The Mechanism for the Conversions of Uric Acid into Allantoin and Glycine.

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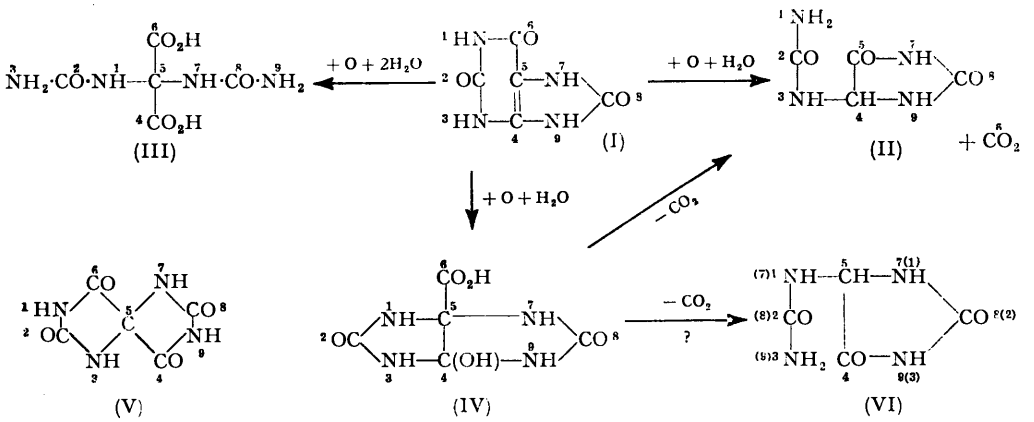
[Reprint Order No. 5372.]

Uric acids labelled with ^{14}C in positions 4, 5, and 6 have been prepared. Oxidation to allantoin with alkaline permanganate, which is probably mechanistically similar to the action of uricase, is accompanied by a rearrangement, the methylene-carbon atom of the hydantoin moiety of allantoin being derived from $C_{(4)}$ of uric acid and the adjacent carbonyl-carbon atom from $C_{(5)}$. On acid hydrolysis of uric acid to glycine, the glycine methylene-carbon atom is derived from $C_{(5)}$ of uric acid and the glycine carboxyl-carbon atom equally from $C_{(4)}$ and $C_{(6)}$. Possible mechanisms for these transformations are suggested.

Quantitatively, the most important product of the oxidation of uric acid (I) under slightly alkaline, neutral, or weakly acidic conditions is allantoin (II) which was first obtained by Liebig and Wöhler (*Annalen*, 1838, **26**, 285) on treatment of uric acid with lead oxide. Many other oxidising agents were later shown to produce allantoin as the main or even the sole product. It was at first thought that this reaction consisted simply of an oxidation of $C_{(5)}$ associated with the removal of $C_{(6)}$ as carbon dioxide, the hydantoin part of allantoin being derived exclusively from the glyoxaline moiety of uric acid. But this simple explanation was found to be untenable.



(In order to facilitate discussion the atoms of the intermediates and products will be numbered according to the carbon and nitrogen atoms of uric acid from which they are thought to be derived.)

Another oxidation product of uric acid, formed in relatively large quantities if the reaction is carried out in strongly alkaline solution, was discovered by Stadeler (*Annalen*, 1867, **78**, 286) and named by him uroxanic acid. The structure of this compound as diureidomalonic acid (III) was established much later by Behrend (*Annalen*, 1904, **333**, 150; see also Behrend and Schultz, *ibid.*, 1909, **365**, 23), but the mechanism of its formation from (I) is not immediately obvious. It appeared almost certain that the malonic acid portion of (III) is derived from $C_{(4)}$, $C_{(5)}$, and $C_{(6)}$ of uric acid and it thus followed that during or after the oxidation of uric acid to uroxanic acid either $N_{(1)}$ or $N_{(3)}$ had become attached to C_5. Evidence was also obtained to suggest that these two oxidation products are not formed by completely independent pathways. Both Behrend (*loc. cit.*) and Sundwik (*Z. physiol. Chem.*, 1904, **41**, 343) reported that, if a solution of uric acid which had been oxidised in a slightly alkaline medium was acidified with acetic acid at the end of the reaction, an almost quantitative yield of allantoin resulted. If, however, more alkali was

added and the solution later acidified with a mineral acid, a large amount of uroxanic acid with little allantoin was obtained. This suggests that both (II) and (III) are derived from a common, labile intermediate.

