BIOLOGICAL PRECURSORS OF URIC ACID*

I. THE RÔLE OF LACTATE, ACETATE, AND FORMATE IN THE SYNTHESIS OF THE UREIDE GROUPS OF URIC ACID

BY JOHN C. SONNE, JOHN M. BUCHANAN, AND ADELAIDE M. DELLUVA

(From the Department of Physiological Chemistry, School of Medicine, University of Pennsylvania, Philadelphia)

(Received for publication, September 10, 1947)

Search for the biological sources of the ureide carbon atoms of uric acid has centered chiefly around compounds similar in structure to the ureide group of uric acid. One of the earliest studies on the precursors of uric acid was made by Wiener (3), who postulated that uric acid was formed by the condensation of 2 moles of urea with 1 of tartaronic acid. Although this hypothesis was at first attractive, it has, however, been disproved as the mechanism of the biosynthesis of uric acid by several investigators using both in vivo and in vitro techniques (4–10).

Pyrimidines (11), which are likewise related to purines, have also been excluded as precursors of uric acid, since they are oxidized almost quantitatively to urea by the mammal. More recent isotopic experiments of Plentl and Schoenheimer (12) also provide convincing evidence that pyrimidines do not participate in uric acid synthesis.

A more serious difference of opinion, however, has persisted among investigators until recently concerning histidine and arginine as the precursors of purines or purine derivatives. The opening investigations on this subject were made by Ackroyd and Hopkins (13), who postulated that the imidazole nucleus of allantoin could be derived from either of these amino acids. Subsequent data obtained by comparing uric acid or allantoin excretion of animals fed diets high and low in these amino acids have not been altogether conclusive or in agreement (14–19). Results obtained by the isotope tracer technique, however, do not confirm the point of view that histidine or arginine may be precursors of uric acid (10). The feeding of N15-labeled ammonium citrate to rats and pigeons led to the incorporation of N15 into purines of the tissues and uric acid or allantoin of the excreta in much greater concentrations than in histidine or arginine of the tissue proteins. Moreover, uric acid isolated after the administration to the pigeon of arginine labeled with N15 did not contain significant amounts of N15 (20). These findings have led to the conclusion that the

* A preliminary report of these studies has been published (1, 2). Aided by a grant from the American Cancer Society.
reactions proposed by Krebs and Henseleit (21) for the synthesis of urea in the rat do not apply to the synthesis of a structurally related substance, the ureide group of uric acid.

The demonstration by Barnes and Schoenheimer (10) that N\(^{15}\) of ammonium citrate is readily incorporated into uric acid has suggested that the ureide carbon might be derived from some simple metabolic unit. In order to test this possibility several isotopic compounds important in intermediary metabolism, namely C\(^{13}\)O\(_2\), HC\(^{18}\)OOH, CH\(_3\)C\(^{13}\)OOH, NH\(_2\)-CH\(_2\)C\(^{14}\)OOH, CH\(_3\)CHOHC\(^{13}\)OOH, and C\(^{13}\)H\(_3\)C\(^{13}\)HOHCOOH, were prepared and administered to pigeons. The excreted uric acid was degraded by chemical procedures and information has been gained concerning the origin of each of the carbon atoms of uric acid. In the present paper experiments dealing with the sources of the ureide carbon will be reported. In the following paper experiments concerning the precursors of the carbon chain of uric acid are described.

**EXPERIMENTAL**

*Organic Syntheses*—Isotopic compounds were synthesized for the most part by procedures described by Sakami, Evans, and Gurin (22). These procedures consisted of standard organic reactions modified for synthesis of compounds with C\(^{13}\). Carboxyl-labeled acetate was prepared from isotopic CO\(_2\) and methyl iodide by the Grignard reaction, carboxyl-tagged glycine by the hydrolysis of ethyl phthalimidoacetate (23) prepared from potassium phthalimide and carboxyl isotopic ethyl bromoacetate. This latter compound was synthesized according to the method of Auwers and Bernhardi (24). Carboxyl-labeled lactate was synthesized according to the method of Cramer and Kistiakowsky (25), \(\alpha\)- or \(\beta\)-tagged lactate by the method of Cramer and Kistiakowsky (25) as modified by Sakami, Evans, and Gurin (22). Formic acid was prepared by the hydrolysis of hydrogen cyanide (26).

