Dietary Methionine and the Excretion of Formiminoglutamic Acid by the Rat

MILTON SILVERMAN* AND ANNA J. PITNEY

From the Arctic Health Research Center, Anchorage, Alaska, and the National Institutes of Health, United States Public Health Service, Bethesda, Maryland

(Received for publication, July 17, 1958)

Formiminoglutamic acid is a product derived from the metabolism of histidine by mammalian liver and several microorganisms (1-3). The metabolism of FGA1 is dependent upon the presence of tetrahydrofolic acid (4, 5), and consequently this formiminog compound is excreted in the urine of human subjects or laboratory rats which have a deficiency of folic acid (6-8). Daft (6) has reported that suitable amino acid mixtures will partially replace the need for folic acid in the growth of the laboratory rat. Therefore, a study was undertaken to determine the influence of amino acids on the excretion of FGA in the urine of the rat. It was found that dietary methionine significantly reduces the excretion of FGA. We shall describe these observations and the implications derived therefrom.

EXPERIMENTAL

Weanling male rats of the Sprague-Dawley strain, weighing between 38 and 58 gm., were used. The observations recorded with diets that contained no added folic acid were made during a period of 8 to 16 weeks after the rats had been consuming this diet and the supplements indicated. The observations concerned with the influence of vitamin B12 were made on rats which were fed for 4 to 6 weeks after weaning the diet that lacked added vitamin B12. All diets were fed ad libitum and rats were kept in individual cages.

The method for the determination of FGA has been described recently. However, 20 µmoles each of citric acid and MgSO4 were used instead of the 200 µmoles reported (9). The latter value was published in error.

The ingredients of the "complete" diet, expressed on a per kg. basis, included the following: casein ("Vitamin Free," Nutritional Biochemicals Corporation), 90 gm.; sucrose, 670 gm.; hydrogenated vegetable oil ("MFB," Wesson Oil and Snowdrift Sales Company), 200 gm.; salts (Jones and Foster (10)), 40 gm.; and vitamins. The vitamins, in amounts added per kg. of diet, were as follows: thiamine, 30 mg.; riboflavin, 30 mg.; pyridoxine, 30 mg.; calcium pantothenate, 40 mg.; choline chloride, 800 mg.; vitamin A, 52,000 i.u.; vitamin D, 10,400 i.u.; α-tocopherol, 200 mg.; niacin, 40 mg.; biotin, 3 mg.; ε-inositol, 800 mg.; 2-methyl-1-naphthaquinone, 60 mg.; vitamin B12, 12 µg.; and folic acid, 20 mg. For the preparation of diets lacking added folic acid or vitamin B12, the vitamins concerned were omitted.

RESULTS

The incorporation of methionine into the diet of rats which were fed a ration that had a low protein (9 per cent casein) content and contained no added folic acid has a profound effect on the excretion of FGA (Table I). In the presence of 2 per cent of the added amino acid, the excretion of the formimino compound is almost completely eliminated. The effect is significant at a concentration of 0.5 per cent and is detectable at the 0.1 per cent level.

Both the D- and L-isomers of methionine are active (Table II). They appear to be equally effective. These results are consistent with those of Wretlind (11) and Gibson and Smyth (12) who have reported (a) that the d-isomer of methionine is almost as effective as the L-isomer in supporting the growth of the rat (11), and (b) that an active racemase is present in rat kidney which converts the D- to the L-isomer (12).

A number of other amino acids2 were examined for their influence on FGA excretion in rats maintained on the low folic acid diets. Except for homocysteine and homocystine, results with which were irregular, none of the other amino acids tested significantly reduced the excretion of FGA. Homocysteine seemed to be more effective than homocystine in reducing the excretion of the formimino compound, but in this respect its action was not as consistent as that of methionine. The results obtained with cystine and cysteine were erratic. In some trials, slight decreases in FGA excretion occurred. In others, no influence of these amino acids could be detected.