The formation of a symmetrical intermediate was also suggested by the work of Fischer and Ach (*Ber.*, 1899, **32**, 2731) who found that the oxidation of both 1- and 7-methyluric acid gave the same methylallantoin, *i.e.*, 3-methyl-5-ureidohydantoin, whilst 3- and 9-methyluric acid yielded 1-methyl-5-ureidohydantoin. Fischer and Ach concluded that the glyoxaline ring must have been opened during the oxidation giving as the primary product an open-chain system of unknown structure, but containing the necessary element of symmetry with respect to the two ureido-groups. Behrend (*loc. cit.*) suggested that the primary product was the symmetrical bicyclic acid (IV) which he called " hydroxyacetylene diureide carboxylic acid " and which is systematically named 5-hydroxy-3 : 7-dioxo-2 : 4 : 6 : 8-tetra-aza*bicyclo*[3 : 3 : 0]octane-1-carboxylic acid. This acid might be hydrolysed by attack of a hydroxyl ion on $C_{(4)}$, giving uroxanic acid, or be decarboxylated in an acidic medium with simultaneous opening of one ring to give allantoin. Behrend was unable to isolate (IV) or a derivative thereof, but Schuler and Reindel (*Z. physiol. Chem.*, 1932, **208**, 248) obtained a trisilver salt of (IV), giving a reasonably good analysis, which on treatment with hydrogen sulphide lost carbon dioxide readily to yield allantoin However, this salt was rather unstable and could not be recrystallised. The structure (IV) for this intermediate is based on inference and exclusion of other possibilities. Uroxanic acid itself cannot be the primary product since it is much less easily decarboxylated in acid than the immediate product of the oxidation and gives allantoin in rather poor yield. Moreover, if (III) were an intermediate in the formation of (II), 1- and 7-methyluric acid should give a derivative of 1-methylhydantoin, and 3- and 9-methyluric acid should yield the ureido-compound of 3-methylhydantoin. If *spiro*hydantoin (V) were an intermediate, the same methylallantoin should arise from 1- and 9-methyluric acid, and the isomeric methylallantoin should be formed from 3- and 7-methyluric acid.

More recently, uric acid labelled with ^{15}N either on $N_{(1)}$ and $N_{(3)}$, or on $N_{(7)}$ and $N_{(9)}$ has been prepared. It was found that, on chemical oxidation or reaction with uricase, allantoin was formed in which the ^{15}N was distributed to an equal extent between the ureido-group and the hydantoin part of (II) (Brown, Roll, and Cavalieri, *J. Biol. Chem.*, 1947, **171**, 835; Cavalieri and Brown, *J. Amer. Chem. Soc.*, 1948, **70**, 1242).

The reaction mechanism suggested by Behrend is reasonably well established, but certain doubts remain. In the first place the intermediate (IV) is poorly characterised and attempts on our part to prepare derivatives or salts other than the trisilver salt, referred to above, have been without success. It is also, at first sight, not easy to see why an acid such as (IV) should be decarboxylated so readily. But the greatest difficulty is the following : The simplest mechanism by which one of the two rings is opened, before, during, or after decarboxylation, is the rupture of one of the C–N bonds attached to $C_{(4)}$, yielding an allantoin the carbon atoms of which are derived from uric acid in the manner shown in formula (VI). Such a hydrolysis would be analogous to the reaction leading to uroxanic acid. However, the results with the *N*-methyluric acids show that, at least with these compounds, this formulation cannot be correct and it is likely, therefore, that the simple mechanism discussed does not apply to uric acid either. Fischer and Ach's results strongly suggest that the origins of the carbon atoms in allantoin are represented by (II), but it was felt that further, more direct evidence, such as can be provided by isotope experiments, was desirable before an attempt was made to explain the mechanism of the reaction. A better understanding of the detailed mechanism by which uric acid is converted into allantoin is not only of intrinsic chemical interest, but is also of biochemical importance. It appears highly probable (for review, see Bentley and Neuberger, *Biochem. J.*, 1952, **52**, 694) that the mechanisms of oxidation of uric acid by uricase and by permanganate are almost identical; in particular it has been shown that the primary product of enzymic oxidation has the same number of carbon atoms as uric acid. Schuler and Reindel (*Z. physiol. Chem.*, 1933, **215**, 258), moreover, succeeded in isolating, after oxidation of uric acid by uricase, a trisilver salt apparently identical with that obtained after chemical oxidation. In addition, the oxidation of uric acid by chemical means at an alkaline pH has been used

