CIV. MICRO-DETERMINATION OF URIC ACID.

BY NORMAN LOWTHER EDSON AND HANS ADOLF KREBS.

From the Biochemical Laboratory, Cambridge, and the Department of Pharmacology, University of Sheffield.

(Received February 29th, 1936.)

PRINCIPLE OF THE METHOD.

In studying uric acid synthesis in avian tissues a reliable micro-method for the determination of uric acid is desirable. Folin's colorimetric method and its modifications which are fairly reliable for blood, and possibly for normal urine, are very unsatisfactory in presence of tissue extracts. The colour-producing reaction lacks specificity [Fujiwara and Kataoka, 1933] and the colour developed is not wholly proportional to uric acid concentration. A second method developed by Fosse *et al.* [1930] depends on the ultimate quantitative conversion of uric acid into urea and glyoxylic acid by the enzymes uricase and allantoinase. With the enzyme preparations at our disposal, however, the analysis takes too long (12 hours). We first tried to improve this enzymic method but finally abandoned it in favour of a similar but non-enzymic procedure.

In the method described in this paper uric acid is decomposed by the following reactions:

1.	Uric acid
↓
Allantoin	shaken in air with MnO_2 ($p_{\text{H}} \sim 13$; 40°)
2.	↓
Allantoic acid	alkaline hydrolysis (100° ; $p_{\text{H}} \sim 10$; 20 min.)
3.	↓
Urea + glyoxylic acid	acid hydrolysis (100° ; $p_{\text{H}} \sim 2$; 3 min.)

Either end-product can be determined with ease, glyoxylic acid colorimetrically [Ro, 1932; Mourot, 1935] or by bisulphite-titration, urea by many methods. We prefer to determine urea manometrically by means of urease, since this method is specific, simple and accurate. Though the yield of urea is not theoretical, the deficit is constant ($8 \pm 3\%$) and can be allowed for by a correction factor. In practice $237 \mu\text{l. CO}_2$ per mg. uric acid are obtained instead of $265 \mu\text{l. CO}_2$. Since the error in determining CO_2 manometrically is $1\text{--}2 \mu\text{l.}$, the method allows $0.1\text{--}1.0$ mg. uric acid to be determined with reasonable accuracy (Table III).

The method is particularly suitable for use in conjunction with tissue slice work [Edson *et al.* 1935]. It is not suitable for blood analysis, nor is it recommended for very dilute solutions containing less than 0.1 mg. uric acid in 5 ml. of fluid.

EXPERIMENTAL.

I. Reagents.

1. *Manganese dioxide.* 10 ml. 20 vols. $\%$ H_2O_2 are added to 10 ml. of 1.82% KMnO_4 solution. When effervescence has subsided, the precipitate is separated by centrifuging, washed first with water and then with 0.1 M NaOH and finally suspended in 100 ml. 0.1 M NaOH . When the precipitate is uniformly distributed 0.1 ml. of this suspension contains 0.1 mg. MnO_2 . The suspension retains its activity for several weeks; it has to be uniformly distributed before use.

2. *2 N sodium hydroxide.*

3. *N sodium bicarbonate.*

4. *Thymol blue* (0.2% in 70% alcohol).
5. 6 N *sulphuric acid*.
6. 3 N *sodium acetate*.
7. 3 M *acetate buffer*, 27.2 g. sodium acetate ($\text{NaC}_2\text{H}_3\text{O}_2$, $3\text{H}_2\text{O}$) and 6.0 g. glacial acetic acid in 100 ml. p_{H} 5.0.
8. *Naphthyl red* (0.25 % in 70 % alcohol).
9. *Urease*: 1 part of jack bean meal (Arlco or British Drug Houses, Ltd.) is shaken for 30 min. with 4 parts of water. After centrifuging, 0.1 volume of acetate buffer is added to the separated supernatant fluid, which contains the enzyme. The solution retains its activity for several months if kept cool and if a drop of octyl alcohol is added to maintain sterility.

