MICROBIOLOGICAL DETERMINATION OF CYSTINE, CYSTEINE, AND GLUTATHIONE IN PLASMA*

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In previous reports from this laboratory, a method for the assay of D- and L-methionine in biologic fluids with Leuconostoc mesenteroides P-60 and Lactobacillus fermenti 36 has been described (1, 2). The present communication describes a method for the determination of cysteine plus cystine in plasma.

In a report by other workers, using the medium of Dunn et al. (3), the organisms Lactobacillus brevis (8257), Lactobacillus pentoaceticus (367), Lactobacillus buchneri, Lactobacillus lycopersici (4005), Leuconostoc mesenteroides P-60 (8042), and Leuconostoc dextranicum (8359) have been described as requiring cysteine or cystine for adequate growth. These organisms have been methodically evaluated by us in a variety of additional media, including fortified peroxide-treated gelatin or casein hydrolysate (1, 2), the medium of Henderson and Snell (4), and that of Steele et al. (5). Only L. lycopersici and L. buchneri have been found to be satisfactory for quantitative determination of these amino acids under the conditions to be described.

Methods

Assays were carried out in 13 x 100 mm. culture tubes. Each tube received 1 ml. of a basal medium with supplements to a total volume of 2 ml. The standard curve of reference was constructed by the use of a cystine solution containing 5 γ per ml. for L. lycopersici or 4 γ per ml.

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1 The numbers in parentheses refer to those of the American Type Culture Collection.
for *L. buchneri* and by adding 0.0, 0.2, 0.4, 0.6, 0.8, or 1.0 ml. of the standard solution to a series of tubes (Fig. 1). The assay of normal plasma was performed in a similar manner. The protein-free filtrate, prepared as previously described (1), results in a 1:2 dilution of the original plasma. This was further diluted with water to result in a final dilution of plasma of 1:5. 0.5 ml. of this solution was added to each assay tube when *L. lycopersici* was used, or 0.4 ml. when *L. buchneri* was the test organism. Water was then added to all tubes to make a final volume of 2 ml. Samples of abnormal blood which were suspected of containing excessive amounts of cystine were diluted accordingly. All tubes were then covered with a

![Standard reference curves for cystine (medium of Steele et al.)](http://www.jbc.org/)

Fig. 1. Standard reference curves for cystine (medium of Steele et al.)

towel and were sterilized by autoclaving at 10 pounds for 5 minutes (media containing glucose) or steaming for 5 minutes (media containing arabinose). After cooling, the tubes were prepared for inoculation as described below.

The stock cultures of the test organisms were maintained on tomato juice agar stabs (Difco) and a 24 hour broth culture for use in the assay was subcultured in an enriched peptone medium. This culture was centrifuged, washed, and resuspended in sterile saline. 1 drop from a sterile pipette was then used to inoculate each assay tube. Incubation at 37° for 72 hours was followed by electrometric titration (of the lactic acid produced) with 0.02 N NaOH. The cystine concentration was estimated by the use of a reference curve in the customary manner. Incubation at temperatures below 37° resulted in decreased acid production; at 32° there was no growth. Good growth was obtained at temperatures as high as 40°.
Results

As stated above, a number of media were evaluated for use in the assay of cystine. Glucose was used as the carbohydrate source for *L. lycopersici* but arabinose was necessary for *L. buchneri* (6). The pentose sugar also enhances acid production by the former organism but results were erratic when compared to those obtained with glucose. The medium of Henderson and Snell (4) supported excellent growth of both organisms if 2.4 gm. of sodium acetate were substituted for the citrate buffer and if the original Salts A and B of Snell and Wright (7) were substituted for the Salts C and KH₂PO₄. Excellent results were also obtained with the medium for *Leuconostoc* recently published by Steele et al. (5), the only modification being the use of arabinose instead of glucose when *L. buchneri* was the test organism. When the amino acids of the modified medium of Henderson and Snell were replaced with a supplemented acid hydrolysate of casein or with hydrogen peroxide-treated gelatin, good growth of both organisms resulted.²

Both organisms responded identically to cysteine or cystine in equivalent quantities. The basal media contained considerable excesses of methionine (up to 400 γ per tube). The addition of as much as 200 γ of methionine to various concentrations of added plasma filtrates did not produce a deviation of more than ±1.25 per cent in the predicted cystine values. This is within the experimental error of the procedure.

Rabinowitz and Snell (8) and Riesen, Spengler, Robblee, Hankes, and Elvehjem (9) reported that the autoclaving of media containing cystine-cysteine at 15 pounds pressure for 15 minutes resulted in destruction of these amino acids. This has been confirmed in this laboratory.

To determine whether autoclaving at 10 pounds pressure for 5 minutes resulted in any destruction of cystine-cysteine, the following procedures were carried out. A standard reference curve was obtained by the method described above. A second curve was obtained by adding equal amounts of cystine, which had been passed through a Seitz filter, to previously sterilized cystine-free medium. The growth curves were identical.

