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 N¹⁶-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 tetrahydrofolate formylase,¹ which catalyzes the formation of 10-formyltetrahydrofolate in the presence of adenosine triphosphate, tetrahydrofolate, and formic acid. The 10-formyltetrahydrofolate formed was converted by acid to 5,10-methylenetetrahydrofolate, which was quantitatively determined by measuring its absorption at 350 mµ. Formimino-L-glutamic acid was measured in a similar manner by the formation of 10-formyltetrahydrofolate in the presence of formiminoglutamic acid transferase, cyclodeaminase, tetrahydrofolate acid, and phosphate buffer (7). Details of these procedures have been presented elsewhere (7, 12, 13).

Formiminoglutamic acid was synthesized as previously described (14). Isotopic histidine (imidazole-2-C¹⁴) was synthesized by a modification of the method of Tesser and Rittenberg (15). Radioactivity determinations were carried out at infinite thinness by means of a gas flow counter.

* A preliminary report on this work was presented by J. C. Rabinowitz at the Gordon Conference on Vitamins and Metabolism, August 12, 1957.
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¹ The preparations used in these experiments were supplied by Mr. W. E. Pricer, Jr.

The folic acid-deficient, succinylsulfathiazole-containing diet previously used (16) was modified as recommended by E. McDaniel;² it contained sucrose (710 gm.), casein (180 gm.), salt mixture (40 gm.), ferric citrate·3 H₂O (10 gm.), CuSO₄·5 H₂O (400 mg.), Crisco (Proctor and Gamble) (50 gm.), Natola (Parke-Davis) (2.5 ml.), choline chloride (2 gm.), folic 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 µmole 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° 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 0.23 µmole (range 0 to 0.7 µmole) to 36.5 µmole. After supplementation with folic acid, this excretion decreased to 0.07 µmole. 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 µmoles per day), whereas the folic acid-supplemented rats consumed an average of 9 gm. of food per rat (calculated histidine content, 300 µmoles per day).

² Personal communication.
The excretion of formic acid in folate-deficient rats rose from a normal value of 1.5 μmoles to a value of 49 μmoles per 7 hour collection period. The increase in the excretion of formic acid preceded the increase in the excretion of formiminoglutamic acid. After supplementation with folic acid the excretion of formic acid decreased to 1.5 μmoles. No decrease in either the formiminoglutamic or the formic acid level was found in comparable experiments after the administration of vitamin B₉.

The administration of histidine caused a large increase in the excretion of formiminoglutamic acid, but not of formic acid (Fig. 1B, Table I). The increase in excretion of formiminoglutamic acid was particularly marked in the folate-deficient group, even when only small quantities of histidine were administered; large increases in the excretion of formiminoglutamic acid (30 to 90 μmoles) were found in normal rats only when very large doses of histidine (400 μmoles) were administered. The increase in excretion of formiminoglutamic acid without any

**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 tetrahydrofolate 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>μmoles</td>
<td>μmoles</td>
<td>μmoles</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>180</td>
<td>39.8</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 μmoles of histidine by stomach tube; the remaining three rats received 400 μmoles of histidine. The 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 diet containing 0.25 μmole of folic acid was administered by oral pipette three times weekly. After 25 days (average weight, 130 gm.), the formiminoglutamic acid and formic acid in the urine, collected over a 7 hour period, averaged 0.13 μmole (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 μmoles of histidine by stomach tube. The formiminoglutamic acid excretion over a 7 hour period averaged 0.21, 8.8, and 36.5 μmoles, respectively.
We have recently tested this postulation in vivo, and, as indicated by Hyde to formic acid (22).

These studies are consistent with the fact that in various enzymatic reactions that do not require folic acid, since no folic acid cofactor has as yet been demonstrated for these enzymes.

Thus, in folic acid deficiency, formiminoglutamic acid accumulates, 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 being formed in the normal animal, not only by pathways involving tetrahydrofolic acid, but by hydrolytic and oxidative reactions that do not require folic acid cofactors. In the normal animal, this formic acid 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.

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-$\text{C}^4\text{H}_3$, choline-$\text{C}^4\text{H}_3$, and sarcosine-$\text{C}^4\text{H}_3$, 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 studies folic acid cofactors have not been demonstrated as a requirement for the formation of formaldehyde from choline, dimethylglycine, or sarcosine (20), or in the oxidation of formaldehyde to formic acid (22).

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 as yet been demonstrated for these enzymes. We have recently tested this postulation in vivo, and, as indicated in Table III, the administration of tryptophan led to an increase in the level of urinary formic acid. This increase was much greater in folic acid-deficient rats than in the folic acid-supplemented group.

