A COMPARATIVE STUDY OF THE END-PRODUCTS OF URIC ACID OXIDATION BY PEROXIDASES*

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Uric acid has long been considered to be a metabolically inert end-product of purine metabolism in the human (1, 2). This view has been challenged by Folin, Berglund, and Derick (3), who, in an extensive review of the subject, reported that only about 50 per cent of intravenously administered uric acid in man could be recovered. More recently, with the use of N15-labeled uric acid, Benedict, Forsham, and Stetten (4) calculated that only 70 to 80 per cent of the uric acid formed each day by normal humans could be isolated in the urine. In more recent experiments, Wyn-gaarden and Stetten (5) have shown that, after administering 1000 mg. of uric acid-1,3-N15 to a normal human, 17 per cent of the administered N15 could be recovered as urinary urea and 1 per cent as urinary ammonia, thus establishing uriculysis in the human. These may be considered to be low values, since they do not take into consideration the initially high elimination of the administered uric acid owing to the large dose administered. The questions are therefore raised as to what enzyme systems are involved, the mechanism of uric acid oxidation, and the end-products of this degradation.

In the present investigation it will be shown that uric acid can be oxidized in the presence of the following enzyme systems: lactoperoxidase, verdoperoxidase, horseradish peroxidase, and catalase. Some aspects of the mechanism of these oxidations have been studied and the end-products identified.

EXPERIMENTAL

2-C14- and 8-C14-labeled uric acids with specific activities of $1.02 \times 10^6$ c.p.m. per mg. and $0.9 \times 10^6$ c.p.m. per mg., respectively, were prepared as described in a previous communication (6). Lactoperoxidase was prepared by the method of Polis and Shmukler (7) up to the stage prior to

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crystallization. The protein concentration was 7.5 mg. per ml. of activity, and 1056.6 units per ml. were measured according to the method of Schultz et al. (8). Because of the method of preparation, borate is present as a contaminant in this lactoperoxidase preparation. Verdoperoxidase prepared from rat chloroma tissue (8) was obtained through the courtesy of Dr. J. Schultz, Temple University, Philadelphia. The protein concentration of this preparation was 3.7 mg. per ml.; activity, 370 units per ml. (8). Crystalline catalase was prepared by a slight modification of the method of Tauber and Petit (9). It had an activity of 14 catalase units per ml. (10). Horseradish peroxidase was obtained through the courtesy of Dr. R. H. Kenten, Rothamsted Experimental Station, Harpenden, Herts, England. It was prepared by the method of Kenten and Mann (11), and had an activity of purpurogallin number = 1040. Notatin (glucose oxidase) (batch No. N/14) was obtained from Boots Pure Drug Company, Ltd., Nottingham, England. Ethyl hydrogen peroxide (EtOOH) was prepared according to the method of Baeyer and Villiger (12). The initial distillate was made basic with KOH and redistilled until one-third of the volume was left. The residue was then acidified, distilled, and collected in three fractions. It was used as a 10 to 15 per cent aqueous solution. Oxalyl diurea was prepared from allantoin according to the method of Biltz and Topp (13) (N found, 30.0%; theory, 30.4). Carbonyl diurea was prepared from uric acid according to the method of Ohta (14) (N found, 38.3%; theory, 38.6). Cyanuric acid was prepared by refluxing cyanuryl chloride for 5 hours with a large excess (1:30) of glacial acetic acid. It was allowed to stand at room temperature overnight, filtered, and recrystallized from water (N found, 32.5%; theory, 32.6). Parabanic acid was prepared by Asche's (15) modification of Menschutkin's (16) procedure (N found, 24.1%; theory, 24.5). Ammonium oxalurate was prepared from parabanic acid according to the method of Wöhler and Liebig (17) (N found, 28.5%; theory, 28.2). Oxaluric acid was obtained from ammonium oxalurate by acidification (17) (N found, 21.4%; theory, 21.2).

The chromatographic techniques previously described (18) as well as crystallization to constant specific activity were used for the identification of the uric acid breakdown products.

**Incubation Conditions; Catalase—**0.4 ml. of catalase and 0.4 ml. of EtOOH were added to a 2.5 ml. solution of 1.5 mg. of labeled uric acid in 0.1 M phosphate buffer, pH 7.2; final pH, 6.7 to 6.8; incubation at 38° for 30 minutes.

**Lactoperoxidase and Verdoperoxidase—**110 units of lactoperoxidase or verdoperoxidase were incubated with 5 mg. of notatin, 10 mg. of glucose,

1 The purified lactoperoxidase was prepared by Mr. E. A. Steberl of this Department.
and 1 mg. of labeled uric acid in 3.0 mg. of 0.1 M phosphate buffer, pH 7.3, for 1 hour at 38°; final pH, 7.1.

