Urinary Phenolic Acid Metabolites of Tyrosine

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More than 40 phenolic acids are known to be excreted in the urine of humans ingesting ordinary diets (1). The number can be appreciably reduced by the feeding of a purified diet during the period of urine collection. However, rats continue to excrete several phenolic compounds during starvation; undoubtedly, the endogenous metabolism of aromatic amino acids is involved.

One of the metabolites excreted by rats on a purified diet is p-hydroxyphenylpropionic (phloretic) acid. Recently, a clew as to the origin of phloretic acid was found. Specifically, o-hydroxyphenylpropionic acid was detected in the urine of rats given o-tyrosine by stomach tube. When similar studies were conducted with tyrosine, an increase in the excretion of phloretic acid was observed.

According to Bernhart and Zilliken (2), a 7- to 10-fold increase in the urinary excretion of phenolic acids occurs when rats are fed a diet containing 10% tyrosine with or without the addition of chlorotetracycline. p-Hydroxyphenylpyruvic acid, p-hydroxyphenylacetic acid and traces of homogentisic acid were specifically implicated in the phenolic acid fraction.

In this present report it will be shown that p-hydroxyphenylpyruvic, p-hydroxyphenyllactic, p-hydroxyphenylactic, phloretic, p-coumaric, and p-hydroxybenzoic acids are all urinary metabolites of tyrosine in the rat and rabbit. A suggested scheme of sequential metabolic relationships for these compounds is also presented.

EXPERIMENTAL PROCEDURE

Methods—Collection of the urine and the procedures used for the two-dimensional paper chromatographic detection of the urinary phenolic metabolites have been described previously (3, 4). To facilitate detection of conjugated phenolic compounds, all urine samples were routinely screened by preparing chromatograms of the ether extract of the urine, hydrolyzed ether extract (2 N HCl reflux for 1 hour), ether-extracted urine, and hydrolyzed ether-extracted urine. Frequently, unidentified areas were cut out and eluted from unsprayed chromatograms and various qualitative tests carried out for purposes of identification.

The composition of the diets fed to rats and rabbits has been described previously (5). However, cornstarch was substituted for corn meal in the rabbit diet. All compounds studied were given by stomach tube at a level of 100 mg per rat unless otherwise indicated.

Materials—o-Tyrosine, m-tyrosine, tyrosine, p-hydroxyphenylacetic acid, and p-hydroxybenzoic acid were purchased from commercial sources. Phloretic acid was prepared by hydrogenation of p-coumaric acid in ethanol solution with palladium as a catalyst. The p-coumaric acid was prepared by the method of Vorotz (6). The ethereal sulfate of p-hydroxybenzoic acid was made by treating p-hydroxybenzoic acid with chlorosulfonic acid and chloroform in pyridine (7). p-Hydroxyphenylpyruvic acid was prepared by a method described by Herbst and Shemin (8). A sample of p-hydroxyphenylactic acid was obtained by hydrogenation of p-hydroxyphenylpyruvic acid with sodium amalgam (9). The chromatographic behavior of these compounds is shown in Table I. All compounds were checked for purity by two-dimensional paper chromatography, and were subjected to the same conditions of acid hydrolysis as the urine fractions in order to distinguish between chemical degradation and metabolic change. The sensitivity of the chromatographic technique used is such that 2 μg of a phenolic acid can be detected in a 5-mg sample of L-tyrosine.

RESULTS

An ether extract of the urine of rats receiving the purified diet described above was subjected to two-dimensional paper chromatography. Three phenolic acids, p-hydroxyphenylactic, phloretic, and p-hydroxybenzoic, were readily detected. The identity of these compounds was based on their \( R_F \) values in two dimensions, their appearance under ultraviolet (Mineralight), and their colors after spraying with diazotized sulfanilic acid followed by sodium carbonate. The amounts excreted of these three compounds are shown in Table II (control). These data were obtained by visual comparison of the color intensity of spots on the chromatogram containing appropriate aliquots of acid-hydrolyzed urine and known amounts (5 μg or less) of the authentic compounds.