The influence of vitamin B12 on the excretion of FGA by the rat was examined because of (a) the apparent specificity of methionine; (b) the fact that under suitable conditions vitamin B12 spares the requirement for methionine in the growth of the chick, rat, and some microorganisms (13-16); and (c) the known requirement for vitamin B12 in the synthesis of methionine (17, 18). The results (Table III) indicate that deprivation of vitamin B12 induces FGA excretion. The amounts of formiminog compound excreted after 4 weeks by rats deprived of dietary vitamin B12 are comparable to those excreted by animals fed diets with a low folic acid content. On the addition of vitamin B12 to the diet, FGA excretion was almost eliminated after 1 week (Table IV).

1 The abbreviation used is: FGA, formiminoglutamic acid.

2 Histidine, tryptophan, threonine, cysteine, cystine, serine, valine, phenylalanine, homocysteine, homocystine, arginine, leucine, proline, lysine, hydroxyproline, alanine, norvaline, tyrosine, glycine.
Methionine and Formiminoglutamic Acid

Vol. 233, No. 5

Vitamin B_{12} results in a diminished excretion of FGA (Table VI). The effect of homocysteine is quite marked and its magnitude approaches that of methionine. The addition of cysteine to the same diet did not consistently result in decreases. If nicotinic acid is omitted from the "complete" diet, some growth retardation results, but FGA excretion does not occur. 15 rats were divided into three groups and fed diets that (a) were

**TABLE I**

Influence of added methionine on excretion of FGA by rats fed diet that lacked added folic acid*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Added DL-methionine</th>
<th>Rat No.</th>
<th>Added DL-methionine</th>
<th>Rat No.</th>
<th>Added DL-methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% 2%</td>
<td>0% 1%</td>
<td>0% 0.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>4.5 0.9 16 17.7 7.8 21</td>
<td>4.4 2.4 21</td>
<td>4.5 0.9 16 17.7 7.8 21</td>
<td>4.4 2.4 21</td>
<td>4.5 0.9 16 17.7 7.8 21</td>
</tr>
<tr>
<td>22</td>
<td>10.0 0.9 17 12.6 3.0 22</td>
<td>7.4 2.7 22</td>
<td>10.0 0.9 17 12.6 3.0 22</td>
<td>7.4 2.7 22</td>
<td>10.0 0.9 17 12.6 3.0 22</td>
</tr>
<tr>
<td>23</td>
<td>2.0 0.0 18 10.4 2.4 23</td>
<td>2.7 1.5 23</td>
<td>2.0 0.0 18 10.4 2.4 23</td>
<td>2.7 1.5 23</td>
<td>2.0 0.0 18 10.4 2.4 23</td>
</tr>
<tr>
<td>24</td>
<td>9.2 0.9 19 5.1 1.5 24</td>
<td>10.5 6.0 24</td>
<td>9.2 0.9 19 5.1 1.5 24</td>
<td>10.5 6.0 24</td>
<td>9.2 0.9 19 5.1 1.5 24</td>
</tr>
<tr>
<td>25</td>
<td>6.0 0.9 20 4.5 2.1 25</td>
<td>6.9 3.6 25</td>
<td>6.0 0.9 20 4.5 2.1 25</td>
<td>6.9 3.6 25</td>
<td>6.0 0.9 20 4.5 2.1 25</td>
</tr>
</tbody>
</table>

* Values are μmoles of FGA in urine excreted per day.

The control urine (no dietary methionine added) was obtained 1 day before the experimental collection. 24-hr. collection periods were used.

Food consumption ranged from 7 to 10 gm. per rat per day.

**TABLE II**

Comparison of influence of D- and L-methionine on FGA excretion by rats fed diet that lacked added folic acid*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Added D-methionine</th>
<th>Rat No.</th>
<th>Added L-methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% 0.5%</td>
<td>0% 0.5%</td>
<td>0% 0.5%</td>
<td>0% 0.5%</td>
</tr>
<tr>
<td>16</td>
<td>17.9 6.7 21</td>
<td>4.9 1.2 21</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>15.0 4.4 22</td>
<td>10.4 2.5 22</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>11.6 3.3 23</td>
<td>3.3 0.8 23</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>7.4 1.5 24</td>
<td>12.1 6.7 24</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>5.3 1.5 25</td>
<td>5.3 1.5 25</td>
<td></td>
</tr>
</tbody>
</table>

* Values are μmoles of FGA in urine excreted per day. See Table I for additional details.

