Some observations and improvements on SMA 6/60 determinations of creatinine

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SYNOPSIS Modifications to an earlier SMA 6/60 method for creatinine determinations are reported. The suggested procedure is linear for concentrations up to 15 mg/100 ml, shows no zero drift, and has a satisfactory analytical precision. Effects have been determined upon the analysis for a number of non-creatinine constituents of biological fluids. Recommendations are made for the optimum determination of plasma and urine creatinine.

Conversion of the glucose channel on the basic Technicon SMA 6/60 to creatinine determinations was reported earlier (Moll and Pring, 1971). Now a number of alternatives, including creatinine, are available at the time of purchase for the two non-electrolyte channels. The analytical range of the creatinine method (0-15 mg/100 ml) differs from that described by Moll and Pring (1971). Expansion to a scale of 0-15 mg/100 ml in our own laboratory presented two problems experienced by other users of the 6/60, which had not been apparent with the earlier SMA system. First, the analytical performance was unsatisfactory, baseline drift occurred, and plateaux were unstable; and secondly, the validity of many determinations was in doubt. The solution of the former problem is reported.

The second problem might have been due to the long recognized (Hunter, 1928) non-specificity of the alkaline picrate reaction (Jaffé, 1886). Sugars, ketones, keto-acids, proteins, and ascorbic acid are amongst the substances which may contribute to the reaction (Barclay and Kenney, 1947; Kostir and Rabek, 1950; Hare, 1950; Taussky, 1954) and none of the techniques employed to overcome interference from such chromogens has been conveniently amenable to continuous flow automated analysis, so that the Jaffé reaction has had to be applied directly to a dialysate of the sample. However, interference problems on the 6/60 appeared to differ from those experienced with earlier AutoAnalyzer methods, because urine samples and aqueous solutions of creatinine gave only 80% of the expected result when using plasma as the reference material, or yielded proportionately falsely high plasma results when using an aqueous solution for reference. This finding required explanation and correction when, for economic reasons, it was decided to replace

Fig. 1 Flow diagram for the determination of creatinine on the SMA 6/60 (range 0-13 mg/%)
plasma as the reference material with an aqueous solution of NaCl, Na₂CO₃ and KCl, urea, and creatinine.

Results

Procedure
Extending the sensitivity to an analytical range of 0 to 15 mg/100 ml required increasing the strength of the saline diluent to 1-8%, dialysis through a 24 in. dialyser (previously 3 in.) and developing the Jaffé colour at 37°C instead of at ambient temperature. Figure 1 shows the flow diagram. Baseline drift is negligible over 30 minutes and plateaux are steady; its analytical characteristics are given in Table I. Linearity was determined for aqueous salt solutions and plasma fortified with creatinine up to 15 mg/100 ml at 2 mg/100 ml intervals. The precision of the method was assessed by replicate analyses of three plasma samples both during a single analytical run (maximum reproducibility) and at intervals over a three-day period (normal reproducibility). The recovery results reported are the mean of triplicate analyses in each case. Urine was diluted 1:10 with 0-9% NaCl for analysis.

Table Ia

<table>
<thead>
<tr>
<th>Analytical Precision (CV, %)</th>
<th>Creatinine Concentration (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Maximum reproducibility</td>
<td>0.71</td>
</tr>
<tr>
<td>Normal reproducibility</td>
<td>3.62</td>
</tr>
</tbody>
</table>

Table Ib

| Recovery (%) Blood Plasma Urine (diluted 1:10) |
|----------------------------------------------|--------|---------|
| Plus 2 mg/100 ml                            | 92.5   | 93.8    |
| Plus 4 mg/100 ml                            | 92.6   | 94.7    |
| Plus 8 mg/100 ml                            | 93.1   | 94.1    |
| Plus 12 mg/100 ml                           | 92.9   | 93.4    |