to identify the biogenetic source of each carbon atom of purines (Buchanan, Sonn, and Delluva, *J. Biol. Chem.*, 1948, **173**, 81).

Samples of uric acid which were labelled with ^{14}C at positions 4, 5, and 6 severally were therefore prepared. The methods were essentially those employed in an earlier investigation (Bentley and Neuberger, *loc. cit.*), but with certain modifications giving improved yields. The three labelled uric acids were then converted into allantoin and hydantoin by standard methods. The hydantoin was hydrolysed with alkali to yield glycine which was isolated as its *N*-benzoyl derivative. The hippuric acid was hydrolysed and the free glycine was oxidised with ninhydrin to yield formaldehyde and carbon dioxide; these are known to be derived from the methylene and the carboxyl group of glycine respectively.

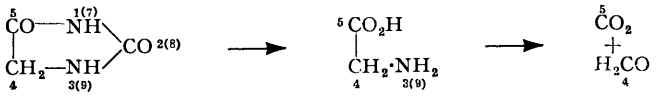


Table 1 shows clearly that $C_{(6)}$ of uric acid is completely lost on conversion of the latter into allantion; this confirms earlier results (Bentley and Neuberger, *loc. cit.*) which showed that the carbon dioxide produced on oxidation of [6-^{14}C]uric acid with uricase had a high

TABLE 1. *Activities of allantoin and hydantoin derived from uric acid.*

(Expressed as counts per carbon atom at infinite thickness on 1 sq. cm. discs; dilutions with non-isotopic material are allowed for in the calculated values.)

Expt.	Starting material and its activity		Activity of allantoin: obs.	calc.*	Activity of hydantoin: obs.	calc.*
1	[4-^{14}C]Uric acid	2230	2640	2780	1780	1730
2	,,	2230	2680	2780	3570	3450
3	[5-^{14}C]Uric acid	840	1010	1050	700	670
4	,,	840	1010	1050	1400	1350
5	[6-^{14}C]Uric acid	370	0	0	—	—
6	,,	370	0	0	—	—

* Calc. by assuming that $C_{(6)}$ of the initial uric acid is lost.

radioactivity. It can be deduced from Table 2 that the methylene group of glycine is derived from $C_{(4)}$ of uric acid and the carboxyl group from $C_{(5)}$. It thus follows, in agreement with Fischer and Ach's results (*loc. cit.*) with methyluric acids, that the derivation of the carbon atoms in allantoin from those of uric acid is correctly presented by formula (II).

Probable Mechanism of the Conversion of Uric Acid into Allantoin.—In earlier experiments (Bentley and Neuberger, *loc. cit.*) the oxidation of uric acid by uricase was studied by using water and gaseous oxygen labelled with ^{18}O. The conclusion was reached that the enzyme catalyses the transfer of two electrons from the urate ion to oxygen, yielding as the first product the bicyclic acid (IV). A similar mechanism is now suggested for the oxidation by chemical agents in an alkaline medium: the urate ion which may be represented by the formula (VII) or (VIII) loses two electrons and is thus converted into the

TABLE 2. *Degradation of glycine derived from uric acid by way of allantoin and hydantoin.**

