The Urinary Excretion of Formic Acid and Formiminoglutamic Acid in Folic Acid Deficiency*

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In previous investigations it was shown that in folic acid deficiency there is a large increase in the urinary excretion of formimino-L-glutamic acid (1-6). This increase has been explained by recent enzymatic studies demonstrating that the first step in the metabolism of formimino-L-glutamic acid is the transfer of the formimino group to tetrahydrofolic acid (7-9). Formimino-L-glutamic acid is an intermediate in histidine metabolism (10, 11), and the urinary excretion of this compound in folic acid deficiency can be increased by the administration of L-histidine (3). Experiments with N\(^1\)-labeled histidine demonstrated that the urinary formimino-L-glutamic acid was derived from histidine (3).

In the present work we have demonstrated that, in addition to formimino-L-glutamic acid, there is a large increase in the excretion of formic acid in the urine of folic acid-deficient rats; in contrast to the results with formiminoglutamic acid, this formic acid is not derived from histidine. Our current studies have been facilitated by the recent development of sensitive spectrophotometric methods for the determination of formic acid (12) and formiminoglutamic acid (13).

EXPERIMENTAL

Formic acid was assayed enzymatically (12) with tetrahydrofolic acid formylase, which catalyzes the formation of 10-formyltetrahydrofolic acid in the presence of adenosine triphosphate, tetrahydrofolic acid, and formic acid. The 10-formyltetrahydrofolic acid formed was converted by acid to 5,10-methenyltetrahydrofolic acid, which was quantitatively determined by measuring its absorption at 350 m\(\mu\) by means of a gas flow counter.

The folic acid-deficient, succinylsulfathiazole-containing diet previously used (16) was modified as recommended by E. McDaniel; it contained sucrose (710 gm.), casein (180 gm.), salt mixture (40 gm.), ferric citrate-5 H\(_2\)O (10 gm.), CuSO\(_4\)-5 H\(_2\)O (400 mg.), Crisco (Proctor and Gamble) (50 gm.), Natola (Park-Davis) (2.5 ml.), choline chloride (2 gm.), folate acid-free vitamin mixture (5 gm.), and succinylsulfathiazole (10 gm.).

A total of 12 female rats (Sprague-Dawley strain) were divided into two equal groups at weaning ("deficient" and "supplemented") and placed on the succinylsulfathiazole-containing, folic acid-deficient diet. Each rat in the "supplemented" group received three times each week by pipette an oral supplement of 0.25 \(\mu\)mole of folic acid. The diet was fed ad libitum except as indicated below.

Urine collections were made each week in accordance with the following procedure. Each rat received 1 ml. of water by stomach tube and 5 ml. of 0.15 M NaCl by an intraperitoneal injection (to increase the urine volume); the urine was then collected for 7 hours in a metabolism cage. After an interval of 3 days another urinary collection was made for 7 hours from the same rats after the administration of 1 ml. of 0.05 M L-histidine by stomach tube and 5 ml. of 0.15 M NaCl by an intraperitoneal injection. After collection, the urine was stored at \(-20^\circ\) until it was assayed.

Average values for the excretion of formiminoglutamic acid and of formic acid in the normal and the folic acid-deficient rats are presented in Fig. 1A. The excretion of formiminoglutamic acid (per 7 hour collection period) increased during the course of the deficiency from a normal value of 0.22 pmole (range 0 to 0.7 pmole) to 36.5 pmoles. After supplementation with folic acid, this excretion decreased to 0.07 pmole. The validity of the enzymatic assay for formiminoglutamic acid in these urines was confirmed by paper chromatography, by alkaline hydrolysis, and by colorimetric assay with the alkaline ferrocyanide-nitroprusside reagent (13, 17).

The increase in the excretion of formiminoglutamic acid was observed in the folic acid-deficient rats, even though the dietary intake (and consequently the histidine intake) was considerably less in these rats than in the folic acid-supplemented rats. On the 38th day, for example, the folic acid-deficient rats consumed an average of 8 gm. of food per rat (calculated histidine content (18), 100 \(\mu\)moles per day), whereas the folic acid-supplemented rats consumed an average of 9 gm. of food per rat (calculated histidine content, 300 \(\mu\)moles per day).

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1 The preparations used in these experiments were supplied by Mr. W. E. Pricer, Jr.
increase in excretion of the free formic acid indicated that the free formic acid found in the urine of folic acid-deficient rats did not arise from histidine or from formiminoglutamic acid. The origin of the formic acid from compounds other than histidine was demonstrated definitively by an experiment (Table II) in which 50 μmole of L-histidine, labeled with C₁⁴ in position 2 of the imidazole ring (specific activity, 8000 c.p.m. per μmole), were administered by stomach tube to a folic acid-deficient rat. During the standard 7 hour collection period, 68.5 μmole of formiminoglu-tamic acid were excreted, with a specific activity of 3300 c.p.m. per μmole. 49 μmole of formic acid were excreted, with essentially no (less than 0.5 per cent) radioactivity.