**Table II**

| Distribution of $\text{C}^4$ in urinary formiminoglutamic and formic acids after administration of histidine (imidazole $\text{C}^4\text{H}_5$) to a folic acid-deficient rat | Specific activity |
|---|---|---|
| Histidine-$\text{C}^4$ administered | $\mu$moles | c.p.m. | c.d.m. / $\mu$moles |
| Urinary formiminoglutamic acid | 68.5 | 296,000 | 3500 |
| Urinary formic acid | 49 | <2,000 | <40 |
| $^* 5$0 $\mu$moles of $L$-histidine, labeled with $\text{C}^4$ in position 2 of the imidazole ring, were administered by stomach tube in a volume of 1 ml. to a folic acid-deficient rat (53rd day of deficient diet; weight of rat, 90 gm.). 5 ml. of isotonic saline were administered intraperitoneally. Urine was collected for 7 hours. |

† The urine was assayed for formic acid enzymatically; this determination was confirmed by analysis of the distillate after addition of acid to the urine and lyophilization. The distillate, after neutralization, was also assayed for radioactivity.

Urinary formiminoglutamic acid was determined enzymatically. To determine the $\text{C}^4$ content of the formiminoglutamic acid, an aliquot of urine was subjected to alkaline hydrolysis (19); after acidification, the formic acid was distilled (by lyophilization), and the neutralized distillate was assayed for radioactivity. These results were confirmed by similar assays after chromatography on Dowex-1 acetate.

**Table III**

<table>
<thead>
<tr>
<th>Urinary excretion of formic acid after administration of tryptophan</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan administered</td>
<td>Folic acid-deficient rats</td>
<td>Normal rats</td>
</tr>
<tr>
<td>$\mu$moles</td>
<td>$\mu$moles</td>
<td>$\mu$moles</td>
</tr>
<tr>
<td>0</td>
<td>38.6</td>
<td>1.9</td>
</tr>
<tr>
<td>200$^\ddagger$</td>
<td>126</td>
<td>6.4</td>
</tr>
<tr>
<td>400$^\ddagger$</td>
<td>182</td>
<td>38.0</td>
</tr>
</tbody>
</table>

$^*$ The diet and conditions in the experiment were essentially the same as those described in Fig. 1. Six rats (average weight, 100 gm.) were used after they had received a folic acid-deficient diet for 25 days. The experiment was repeated 3 days later, and the values presented represent the average of both experiments.

† The urinary samples were also analysed for formiminoglutamic acid. After 0, 200, and 400 $\mu$moles of tryptophan, the urine contained 12.5, 20.1, and 10.5 $\mu$moles of formiminoglutamic acid, respectively.

‡ The normal diet used in these experiments was essentially the same as the folic acid-deficient diet, except for the omission of succinylsulfathiazole. Each rat received an oral supplement of 0.25 $\mu$mole of folic acid three times weekly. Each figure represents the average value from a group of three to six rats.

§ Administered as a 0.2 $\mu$ suspension in 2 per cent gum arabic.

**Table IV**

<table>
<thead>
<tr>
<th>Urinary formate-$\text{C}^4$ after administration of formic acid intraperitoneally</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Formate-$\text{C}^4$ administered</td>
<td>Counts recovered in urinary formate $^*$</td>
<td></td>
</tr>
<tr>
<td>$\mu$moles</td>
<td>Folic acid-deficient rats</td>
<td>Folic acid-supplemented rats</td>
</tr>
<tr>
<td>1</td>
<td>38</td>
<td>%</td>
</tr>
<tr>
<td>10</td>
<td>53</td>
<td>1.5</td>
</tr>
<tr>
<td>100</td>
<td>63</td>
<td>1</td>
</tr>
<tr>
<td>600</td>
<td>45</td>
<td></td>
</tr>
</tbody>
</table>

$^*$ Each rat received 800,000 c.p.m. of sodium formate-$\text{C}^4$, diluted with sufficient nonradioactive sodium formate (0.1 $\mu$ mole solution) to give the dose indicated. The volume was adjusted to 6 ml. with 0.15 $\mu$ NaCl and injected intraperitoneally. Each figure represents the average value from a group of three to six rats.

† Per cent of administered counts.

‡ The diet and urinary collection period (7 hours) were the same as those described in Fig. 1. The rats (average weight, 100 gm.) had been fed the succinylsulfathiazole-containing diet for 31 days before this experiment was carried out.

§ The diet for this group was the same as that used in the folic acid-deficient group, except for the omission of succinylsulfathiazole. Each rat received 0.25 $\mu$moles of folic acid orally three times weekly. The average weight at the time of the experiment was 95 gm.
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.

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.

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REFERENCES

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