**TABLE III**

Deprivation of dietary vitamin B_{12} and FGA excretion by rats

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>&quot;Complete&quot; diet</th>
<th>Diet minus folic acid</th>
<th>Diet minus vitamin B_{12}</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>0.0*</td>
<td>15.9*</td>
<td>11.8*</td>
</tr>
<tr>
<td>37</td>
<td>2.6</td>
<td>13.2</td>
<td>14.8</td>
</tr>
<tr>
<td>38</td>
<td>1.0</td>
<td>9.0</td>
<td>17.0</td>
</tr>
<tr>
<td>39</td>
<td>0.6</td>
<td>10.6</td>
<td>17.0</td>
</tr>
<tr>
<td>40</td>
<td>0.0</td>
<td>7.9</td>
<td>14.8</td>
</tr>
</tbody>
</table>

Average food intake/rat/day... 4.6 gm., 4.8 gm., 5.0 gm.

* Values are μmoles of FGA in urine excreted per day after 4 weeks on diets.

Since FGA is derived from histidine, it could be anticipated that histidine added to the diet would give rise to an increase in FGA. Such indeed occurs (Table V). Furthermore, as in the case of the animal fed a diet that lacked added folic acid, addition of methionine to the diet with a low vitamin B_{12} content leads to a marked decrease in FGA excretion (Table V). This decrease in excretion occurred despite the increase in food intake observed with the diet that contained 1 per cent L-methionine.

The addition of homocysteine to diets that contain no added vitamin B_{12} results in a diminished excretion of FGA (Table VI). The effect of homocysteine is quite marked and its magnitude approaches that of methionine. The addition of cysteine to the same diet did not consistently result in decreases.

If nicotinic acid is omitted from the "complete" diet, some growth retardation results, but FGA excretion does not occur. 15 rats were divided into three groups and fed diets that (a) were

**TABLE IV**

Influence of dietary vitamin B_{12} on FGA excretion by rats*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>100 μg. of vitamin B_{12} per kilo added to diet lacking vitamin B_{12}</th>
</tr>
</thead>
<tbody>
<tr>
<td>66</td>
<td>26.0 22.4 10.8 6.8 5.6 4.0 3.1</td>
</tr>
<tr>
<td>67</td>
<td>32.6 23.8 17.2 6.5 4.1 3.3 2.3</td>
</tr>
<tr>
<td>68</td>
<td>35.6 23.6 24.0 11.0 5.8 5.8 2.6</td>
</tr>
<tr>
<td>69</td>
<td>37.6 28.4 24.5 13.3 9.7 5.4 5.0</td>
</tr>
<tr>
<td>70</td>
<td>35.6 22.0 13.1 8.2 4.2 2.3 1.7</td>
</tr>
</tbody>
</table>

Collection day... 1 2 3 4 5 6 7

* Values are μmoles of FGA in urine excreted per day.

Food intake was 9.9 gm. per rat per day.

Animals were fed a diet that lacked vitamin B_{12} for 7 weeks before vitamin B_{12} was added.

**TABLE V**

Influence of methionine and histidine on FGA excretion by rats fed diets that lacked added vitamin B_{12}*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Control diet</th>
<th>Diet plus 1% D-methionine</th>
<th>Diet plus 0.5% L-histidine- HCl</th>
<th>Diet plus 0.5% L-histidine-HCl and 1% L-methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>21.6</td>
<td>3.1</td>
<td>44.4</td>
<td>4.8</td>
</tr>
<tr>
<td>82</td>
<td>21.6</td>
<td>1.6</td>
<td>79.2</td>
<td>5.4</td>
</tr>
<tr>
<td>83</td>
<td>25.0</td>
<td>2.6</td>
<td>59.0</td>
<td>6.0</td>
</tr>
<tr>
<td>84</td>
<td>16.8</td>
<td>2.4</td>
<td>87.6</td>
<td>7.0</td>
</tr>
<tr>
<td>85</td>
<td>22.9</td>
<td>4.6</td>
<td>87.6</td>
<td>8.3</td>
</tr>
</tbody>
</table>

Average food intake/rat/day... 6.8 gm., 8.7 gm., 5.0 gm., 9.0 gm.

* Values are μmoles of FGA in urine excreted per day. 3-day collection periods were used.