The administration of o-tyrosine to rats by stomach tube (100 mg per rat) led to the detection in the urine of p-hydroxyphenylpropionic and o-hydroxyphenyllactic acids. m-Tyrosine gave rise to m-hydroxyphenylpropionic and m-hydroxyphenyllactic acids. These findings prompted us to investigate the urinary phenolic acid metabolites of tyrosine. The metabolic fate of o-tyrosine and m-tyrosine will be described in more detail in a subsequent report.

When tyrosine itself was given to rats and the 48-hour urine sample was examined chromatographically, distinct increases in p-hydroxyphenyllactic and p-hydroxyphenylpropionic acids were evident when compared with the ether extract of the control urine (Table II). In addition, p-coumaric acid and the ethereal sulfate of p-hydroxybenzoic acid were detected on the chromatogram (Table I).

An understanding was obtained regarding the sequential rela-
Phlorotic and p-coumaric acids was increased, whereas the excretion of p-hydroxyphenylacetic acid was decreased and the excretion to p-hydroxyphenylpyruvic acid, the excretion of both lactic acid was also observed on the chromatogram.

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Some of these phenolic acids arising from tyrosine by studying the metabolic fate of each compound separately. Since p-hydroxyphenylpyruvic and p-hydroxyphenyllactic acids have previously been identified as tyrosine metabolites (10), they were included in this investigation.

Rats were given p-hydroxyphenylpyruvic acid. The results were comparable to those obtained for tyrosine, except the increase amounts of p-hydroxybenzoic acid and phloretic acid were excreted (Table II). The presence of p-hydroxyphenyllactic acid was also observed on the chromatogram.

When p-hydroxyphenyllactic acid was given to rats, in contrast to p-hydroxyphenylpyruvic acid, the excretion of both phloretic and p-coumaric acids was increased, whereas the excretion of p-hydroxyphenyllactic acid was decreased and the excretion of p-hydroxybenzoic acid was unchanged (Table II). From these findings and the observations to follow it was concluded that p-hydroxyphenylpyruvic acid is converted to p-hydroxyphenyllactic acid which in turn is then converted to p-coumaric and phloretic acids.

Four of these acids, phloretic, p-coumaric, and p-hydroxybenzoic and its ethereal sulfate, were previously encountered and identified as urinary metabolites of naringin (11). Phloretic acid administration resulted in the excretion of p-coumaric acid, the ethereal sulfate of p-hydroxybenzoic acid, and an increase in the excretion of p-hydroxybenzoic acid. In this same report (11) it was also found that the only metabolite of p-hydroxybenzoic acid was its ethereal sulfate.

When p-coumaric acid was administered to rats, large amounts of p-hydroxybenzoic acid and its ethereal sulfate derivative were excreted in the urine. The compound given, p-coumaric acid, and its glycine conjugate were also readily detected. Saturation of the double bond in the side chain of the p-coumaric acid would account for the observed increase in the excretion of phloretic acid.

When two antibiotics were added to the basal diet (streptomycin, 23 mg per 100 g and sulfathiazole, 750 mg per 100 g) and fed to rats without added tyrosine, there was no change (decrease) in the normal excretion of phloretic, p-hydroxyphenyllactic, or p-hydroxybenzoic acids. Furthermore, when 10% tyrosine was added to the above antibiotic containing diet, large increases in the excretion of phenolic acids were measured. For example, the ingestion of 1.3 g per rat per day of tyrosine resulted in the minimal excretion of 75 mg of p-hydroxyphenylpropionic acid, 75 mg of p-hydroxyphenyllactic acid, 50 mg of p-hydroxybenzoic acid, and 15 mg of p-coumaric acid. Thus, more than 14% (215 mg) of the extra tyrosine fed was excreted in the urine as phenolic acids.

A species difference exists between rats and rabbits, in that the excretion of phloretic acid is not normally detected in the urine of rabbits on a purified diet, whereas it is readily detected in the case of the rat. Both species, however, excrete p-hydroxyphenyllactic acid and p-hydroxybenzoic acid on purified diets containing casein as a protein source. Urine from humans ingesting a high protein diet, is similar to rabbit urine with respect to the absence of phloretic acid and the presence of p-hydroxyphenyllactic and p-hydroxybenzoic acids.

