change in absorbance (ΔA) was obtained by subtraction (A80 - A20). The reaction temperature was held constant at 30°C, and absorbance was measured at 515 nm. A standard curve was prepared each day for reference information; however, we noted that only a single standard was required to calculate results, according to the following equation:

\[
\text{Concentration of unknown} = \frac{\Delta A \text{ unknown}}{\Delta A \text{ standard}} \times \frac{\text{concentration of standard}}{100}
\]

To monitor the reaction in greater detail over a longer time interval, we switched the Data Lister to automatic mode to observe ΔA every 10 s.

Temperature effects were investigated by resetting the thermocuvet to various values: 22°, 30°, 32°, 37°C.

We studied the ΔA in relation to wavelength, to select the optimal spectral range, and with regard to creatinine concentrations, to evaluate the linearity of the reaction.

Recovery was measured by adding small volumes of concentrated NBS creatinine solution to an assayed serum pool, to effect final creatinine concentrations of 60 to 110 mg/liter.

Precision was evaluated from the results of a single analysis, replicated three times each week for two months.

We questioned the need to prepare fresh working reagent for each run. Alkaline picrate reagent was premixed and its reactivity with creatinine was determined after two weeks exposure to bright sunlight at room temperature.

Various substances were studied for their possible interference in a creatinine-alkaline picrate rate reaction (see Table 3).

Results

The reaction kinetics were identical for aqueous creatinine solutions and for sera of equivalent creatinine concentrations.

A wavelength of 515 nm was optimal; at lower wavelengths the absolute background absorbance of the reagent was very great, whereas higher wavelengths produced a smaller ΔA.

The relationship between ΔA/minute and creatinine concentration was linear to 250 mg/liter (Figure 1).

Reactions run at three different temperatures showed linearity of ΔA/minute with concentration (Figure 2). A Q10 value of about 1.3 was observed.1

Results of assays of patient sera by the kinetic and the reference methods correlated excellently (Figure 3). Patient specimens (60 pairs), and replicated (80) analyses on serum pools of creatinine concentrations of 12, 59, and 113 mg/liter, showed no significant differences in mean values by t-tests (P = .05). There was significant difference in variation by F tests at all concentrations for patient and pool material (P = .05). The coefficient of variation for day-to-day replicate analyses was about twice that for the automated reference procedure (Table 1).

Recovery data for added creatinine for the reaction-rate and automated procedures are shown in Table 2. Average recovery, at concentrations of 60 and 110 mg/liter was 95.5% and 101.0%, respectively.

The effects of interferants are shown in Tables 3 and 4. Acetone produces artifically high values with the kinetic method.

The reactivity of alkaline picrate was unchanged after two weeks storage at room temperature in bright sunlight. Although the absorbance of the reagent increased markedly, its reactivity with creatinine was unaffected. Creatinine concentration values were the same, whether determined with two-week-old reagent or freshly prepared reagent, so long as a standard was run at the same time as the unknown; the usefulness of aged reagent was not diminished in the kinetic method. During one 8-h working day there was no appreciable change in reagent absorbance.

Discussion

Our procedure has many advantages over the endpoint Jaffé reaction: deproteinization is unnecessary; a single, stable reagent is used; results can be obtained

---

1 Q10 of a reaction is the ratio of reaction rates at T° + 10°C and at T°C; e.g., a Q10 of 2 is a doubling of the rate of reaction.
Table 1. Precision of Kinetic and Reference Methods Compared*

<table>
<thead>
<tr>
<th>Mean creatinine concentration of pools</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within-day</td>
</tr>
<tr>
<td>Kinetic</td>
<td>Reference</td>
</tr>
<tr>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>59</td>
<td>60</td>
</tr>
<tr>
<td>113</td>
<td>110</td>
</tr>
</tbody>
</table>

* Twenty replicate analyses on three pools by each method.

Table 2. Comparison of the Recoveries of Creatinine by Reference and Kinetic Methods

<table>
<thead>
<tr>
<th>Method</th>
<th>Recovery at 60 mg/liter concn, 5.0 mg creatinine added</th>
<th>Recovery at 110 mg/liter concn, 10.0 mg creatinine added</th>
</tr>
</thead>
<tbody>
<tr>
<td>AutoAnalyzer</td>
<td>mean: 99.1</td>
<td>range: 98-102</td>
</tr>
<tr>
<td>Kinetic</td>
<td>mean: 95.5</td>
<td>range: 92-98</td>
</tr>
</tbody>
</table>

* All values are mean of 20 determinations.