**TABLE VI**

Effect of homocysteine and methionine on FGA excretion by rats fed diets that lacked added vitamin B_{12}*

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Control diet</th>
<th>Diet plus 1% L-homocysteine</th>
<th>Diet plus 1% L-methionine</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>21.1</td>
<td>13.8</td>
<td>21.4</td>
</tr>
<tr>
<td>82</td>
<td>19.4</td>
<td>14.0</td>
<td>17.8</td>
</tr>
<tr>
<td>83</td>
<td>27.4</td>
<td>15.6</td>
<td>22.6</td>
</tr>
<tr>
<td>84</td>
<td>19.4</td>
<td>9.7</td>
<td>12.7</td>
</tr>
<tr>
<td>85</td>
<td>23.3</td>
<td>14.0</td>
<td>21.4</td>
</tr>
</tbody>
</table>

Average food intake/rat/day... 6.6 gm., 6.0 gm., 5.0 gm., 7.5 gm.

* Values in μmoles FGA in urine excreted per day. 3-day collection periods were used.
"complete," (b) lacked folic acid, or (c) lacked niacin. The urine of each rat was examined for the presence of FGA at 4 and 6 weeks. Only those animals which were fed the diet with a low folic acid content excreted significant amounts of this compound. After the incorporation of 0.5 per cent histidine in the diet, FGA could be detected in the urine of each animal. The average values (molecules of FGA excreted per day) found for each group at 4 weeks were the following: Group A, 0.5; Group B, 6.4; Group C, 0.5. After the incorporation of histidine into the diet, the following were found: Group A, 8.5; Group B, 43.1; and Group C, 7.0.

The weight gains made by animals fed the various regimes are shown in Table VII. With one exception (first trial), the presence or absence of folic acid or vitamin B12 had no influence on growth. We are unable to account for the apparent beneficial influence of these vitamins in the first trial. The omission of niacin from the diet resulted in some retardation of growth. The groups fed the "complete" diet or that lacking niacin excreted insignificant amounts of FGA, those fed the diets lacking folic acid or vitamin B12 did excrete significant amounts.

The FGA in the urine of the rats deprived of dietary vitamin B12 was characterized as a compound which (a) in the presence of tetrahydrofolic acid and chick liver extract and in the absence of adenosine triphosphate gave rise to N5-formyltetrahydrofolic acid; (b) was retained by Dowex 50-H+; (c) was inactivated on autoclaving at pH 6.0 to 8.0; (d) was degraded by alkali; (e) was excreted in significantly increased amounts after the ingestion of histidine (9). Fractions separated from Dowex 50-H+ migrated on paper (in buanol-acetic acid-water) as did authentic FGA and were degraded to glutamic acid by autoclaving or alkaline treatment.

**DISCUSSION**

Neither folic acid nor vitamin B12 have any marked effect on the growth rate of the rats which were fed a 9 per cent casein diet. However, under these conditions, the two B vitamins have a demonstrable influence on the metabolism of FGA. Daft (8) has reported that rats fed a 4 per cent casein diet excrete FGA and present the typical symptoms of folic acid deficiency. More recently he has shown that the conventional rat fed an 8 per cent casein diet does excrete FGA yet does not exhibit the signs of a frank folic acid deficiency. Although the rat fed an 8 to 9 per cent casein diet does not appear to have any frank deficiency symptoms directly ascribable to the absence of folic acid or vitamin B12, it is nonetheless a useful animal for studies concerned with the involvement of these two vitamins in FGA metabolism.

With the data at hand, no direct conclusion can be drawn as to the mechanism through which folic acid, vitamin B12, methionine, and homocysteine influence the excretion of FGA. Although the diet employed was deficient in methionine (19), retardation of growth per se does not appear to be a significant factor in this relationship. Neither added folic acid nor vitamin B12 had any great influence on growth. Furthermore, retardation of growth by the elimination of nicotinic acid from the diet did not induce excretion of FGA. It seems more likely that a specific metabolic relationship exists between FGA and methionine (or a metabolic derivative) in which the two B vitamins are involved as cofactors. A rather direct metabolic relationship is indicated by the observation that if histidine is added to a ration containing no added vitamin B12, the excretion of the excess FGA is abolished almost immediately by the presence of dietary methionine (Table V).

