The Effect of Ammonia Administration on Orotic Acid Excretion in Rats

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Ammonia fixation has been shown to proceed through at least three major pathways in ureotelic animals (1, 2). These are carbamyl phosphate synthetase, glutamic dehydrogenase, and glutamine synthetase. Perhaps the most significant means for removal of exogenous ammonia is by its conversion to urea (3). The initial step in this conversion is the production of carbamyl phosphate in the liver. Carbamyl phosphate, in addition to being a precursor for urea synthesis, is also believed to be a precursor for pyrimidine synthesis via the orotic acid pathway (2).

Although orotic acid is not found in urine from normal humans or rats, its excretion has been observed in conditions of altered pyrimidine metabolism (4, 5). In the course of studies relating urinary organic acid excretion to acid-base balance, it was noted that an ultraviolet-absorbing acidic substance was rapidly excreted into the urine in large quantities after ammonium salt administration. Methods were devised to identify this substance. It was found to be orotic acid, and experiments were undertaken to determine some of the conditions under which it is excreted.

EXPERIMENTAL PROCEDURE

Materials and Methods—Male Wistar rats weighing 250 to 300 g were used in all experiments. They were routinely maintained on a diet of Purina rat chow ad libitum unless otherwise stated. Chronic alkalosis and acidosis were induced by substitution of 1% NaHCO₃ and 1.5% NH₄Cl, respectively, for drinking water over a period of 7 days. In acute experiments, groups of three rats were fasted for 18 hours and then given the test substance by stomach tube. After a 90-minute urine collection period, 3 mmoles of NH₄Cl contained in 3 ml of solution were given by stomach tube. Another urine specimen was then collected over a period of 7 days. In acute experiments, groups of three rats were fasted for 18 hours and then given the test substance by stomach tube. After a 90-minute urine collection period, 3 mmoles of NH₄Cl contained in 3 ml of solution were given by stomach tube. Another urine specimen was then collected over a period of 7 days.

Ammonia and urea were determined by the aeration method of Conway (6). Orotic acid was determined by silica gel chromatography (7) with the use of automatic indicator titration. Ion exchange chromatography was performed on a Dowex 1 X8 (formate) column (200 to 400 mesh, 0.9 × 20 cm) with a 0 to 4 M formic acid gradient. The eluate was continuously monitored at 280 nm on a Beckman model DB spectrophotometer.

RESULTS

Identification of Orotic Acid in Urine—Fig. 1 is a representation of the organic acid pattern obtained from analysis of a 0.2-ml aliquot of urine collected from a fasted rat during an interval of 30 minutes following the administration of 3 mmoles of NH₄Cl. This is superimposed on a similar chromatogram of a synthetic mixture of commonly occurring metabolic acids including authentic orotic acid. The largest urinary organic acid peak in this specimen corresponds to the added orotic acid. Further, anion exchange chromatography of a similar urine specimen on Dowex 1 showed two major ultraviolet-absorbing peaks. After concentration and removal of the formic acid used for elution, the substances corresponding to the two peaks were separately subjected to silica gel column chromatography for organic acid analysis. One of the substances gave a peak corresponding to orotic acid. Spectral studies of this substance yielded absorption spectra which closely resembled those of orotic acid (8).

Azauridine, a potent pyrimidine synthesis inhibitor, has been shown to produce orotic aciduria (5). As further evidence that the acidic material that appeared in the urine after ammonium chloride administration was orotic acid, two normal fasted rats were given by intraperitoneal injection a dose of 60 mg per kg of azauridine. After 90 minutes of urine collection, 3 mmoles of NH₄Cl in 3 ml of solution were given by stomach tube. Urine was then collected for 3 hours. A peak corresponding to orotic acid was present in both urines. The 90-minute excretion after azauridine contained 1.5 μmoles of orotic acid per rat, whereas the 3-hour urine after NH₄Cl contained 10.3 μmoles of orotic acid per rat.