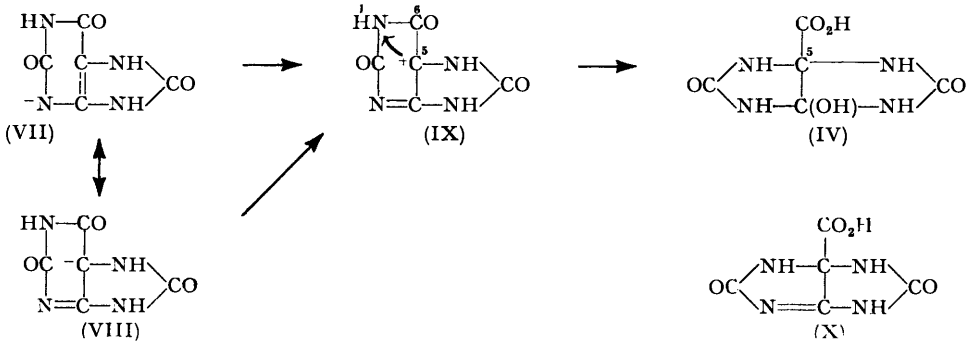
Expt.	Starting material	Calc. activity of glycine based on observed activity of hydantoin	Activity of formaldehyde–dimedone derived from glycine $-CH_2-$: obs.	calc.†	Activity of $BaCO_3$ derived from glycine $-CO_2H$: obs.	calc.†
1	[4-^{14}C]Uric acid	2590	4280	5180	0	0
2	,,	5180	8520	10360	0	0
3	[5-^{14}C]Uric acid	1000	0	0	1890	2000
4	,,	2030	0	0	4000	4060

* Activities expressed as in Table 1.

† Values calculated assuming C_5 of initial uric acid becomes the glycine carboxyl carbon atom and C_4 the glycine α-carbon atom.

carbonium ion (IX). Interaction of the strongly electrophilic 5-carbon atom with the 1-nitrogen atom, followed by the rupture of the bond joining $N_{(1)}$ to $C_{(6)}$ and loss of proton will give (X). This structure (X) was originally suggested by Bentley and Neuberger for

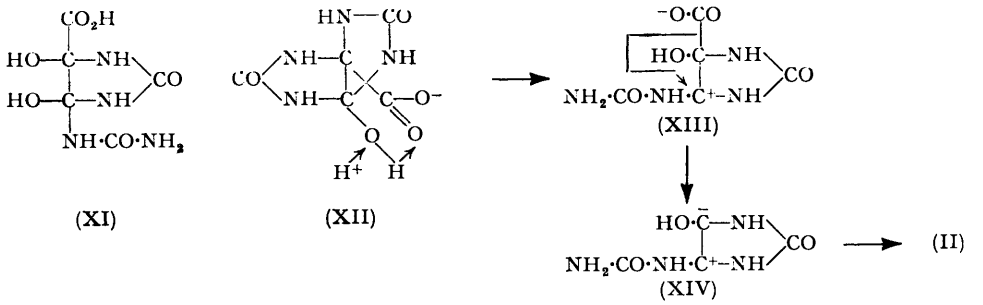
the bicyclic acid isolated by Reindel and Schuler (*loc. cit.*). However, such a structure appears unlikely since it contains a double bond at the bridgehead of two five-membered rings. It is now recognised (for references, see Fawcett, *Chem. Reviews*, 1950, **47**, 219) that Bredt's rule applies to carbon–nitrogen bonds as well as to carbon–carbon bonds. The " glycoluril " system is almost certainly, unlike that of uric acid, non-aromatic and the presence of a double bond in the position indicated by (IX) would involve a great distortion of bond angles and thus raise the energy of the molecule. It is therefore suggested that concomitant with the rearrangement proposed above, there is addition of water to the double bond leading to the acid (IV).