II. Details of the reactions involved.

Oxidation of uric acid. Schuler and Reindel [1933] have shown that uric acid is oxidised catalytically by molecular oxygen in the presence of MnO_2 ; the reaction depends on temperature, p_{H} of the medium and the concentration and method of preparation of the catalyst. The dried MnO_2 of British Drug Houses, Ltd. is almost completely inactive under conditions in which freshly precipitated catalyst is highly active. 0.3 ml. of the suspension described in Section I oxidises, under the given conditions, 1–2 mg. uric acid in 30 min. (Table I).

Table I. *Catalytic oxidation of uric acid with MnO_2 .*

Manometric experiment; conical cup with side bulbs; main part of cup contained 2 ml. uric acid solution (= 1.895 mg. uric acid) + 0.1 ml. 2N NaOH; side bulbs, 0.3 ml. MnO_2 suspension; 40°; gas, air; MnO_2 and uric acid mixed after equilibration.

Time after mixing (min.)	5	10	20	40	80	
Oxygen absorbed ($\mu\text{l.}$)	33.4	65	123	126.5	126.5	(calculated 126.5)

Hydrolysis of allantoin. The conditions established by Ro [1932] for the alkaline and acid hydrolysis of allantoin have been found satisfactory for our purpose. Table II shows that the method yields 96–98 % of the theoretical amount of urea. Allan and Cerecedo [1931] showed that the yield of urea can be increased to 100 % if the hydrolysis is carried out at 70°. Allan and Cerecedo's procedure, however, requires 2.5 hours.

Table II. *Hydrolysis of allantoin (20 min. p_{H} 10; 3 min. p_{H} 2).*

Allantoin mg.	Urea found (expressed in $\mu\text{l. CO}_2$)	Yield %
0.284	77.7	97.0
0.448	123.0	97.3
0.493	135.0	97.2
0.697	197.0	96.6

III. Procedure.

3–5 ml. of solution containing uric acid are transferred to a conical Warburg cup. If these cups are being used in a tissue slice experiment, the slices are simply removed from the solution. Sufficient 2N NaOH is added to the solution to make the fluid approximately 0.1 N with respect to OH^- ; 0.3 ml. MnO_2 suspension is then added, and the vessel is attached to the manometer and shaken for 40 min. at 37–40°. The gas space contains air. The MnO_2 is then separated from the solution by filtration, the filtrate is collected in a 10 ml. graduated test-tube and its volume read carefully. If the diameter of the filter-paper is 4 cm. and the filtrate is allowed to drain thoroughly, the loss of fluid is about 0.3–0.4 ml.

Next 0.1 volume of N NaHCO_3 solution is added to reduce alkalinity. The tube is heated for 20 min. in a boiling water-bath, a drop of thymol blue is added, and when cool the fluid is cautiously acidified by addition, drop by drop, of $6N$ H_2SO_4 until the indicator is distinctly red (p_{H} 2). The tube is returned to the boiling water-bath for 3 min. and cooled. A drop of naphthyl red is added and p_{H} is adjusted to 5.0 by $3 M$ sodium acetate; a drop of both indicators added to acetate buffer of p_{H} 5.0 serves as a standard colour. An aliquot of the solution is then transferred to a conical Warburg flask and urea is determined with urease by the method of Krebs and Henseleit [1932].

IV. Example.

3 ml. of solution containing 1.450 mg. uric acid were shaken with 0.3 ml. MnO_2 suspension and 0.15 ml. 2 *N* NaOH (40 min.; 40°).

Filtrate = 3.0 ml. $\left(\text{factor for correction } \frac{3.45}{3.00} \right) + 0.3 \text{ ml. } N \text{ NaHCO}_3$.

↓

Heated to 100° for 20 min.

↓

cooling, + 1 drop thymol blue + 6 $N \text{ H}_2\text{SO}_4$ until red.

Heated to 100° for 3 min.

↓

cooling, + 1 drop naphthyl red + 40% sodium acetate until the colour of the control is reached.

↓

Total volume now 5.1 ml. Transferred into manometric flask for urea determination: 5.0 ml. $\left(\text{factor for correction } \frac{5.1}{5.0} \right)$.

