THE SULFUR BALANCE OF RATS FED EXCESS D L-METHIONINE PLUS GLYCINE OR D L-ALANINE*

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It has been demonstrated that when mature, male, Sherman strain rats are fed 4.8 per cent D L-methionine while on a 12 per cent casein diet the animals soon attain nitrogen equilibrium or a small positive nitrogen balance (1, 2). The rats lose weight, however, even in positive balance, this loss resulting from a decrease in the fat stores of the body. At the end of 20 days the fat deposits are markedly reduced. A hypertrophy of the kidney of approximately 30 per cent over control values is also noted.

The addition of 4.8 per cent glycine to this diet partly counteracts the loss in weight and kidney hypertrophy. It has been suggested (2) that the glycine may serve as an additional source of the serine (3, 4) needed for methionine catabolism. If this is so, the effect of the glycine should be fairly specific. To test this point extra D L-alanine was fed, and to study further the relationships between the metabolism of fat and methionine and the effects of added glycine, sulfur balances and urinary distribution of sulfur were determined in four groups of rats.

EXPERIMENTAL

Four groups of male, Sherman strain rats, weighing approximately 250 gm. each, were utilized. There were ten rats in each group. The basal diet contained, in addition to the ingredients previously described (2), 1 gm. of choline chloride per 1000 gm. of dry material.

Group I received the basic diet alone, while the other three groups received, in addition to the basic diet, the following ingredients per 1000 gm. of dry material: Group II, 48 gm. (4.8 per cent) of D L-methionine; Group III, 48 gm. (4.8 per cent) of D L-methionine plus 48 gm. (4.8 per cent) of glycine; Group IV, 48 gm. (4.8 per cent) of D L-methionine plus 50 gm. (5.0 per cent) of D L-alanine.

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All of the animals were fed the same weight of diet as was consumed by Group II. Since the animals in this group restrict their intake, the caloric and protein intakes of the other groups are similarly limited. The rats were housed in metabolism cages, two in a cage, and urine and fecal collections made daily for the last 16 days of a 20 day experimental period. The urine samples were preserved with dilute hydrochloric acid and toluene until analyzed. The fecal samples were homogenized with water and an aliquot taken for nitrogen analysis. The remainder was preserved with concentrated nitric acid for sulfur determinations.

Dietary sulfur was determined by analysis of the diets and expressed in terms of gm. of sulfur per gm. of diet. This, multiplied by the food intake per day, gave the daily sulfur ingestion. The sulfur contents of the diets, determined by analysis, were found to agree with those values calculated from the sulfur contents of the protein, methionine, and salts.

The urine and fecal samples from each cage were divided into four consecutive 4 day periods, and analysis of each sample performed in duplicate. Pseudocholinesterase was determined by the method of Ammon (5), as modified by Mendel and Mundell (6). Liver fat was determined by the method of Hsiao (7) on the dried livers.

Results

The nitrogen balances for the four groups and the weight losses over the 20 day period are listed in Table I.

All of the groups lost considerable weight in this experiment, but Group III lost 30 per cent less weight than the others. That the effect of the glycine is not due to the extra nitrogen it affords becomes apparent from a consideration of the data in Table I. The rats in Group IV, fed excess methionine plus alanine, with approximately the
same nitrogen intake as Group III (methionine plus glycine), still lost as much weight as the group fed excess methionine alone (Group II).

The ingestion of 200 mg. per kilo of extra nitrogen in the form of DL-alanine had no significant effect on the nitrogen balance. The ingestion of approximately the same amount of nitrogen as glycine, however, reduced the weight loss and favored a more positive nitrogen balance.

Further confirmation that weight loss takes place largely in fat stores is given by comparison of Group I (casein alone) with Groups II and IV (excess methionine and excess methionine plus alanine respectively). The latter two have less negative nitrogen balances than Group I, but lost more weight, the extra weight representing fat.

Values for plasma pseudocholinesterase, the liver and kidney weights, and liver fat for the four groups are recorded in Table II.