The conversion of the acid (IV) into allantoin involves opening of one of the two rings, decarboxylation, and a shift of the oxygen from position (4) to position (5). The last-mentioned step is most likely to be a pinacol type of rearrangement, as already suggested by Biltz and Max (*Ber.*, 1921, **54**, 2451). The order in which the various changes take place is uncertain, but it is possible that the first step is hydrolysis of the bond $N_{(1)}$–$C_{(5)}$, giving the dihydroxy-acid (XI). A more reasonable mechanism however, involves as the initial step an electrophilic attack of a proton on the β-hydroxy-group of the anion of (IV), giving a transitory intermediate of carbonium ion-type. However, the steric requirements of the tervalent carbon atom will impose a considerable steric strain on the bicyclic system and it is therefore suggested, that the ionisation of the β-carbon atom is associated with the opening of one of the two rings. The attack of the proton is probably facilitated by the existence of a hydrogen bond between the carbonyl-oxygen atom of the carboxylate group and the hydrogen atom of the β-hydroxyl group, as in (XII). Inspection of a Stuart model of (IV) shows that hydrogen bonding of this type (XII) is probable on steric grounds and that the three rings (the two five-membered nitrogen-containing rings and the six-membered hydrogen-bonded ring) are approximately equally disposed in space round the common C–C axis. Removal of water involving carbonium-ion formation is presumably assisted by the possibility of resonance of the type $HN{-}C^{+}{-}NH \longleftrightarrow {}^{+}NH{=}C{-}NH$. The zwitterion (XIII) would be expected to lose carbon dioxide readily; the formation of zwitterions as intermediates in the decarboxylation of heterocyclic acids has been postulated on good grounds by Brown and Hammick (*J.*, 1949, 659) and the transitory existence of β-carbonium ions in the decarboxylation of substituted cinnamic acids has been suggested by Johnson and Heinz (*J. Amer. Chem. Soc.*, 1949, **71**, 2913). Decarboxylation produces the zwitterion (XIV) which, it is suggested, is transformed into allantoin by a shift of an electron pair and of a proton from position 5 to position 4.

This last transformation is of course a shift similar in type to that postulated in the Whitmore hypothesis in pinacol, Wagner–Meerwein, and other rearrangements, but it differs from the usual pinacol rearrangement in that it is a hydride ion or an electron pair, and not an alkyl or an aryl group, which migrates. Ingold (" Structure and Mechanism in Organic Chemistry," London, G. Bell & Sons, 1953, p. 474) has already suggested that the frequently observed transformations of αβ-diols to carbonyl compounds without change in the carbon skeleton are essentially pinacol rearrangements. An even closer

analogy to the present case is probably the decarboxylation of an αβ-epoxy-acid to a ketone which is found in the Darzens reaction. This change is best visualized as decarboxylation of a carbonium ion or zwitterion. This proposed mechanism of the transformation of uric acid into allantoin is admittedly speculative, but it explains all the observed facts and the individual steps suggested appear, in the light of other knowledge available, to be reasonable.



Degradation of Uric Acid by Strong Acids.—It was found by Strecker (*Annalen*, 1868, **146**, 1427) that uric acid and concentrated hydrochloric acid at 160—170° yield glycine. Glycine was also obtained under similar conditions from a dimethyluric acid (Mabery and Hill, *Ber.*, 1878, **11**, 1329), later shown to be 3 : 9-dimethyluric acid and also from 9-methyluric acid (Fischer, *Ber.*, 1884, **17**, 1776). The other products obtained from the methyl derivatives were identified as carbon dioxide, ammonia, and methylamine. Fischer (*Ber.*, 1895, **28**, 2480) later obtained sarcosine by hydrolysis of 7-methyluric acid and this clearly showed that the nitrogen atom of the glycine was derived from $N_{(7)}$ of uric acid. Shemin and Rittenberg (*J. Biol. Chem.*, 1947, **167**, 875), in isotope studies on the biogenesis of uric acid in man, used this reaction for the separation of $N_{(7)}$ from the other three nitrogen atoms of uric acid. It is reasonable to assume on the basis of Fischer's observations that the methylene-carbon atom of glycine is derived from $C_{(5)}$, but the carboxyl group could originate from $C_{(4)}$ or $C_{(6)}$ or from both. We have therefore subjected the three types of labelled uric acid to the conditions of hydrolysis first used by Strecker and have determined the radioactivities of the products.

Preliminary experiments with non-labelled uric acid showed that the production of ammonia exceeded the theoretical 3 moles per mole of uric acid. Since glycine was found to be stable under the conditions employed, it follows that the conversion into glycine is accompanied by other, unknown, side reactions. However, no nitrogenous substance other than glycine and ammonia could be detected in the hydrolysates. The calculated yield of glycine, based on liberated ammonia, varied in different experiments between 55% and 75%.