↓

Urea determination: 0.4 ml. urease; V_F 5.4 ml.; V_G 11.17; 38°. K_{CO_2} 1.273.

Pressure changes after mixing: time

20	40	60 min.
mm. 213.5	238	238.5

$v_{\text{CO}_2} = 238.5 \times 1.273 \times \frac{3.45}{3.00} \times \frac{5.1}{5.0} = 357 \mu\text{l.}$

(theoretical value 385; yield 92.7 %).

V. Recovery.

In Table III a number of recovery experiments, carried out on pure uric acid solutions, are listed. The average yield is 92.1 %, if the first two determinations, in which the amount of uric acid was small and the accuracy therefore less, are excluded. The last column of Table III shows that the deviations from the average figure are, with one exception, less than 3 %. We correct this deficit in the uric acid determinations by dividing the CO_2 found by 0.92.

Table III. *Recovery of uric acid.*

Uric acid added mg.	CO ₂ found μl.	Yield %
0.073	18.8	97
0.146	39.1	101
0.182	45.7	94.8
0.241	59.7	93.3
0.253	63	94.0
0.255	62.5	92.5
0.292	69.2	89.2
0.328	82.0	94.3
0.484	112	87.5
0.507	122	91.1
0.730	179	92.4
0.967	236	92.2
1.450	357	92.7
1.930	482	94.0
1.930	469	91.5
2.42	577	90.5

Average
92.1%

VI. *Interference by other substances.*

The recovery of uric acid is equally satisfactory, if liver slices, liver extract or dilute (1 : 10) serum are present. Higher concentration of proteins, above 1–2 %, interfere with the filtrations. Agglomeration of MnO_2 , which may occur in certain protein-containing solutions, does not significantly affect the catalytic activity.

If the solution contains glucose, and possibly other substances, autoxidisable products are formed in the course of the alkaline hydrolysis. These cause an absorption of oxygen during the urea determination, but this difficulty can be overcome by filling the manometric flask with nitrogen.

If urea is present, it is advisable either to determine it directly in an aliquot or to destroy it by means of urease before the determination of uric acid.

VII. *Specificity.*

The following purines and pyrimidines do not yield urea when treated according to Section III and do not absorb oxygen with MnO_2 in alkaline solution: hypoxanthine, xanthine, adenine, guanine, cytosine, uracil, thymine.

Substances which may interfere with the determination of uric acid are a number of guanidine derivatives (arginine, glycoxyamine, guanidine and creatinine) and some ureides (such as allantoin, allantoic acid, parabanic acid, oxaluric acid and dialuric acid). Table IV shows the extent to which urea may

Table IV. *Production of urea from substances other than uric acid.*

Substance	Urea formed when treated according to section III. % maximum yield
Allantoin	98
Allantoic acid	98
Parabanic acid	98
Dialuric acid	98
Arginine	9
Guanidine	11
Creatinine	10
Glycoxyamine	10
Oxaluric	98

be formed from these substances under the conditions of the determination. In all these cases the urea is produced during the alkaline or acid hydrolysis so that uric acid can be determined in their presence if the urea formed in an aliquot, without previous treatment with manganese dioxide, is measured.

SUMMARY.

A method is described, by which 0.1–2.0 mg. uric acid can be determined with reasonable accuracy. Uric acid is converted into urea and glyoxylic acid and the urea produced is estimated manometrically by means of urease. The method is especially suitable for use in conjunction with tissue slice work.

REFERENCES.

- Allan and Cerecedo (1931). *J. Biol. Chem.* **93**, 193.
 Edson, Krebs and Model (1935). *Chem. Ind.* **54**, 1026.
 Fosse, Brunel and de Graeve (1930). *Compt. Rend. Acad. Sci.* **190**, 693.
 Fujiwara and Kataoka (1933). *Z. physiol. Chem.* **216**, 133.
 Krebs and Henseleit (1932). *Z. physiol. Chem.* **210**, 33.
 Mourot (1935). *Bull. Soc. Chim. Biol.* **17**, 1845.
 Ro (1932). *J. Biochem.* **14**, 391.
 Schuler and Reindel (1933). *Z. physiol. Chem.* **215**, 258.