### Table II

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Supplement</th>
<th>Plasma Pseudocholinesterase</th>
<th>Fresh liver weight</th>
<th>Fresh kidney weight</th>
<th>Fat in dried livers</th>
<th>Liver fat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methionine</td>
<td>Glycine</td>
<td>Alanine</td>
<td>per cent</td>
<td>CO₂ per hr. per ml. plasma</td>
<td>gm. per 100 gm. body weight</td>
</tr>
<tr>
<td>I</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>67.1</td>
<td>2.70</td>
<td>0.578</td>
</tr>
<tr>
<td>II</td>
<td>4.8</td>
<td>0.0</td>
<td>42.6</td>
<td>35.6</td>
<td>3.38</td>
<td>0.909</td>
</tr>
<tr>
<td>III</td>
<td>4.8</td>
<td>5.0</td>
<td>64.8</td>
<td>42.6</td>
<td>3.10</td>
<td>0.746</td>
</tr>
<tr>
<td>IV</td>
<td>4.8</td>
<td>0.0</td>
<td>5.0</td>
<td>64.8</td>
<td>3.22</td>
<td>0.869</td>
</tr>
</tbody>
</table>

The effect of glycine in reducing kidney hypertrophy caused by excess methionine is illustrated again in Table II. Alanine reduced kidney hypertrophy only slightly.

There is no correlation of values for pseudocholinesterase with those for liver fat or liver and kidney weights. The functions of this enzyme, therefore, remain obscure.

### Sulfur Balance

Fecal and urinary sulfur was determined essentially as described by Eckert (8), with methods by which 99 per cent of added methionine sulfur is recovered. The sulfur balances for the four groups are recorded in Table III.

Consideration of the data in Table III shows excellent absorption of methionine, even at the high levels fed. Groups II and IV have very similar sulfur balances, which again point to the lack of action of alanine.
The addition of the extra glycine, however, increases the total urinary sulfur in comparison to Groups II and IV.

Interestingly enough, Groups II and IV, while both in positive sulfur balance, are in slight negative nitrogen balance. The converse is true of Group III (methionine and glycine) in which the sulfur balance is near equilibrium and yet the nitrogen balance is increased to a positive value.

In order to determine the distribution of the urinary sulfur, sulfur partition was performed on the urine samples. The results are presented also in Table III.

The excretion of inorganic and organic sulfur in Group III (methionine plus glycine) is increased over that of Group II (methionine), even though the latter group ingested more sulfur. The addition of extra alanine to

**TABLE III**

*Effect of Dietary Supplements of DL-Methionine, Glycine, and DL-Alanine on Sulfur Balance and Urinary Sulfur Partition of Rats during 20 Days*

Average of twenty values obtained on ten rats in each group.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Supplement</th>
<th>Ingested S</th>
<th>Urinary S</th>
<th>Fecal S</th>
<th>Sulfur balance</th>
<th>Organic S*</th>
<th>Total inorganic S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Methionine</td>
<td>Glycine</td>
<td>Alanine</td>
<td>mg. per kg.</td>
<td>mg. per kg.</td>
<td>mg. per kg.</td>
<td>mg. per kg.</td>
</tr>
<tr>
<td>I</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>36.5</td>
<td>33.7</td>
<td>22.5</td>
<td>-20</td>
</tr>
<tr>
<td>II</td>
<td>4.8</td>
<td>0.0</td>
<td>0.0</td>
<td>313.0</td>
<td>246.0</td>
<td>29.1</td>
<td>+38</td>
</tr>
<tr>
<td>III</td>
<td>4.8</td>
<td>4.8</td>
<td>0.0</td>
<td>302.0</td>
<td>270.0</td>
<td>31.5</td>
<td>+0.5</td>
</tr>
<tr>
<td>IV</td>
<td>4.8</td>
<td>0.0</td>
<td>5.0</td>
<td>294.6</td>
<td>235.0</td>
<td>29.6</td>
<td>+30</td>
</tr>
</tbody>
</table>

*Ethreal sulfur was negligible in all four groups.

excess methionine actually depresses somewhat the sulfur excretion, although this cannot be considered significant, since the sulfur intake of Group IV was slightly less than that of Group II.

