Determination of Urinary Creatine

Samuel S. Kurohara*

A method of separation of creatine using ion-exchange chromatography is described. This method is suitable for serial determinations of urinary creatine despite a small contribution by creatinine and guanidino compounds to the color value of the α-naphthol and diacetyl reaction. The creatine coefficients of four patients, determined in duplicates, before and after the induction of creatinuria by X-irradiation are presented to illustrate the applicability of the method described.

The use of the ion-exchange resins for the separation of creatine from substances which interfere with the diacetyl-α-naphthol reaction for the determination of creatine in animal urine has been described by several investigators (1, 2). These procedures were found by us to be cumbersome and not readily reproducible when applied to human urine in serial determinations on a large scale.

A method suitable for serial determination of creatine in human urine will be described below and examples of applications to normal and creatinuric urine will be presented.

Experimental Methods

Reagents and Materials

1. Creatine. Reagent grade creatine (California Foundation for Biochemical Research, Los Angeles, Calif.) was dried to constant weight at 115 to 120°C. One milligram was dissolved in 100 ml. of distilled water for use as a stock solution.

2. Creatine-2-C14. This compound was synthesized in our laboratory by Dr. E. Rauenbusch.

3. Sodium hydroxide solution. An aqueous solution, 1.5N with respect to NaOH and Na2CO3, was prepared.

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4. \(\alpha\)-Naphthol. Immediately before use, a 3% solution of \(\alpha\)-naphthol (Eastman Kodak Co., Rochester, N.Y.) was prepared by dissolving the powder in the alkali solution described in 3.

5. Diacetyl. This reagent as obtained commercially from Eastman Kodak Co. was made up as an aqueous 0.05% solution according to the modification of \(\alpha\)-naphthol-diacetyl reaction of Anderson et al. (3).

6. Amberlite IRC 120. The resin—analytical grade of 30-60 mesh (Rohm and Haas Co., Philadelphia, Pa.)—was treated, before use, with 1N NaOH and washed with distilled water until the water attained a constant pH. After drying in air, 8 gm. of the resin were placed in a glass column, 200 \(\times\) 15 mm., and treated before each determination with 25 ml. of 2N HCl, followed by 250 ml. of distilled water.

7. Solvent system for paper chromatography. The butyl acetate-acetic acid-ethanol-water solvent system (3:2:1:1, \(v/v/v/v\)) described by Masamune and Yosizawa (4) was used.

8. Urine. Two milliliters of urine were diluted with 200 ml. of distilled water prior to the analysis.

9. Chromatographic paper. Whatman No. 3 MM paper, 5 in. wide, was used in the chromatographic purification of urinary creatine.

Procedure

Determination of Urinary Creatine

The diluted urine was passed through a column of Amberlite 120 at a rate of about 20 drops per minute. The creatine remaining on the column was eluted with 40 ml. of 2N NH\(_4\)OH at a rate of 40-60 drops per minute. Other eluents, such as 1N or 2N sodium acetate (3), sodium chloride, potassium chloride, sodium formate, or ammonium formate were less efficient in recovering creatine. The volume of effluent solution was brought to 50 ml. by the addition of 2N NH\(_4\)OH. Although it had been observed that NH\(_4\)OH inhibits to some extent the development of color with the \(\alpha\)-naphthol-diacetyl reagent, this does not represent an obstacle in the present situation for the following reason. Although the inhibition of the color reaction follows an exponential course with respect to increasing concentrations of NH\(_4^+\), the average concentration present in the effluent solution was 1.6 \(\pm\) 0.1N at the termination of the elution and falls on the asymptotic portion of this inhibition curve. To correct for the
inhibition at this concentration, the determination of creatine was therefore always carried out in the presence of 1.6N NH₄OH.

One milliliter each of Reagents 3 and 4 was added to a 7-ml. portion of the eluate, containing 10-60 μg. of creatine, and gently swirled. This was followed by adding 1 ml. of Reagent 5 with mixing. The optical density was measured at 525 m.μ. 7-10 min. after the addition of the last reagent. The color was stable during this interval but diminished in intensity after 10 min.

Paper Chromatographic Procedure

The identification of the compound present in the effluent solution and reacting with the α-naphthol-diacyl reagent was ascertained by paper-chromatography in the solvent system described above and by the procedure described by Eden et al. (5).