Adult albino rabbits were given L-tyrosine by stomach tube (2 g each), and urine was collected for a 24-hour period. Ether extracts of the acidified urine were chromatographed as described above for rat urine. No phloretic acid was detected on the chromatogram, but slight increases in the excretion of both p-hydroxyphenyllactic and p-hydroxybenzoic acids were observed.

When p-hydroxyphenylpyruvic acid was administered to rabbits (0.9 g each), an area corresponding to phloretic acid (Table II) was clearly evident. The amount of phloretic acid excreted in the 24 hour urine sample was in excess of 3 mg per rabbit. The amounts of p-hydroxyphenyllactic and p-hydroxybenzoic acids were increased in this same urine from control values of 18 and 9 mg to 36 and 45 mg, respectively. An increase in the excretion of p-hydroxyphenyllactic acid was also noted.

Finally, when p-hydroxyphenyllactic acid was given to rabbits (0.9 g each), more than 3 mg of phloretic acid were excreted. In the same urine the amount of p-hydroxybenzoic acid including

<table>
<thead>
<tr>
<th>Compound given</th>
<th>Amounts of phenolic acids excreted in rat urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (control)</td>
<td>0.4 mg tyrosine, 2.0 mg p-hydroxyphenylpyruvic acid, 5.2 mg p-hydroxyphenyllactic acid, 0.6 mg p-coumaric acid</td>
</tr>
<tr>
<td>2.0 mg tyrosine</td>
<td>0.2 mg tyrosine, 0.2 mg p-hydroxyphenylpyruvic acid, 0.4 mg p-hydroxyphenyllactic acid, 1.0 mg p-coumaric acid</td>
</tr>
<tr>
<td>0.5 mg tyrosine</td>
<td>0.2 mg tyrosine, 0.4 mg p-hydroxyphenylpyruvic acid, 0.6 mg p-hydroxyphenyllactic acid, 25.0 mg p-coumaric acid</td>
</tr>
</tbody>
</table>

These results indicate that the excretion of phloretic acid is not normally detected in the urine of rabbits on a purified diet, whereas it is readily detected in the case of the rat. Both species, however, excrete p-hydroxyphenyllactic acid and p-hydroxybenzoic acid on purified diets containing casein as a protein source. Urine from humans ingesting a high protein diet, is similar to rabbit urine with respect to the absence of phloretic acid and the presence of p-hydroxyphenyllactic and p-hydroxybenzoic acids.

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**TABLE I**

**Chromatographic behavior of tyrosine and related compounds**

<table>
<thead>
<tr>
<th>Compound</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Tyrosine</td>
<td>0.00</td>
<td>0.84</td>
<td>0.00</td>
</tr>
<tr>
<td>p-Hydroxyphenylpyruvic acid</td>
<td>0.13</td>
<td>0.90</td>
<td>0.13</td>
</tr>
<tr>
<td>p-Hydroxyphenyllactic acid</td>
<td>0.11</td>
<td>0.76</td>
<td>0.11</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>0.61</td>
<td>0.70</td>
<td>0.61</td>
</tr>
<tr>
<td>p-Coumaroylglycine</td>
<td>0.50</td>
<td>0.27</td>
<td>0.50</td>
</tr>
<tr>
<td>p-Hydroxyphenyllactic acid</td>
<td>0.16</td>
<td>0.50</td>
<td>0.16</td>
</tr>
<tr>
<td>2,5-Dihydroxyphenylacetic acid (homogenticetic)</td>
<td>0.44</td>
<td>0.78</td>
<td>0.44</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid</td>
<td>0.09</td>
<td>0.76</td>
<td>0.09</td>
</tr>
<tr>
<td>p-Hydroxybenzoic acid-OSOsH</td>
<td>0.08</td>
<td>0.77</td>
<td>0.08</td>
</tr>
</tbody>
</table>

- **A** represents the absorbance at 254 nm.
- **B** represents the absorbance at 280 nm.
- **C** represents the color with 1% sulfuric acid-sodium carbonate.
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its ethereal sulfate was increased to 45 mg, but the amount of p-hydroxyphenylacetic acid was not increased (18 mg). Approximately one-fifth of the p-hydroxyphenyllactic acid administered was excreted unchanged.