**Procedure**

Pigeons used in this experiment weighed 295 to 323 gm. with an average weight of 310 gm. All birds had been fasted for 1 day. During the experiment the pigeon was kept on a wire screen in a large desiccator through which was passed a stream of CO\(_2\)-free air. Collections of respiratory CO\(_2\) were made at 15, 30, 60, or 120 minute intervals during the experiment by passing the respiratory gases through a solution of NaOH and by precipitating the carbonate as BaCO\(_3\).

3 cc. of a solution containing 20 mg. of N\(^{14}\)H\(_4\)Cl (3.7 atoms per cent excess) and from 0.5 to 1.0 mm of the isotopic compound were alternately fed or injected intraperitoneally at intervals of 2 hours. Acidic substances
were administered as the sodium salt. Glycine and the two types of isotopic lactate were administered at the rate of 0.5 mM per hour, NaHCO₃ and formate at the rate of 0.75 mM per hour, and acetate at the rate of 1 mM per hour. The method of administration of solutions was changed in the experiment with NaHC¹³O₃. In this case 2.5 cc. of a solution containing 0.38 mM of NaHC¹³O₃, 10 mg. of N¹⁵H₄Cl, and 25 mg. of glucose were administered every 30 minutes. The duration of the experiments was from 16 to 20 hours, except in the experiments with glycine and formate. Because of the small amount of glycine available to us, four administrations were made over a period of 8 hours and the experiment was not concluded until the end of the 10th hour. The experiment with formate was concluded at the end of the 12th hour. In the experiments with acetate, formate, and lactate the pigeons were sacrificed and the contents of the peritoneal cavity and enteric tract collected. These washings were cleared with colloidal iron and copper-lime and the unabsorbed volatile acids or lactate were determined by the methods of Friedemann (27) and Friedemann and Graeser (28) respectively. From these determinations the amounts of these substances absorbed during the experimental period were calculated. 93, 94, 78, and 91 per cent of the administered acetate, formate, carboxyl-labeled lactate, and α,β-tagged lactate respectively were absorbed.

In a supplementary experiment performed with a rat, 3 cc. of a solution containing 1 mm of carboxyl-labeled acetate, 50 mg. of glucose, and 30 mg. of NH₄Cl were fed every hour for a period of 4 hours. At the conclusion of the experiment urea carbon was liberated from the urine as CO₂ by urease. Samples of respiratory CO₂ were also collected.

In the experiments with pigeons uric acid was isolated from the excreta and purified by the method of St. John and Johnson (29) as modified by Fisher (30). Uric acid obtained by this procedure was suspended in 40 cc. of water, dissolved with a minimum amount of NaOH, decolorized with carboraffin, and reprecipitated with HCl. This procedure was repeated until a pure white product was obtained.

**Degradation of Uric Acid**—The conventional numbering system, shown in Scheme 1 of the following paper, has been used to designate the position of the carbon atoms of the uric acid molecule. Carbons 2 and 8, representing the ureide carbons of the pyrimidine and imidazole rings of uric acid respectively, may be separated from the other carbons of uric acid and from each other by appropriate degradation procedures. The first procedure is a modification of a method described by Edson and Krebs (31). The exact details of the method of degradation of uric acid with alkaline MnO₂ into CO₂, glyoxylic acid, and urea may be found in the accompanying paper. Urea produced by the alkaline oxidation of uric acid was extracted
by a suitable procedure and converted into CO₂ by urease (32). Since this urea is derived equally from carbons 2 and 8, isotopic analysis of this fraction gives an average of the C¹³ concentration of these 2 carbon atoms. When this urea contained no excess of isotope, it was concluded that neither carbon 2 nor 8 was isotopic and further degradation procedures were usually not employed. If, however, this fraction contained C¹³, a second oxidation procedure was used by which it was possible to obtain carbons 2 and 8 separately (33). In this procedure urea containing carbon 8 was obtained by oxidizing uric acid with KClO₃ in strong HCl (34). Alloxan, the other product of the reaction, was reduced to alloxantin with H₂S (34) and the insoluble crystalline alloxantin was isolated. Alloxantin was subsequently oxidized by PbO₂ to urea containing carbon 2 (34). The oxidation with PbO₂, a modification of a procedure described by Liebig and Wöhler, was carried out at 100° for 20 minutes. The urea fractions obtained in each case were treated with urease to liberate CO₂.