Table 3. Effect of Adding Various Substances on Creatinine Methods

<table>
<thead>
<tr>
<th>Substance added</th>
<th>Final concentration mg/liter</th>
<th>Apparent creatinine mg/liter</th>
<th>AutoAnalyzer</th>
<th>Kinetic method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>50</td>
<td>5.8 (6.0)*</td>
<td>5.9 (5.9)*</td>
<td></td>
</tr>
<tr>
<td>Acetoacetic acid</td>
<td>1000</td>
<td>7.1</td>
<td>5.7</td>
<td></td>
</tr>
<tr>
<td>ethyl ester</td>
<td>1000</td>
<td>6.2</td>
<td>6.7</td>
<td></td>
</tr>
<tr>
<td>Acetone</td>
<td>250</td>
<td>5.8</td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>BSP</td>
<td>2350</td>
<td>5.8</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>4700</td>
<td>5.7</td>
<td>5.9</td>
<td></td>
</tr>
</tbody>
</table>

* Values in parentheses: pool mean concentration before addition.

Table 4. Comparison of the Effects of Icterus, Lipemia, Hemolysis, and Supranormal BUN and Sugar on Creatinine in Sera from Patients

<table>
<thead>
<tr>
<th>Component elevated</th>
<th>Apparent creatinine mg/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN 640*</td>
<td>7.2</td>
</tr>
<tr>
<td>Glucose 3080</td>
<td>0.9</td>
</tr>
<tr>
<td>Total bilirubin 132</td>
<td>0.7</td>
</tr>
<tr>
<td>Lipemia</td>
<td>1.1</td>
</tr>
<tr>
<td>Lipemia (gross)</td>
<td>0.7</td>
</tr>
<tr>
<td>BUN 1360, bilirubin 69</td>
<td>2.9</td>
</tr>
<tr>
<td>Total bilirubin 90 mg/dl</td>
<td>0.8</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>1.5</td>
</tr>
<tr>
<td>Hemolysis (gross)</td>
<td>1.0</td>
</tr>
<tr>
<td>Glucose 2290, BUN 1000</td>
<td>17.6</td>
</tr>
</tbody>
</table>

* Figures are mg/liter.
in a few minutes; and there is linearity up to 250 mg/liter, so that a standard curve is not needed.

The simplicity, accuracy, and speed of the procedure make it useful for hemodialysis units and nephrology services. Serum creatinine better correlates with the course of uremia than serum urea, and is almost independent of diet, catabolic effects on body protein, and other nonrenal causes of azotemia (5). During dialysis, urea concentrations may decrease rapidly while the patient's condition is not substantially bettered, and serum creatinine often parallels the observed clinical effectiveness of therapy better than does urea (6).

When the present technique is compared with previous kinetic procedures, other advantages may be noted. No reagent blank is required. Fabiny and Ertingshausen's (4) test offers batch analysis but requires a centrifugal fast analyzer, which is not generally available. Cook's (3) test requires 1.0 ml of serum, a serum blank, and 5 min of continuous monitoring; and pre-buffering is necessary to decrease variation produced by different protein concentrations.

The essentials of this method are a quality spectrophotometer, accurate timing, and temperature control if available. The spectrophotometer we used was convenient, but similar results are obtained with a Coleman Jr. II 6/35 with a 2-min timed interval. Timing with a stopwatch is sufficiently accurate. Temperature control in the spectrophotometer was not critical to reproducibility as long as standard and unknown are preincubated at the same desired temperature. During the reaction, little variation in cuvet temperature occurs. For a $Q_0$ of 1.3, each degree of temperature change results in only 3% change in rate of reaction. The automatic pipet we used was an additional convenience, but is is not essential.

Insignificant differences from the reference method in mean values obtained for patient material and excellent correlation in all clinical ranges indicate that "normal" values for the kinetic test are similar to those for the reference method. The poorer day-to-day precision of the manual rate-reaction method, however, decreases its usefulness in detection of subclinical glomerular disease. At the low creatinine concentration of a normal individual's serum, the kinetic method would produce more falsely positive and falsely negative results. On the other hand, the rate-reaction procedure is quite simple and rapid (3 min), and reagent may be premixed and stored. These features are especially attractive for use in monitoring patients undergoing hemodialysis.

The kinetic reaction, under the study conditions, appears to be as accurate as the common AutoAnalyzer technique, and is probably no more or less specific. We did not observe separation of fast and slow components of the reaction. Others (4) have reported this separation. The kinetics of the reaction of aqueous standards (presumably containing a single species of creatinine) were identical to those for sera containing equivalent creatinine concentrations.

The rate-reaction method has been successfully applied to a number of diluted urine samples, allowing for rapid determination of creatinine clearance.

References


2. List of Test Kits for Clinical Laboratories, U. S. Dept. HEW, USPHS, Health Services and Mental Health Administration, Center for Disease Control, Atlanta, Ga. 30333, 1971.