**DISCUSSION**

When excess DL-methionine (up to 7 per cent of the diet) is fed to Sherman strain rats, practically all of the methionine is absorbed from the gut. The methionine absorbed must either be metabolized, stored, excreted in the urine, or used in the synthesis of protein. The last possibility appears quite unlikely, since it has been demonstrated (9) that the essential amino acids are not utilized for protein synthesis unless all of them are presented to the tissues simultaneously and in proper quantities.

Thus far, the storage of single amino acids has not been demonstrated, although in this work rats fed excess methionine (Group II) or excess methionine plus alanine (Group IV) are in positive sulfur balance and
therefore must be storing sulfur, in some form, to the extent of the respective positive balances. The amount of sulfur stored represents approximately 10 per cent of the ingested sulfur in these two groups. The remaining 90 per cent, and, in the case of Group III (methionine plus glycine) nearly 100 per cent, must be metabolized or eliminated unchanged.

A consideration of the urinary sulfur partition of the control group fed casein alone (Group I) shows that approximately two-thirds of the eliminated sulfur is oxidized to sulfate, while the other one-third is excreted in organic form. This same proportion is maintained in Group II (methionine) and Group IV (methionine plus alanine), indicating that no single mechanism for sulfur metabolism or elimination is favored, but that all mechanisms for these processes are increased concurrently and by the same amounts. In Group III (methionine plus glycine) approximately 10 per cent more sulfur is excreted, most of the increase being in the organic sulfur fraction. It would seem, therefore, that the glycine facilitates, somewhat, the excretion of the extra sulfur of added methionine, particularly in the organic form.

It has been suggested previously (2) that the beneficial effects of glycine may be due to its direct participation in the catabolism of methionine, since it has been shown (3) that glycine may be the precursor of serine in vivo and it has been demonstrated (10-12) that one pathway for the metabolism of methionine is by combination of homocysteine and serine to form cystathionine, which then is cleaved to cysteine and a 4-carbon fragment. When methionine metabolism is channeled, to a greater extent, in this way through organic derivatives, with their subsequent excretion in the urine, this may cause less of a drain on the fat reserves than does complete oxidation to sulfate.

It should be emphasized that methionine fed at high levels does not necessarily exert a marked lipotropic action on liver fat. Best and Ridout (13) have reported that feeding levels greater than 0.5 per cent methionine did not produce a correspondingly greater decrease in liver fat, and they still found a considerable amount in the liver (10 to 17 per cent). This has been confirmed by Channon et al. (14).

The liver fat of the animals in Group IV (methionine plus alanine) was higher than in the other groups; three of the animals had fatty livers with fat content up to 38 per cent on a dry weight basis. It is possible that excess alanine favors the oxidation of methyl groups made available by demethylation of methionine in the liver. The net effect would be similar to that obtained by feeding extra cystine or cysteine, which have been shown to cause a large increase in liver fat of rats fed on a low casein-high fat diet (15, 16). Homocystine also was reported to act in the same manner as cystine on liver fat (17).
At the present time a point has been reached where a more critical eval-
uation of some of these factors can be made.

SUMMARY

1. Nitrogen and sulfur balances have been determined on four groups
   of Sherman strain rats fed 4.8 per cent dL-methionine, 4.8 per cent dL-
   methionine plus 4.8 per cent glycine, and 4.8 per cent dL-methionine plus
   5 per cent dL-alanine in addition to a 12 per cent casein diet. Liver fat
   and plasma pseudocholinesterase values have been reported for the four
   groups.
2. On a control 12 per cent casein diet, two-thirds of the urinary sulfur
   excretion is as sulfate and one-third as organic sulfur. This proportion is
   maintained in the groups fed excess methionine or excess methionine plus
   alanine.
3. Rats fed excess methionine plus glycine excrete 10 per cent more sul-
   fur than those fed the same quantity of excess methionine alone.
4. A storage of 10 per cent of the ingested sulfur is indicated in the
   groups fed excess methionine or excess methionine plus alanine.
5. High liver fat is observed in rats fed excess methionine plus alanine.
6. An explanation for these results is advanced.

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