**Horseradish Peroxidase**—1 mg. of horseradish peroxidase was incubated with 1 mg. of uric acid-2-C\(^{14}\) under the same conditions as described for lactoperoxidase and verdoperoxidase.

**Results**

*Catalase Plus Ethyl Hydrogen Peroxide*—No appreciable decomposition of uric acid could be demonstrated spectrophotometrically in the presence of catalase and a hydrogen peroxide-generating system composed of notatin and glucose at pH 7.2. Keilin and Hartree (19) reported that ethyl hydrogen peroxide could be used in the presence of catalase to oxidize ethanol to acetaldehyde. Preliminary experiments indicated no effect of catalase or EtOOH alone on uric acid. Spectrophotometric data revealed that incubation of uric acid with catalase and EtOOH in 0.1 M phosphate buffer, pH 7.2, led to a rapid disappearance of the 293 m\(\mu\) uric acid peak and to the concurrent appearance of an absorption peak at 325 m\(\mu\) (20).

Incubation of uric acids-2-C\(^{14}\) and -8-C\(^{14}\) with the catalase-EtOOH system led to a large number of oxidation products (Table I) which were identified. It will be noticed that a common characteristic of these compounds is the absence of C-6 of uric acid, indicating that their formation took place subsequent to the loss of this carbon.

Radioactive parabanic acid and its open ring form, oxaluric acid, could be isolated only from the catalase-EtOOH system in which uric acid-8-C\(^{14}\) was oxidized.

*Lactoperoxidase*—Lactoperoxidase (which contains borate as a contaminant), when incubated in the presence of an \(\text{H}_2\text{O}_2\)-generating system and labeled uric acid in phosphate buffer, yields among the oxidation products 5-ureido-2-imidazolidone-4,5-diol-4-carboxylic acid (UIDC, Formula II, Fig. 1), alloxanic acid, and a radioactive compound responsible for Peak A (Table II). Similar results have been previously obtained (18) from experiments in which borate buffer was present in a uricase system. The radioactive UIDC has the same properties as UIDC obtained from the uricase-borate system. It decomposes to C\(^{14}\)-labeled urea when obtained from an incubation mixture containing uric acid-8-C\(^{14}\), and to C\(^{14}\)-labeled alloxanic acid when obtained from an incubation mixture containing uric acid-2-C\(^{14}\). These results have been previously summarized (18).

It will be noted that an acidic component is present in the lactoperoxidase system, accounting for about 40 per cent of the total radioactivity. This acidic component can be eluted from the Dowex 1 column with concentrated formic acid. Initial attempts at purification have indicated that more than one component is present.

*Verdoperoxidase and Horseradish Peroxidase*—When C\(^{14}\)-uric acid is
<table>
<thead>
<tr>
<th>Oxidation Products of Uric Acid-2-C$^{14}$ and Uric Acid-8-C$^{14}$ by Catalase-Ethyl Hydrogen Peroxide System*</th>
<th>URIC ACID 2-C$^{14}$</th>
<th>URIC ACID 8-C$^{14}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>UREA</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>$\text{NH}_2$ $\text{CO}$ $\text{NH}_2$</td>
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</tr>
<tr>
<td>ALLANTOIN</td>
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<td>3</td>
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<tr>
<td>$\text{HN}-\text{CO}$ $\text{OC}$ $\text{HN CH}$ $\text{H}_2\text{NOCNH}$</td>
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<td></td>
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<tr>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>$\text{OCONHCONH}_2^-$ $\text{OCONHCONH}_2^-$</td>
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<td></td>
</tr>
<tr>
<td>CARBONYL DIUREA</td>
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<td>16</td>
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<td>$\text{HNCONH}_2^-$ $\text{CO}$ $\text{HNCONH}_2^-$</td>
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<td></td>
</tr>
<tr>
<td>CYANURIC ACID</td>
<td>24</td>
<td>21</td>
</tr>
<tr>
<td>$\text{NH}$ $\text{OC'-CO}$ $\text{HN}$ $\text{NH}$ $\text{CO}$</td>
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<td></td>
</tr>
<tr>
<td>PARABANIC ACID</td>
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</tr>
<tr>
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<tr>
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<td>$\text{COOH}$ $\text{OC'}$ $\text{CO}$ $\text{NH}$ $\text{N}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO$_2$</td>
<td>10</td>
<td>2</td>
</tr>
</tbody>
</table>

* Values expressed as per cent of the total eluted radioactivity.
incubated with verdoperoxidase or horseradish peroxidase, the end-products consist only of urea and allantoin (Table III). This finding is similar to the results obtained with uricase in the presence of phosphate buffer (18). In the presence of borate buffer, the verdoperoxidase system produces alloxanic acid.