Excretion of Orotic Acid after Single Dose of Ammonium Chloride—Two fasted rats were given 3 mmoles of NH₄Cl in 3 ml of solution by stomach tube, and individual urine specimens were collected as voided. Assays were performed on pooled samples. The data from an illustrative experiment are presented in Table I, showing that maximum orotic acid excretion occurred during the first 105 minutes and then rapidly tapered off. Because of the highly insoluble nature of orotic acid and its salts, large numbers of crystals were observed in the urine. Therefore, the possibility of contamination exists for the second and third urines by insoluble orotic acid in the urinary tract of the rats. In comparable experiments, the orotic acid excretion during the first 180 minutes after NH₄Cl administration was much less when fed rats rather than fasted rats were employed.

The most marked changes in urinary pH and in maximum urinary ammonia and urea excretion rates consistently occurred after orotic acid excretion had subsided.
FIG. 1. Chromatographic separation of organic acids. The upper curve is a representation of the acids present in 0.2 ml of urine obtained from a fasted rat 30 minutes after administration of 3 mmoles of ammonium chloride. The lower curve represents the separation of approximately 0.5-peq quantities of a number of metabolite acids. The separation was achieved on a hydrated silica gel column (0.9 X 140 cm) with a gradient elution consisting of chloroform and tert-amyl alcohol (7).

Analysis of 1 ml of plasma 30 minutes after 3 mmoles of NH₄Cl administration showed a small detectable amount of circulating orotic acid. However, its concentration was too low to be accurately determined. At the end of 3 hours, no orotic acid could be detected in the plasma.

**Excretion of Orotic Acid after Graded Doses of Ammonium Chloride**—Individual rats fasted for 18 hours and given water ad libitum were given, by stomach tube, 0.5, 1.0, 2.0, and 4.0 mmoles of NH₄Cl in 5 ml of solution. Urine collections were made over a 3-hour period. The data presented in Table II indicate that a relationship exists between the dose of ammonium chloride administered and the quantity of orotic acid excreted.

**Excretion of Orotic Acid after Chronic Administration of NH₄Cl and NaHCO₃**—Rats were allowed solutions of 1.5% NH₄Cl or 1% NaHCO₃ instead of drinking water for periods up to 7 days. No significant amounts of orotic acid were found in urine specimens obtained from unfasted rats after 1 and 3 days of such treatment nor in specimens from fasted animals collected after 7 days. Both groups of chronically treated rats were given 3 mmoles of NH₄Cl in 3 ml of solution by stomach tube 18 hours after the food, but not the drinking solution, had been withdrawn. Urine was collected during the 3-hour period after the NH₄Cl treatment. The average of four animals in each group was 1.4 μmoles of orotic acid excreted per rat by the chronic NH₄Cl group and 24.3 μmoles of orotic acid excreted by the NaHCO₃-treated animals.

**Excretion of Orotic Acid as Affected by Pretreating Fasting Animals**—As noted previously, the orotic acid excretion following NH₄Cl administration was much less when fed rats instead of fasted rats were employed. A number of metabolites were given to fasted rats in an attempt to influence the excretion of orotic acid before and after ammonium chloride administration.

No orotic acid could be found in the urines of fasted rats collected during a 90-minute interval after the administration of 1.5-mmole quantities of either glutamic acid, aspartic acid, citric acid, α-ketoglutaric acid, ornithine, citrulline, or arginine. The administration of a similar quantity of glutamine was accompanied by the excretion of 2.3 μmoles of orotic acid during the 90-minute interval. It is known that exogenous glutamine is readily deamidated in vivo. Consequently, the orotic acid excretion in this case can probably be related to endogenous ammonia produced by deamination.

