New Method for Measurement of Guanidinosuccinic Acid in Serum and Urine

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We describe a new method for measurement of guanidinosuccinate in serum and urine. To separate it from interfering guanidine compounds, it is adsorbed on and eluted from an anion-exchange column. The modified method requires less serum and is considerably faster than previous ones.

Additional Keyphrases: hemodialysis • anion-exchange chromatography • Sakaguchi reaction • uremia

In 1963 Bonas et al. (1) isolated a guanidine derivative from the urine of uremic patients. Subsequent investigations proved that it was guanidine mono-substituted with succinic acid, guanidinosuccinate (GS) (2). Later it was demonstrated that both the sera of uremic patients and the urine of healthy humans contain this compound (3). Several communications attributed a role to GS in the development of uremic toxemia (3-6).

Because no method was quick and simple enough for routine laboratory use, we have developed a new method that can be used to determine serum and urine GS concentrations more quickly and sensitively.

Materials and Methods

GS was determined in sera of patients on dialysis therapy in our institution's Artificial Kidney Unit.

The patients were being dialyzed for 7–9 h with a Kiil dialysis unit, twice or three times per week.

Procedure

GS concentration was determined by the Sakaguchi color reaction after the serum was fractionated on a 10 × 0.8 cm column of anion-exchange resin (Dowex 1 × 8, 200–400 mesh, No. 41111; SERVA Feinbiochemica). The resin was washed with 100 ml of NaOH solution (100 g/liter), converted to acetate form by successive washing with 100-ml portions of dilute acetic acid (100 ml/liter) and sodium acetate (3 mol/liter), and stored in dilute (1 mol/liter) acetic acid. Before use, the resin was washed with 50 ml of 1 molar acetic acid and equilibrated with 50 ml of 0.1 molar acetic acid. Four milliliters of serum was adjusted to pH 6.0 with less than 0.1 ml of glacial acetic acid, and then pumped through the column with a peristaltic pump. Elution was started with 25 ml of 0.1 molar acetic acid, removing in this way all the Sakaguchi-positive materials except GS, which was eluted by subsequent washing with 25 ml of acetic acid (0.5 mol/liter). The flow rate of the sample and of the eluting solvents was adjusted to 2 ml/min.

Fractions of 2 ml, collected with a fraction collector, were assayed at 4 °C as follows: 2 ml of sample, 0.5 ml of sodium hydroxide solution (100 g/liter), 0.5 ml of a 1:1 mixture of thymine and α-naphthol solutions (2 g of thymine in 100 ml of 10 g/100 ml NaOH and 2 g of α-naphthol in 100 ml of ethanol), and 2 ml of sodium hypochlorite (2.6 g/liter). After 1 min, 0.5 ml of sodium thiosulfate (20 g/liter) was added. The resulting pink color was measured at 515 nm and the results were calculated from a standard curve prepared with a GS preparation from Sigma Chemical Co., St. Louis, Mo. 63178.

Results

The recovery of known amounts of GS was measured by our method and that of Stein et al. (3). In both cases recovery ranged from 93–102%. For 10 measurements by each method, the coefficients of variation were 3.35% and 2.62% on the 25 × 1 cm column (3) and ours, respectively. Two representative elution curves are shown in Figure 1. In the first case (curve A) 10 ml of a solution of GSA (30 mg/liter) was applied to the 25 × 1 cm column. The maximal absorbance was 0.480. In the second case (curve B) 4 ml of this same solution was introduced into the short column and the maximal absorbance was 0.510. The short-column method enabled us to obtain a relatively high peak for sera having lower GS concentrations, which are the more common in routine clinical investigations.

We did parallel determinations of GS in the sera of 10 uremic patients by both methods. The results agreed well (Table 1) and supported our previous findings that the serum nonprotein nitrogen/GS ratio is 150 or more in acute uremia, where the uremic state persists no longer than a week. On the other hand, the sera of chronically uremic patients gave ratios of 150 or less (7).
Table 1. Parallel Determinations of Guanidinosuccinate in the Sera of 10 Uremic Patients, by the Method of Stein et al. (3) and Our Method

<table>
<thead>
<tr>
<th>Serum GS, mg/liter Method</th>
<th>Present</th>
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<tbody>
<tr>
<td>Ref s</td>
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<tr>
<td>18.5</td>
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<tr>
<td>8.0</td>
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<tr>
<td>9.0</td>
<td>9.5</td>
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<tr>
<td>10.0</td>
<td>10.0</td>
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<tr>
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<td>8.0</td>
<td>7.0</td>
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<td>16.0</td>
<td>17.0</td>
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<tr>
<td>11.0</td>
<td>10.0</td>
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$S_a = \pm 2.06\%$

$0.25 > P > 0.20$

Discussion

Horowitz (4) established that among other causes of hemostatic disorders in chronic renal failure, there is a toxin that competitively inhibits the internal platelet transformation induced by ADP. With normal platelet-rich plasma he demonstrated that GS could inhibit the aggregation of platelets and that this effect could be prevented by higher ADP concentration.

Lonergan et al. (5) suggested another possible effect of GS that may play a role in development of uremic neuropathy. They showed that GS significantly inhibited normal erythrocytic transketolase activity, while urea, methylguanidine, guanidinoacetic acid, sodium, or potassium did not influence it. Transketolase, a member of the pentose-phosphate cycle, plays an important role in maintenance of the myelin sheath.

Unfortunately, the exact route for GS biosynthesis has not been clarified, despite intensive investigations by Cohen's group (1, 3) and Takahara et al. (8, 9). Earlier reports showed that GS is dialyzable, so that the determination of its serum concentration in patients undergoing dialysis can indicate the effectiveness of treatment. Moreover, the urinary guanidinoacetic acid/GS ratio of patients suffering from chronic renal failure correlates well with the severity of the illness (9). On the basis of these data, clinicians dealing with chronic renal failure have directed more attention to this newly isolated metabolite in the past few years. However, present methods for detecting this compound in biological fluids are generally too cumbersome for routine use and require an inconveniently large volume of serum. In the method of Stein et al. (3), for example, with a 25 x 1 cm column of resin 10 ml of serum is required, which is eluted by successively washing the column with 100 ml portions of 0.1, 0.25, and 0.5 molar acetic acid. With a 2 ml/min flow rate, the procedure takes nearly 3 h. Recently Kamoun et al. (10) proposed a semiautomated method that decreased the time to 50 min, but still required 10 ml serum.

Our new method equals the previous ones in recovery and accuracy, but by using a column of 10 x 0.8 cm and changing the elution technique we can detect GS in 4 ml of serum, and the procedure takes only 30 min.

References