Creatinine Determination

Urinary creatinine was determined according to the method of Peters (6).

Calculation

To correct for possible loss of urine during collection, the creatine excretion was expressed in terms of the creatine coefficient defined according to Wilder and Morgulis (7):

\[
\text{Creatine coefficient} = \frac{\text{mg. of creatine} \times 100}{\text{mg. creatine} + \text{mg. creatinine}}
\]

Results

Recovery

The recovery of different amounts of creatine (25-100 μg.) added to the diluted urine was about 100 per cent using the method described above (Table 1). The recovery was checked further by means

<table>
<thead>
<tr>
<th>Creatine added (μg.)</th>
<th>Total creatine calculated (μg.)</th>
<th>Total creatine determined (μg.)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>....</td>
<td>330</td>
<td>....</td>
</tr>
<tr>
<td>25</td>
<td>355</td>
<td>365</td>
<td>103</td>
</tr>
<tr>
<td>25</td>
<td>355</td>
<td>355</td>
<td>100</td>
</tr>
<tr>
<td>50</td>
<td>380</td>
<td>384</td>
<td>101</td>
</tr>
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<td>50</td>
<td>380</td>
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</tr>
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<td>430</td>
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<tr>
<td>100</td>
<td>430</td>
<td>440</td>
<td>102</td>
</tr>
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</table>
of the isotope dilution technic. When creatine-2-C\textsuperscript{14} was added to the diluted urine, about 97 per cent of the added C\textsuperscript{14} could be recovered in the eluate. Creatine was brought to a constant C\textsuperscript{14} activity, as ascertained by paper chromatography according to the method described by Eden et al. (5).

Accuracy and Sensitivity

The mean value for the creatine coefficient based on studies of 11 women is 6.8 ± 0.6 (standard error), the range of values being 3.3–11.0. This is less than the composite range reported by Wilder and Morgulis (7). If determinations are done in duplicate, the concentration of creatine can be estimated with an accuracy of 4–5 per cent for concentrations of creatine of about 100 µg./ml. At concentration of 30 µg./ml., the accuracy is about 8–10 per cent.

Table 2 shows the urinary creatine coefficients of patients before and after the induction of creatinuria by X-irradiation. Values for duplicate determination are shown and indicate that the results of parallel determinations are in better agreement when creatinuria is present.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Before induction of creatinuria</th>
<th>After induction of creatinuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.0, 4.3</td>
<td>7.6, 8.0</td>
</tr>
<tr>
<td>2</td>
<td>7.6, 8.1</td>
<td>49.5, 50.0</td>
</tr>
<tr>
<td>3</td>
<td>5.6, 6.1</td>
<td>27.0, 27.8</td>
</tr>
<tr>
<td>4</td>
<td>9.8, 11.0</td>
<td>26.0, 26.0</td>
</tr>
</tbody>
</table>

*All had malignancies and were given partial-body X-irradiation for therapeutic reasons. Values of duplicate determinations are shown.

Discussion

Numerous modifications of the reaction between \(\alpha\)-naphthol, diacetyl, and creatine or similar compounds have been devised in an effort to develop an adequate clinical test for creatine in biologic fluids. Most of these tests suffer from two major defects: the slowness of conversion of creatinine to creatine at alkaline pH (8), and nonspecificity of the color reaction. The latter defect is caused by substances in the reaction mixture which act in the following manner: (1) They may react with \(\alpha\)-naphthol and diacetyl to produce a chromophoric compound whose maximal light absorption is located
at the same wave length as that of the creatine chromophore. The absorption coefficient of such compounds is, however, lower than that of creatine. (2) They may inhibit color production by competing with creatine for diacetyl. Guanidino compounds (9, 10), such as glycoamyamine, are examples of the first type of interfering substances whereas NH₄⁺ serves as an example of the second.

The use of sodium carbonate as a component of the alkali solution (Reagent 3) reduces the rate of conversion of creatinine to creatine during the development of color with the chromogenic reagent. The present method is applicable in situations where the induction of creatinuria does not invoke a concomitant increase in the urinary excretion of glycoamyamine—as in postirradiation creatinuria (11).

References

8. Walpole, G. S., J. Physiol. 42, 301 (1911).