**Results**

**Oxidation of Administered Substances**—No attempt was made in these studies to follow in detailed manner the rate of oxidation of the various substances administered to pigeons. In previous studies on rats it has been found that 2 hours after the administration of carboxyl-labeled acetate (35), carboxyl-labeled lactate (36), α- and β-labeled lactate (37), and carboxyl-labeled glycine (38) respectively, 36, 16, 8, and 4 per cent of the administered isotope are liberated as CO₂ in the respiratory gases. Since in the present experiments measurement was not made of the total amount of C¹³ excreted, it is not possible to express C¹³ excreted in terms of per cent of the amount administered. However, an approximate estimation of the rate of oxidation of the administered substance may be made by comparing their “coefficients of oxidation.” The “coefficient of oxidation” is arbitrarily defined as

\[
100 \times \frac{\text{C}^{13}\text{ concentration of CO}_2 \text{ of respiratory gases}}{\text{mm excess CO}_2 \text{ given per hr.}}
\]

where millimoles of excess C¹³ given per hour \(\times 100 = \text{C}^{13}\) concentration (atoms per cent excess) of carbon atoms labeled \(\times\) the number of atoms labeled \(\times\) the rate of administration of isotopic compound.

As may be seen in Column 8 of Table I, the carboxyl carbon atoms of acetate and glycine and the α- and β-carbon atoms of lactate are all excreted as CO₂ by the pigeon to approximately the same extent. There is the possibility that the carboxyl carbon atom of lactate is excreted at a rate somewhat greater but in the same order of magnitude as the labeled carbon atoms of the other compounds.
Since the metabolism of isotopic formate has not been studied previously in vivo, a more thorough investigation was made of the rate of oxidation of this substance. In Table II is reported the $^{13}$C concentration of the

**Table I**

**Precursors of Ureide Carbon Atoms of Uric Acid in Pigeon**

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Precursor</th>
<th>Rate given (mM per hr)</th>
<th>$^{13}$C concentration (atoms per cent excess)</th>
<th>Oxidation respiratory coefficient (CO$_2$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>(2)</td>
<td>(3)</td>
<td>(4) (5) (6)</td>
<td>(7)</td>
</tr>
<tr>
<td>1†</td>
<td>C*O$_2$</td>
<td>0.75</td>
<td>8.13 0.02</td>
<td>0.28</td>
</tr>
<tr>
<td>2†</td>
<td>HC*OOH</td>
<td>0.75</td>
<td>3.34 2.41</td>
<td>0.01</td>
</tr>
<tr>
<td>3</td>
<td>CH$_3$C*OOH</td>
<td>1.00</td>
<td>5.82 2.10</td>
<td>0.26</td>
</tr>
<tr>
<td>4</td>
<td>NH$_2$CH$_2$C*OOH</td>
<td>0.50</td>
<td>5.20 0.00</td>
<td>0.12</td>
</tr>
<tr>
<td>5†</td>
<td>dl-CH$_3$CHOHC*OOH</td>
<td>0.50</td>
<td>5.80 0.01</td>
<td>0.25</td>
</tr>
<tr>
<td>6</td>
<td>dl-C<em>H$_3$C</em>HOHCOOH</td>
<td>0.50</td>
<td>5.40 0.10</td>
<td>0.11</td>
</tr>
</tbody>
</table>

C* = carbon atom labeled with $^{13}$C.
† The values reported for the $^{13}$C concentrations of uric acid carbons 2 and 8 in Experiments 1, 2, and 5 are the $^{13}$C concentrations of the mixed urea carbons. Further degradation procedures to separate carbons 2 and 8 were not performed in these experiments.

**Table II**

**Appearance of Isotopic CO$_2$ in Respiratory Gas after Administration of Isotopic Formate to Pigeon**

3 cc. of a solution containing 8.7 mM of isotopic formate (3.34 atoms per cent excess $^{13}$C), 0.75 gm. of glucose, and 6.3 mM of NH$_4$Cl in a total volume of 18 cc. were administered six times at 2 hour intervals to a 360 gm. pigeon.