Because of the presence of glutathione in biological fluids including blood, it was of interest to investigate the response of the test organisms to this cysteine-containing tripeptide.

When glutathione was added to cystine-free medium, and heat steriliza-

² In the preparation of the casein and gelatin hydrolysate media, the following, in amounts per 100 ml. of basal medium, may be used to replace the amino acid mixture of the modified medium of Henderson and Snell: (1) 20 ml. of 5 per cent casein or 20 ml. of 5 per cent peroxide-treated gelatin hydrolysates; (2) 10 mg. of L-tryptophan; (3) 40 mg. of DL-methionine; (4) 10 mg. of L-tyrosine; (5) 40 mg. of L-glutamic acid.
tion was performed as noted above, growth equivalent to the cysteine content of the glutathione occurred.

Repetition of the procedure with glutathione which had been passed through a Seitz filter, resulted in growth which at all times was equivalent to less than 20 percent of the cysteine content of the added glutathione. These findings suggest that (a) autoclaving under the conditions noted results in hydrolysis of glutathione and (b) the organisms are able only feebly to utilize previously unhydrolyzed glutathione. Consequently the combination of the two procedures provides a method for determination of free cystine-cysteine and of glutathione.

The addition of 10 \( \gamma \) each of homocystine, cystathionine, and homolangithionine to cystine- and cysteine-free media, followed by heat sterilization, resulted in no growth.

Cystine-cysteine-glutathione were determined on specimens of blood obtained from different individuals with both test organisms and the various media already described. The values obtained with each organism

<table>
<thead>
<tr>
<th>Patient</th>
<th>Diagnosis</th>
<th>Test organisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>ROM</td>
<td>Acute hepatitis, severe</td>
<td>L. lycopersici</td>
</tr>
<tr>
<td>SHA</td>
<td>Diabetes mellitus</td>
<td>2.7</td>
</tr>
<tr>
<td>ARM, Aug. 2 &quot; &quot; 3</td>
<td>Receiving methionine</td>
<td>1.8</td>
</tr>
<tr>
<td>&quot; &quot; 9</td>
<td>Post methionine</td>
<td>1.6</td>
</tr>
<tr>
<td>BEL, &quot; &quot; 2 &quot; &quot; 3</td>
<td>Cirrhosis</td>
<td>2.3</td>
</tr>
<tr>
<td>&quot; &quot; 9</td>
<td>Receiving methionine</td>
<td>2.1</td>
</tr>
<tr>
<td>TUC, &quot; &quot; 2 &quot; &quot; 3</td>
<td>Post methionine</td>
<td>3.0</td>
</tr>
<tr>
<td>&quot; &quot; 9</td>
<td>Receiving methionine</td>
<td>1.95</td>
</tr>
<tr>
<td>GLE &quot; Normal</td>
<td>1.55</td>
<td>1.55</td>
</tr>
<tr>
<td>LOP, Aug. 2 &quot; &quot; 3</td>
<td>Receiving methionine</td>
<td>1.9</td>
</tr>
<tr>
<td>BRA &quot; &quot; 3</td>
<td>Normal</td>
<td>1.9</td>
</tr>
<tr>
<td>HOO &quot; Cirrhosis</td>
<td>2.7</td>
<td>2.4</td>
</tr>
<tr>
<td>LAR &quot; &quot;</td>
<td>Cholangitis, receiving methionine</td>
<td>2.3</td>
</tr>
<tr>
<td>WHI &quot; Hepatitis with probable cirrhosis</td>
<td>1.8</td>
<td>1.95</td>
</tr>
<tr>
<td>LAN &quot; Acute hepatitis</td>
<td>2.2</td>
<td>2.25</td>
</tr>
</tbody>
</table>
on a given blood sample did not differ significantly (Table I). Recovery experiments on human blood plasma were also carried out. Mean recovery values for eighteen separate determinations with *L. lycopersici* were 101 ± 1.4 per cent (extreme values 89 and 109 per cent). The corresponding figures for fourteen separate determinations with *L. buchneri* were 97 ± 0.97 per cent (extreme values 90 and 103 per cent).

**SUMMARY**

A microbiological method is described for the determination of free cysteine plus cystine and of glutathione-cysteine in human plasma by use of the organisms *L. lycopersici* and *L. buchneri*. Reproducible recoveries were obtained under the various experimental conditions noted. The concentration of total cysteine plus cystine in normal fasting human plasma ranged from 1.55 to 2.0 mg. per 100 ml.

**BIBLIOGRAPHY**


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3 Standard error of the mean = \(\sqrt{(\bar{x} - d)^2/(n(n - 1))}\).
MICROBIOLOGICAL DETERMINATION OF CYSTINE, CYSTEINE, AND GLUTATHIONE IN PLASMA
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