Table Ic

Tables Ia, b, c  Characteristics of the suggested SMA 6/60 creatinine method

Factors Contributing to the Reaction
Using the new SMA method, the apparent creatinine concentration of simple aqueous solutions containing the same concentration of creatinine is shown in Table II. Our intended aqueous reference solution

<table>
<thead>
<tr>
<th>Creatinine (5 mg/100 ml)</th>
<th>Apparent Creatinine Concentration (mg/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.9% NaCl aqueous</td>
<td>5.0</td>
</tr>
<tr>
<td>0.9% NaCl - urea (120 mg/100 ml)</td>
<td>5.1</td>
</tr>
<tr>
<td>0.9% NaCl + Na₂CO₃ (10 mEq/l)</td>
<td>4.7</td>
</tr>
<tr>
<td>Water</td>
<td>4.7</td>
</tr>
<tr>
<td>0.1M HCl aqueous</td>
<td>4.0</td>
</tr>
<tr>
<td>0.1M HCl - urea (120 mg/100 ml)</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table II  Apparent concentration of creatinine in various solutions when the saline solution is used as reference standard

Fig. 2  The effects of dissolved proteins on the apparent creatinine concentration (mg/100 ml) of the aqueous reference solution (actual creatinine concentration 5.0 mg/100 ml).

(Na, K, Cl and HCO₃ at 140, 4.0, 109, and 20 m-equiv/1 respectively, with urea and creatinine at 120 and 5-0 mg/100 ml) had a recorded creatinine concentration of 4.2 mg/100 ml when analysed against blood plasma containing 5-0 mg/100 ml of creatinine. The effects of the addition of several different proteins to the aqueous solution are shown in Figure 2. Neither glucose, uric acid, nor ascorbic acid (up to 400, 15, and 5 mg/100 ml respectively) affected the creatinine result in any way, but acetone had a chromogenicity equivalent to 0.29 mg creatinine per mMol.

Discussion

Creatinine determinations are a natural adjunct to those of plasma electrolytes and urea and with the availability of multiple analysis machines simultaneous determinations of all six parameters has become possible. Plasma creatinine determinations have thus become much more numerous than was usual in the past, making a reassessment of their validity desirable, particularly as interference problems appeared to be greater than had been encountered with earlier AutoAnalyzer systems.
The known non-specificity of the Jaffé reaction was expected to be responsible for the observed differences between analyses of plasma and aqueous solutions. Indeed, it was tempting, because of the 80% chromogenicity of aqueous creatinine compared with plasma standards, to propose that the method presented here measured true creatinine, but that was only a fortuitous result of dialysis as shown by the addition of various proteins. Those effects were presumably due to oncotic phenomena at the membrane because no protein crossed into the dialysate. The small differences between the three different types of protein were probably due to contaminants in the protein source, eg, when the bovine albumin concentration was increased from 2 to 6 g/100 ml in the aqueous reference solution the bicarbonate concentration of the latter decreased by 8 m-equiv/1, which from the results presented in Table II would suggest that the actual effects due to albumin alone were similar to those for freeze-dried plasma.

From the results presented, it is imperative that the full composition of both test and reference solutions should be taken into account when interpreting creatinine results. Unless the effects of individual components are known, the reference solution should be similar in composition to the test. Thus, unless the reference solution has been calibrated in terms of urine and plasma creatinine, for plasma analyses a reference plasma sample should be used and for urine a reference urine or aqueous solution; in all cases the concentration of creatinine in the reference material should be about 5 mg/100 ml. Whether the analyses are on urine or plasma, the linearity of the method and its analytical precision are satisfactory. The difference between the maximum and normal reproducibility figures (Table I) is to be expected, as the latter values included batch-to-batch, day-to-day, reagent, and operator variation, and also electro-mechanical factors within the equipment which from time to time can impose significant limitations on reproducibility. Results of recovery experiments were less satisfactory but may be a reflection of the experimental situation because unfortified quality control plasma samples with high creatinine concentrations give expected results. The method has now been in use for one year.

References
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Madeleine Close-Moll and J. G. Lines

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