<table>
<thead>
<tr>
<th>Hrs. after 1st administration</th>
<th>$^{13}$C concentration of respiratory CO$_2$ (atoms per cent excess)</th>
<th>Hrs. after 1st administration</th>
<th>$^{13}$C concentration of respiratory CO$_2$ (atoms per cent excess)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.01</td>
<td>6</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.02</td>
<td>7</td>
<td>0.00</td>
</tr>
<tr>
<td>3</td>
<td>0.00</td>
<td>8</td>
<td>0.02</td>
</tr>
<tr>
<td>4</td>
<td>0.01</td>
<td>9</td>
<td>0.01</td>
</tr>
<tr>
<td>5</td>
<td>0.02</td>
<td>10</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Average .......................................................... 0.013

respiratory CO$_2$ of samples taken every hour for the first 10 hours of the 12 hour experiment. As may be seen, the $^{13}$C concentration of the CO$_2$ is very low throughout the entire experiment and within the experimental
error of the mass spectrometer. When the average of the excess C\textsuperscript{13} found in these samples is used in the calculation of the “oxidation coefficient” (Table I), a value for the “oxidation coefficient” is obtained which is far below that of the other compounds. It would thus seem that under the conditions of this experiment formate is not further oxidized or, at most, very slowly oxidized to CO\textsubscript{2} by the pigeon. The absence of isotopic CO\textsubscript{2} in the respiratory gases was not due to lack of absorption of the isotopic formate. The inability of the pigeon to oxidize formate, however, does not mean that formate may not enter into metabolic reactions. As shown in Table I, formate participates in the synthesis of uric acid.

Metabolic Origin of Carbons 2 and 8 of Uric Acid—After the administration to pigeons of the six compounds listed in Table I, it was found that the carboxyl carbon atoms of acetate and formate and the α- or β-carbon atom of lactate contributed the ureide carbon atoms in the synthesis of uric acid, but that CO\textsubscript{2} and the carboxyl carbon atoms of lactate and glycine did not participate to any measurable extent. In two instances where C\textsuperscript{13} appeared in ureide carbon of uric acid after the administration of isotopic compounds (i.e. Experiments 3 and 6), uric acid was degraded by the procedure which permitted the separation of carbon atoms 2 and 8 for isotopic analysis. The fact that the concentrations of C\textsuperscript{13} of carbon atoms 2 and 8 in both experiments are essentially equal argues for the belief that both ureide carbon atoms are formed from the same source (i.e., acetate, lactate, or formate) and that a part of the uric acid molecule is not derived preferentially from a pyrimidine or imidazole ring compound of the body tissues or diet.

From the concentration of C\textsuperscript{13} in the carbon of the substances administered and in carbon atoms 2 and 8 of the uric acid isolated and from other data reported in the literature, an estimation may be made of the extent to which the above compounds participate in uric acid synthesis. After the administration of formate containing 3.34 atoms per cent C\textsuperscript{13} to a pigeon, uric acid was isolated containing 2.41 atoms per cent excess C\textsuperscript{13} in the ureide carbon atoms. It may thus be calculated that 72 per cent of the ureide carbon of the newly formed uric acid was derived from the administered formate. In addition to formate which is readily utilized in the synthesis of ureide, the carboxyl carbon of acetate is also an important precursor of this group, an indication that formate and acetate may be closely linked in avian metabolism. After the administration of acetic acid labeled in the carboxyl group with 5.82 atoms per cent excess C\textsuperscript{13}, it was found that the uric acid isolated contained 2.02 and 2.10 atoms per cent excess C\textsuperscript{13} in the 2 and 8 positions respectively. This demonstrates that the \((2.10/5.82) \times 100\) or approximately 35 per cent of the ureide carbon of uric acid was derived from the acetate administered.
From the experiments of Bloch and Rittenberg (39) it is known that a 300 gm. rat may form 50 mm of acetate per day. Although the difference of experimental conditions makes it difficult to estimate from these data the amount of acetate formed by a 300 gm. pigeon, a rough approximation may be made to ascertain the order of magnitude. Since Experiment 3 was of 16 hour duration, the pigeon may have formed as much as 33 mm of acetate. Since 16 mm of isotopic acetate were administered during this time, 1 part of isotopic acetate may have been diluted by 2 parts of metabolically produced non-isotopic acetate. The $^{13}C$ concentration of the carboxyl carbon of acetate which takes part in metabolic reactions would be approximately one-third that of the carboxyl carbon of the administered acetate or 1.91 atoms per cent excess. This latter value approximates very closely the value of the $^{13}C$ concentration of the ureide carbon atoms of uric acid. These calculations may indicate that acetate is the mother substance from which ureide carbon is derived and suggest that formate is either an intermediate between acetate and the ureide group or may readily be converted into an intermediate of this reaction.