TABLE 3. *Acid hydrolysis of uric acids.**

Starting material and its activity		Activity of $BaCO_3$ from CO_2 released on hydrolysis: obs.	calc.†	Activity of formaldehyde–dimedone derived from glycine CH_2 group: obs.	calc.‡	Activity of $BaCO_3$ derived from glycine-CO_2H group: obs.	Calc. for origin at: $C_{(1)}$ only	$C_{(1)}$ and $C_{(6)}$ equally
[4-^{14}C]Uric acid	22,280	16,690	18,550	2800	0	55,000	111,400	55,700
	,,	17,270	,,	2060	,,	56,290	,,	,,
	3800	Not measured		390	0	8700	19,000	9500
[5-^{14}C]Uric acid	8400	690	0	39,330	42,000	1910	0	0
	1400	Not measured		5990	7000	320	0	0
[6-^{14}C]Uric acid	3690	3600	3080	650	0	8870	0	9220
	720	Not measured		70	0	1690	0	1800

* Activities expressed as in Table 1.

† Calc. on the assumption that the glycine-carboxyl is derived from $C_{(4)}$ and $C_{(6)}$ equally (*i.e.*, CO_2 derived from $C_{(2)}$ and $C_{(8)}$ + ½ mole each from $C_{(4)}$ and $C_{(6)}$).

‡ Calc. on the assumption that CH_2O is derived from $C_{(5)}$.

In the isotope experiments the ammonia and carbon dioxide were removed and the glycine without further purification degraded as described above. The radioactivities of the

carbon dioxide formed on hydrolysis, and of the formaldehyde and of the carbon dioxide produced by the degradation of glycine, were measured. The results (Table 3) show that the carbon dioxide samples evolved during the hydrolysis had high activities compared with corresponding uric acid specimens, in the case of both [4-^{14}C]uric acid and [6-^{14}C]uric acid. By comparison with calculated values, the figures suggest that, while $C_{(2)}$ and $C_{(8)}$ give rise to one mol. of carbon dioxide each, $C_{(4)}$ and $C_{(6)}$ produce half a mol. each. The small amount of radioactivity in the carbon dioxide derived from [5-^{14}C]uric acid is almost certainly due to the side-reactions referred to above. The values of the radioactivities of the formaldehyde–dimedone complex and of the barium carbonate derived from the degradation of glycine show clearly that almost all the α-carbon atom of glycine originates from $C_{(5)}$ of uric acid, whilst the carboxyl-carbon atom is derived equally from $C_{(4)}$ and $C_{(6)}$. There is, however, a small amount of radioactivity in the carbonate obtained from [5-^{14}C]-uric acid. This may be due to formation of small amounts (approx. 5%) of glycine by a side reaction. But it is also possible that traces of carbon dioxide and formaldehyde are produced by ninhydrin from an unknown impurity present in the unpurified glycine. However, the main reaction appears to consist of a hydrolysis of uric acid to yield, in the first place, two molecules of carbon dioxide, three of ammonia, and one of aminomalonic acid. The latter is then decarboxylated, to give glycine and a further mole of carbon dioxide which is derived to an approximately equal extent from $C_{(4)}$ and $C_{(6)}$.

Experimental

Counting.—Samples were counted on 1 sq. cm. Polythene discs at "infinite thickness" (Popják, *Biochem. J.*, 1950, **46**, 560), with the exception of some of the formaldehyde–dimedone derivatives, of which the amounts available were insufficient. Appropriate corrections were made to the observed count of these specimens by reference to a standard curve relating weight of sample to activity. The observed counts, corrected where appropriate, were converted into counts per carbon atom at "infinite thickness" on 1 sq. cm. discs by multiplying the sample count by the factor (mol. wt.)/12n, where n = number of carbon atoms in the substance (cf. Cornforth, Hunter, and Popják, *Biochem. J.*, 1953, **54**, 597). To make the results for the formaldehyde–dimedone derivatives comparable with those for barium carbonate n was taken as 1 for these compounds, as only one of the carbon atoms is derived from the labelled substance.

^{14}C-*Labelled Uric Acids.*—These were prepared by the route outlined by Bentley and Neuberger (*loc. cit.*) with certain improvements exemplified in the following preparation of [4-^{14}C]uric acid.