Leucocyte suspensions prepared from sterile abscesses in rats behave like the verdoperoxidase system in phosphate buffer in that they oxidize uric acid to urea and allantoin (unpublished experiments).

When C\textsuperscript{14}-allantoin, isolated from the enzymatic decomposition of uric acid, was incubated in the presence of verdoperoxidase and notatin under conditions similar to those already described, no decomposition of allantoin was noted.

**DISCUSSION**

The peroxidases, verdoperoxidase and horseradish peroxidase, seem to attack uric acid in a manner qualitatively similar to that of uricase in phosphate buffer, although quantitative differences may exist. Here, as in the uricase-phosphate system, the only major end-products are urea and allantoin. The lactoperoxidase system which contains borate shows deviations from the other peroxidases similar to those obtained by the addi-
tion of borate to the uricase system. Alloxanic acid and UIDC are again apparent. In contrast to the uricase-borate system, an appreciable percent of the radioactivity can be accounted for in the form of unidentified acidic components in the lactoperoxidase system.

![Chemical structure diagram](image)

**Fig. 1**

The catalase-ethyl hydrogen peroxide system seems to represent a very much stronger peroxidative system than any of the others studied. While urea and allantoin are formed, a large number of allantoin oxidation products are also produced. In this system, in which the oxidation of uric acid proceeds to such an extent as to form a large number of end-products, including CO₂, the relative amounts of compounds formed have little meaning, since these are bound to change with the time of incubation and the amount of EtOOH available. The major difference between this system and the other peroxidases is that with the catalase-ethyl hydrogen peroxide system more than 1 atom of oxygen appears to be effectively utilized for the oxidation of uric acid, while with the other peroxidase systems, by forming end-products similar to those of uricase, only 1 atom of oxygen appears to be used effectively, in spite of the fact that about 10 atoms are available.

The biological significance of these results is at present difficult to evaluate. Folin et al. (3), in their review on uricolysis, had reached the conclusion that a non-dialyzable blood component and a small molecular weight tissue component react in the blood to decompose uric acid. In terms of the present discussion, these components could be thought of as a peroxidase and H₂O₂. Our results indicate that allantoin is a major end-
product of uric acid oxidation in a peroxidase-H₂O₂ system. In contrast, the experiments in vivo of Wyngaarden and Stetten show that, when N¹⁵-uric acid is administered to humans, the major non-uric acid component in the urine is urea. If peroxidases are responsible for the oxidation of uric acid in humans, then systems must be available to carry out the further oxidation of allantoin, since peroxidases in the presence of H₂O₂ will not decompose allantoin as previously mentioned. It is possible that in vivo the oxidation of uric acid goes beyond the allantoin stage, or that a catalase-peroxide system is responsible for the further oxidation of the latter.

An additional uricolytic system to be considered in organisms lacking uricase is cytochrome oxidase. Griffiths (21) has found that uric acid is oxidized by a crude beef heart cytochrome oxidase system at pH 7.9.

The results on the uric acid oxidation at neutral pH seem to exclude the possibility of alloxan being a precursor of alloxanic acid under our conditions. Rather, they indicate UIDC as a more direct precursor. Paul and Avi-Dor (22) have recently postulated the existence of an intermediate formed during the oxidation of uric acid by horseradish peroxidase and H₂O₂ at pH 3 to 5. This is converted to alloxan at pH values <1 or to allantoin at pH 6 to 7. The glycol structure which they propose (Formula I, Fig. 1) is compatible with its being a precursor of UIDC (Formula II). These results indicate that the scheme for uric acid oxidation which these authors propose may be modified as indicated in Fig. 1 in which D would represent the "allantoin precursor" found in the uricase system.

**SUMMARY**

1. The oxidative decomposition of uric acid-2-C¹⁴ and uric acid-8-C¹⁴ by a catalase-ethyl hydrogen peroxide system and by systems of lactoperoxidase-H₂O₂, verdoperoxidase-H₂O₂, and horseradish peroxidase-H₂O₂ has been studied at neutral pH.

2. The catalase-ethyl hydrogen peroxide system oxidizes uric acid beyond the allantoin stage. Among the labeled end-products of uric acid-2-C¹⁴ oxidation found are urea, allantoin, carbonyl diurea, oxalyl diurea, cyanuric acid, oxonic acid, and CO₂. Uric acid-8-C¹⁴ yields in addition labeled parabanic acid and oxaluric acid.

3. The lactoperoxidase-H₂O₂-borate system yields urea, allantoin, UIDC, and alloxonic acid in contrast to the verdoperoxidase-H₃O₂-phosphate and horseradish peroxidase-H₂O₂-phosphate systems, which yield only urea and allantoin.

4. The relationship of these systems, their possible biological significance, and some aspects of the mechanism of uric acid oxidation are discussed.
BIBLIOGRAPHY

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