**Discussion**

**Possible Sources of Urinary Formic Acid in Folic Acid Deficiency**—The increased excretion of formiminoglutamic acid in folic acid-deficient rats can be explained by the demonstration that tetrahydrofolic acid is required for the further metabolism

**Table I**

<table>
<thead>
<tr>
<th>Histidine administered</th>
<th>Folic acid-deficient rats*</th>
<th>Normal rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>μmole</td>
<td>Formiminoglutamic acid</td>
<td>Formic acid</td>
</tr>
<tr>
<td>0</td>
<td>5.3</td>
<td>36.5</td>
</tr>
<tr>
<td>100</td>
<td>24.7</td>
<td>31.0</td>
</tr>
<tr>
<td>200</td>
<td>0.61</td>
<td>2.06</td>
</tr>
<tr>
<td>400</td>
<td>180</td>
<td>39.8</td>
</tr>
</tbody>
</table>

* The diet and other conditions in the experiment were essentially the same as those described in Fig. 1. Six rats (Sprague-Dawley strain, average weight, 95 gm.) were used after they had received a folic acid-deficient diet for 23 days. Urine was then collected for 7 hours in order to obtain the data for formiminoglutamic and formic acid excretion in the absence of histidine administration. On the following day, three of these rats received 100 μmole of histidine by stomach tube; the remaining three rats received 400 μmole of histidine. Urine was again collected for 7 hours. All values are expressed as the average excretion per rat.

† Female rats (Sprague-Dawley strain, average weight, 75 gm.) on a folic acid-supplemented stock diet. Each value represents the average excretion for three rats.

Additional data were obtained in two other experiments with normal rats. In one experiment, six weanling Sprague-Dawley female rats were fed a purified diet without succinylsulfathiazole; in addition, 0.25 μmole of folate acid was administered by oral pipette three times weekly. After 35 days (average weight, 130 gm.), the formiminoglutamic acid and formic acid in the urine, collected over a 7 hour period, averaged 0.13 Mmole (range 0.01 to 0.27 μmole) and 1.89 μmole, respectively. No data were obtained in this group after histidine administration.

In another experiment, five Sprague-Dawley weanling rats were fed the succinylsulfathiazole-containing diet, but received a folic acid supplement (0.25 μmole) by oral pipette once each week. After 43 days (average weight, 121 gm.), the rats were divided into three groups. Each group received either 0, 200, or 400 μmole of histidine by stomach tube. The formiminoglutamic acid excretion over a 7 hour period averaged 0.21, 8.8, and 36.5 μmole, respectively.
We have recently tested this postulation in vivo, and, as indicated by MacKenzie (20) and of Weinhouse and Friedmann (21). These studies are consistent with the fact that in various enzymatic reactions that do not require folic acid, since no folic acid is present.

Formic acid is metabolized poorly, and is excreted in the urine. The explanation for the increased excretion of formic acid in folic acid deficiency, on the other hand, is not so obvious; it can be postulated, however, that formic acid is constantly excreted in the urine of normal animals, and is largely metabolized further, and only a small amount is excreted in the urine (19). In the folic acid-deficient animal, on the other hand, as will be discussed below, formic acid is metabolized poorly, and is excreted in the urine.

The possible reactions that might lead to formic acid without involving folic acid cofactors are suggested by the experiments of MacKenzie (20) and of Weinhouse and Friedmann (21). MacKenzie (20) demonstrated that urinary formic acid was increased after the administration of sarcosine. This study was extended by Weinhouse and Friedmann (21), who administered methionine-C14H3, choline-C14H5, and sarcosine-C14H3, together with large quantities of sodium formate in a "trapping" experiment in vivo. The radioactivity recovered in the formic acid excreted was markedly increased in folic acid-deficient rats. These studies are consistent with the fact that in various enzymatic reactions folic acid cofactors have not been demonstrated as a requirement for the formation of formaldehyde from choline, dimethylglycine, or sarcosine (20). For example, when formaldehyde was fed to rats, the formaldehyde was largely metabolized further, and only a small amount was excreted in the urine (19). In the folic acid-deficient animal, on the other hand, as will be discussed below, formic acid is metabolized poorly, and is excreted in the urine.

The studies of Knox and Mehler (23, 24) on the enzymatic degradation of tryptophan to formic acid and kynurenine indicate another metabolic reaction in which formic acid might be formed without the participation of folic acid, since no folic acid cofactor has been demonstrated as a requirement for the formation of formaldehyde from choline, dimethylglycine, or sarcosine (20). For example, when formaldehyde was fed to rats, the formaldehyde was largely metabolized further, and only a small amount was excreted in the urine (19). In the folic acid-deficient animal, on the other hand, as will be discussed below, formic acid is metabolized poorly, and is excreted in the urine.
Friedmann et al. (26), who demonstrated a marked decrease in the respiratory C1402 excreted after the administration of formic acid-C14 to folic acid-deficient rats. Friedmann et al. (26) were unable, however, to demonstrate an effect of folic acid deficiency on the urinary excretion of formic acid-C4. This was presumably because of the large amount of formic acid (2000 pmoles) administered in their experiments; with smaller doses we have been able to show a marked increase in the urinary formic acid-C14 of folic acid-deficient rats (Table IV).

The data presented in this paper on the accumulation of formic acid in folic acid-deficient rats and on the rapid metabolism of administered formic acid-C14 in the presence of folic acid indicate a large turnover of formic acid in vivo in normal rats.

The role of histidine as a precursor of formiminoglutamic acid was confirmed. Histidine, however, was not a precursor of the urinary formic acid in the folic acid-deficient rats. The administration of tryptophan, on the other hand, resulted in an increased excretion of formic acid, with essentially no change in the excretion of formiminoglutamic acid.

Formic acid can be produced from tryptophan and other precursors by metabolic reactions that do not require folic acid. In the absence of folic acid, the formic acid is metabolized poorly and is excreted in the urine.

SUMMARY

The urinary excretion of formiminoglutamic acid and of formic acid was quantitatively followed during the development of a folic acid deficiency in rats. In addition to the previously reported increase in the excretion of formiminoglutamic acid, there was a marked increase in the excretion of formic acid.

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