Bennett (20) has shown that the methionine requirement for growth of the rat may be replaced by a combination of homocysteine, folic acid, and vitamin B12. Recent studies by Helleiner et al. (21) and Kisliuk and Woods (22) directly implicate both folic acid and vitamin B12 in the synthesis of methionine by extracts of *Escherichia coli*. Their system involved homocysteine as the ultimate acceptor of the l-carbon unit derived from a formaldehyde-tetrahydrofolic acid complex. In their view, vitamin B12 functions in the transfer of the hydroxymethyl group to homocysteine or in its final reduction to a methyl group. The present dietary studies suggest, as a possibility, that, in the rat, a major pathway for the utilization of the available l-carbon unit of FGA involves the over-all conversion of this unit into a methyl group via a similar reaction sequence which involves the two B vitamins and a suitable acceptor. If the normal reaction involves methionine as the acceptor, S-methylmethionine can be considered as a possible reaction product. Although the latter compound can replace methionine for growth purposes (23, 24) and is active metabolically in microbial systems (25, 26), there is no direct evidence for its natural occurrence in animal systems. If homocysteine is the normal acceptor, methionine would result. In this case the role of dietary methionine would be that of providing a source of homocysteine. Should this be so, one would have to account for the greater effectiveness of methionine as compared to its demethylation product. The reaction sequences postulated above may be blocked by the absence of either cofactor (folic acid and vitamin B12 derivatives) or by the absence of a suitable acceptor (methionine, homocysteine, or derived products) and FGA is excreted.

Nakao and Greenberg (27) noted that methionine in the presence of homocysteine had an activating effect on an enzyme system capable of synthesizing methionine methyl from formaldehyde or serine. The mode of action of methionine in their system was not clear. They did, however, exclude methylmethionine from consideration as an intermediate in methionine methyl synthesis from formaldehyde.

It can no longer be assumed that the occurrence of FGA in urine is a specific index of folic acid deficiency (9). Rucknagel et al.,4 observed the excretion of significant amounts of FGA in the

---

The current observations with the rat confirm the findings that vitamin Bl2 deficiency induces FGA excretion.

The effect of methionine on FGA excretion in the rat may help explain the variations observed in the excretion of the formimino compound by subjects receiving anti-folic acid therapy. FGA could be detected in the urine of some treated subjects, yet appeared to be absent from the urine of others (9).

It seems possible that one reason for the absence of FGA might be the presence of relatively high concentrations of methionine in the diet. Thus the extent to which FGA is excreted in the urine depends upon the degree of folic acid or vitamin Bl2 deficiency of the host, and the relative amounts of dietary histidine (the precursor of FGA) and methionine or homocysteine available. In this connection it may be noted that under the stress induced by the incorporation of extra dietary histidine, FGA excretion may be induced in the rat fed a 9 per cent casein diet that contains folic acid and vitamin Bl2.

**SUMMARY**

Formiminoglutamic acid is excreted by the rat which is fed a diet that is low in protein (9 per cent casein) content and deprived of either folic acid or vitamin Bl2. The excretion of the formimino compound is reduced by dietary methionine or homocysteine. It is postulated that a major pathway for the metabolism of the formimino compound in the intact rat involves the conversion of the available l-carbon unit to a methyl group, a reaction sequence involving both folic acid and vitamin Bl2. It is further postulated that an acceptor for the l-carbon unit is supplied by methionine, homocysteine, or metabolic derivatives of these amino acids.

**Acknowledgments**—The authors are indebted to Drs. G. M. Briggs, F. S. Daft, R. L. Kisliuk, and J. E. Seegmiller for many helpful suggestions offered during the course of this work. We wish to thank Dr. H. G. Steinman for a generous gift of L-homocysteine.

**REFERENCES**

6. Daft, F. S., In R. Brauer (Editor), Monograph on the approaches to the quantitative description of liver functions, in press.
Dietary Methionine and the Excretion of Formiminoglutamic Acid by the Rat
Milton Silverman and Anna J. Pitney


Access the most updated version of this article at
http://www.jbc.org/content/233/5/1179.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at
http://www.jbc.org/content/233/5/1179.citation.full.html#ref-list-1