K^{14}CN (20 mg.) was diluted to 6·3 g. with inactive KCN and dissolved in water. The solution was added to a neutralised (Na_2CO_3) solution of bromoacetic acid (13·9 g.) in water, and after 10 min. the mixture was brought to the boil, and boiling continued for 5 min. To the cooled solution was added concentrated hydrochloric acid (10 ml.), and the mixture was taken to dryness at the pump at 60—70°. The residue was extracted with 95% ethanol and the whole was filtered, the residue was re-extracted, and the combined filtrates were evaporated at 40—50° at the pump. To this residue were added 150 ml. of ethanol and 1 ml. of concentrated sulphuric acid, and the whole was boiled under reflux for 3 hr., cooled, filtered, and concentrated. The residue was neutralised with aqueous sodium carbonate and twice extracted with benzene. The combined extracts after drying (Na_2SO_4) were fractionally distilled in a vacuum, to give 7·9 g. (70%) of $^{14}CN{\cdot}CH_2{\cdot}CO_2Et$ (cf. Inglis, *Org. Synth.*, Coll. Vol. I, 2nd edn., p. 254).

The labelled cyanoacetic ester was diluted with inactive ester to 11·3 g. and converted by Bogert and Davidson's procedure (*J. Amer. Chem. Soc.*, 1933, **55**, 1667) into diaminouracil sulphate (11·8 g., 57%). The following procedure for the conversion of diaminouracil sulphate into uric acid (without the necessity for isolating free diaminouracil) had considerable advantages over the procedure of Johnson and Johns (*J. Amer. Chem. Soc.*, 1914, **36**, 545): Diaminouracil sulphate (4·8 g.) and urea (14 g.) were powdered together and then heated in a sealed tube for 1 hr. at 180°. The tube contents were extracted with water. The extract was discarded, and the residue was taken up in hot aqueous sodium hydroxide, filtered, and acidified with hydrochloric acid, to precipitate uric acid (4·15 g., 98% based on diaminouracil sulphate). The product was considerably cleaner than when the Johnson–Johns procedure was used, but was recrystallised from a large volume of boiling water (charcoal) to give almost colourless crystals (85% recovery), the purity of which was checked spectroscopically (cf. Bentley and Neuberger,

loc. cit.). The [4-^{14}C]uric acid so obtained had an activity of 7952 counts per min. per 1 sq. cm. disc.

By similar methods, using bromoacetic acid labelled in the methylene or carboxyl groups (obtained from the Radiochemical Centre, Amersham) there were obtained [5-^{14}C]uric acid and [6-^{14}C]uric acid with activities of 2998 and 1318 counts per min. per 1 sq. cm. disc, respectively. Uric acids labelled with ^{14}C predominantly, but not exclusively, in the 4, 5, or 6 position have previously been prepared biosynthetically by injecting suitably labelled precursors into pigeons (Karlsson and Barker, *J. Biol. Chem.*, 1949, **177**, 597). More recently Brandenberger (*Helv. Chim. Acta*, 1954, **37**, 641) has prepared [6-^{14}C]uric acid by a similar method and [4-^{14}C]uric acid from the corresponding labelled hypoxanthine.

Acid Hydrolysis of Uric Acid.—Unlabelled uric acid (0·25 g.), concentrated hydrochloric acid (6 ml.), and water (3 ml.) were heated for 20 hr. at approx. 185—200°. The tube contents (CO_2 evolution) were taken to dryness in a vacuum on the water-bath, water was added, the whole again evaporated to dryness, and the residue made up to 50 ml. with water. The immediate formation of a clear solution in water showed the complete destruction of uric acid. The amount of ammonia in the hydrolysate was estimated in triplicate on 2-ml. aliquot portions by the micro-Kjeldahl technique of Markham (*Biochem. J.*, 1942, **36**, 790). The number of N atoms liberated as ammonia from each molecule of uric acid was found in four experiments to be 3·24, 3·39, 3·42, 3·44. In control experiments glycine (100 mg.) was treated in the same way and no ammonia was found after "hydrolysis." Ammonia in excess of 3 atoms per molecule is not therefore due to breakdown of preformed glycine. Paper chromatography of the uric acid hydrolysate showed the presence of glycine, but of no other substance reacting with ninhydrin. Neither was there any indication of material absorbing ultra-violet light. A sample of the ammonia formed on hydrolysis was kindly examined by Dr. A. T. James by the gas-partition chromatographic procedure of James and Martin (*Biochem, J.*, 1952, **51**, 323). Only ammonia, and in particular no methylamine, was found to be present.