Isotopic carbon of $\alpha,\beta$-labeled lactate is probably converted into ureide carbon by virtue of the fact that the carboxyl carbon of acetate may be derived from the $\alpha$-carbon of lactate. The utilization of the $\alpha$-carbon of lactate for ureide synthesis is perhaps less than was expected. It should be noted that the experiments with $\alpha,\beta$-tagged lactate and ureide formation have only qualitative importance, in view of the fact that in this one instance the administered lactate was contaminated with non-isotopic formate which may have served to dilute isotopic ureide carbon formed by the metabolism of $\alpha,\beta$-tagged lactate by the bird. It was not appreciated at the time of the experiment with $\alpha,\beta$-tagged lactate that formate played a rôle in avian metabolism. In a subsequent experiment with carboxyl-tagged lactate, contaminating formate was removed from solution by an appropriate procedure.

Administration of Carboxyl-Labeled Acetate to Rat—In an experiment supplementary to those with pigeons, acetate labeled in the carboxyl carbon with 5.20 atoms per cent excess $^{13}C$ was fed to a rat at the rate of 1 mm per hour for 4 hours. Urea was collected at the mid-point of the experiment. It was found that the $^{13}C$ concentration of urea carbon was 0.27 atom per cent excess as compared to 0.50 atom per cent excess for the respiratory CO$_2$. Since CO$_2$ is a source of urea carbon both in vivo (40) and in vitro (21, 41, 42), it was concluded that the carboxyl carbon of acetate does not directly supply urea carbon in the rat. This is in contrast to the finding that the carboxyl carbon of acetate is a major source of the ureide carbon of uric acid in the pigeon.
DISCUSSION

Data presented in the present paper have served to confirm the belief of recent investigators that arginine and histidine are not direct sources of the ureide carbon of uric acid. To the already accepted fact that urea cannot be incorporated into uric acid has been added the information that the ureide group of uric acid in the pigeon and urea carbon in the rat derive their carbon from dissimilar sources. In addition, it has been demonstrated that formate may enter into avian metabolism and that the carboxyl carbon of acetate may be utilized in the biosynthesis of a group containing but 1 carbon atom. Although little is known about the formation of imidazole or pyrimidine rings in the living organism, there is a chemical precedent for the formation of imidazole ring structures by the condensation of diamino compounds with the carboxyl groups of fatty acids. Thus benzimidazole may be formed by the condensation of o-phenylenediamine and formic acid (43). The participation of both acetic and formic acids in uric acid formation in the bird indicates that a similar mechanism is involved in the biosynthesis of not only the imidazole but also the pyrimidine ring. The utilization of formate and the non-utilization of CO₂ suggest that a reduced form of uric acid (i.e., a purine) is an intermediate in uric acid synthesis. Örström, Örström, and Krebs (44) have shown that hypoxanthine may be formed in pigeon liver slices from simple metabolic units and have suggested that hypoxanthine is an intermediate of uric acid formation by the intact organism. Our data are in complete accord with this postulation. The hypothesis that the biosynthesis of the ureide groups of purines involves a condensation of a diamino compound and a fatty acid is supported by the recent work of Stetten and Fox, Shive and Roberts, and Shive et al. Stetten and Fox (45) have isolated in pure form an amine which accumulates in cultures of various bacteria as a result of the addition of sulfonamides. Shive and Roberts (46) have demonstrated that purines are capable of overcoming partially the bacteriostatic action of sulfonamides in certain instances and have postulated that the sulfonamides compete with a factor involved in the conversion of a purine precursor into the purine itself. This hypothesis has been recently borne out by Shive et al. (47), who have characterized the amine isolated by Stetten and Fox as 5(4)-amino-4(5)-imidazolecarboxamide. In view of the work reported from various laboratories, the utilization of fatty acid, CO₂, NH₃, and glycine (1) in the synthesis of 5(4)-amino-4(5)-imidazolecarboxamide and the further condensation of this compound with a second molecule of fatty acid to form hypoxanthine might be predictable steps in uric acid synthesis.