<table>
<thead>
<tr>
<th>Time</th>
<th>Volume</th>
<th>Orotic acid</th>
<th>pH</th>
<th>NH₃</th>
<th>Urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>min</td>
<td>ml</td>
<td>μmoles</td>
<td></td>
<td>μmoles/mg/rat</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6.3</td>
<td>2.27</td>
<td>117</td>
</tr>
<tr>
<td>105</td>
<td>2.7</td>
<td>8.3</td>
<td>6.3</td>
<td>3.32</td>
<td>255</td>
</tr>
<tr>
<td>205</td>
<td>2.6</td>
<td>1.0</td>
<td>5.6</td>
<td>3.58</td>
<td>101</td>
</tr>
<tr>
<td>255</td>
<td>2.5</td>
<td>1.7</td>
<td>5.6</td>
<td>3.36</td>
<td>93</td>
</tr>
<tr>
<td>300</td>
<td>2.8</td>
<td>5.8</td>
<td>5.8</td>
<td>2.45</td>
<td>54</td>
</tr>
</tbody>
</table>

**Table II**

**Orotic acid excretion in relation to ammonium chloride dose**

Ammonium chloride was administered by stomach tube in 5 ml of solution to rats fasted for 18 hours. Urine was collected for 3 hours, and the orotic acid content was determined by silica gel column chromatography.

<table>
<thead>
<tr>
<th>NH₄Cl administered</th>
<th>Orotic acid excreted per rat in 3 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>mmoles</td>
<td>μmoles</td>
</tr>
<tr>
<td>0.5</td>
<td>2.3</td>
</tr>
<tr>
<td>1.0</td>
<td>4.1</td>
</tr>
<tr>
<td>2.0</td>
<td>5.6</td>
</tr>
<tr>
<td>4.0</td>
<td>9.5</td>
</tr>
</tbody>
</table>

At the close of the 90-minute period, the pretreated rats were given 3 mmoles of NH₄Cl, and the urine was collected during the succeeding 3-hour interval. Compared with suitable controls, no significant change in the increased orotic acid excretion following NH₄Cl administration was found in rats treated with either glutamic acid, aspartic acid, citric acid, α-ketoglutaric acid, or glutamine. In contrast, only negligible amounts of orotic acid appeared in the 3-hour urine following NH₄Cl admin-
in the urine as erotic acid, the excretion was found to be proportional to the amount of NH$_4$Cl administered. There is evidence that the amount of erotic acid found in the urine may be a true measure of the total amount of ammonia shunted into the erotic acid pathway. Thus, Hurlbert and Potter (13) observed a 30% excretion of labeled erotic acid within a 2-hour period following administration. Also, von Euler, Rubin, and Handschumacher (14) more recently found only 5 to 20% of the absorbed ring-labeled erotic acid in the urine.

The continuous synthesis of pyrimidines in nonhepatic tissues and tumors devoid of carbamyl phosphate synthetase activity has promoted speculations concerning the source of the carbamyl phosphate required for such synthesis. These suggestions have ranged from alternative pathways for synthesis (1, 2) to markedly diminished but sufficiently rates of production of carbamyl phosphate which would supply pyrimidine requirements (15). It may be significant that a tissue, presumably the liver, has the capacity to produce and release to the circulation relatively large quantities of erotic acid. In view of these findings, the role of hepatic erotic acid as a precursor of pyrimidines in those tissues which lack carbamyl phosphate synthetase activity is presently under investigation.

**SUMMARY**

Orotic acid has been identified as a major organic acid in the urine of fasted rats shortly after treatment with ammonium salts or azauridine. The excretion of erotic acid was found to be roughly proportional to the dose of ammonium chloride administered but accounted for less than 1% of the nitrogen intake. Fed rats and rats pretreated with ornithine, citrulline, or arginine excreted greatly diminished quantities of erotic acid. Rats consuming 1.5% NH$_4$Cl as drinking water for 7 days excreted 1.4 mmoles of erotic acid after a 3-mmmole dose of NH$_4$Cl. Rats using 1% NaHCO$_3$ as drinking water, under similar conditions, excreted 24.3 mmoles of erotic acid. Pretreatment with several Krebs cycle intermediates and amino acids had little effect. Glutamine and ureidosuccinic acid administration both resulted in erotic acid excretion.

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**REFERENCES**

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