Hydrolysis was then carried out in the same way on samples of [4-^{14}C]-, [5-^{14}C]-, and [6-^{14}C]-uric acid. When cool the tubes were frozen in liquid air, opened, and quickly transferred to an apparatus swept with CO_2-free nitrogen. The tubes were allowed to warm slowly, the evolved gases passing through saturated silver sulphate solution to remove any hydrogen chloride and then through saturated barium hydroxide solution. The barium carbonate was collected in the usual way and counted. After liberation of carbon dioxide the residual aqueous solution was taken to dryness, redissolved, again taken to dryness, and then taken up in phosphate buffer, pH 5·5 (K_3PO_4 3·5 g. and KH_2PO_4 20 g./100 ml.; cf. Arnstein, *Biochem. J.*, 1951, **49**, 439). The glycine in the hydrolysate was degraded with ninhydrin as described by Arnstein (*loc. cit.*), the methylene group of glycine being counted as formaldehyde–dimedone complex and the carboxyl group as barium carbonate. The formaldehyde–dimedone complex specimens were recrystallised from aqueous ethanol.

Stepwise Degradation of Uric Acid via *Allantoin.*—Labelled uric acid (*ca.* 0·2 g.; weighed accurately) was diluted with unlabelled uric acid (*ca.* 1·8 g.; weighed accurately) and converted into allantoin (1·5 g. after recrystallisation from water) by the method of Hartmann, Moffett, and Dickey (*Org. Synth.*, Coll. Vol. II, p. 21). Activities of the products are summarised in Table 2. The complete absence of activity in the allantoin from [6-^{14}C]uric acid shows that no appreciable amount of uroxanic acid or other undecarboxylated product can be present in material prepared by this method.

Allantoin (2 g. of labelled, or 1 g. of labelled diluted with 1 g. of unlabelled) was converted into hydantoin by reductive hydrolysis with phosphonium iodide (Gillespie and Snyder, *Org. Synth.*, Coll. Vol. II, p. 489), giving 0·5 g. of recrystallised hydantoin, decomp. 220—222°. The absence of glycine and allantoin was confirmed by paper chromatography. Activities of the products are summarised in Table 2.

The hydantoin was refluxed with 50 ml. of *ca.* N-sodium hydroxide (a considerable excess) for 40 hr. The solution was cooled, filtered, shaken with benzoyl chloride (x ml., where $x = 1{\cdot}25$ times the weight of initial hydantoin in g.), acidified with concentrated hydrochloric acid, and set aside at 0°. The hippuric acid was separated from the benzoic acid by a procedure similar to that described by Arnstein (*loc. cit.*). The precipitated material, after drying, was twice extracted with light petroleum (b. p. 60—80°), and the mother-liquors were twice extracted with toluene. The mother-liquors were then extracted three times with ethyl acetate, the combined ethyl acetate extracts were dried (Na_2SO_4), and the solvent was evaporated. The residue was combined with the solid residue from the light petroleum extraction of the original precipitate, and the combined solids were recrystallised from ethanol. The purity of the

resultant hippuric acid was confirmed by m. p., and in two cases (experiments **3** and **4**) by counting. In these cases the observed counts were **134** and **263**, and the calculated counts were **137** and **268** counts per min. per 1 sq. cm. disc respectively.

The hippuric acid (approx. 300 mg.) was hydrolysed by refluxing with 1 : 1 concentrated hydrochloric acid for 20 hr. The cooled hydrolysate was diluted and extracted once with ethyl acetate and twice with ether. The aqueous fraction was taken down to dryness at the pump on the water-bath, and the resultant glycine degraded with ninhydrin as previously described. These results are summarised in Table **3**.

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