In order to reconcile the utilization of both formate and acetate in uric acid synthesis, the following series of reactions are tentatively proposed to explain the derivation of ureide carbon from acetate.
In these reactions it is postulated that the carboxyl group of acetate is first linked to nitrogen atoms with the formation of a methyl-substituted amidine structure. Upon further oxidation, a carboxyl group might be formed from the methyl carbon. Decarboxylation would yield the ureide carbons attached to hydrogen atoms as they are in the hypoxanthine molecule. This same structure might conceivably be formed directly by the condensation of the carboxyl group of formate with 2 atoms of nitrogen. It is evident that the above reactions are only hypothetical and require further investigation to establish their validity.

If formate enters into uric acid synthesis by virtue of its metabolic relationship to an intermediate in the reactions involved in the conversion of acetate carbon to ureide carbon, it may be concluded that this new oxidative pathway of acetate metabolism does not constitute a second mechanism for the complete oxidation of acetate to CO₂ and H₂O. This conclusion is based on the observation that the carbon of isotopic formate does not appear in the respiratory gases to a significant degree after its administration.

From the data presented in Table I, additional information concerning formate metabolism may be obtained. The fact that formate is an important source of the ureide carbon of uric acid and that CO₂ does not enter into this reaction to a measurable extent demonstrates that CO₂ may not be reduced to formate in the bird as it is in certain bacterial preparations (48). Furthermore the inability of the carboxyl carbon of lactate to serve as a source of ureide carbon demonstrates that formate is not a significant metabolic product of the carboxyl carbon of lactate or pyruvate in the bird as it is in the metabolism of some bacteria (49, 50).

**SUMMARY**

1. Six compounds labeled with C¹³ (i.e. C¹³O₂, HC¹³OOH, CH₄C²⁺OH, NH₂CH₃C¹³OOH, CH₃CHOHC¹³OOH, and C¹³H₂C¹³HOHCOOH) have been prepared and administered to fasted pigeons. The excreted uric acid was isolated, purified, and degraded by procedures which permitted the isolation of both the ureide carbon atoms for isotopic analysis.
2. The carboxyl carbon of acetate and formate and the α- (or β-) carbon of lactate may participate in ureide synthesis, but the labeled carbon atoms of the other compounds studied do not. It is believed that acetate is the biological source of the ureide carbon and that formate is either an intermediate or may readily be converted into an intermediate of this reaction. It is further believed that the isotopic carbon of α,β-labeled lactate is converted into ureide carbon by virtue of the fact that the carboxyl carbon of acetate may be derived metabolically from the α-carbon of lactate.

3. The present experiments demonstrate that formate may be utilized in avian metabolism, even though it is not oxidized to an appreciable extent.

4. The utilization of acetate in ureide formation, although a new oxidative path of acetate metabolism, probably does not constitute a second pathway for the complete oxidation of acetate to CO₂ and H₂O.

5. CO₂ and the carboxyl groups of lactate or pyruvate are not precursors of formate in the pigeon as they are in certain bacterial preparations.

6. In a supplementary experiment it was found that the carboxyl group of acetate is not a direct precursor of urea in the rat. These experiments demonstrate that the carbon of urea and ureide carbon of uric acid have different metabolic origins.

BIBLIOGRAPHY

5. Russo, G., Arch. sc. biol., 19, 384 (1933-34).
BIOLOGICAL PRECURSORS OF URIC ACID: I. THE RÔLE OF LACTATE, ACETATE, AND FORMATE IN THE SYNTHESIS OF THE UREIDE GROUPS OF URIC ACID

John C. Sonne, John M. Buchanan and Adelaide M. Delliua