**DISCUSSION**

A scheme summarizing the proposed pathways by which tyrosine is converted to p-hydroxyphenylacetic and p-hydroxybenzoic acids is shown in Fig. 1. The interrelationships for these compounds are based on the results obtained when the individual compounds were given to rats or rabbits. It should be emphasized that this scheme is simply a working hypothesis which satisfactorily accounts for the observations reported herein (Table II). The directions of the arrows in Fig. 1 indicate only end results, rather than completely balanced reactions.

The conversion of p-hydroxyphenyllactic acid to p-coumaric acid appears to be a new metabolic reaction. Experiments involving radioactive substrates and incubations in vitro are being designed to study in more detail the biological dehydration of a phenolic α-hydroxy acid. Another example of a new metabolic reaction was encountered recently when we discovered the dehydration in vivo of specific phenolic hydroxyl groups (12). Homogentisic acid was not detected, in that m-hydroxyhippuric acid was primarily excreted by humans, whereas the rat preferentially excreted m-hydroxyphenylpropionic acid after the ingestion of chlorogenic acid (3).

Homogentisic acid was not detected in the urine in these experiments even when its immediate precursor, p-hydroxyphenylpyruvic acid, was administered to rats. However, this does not mean that homogentisic acid was not formed; rather it was rapidly metabolized to other products no longer detectable by the procedures used here. Supporting this view is the finding that only traces of homogentisic acid were detected in the urine when a large single dose of it (100 mg per rat) was given. Bernhart and Zilliken also concluded that only small amounts of homogentisic acid were excreted, as compared to the total phenol and phenolic acid fractions when rats were fed up to 10% tyrosine in the diet (2).

The p-hydroxybenzoic and p-hydroxyphenylacetic acid pathways of tyrosine metabolism described here are of fundamental biochemical interest for at least three reasons: (a) a satisfactory explanation for the occurrence of these phenolic acids in the urine of normal animals is now available; (b) at least one-seventh of the extra tyrosine ingested by normal rats was excreted via these two pathways; and (c) in the presence of defects in the metabolism of phenylalanine and tyrosine via homogentisic acid, the newly proposed pathways may assume an even more significant role. Regarding the latter point (c), a report by Aterman et al. (15) indicates that such is the case. These workers found that, "in contrast to normal animals, scorbute guinea pigs excreted in the urine p-hydroxyphenylacetic acid as well as smaller amounts of p-hydroxybenzoic acid."

Abolishment of the urinary excretion of p-hydroxyphenylacetic, p-hydroxyphenyllactic, and phloretic acids by feeding chloretone in the diet of rats was reported by Boscott and Greenberg (14). Since our attempts to confirm this effect were negative, we therefore do not concur with the suggestion that the urinary excretion of p-hydroxyphenylacetic acid may be regarded as an early indication of suboptimal intake of ascorbic acid.

**SUMMARY**

Metabolic pathways involving the conversion of tyrosine to p-hydroxyphenylacetic acid and p-hydroxybenzoic acid in the animal body are presented. At least four urinary phenolic acid intermediates have been identified, including p-hydroxyphenylpyruvic, p-hydroxyphenyllactic, phloretic, and p-coumaric.
Phloretic acid was readily detected in the urine of rats, but not in the urine of rabbits unless p-hydroxyphenylpyruvic acid was given, indicating a more efficient conversion of phloretic acid to p-hydroxybenzoic acid by the latter species. A new metabolic reaction involving the conversion of p-hydroxyphenyllactic acid to p-coumaric acid is suggested by this work.

REFERENCES
Urinary Phenolic Acid Metabolites of Tyrosine
Albert N. Booth, Merle S. Masri, Dorothy J. Robbins, Oliver H. Emerson, Francis T. Jones and Floyd DeEds