TRUE URIC ACID VALUES

BY

J. MAXWELL JOHNSTONE

From the S.A. Courtauld Institute of Biochemistry, the Middlesex Hospital Medical School, London

(RECEIVED FOR PUBLICATION APRIL 15, 1952)

The estimation of uric acid in blood is often unsatisfactory, and substances commonly present in blood, other than uric acid, may react with the reagents to give false values. This difficulty may be overcome by the use of uricase (Blauch and Koch, 1939; Bulger and Johns, 1941; Block and Geib, 1947), although in these methods some uric acid is probably lost during protein precipitation. The error due to unrelated substances may be equivalent to 0.32–1.54 mg. uric acid/100 ml. (Block and Geib, 1947; Blauch and Koch, 1939).

This paper describes uric acid values found in normal and pathological bloods before and after treatment with uricase, and indicates the error due to non-uric acid reacting substances.

Method

The procedure of Block and Geib (1947) has been modified by substituting for their arsenophosphotungstic acid reagent, which gives too weak colours, the colour-developing reagents described by Brown (1945). By this method a linear relationship is obtained between uric acid concentration and optical density up to about 12 mg. uric acid/100 ml. plasma.

As the intensity of the colour gradually increases even after one hour, all colours were read precisely the same number of minutes after adding the reagents. This was achieved by adding the phosphotungstic acid reagent to successive tubes at one-minute intervals.

Reagents.—The following were used: (1) uricase powder (Buchanan, Block, and Christman, 1945); (2) uricase extract (Block and Geib, 1947); (3) 10% (w/v) sodium tungstate; (4) $2/3N$ H_2SO_4 ; (5) lithium chloride solution, made by dissolving 7.5 g. lithium chloride in water to which 35 ml. concentrated HCl has been added, and diluting to one litre; (6) 2.9% (w/v) silver nitrate; (7) 50% (w/v) urea; (8) 12% (w/v) recrystallized sodium cyanide (made immediately before use, otherwise blanks will have too great a density); (9) phosphotungstic acid reagent (Folin, 1934); (10) stock uric acid solution (Folin, 1930), but without methyl orange; (11) standard uric acid solutions made by diluting 2, 4, 6, and 8 ml. each of stock uric acid solution to 1,000 ml., which, when used as described below, are equivalent to 2, 4, 6, and 8 mg. uric acid/100 ml.; (12) $N/3$ HCl.

Procedure

Non-uric Acid Residual Colour.—To 2 ml. of plasma 2 ml. of uricase extract and 12 ml. of water are added. The solution is incubated for two hours in a water-bath at 45° C. and then the proteins are precipitated, adding 2 ml. each of 10% sodium tungstate and $2/3N$ H_2SO_4 . After 20 to 30 minutes the solution is centrifuged and the supernatant filtered through a No. 54 Whatman filter paper. To 8.0 ml. of filtrate 0.8 ml. of lithium chloride solution and 0.8 ml. of silver nitrate solution are added, mixed, and centrifuged at once. In this order, 2 ml. of 50% urea, 2 ml. of sodium cyanide solution, and 1 ml. of phosphotungstic acid reagent are added to 6.0 ml. of the supernatant; the solution is mixed, and then diluted to 15 ml. with water.

Uricase Blank.—Uricase extract, 2 ml., and 14 ml. of water are incubated for two hours at 45° C. and carried through the above procedure.

Total Colour.—Water, 7 ml., and 1 ml. each of 10% sodium tungstate and $2/3N$ H_2SO_4 are added to a tube containing 1 ml. of plasma. After 20 to 30 minutes the solution is centrifuged and the supernatant filtered as above. To 4.0 ml. of filtrate 0.8 ml. each of lithium chloride solution and silver nitrate solution are added, and the resulting solution mixed and centrifuged immediately. Then 2 ml. of 50% urea, 2 ml. of 12% sodium cyanide solution, and 1 ml. of phosphotungstic acid reagent are added to 3.5 ml. of the supernatant and made up to 15 ml. with water.

Standards.—To 2.5 ml. of each of the standard solutions 1 ml. of $N/3$ HCl, 2 ml. of 50% urea, 2 ml. of 12% sodium cyanide, and 1 ml. of phosphotungstic acid reagent are added, and diluted to 15 ml. with water. Throughout this procedure a reagent blank is run.

The colour intensities are read after 45 to 50 minutes in a Spekker absorptiometer, using Ilford red No. 608 filters.

Calculation.—The readings, corrected for blanks, are converted to mg. uric acid by the calibration curve. The non-uric acid values must be halved, as 2 ml. of plasma were originally used for these estimations.

Results

Uric acid values in 105 plasma samples are shown in Table I.

TABLE I
MEAN TOTAL URIC ACID VALUES AND RANGES IN EACH
TYPE OF CASE INVESTIGATED

Condition	No. of Cases	Mean Total Uric Acid Value (mg.%)	Range of Total Uric Acid Values (mg.%)
Normal { Men	17	4.61 ± 0.24	2.80–7.00
	21	3.75 ± 0.22	2.00–6.50
Uraemia	14	7.49	5.30–11.30
Gout	8	7.80	4.25–10.10
Rheumatoid arthritis ..	7	3.88	3.05–4.80
Diabetes mellitus	10	3.84	2.60–6.05
Leukaemia	8	6.92	2.65–26.00
Carcinoma	4	4.27	3.00–6.90
Miscellaneous*	16	4.12	2.25–9.00

* This group comprised two cases each of hypothyroidism and obstructive jaundice, and one case each of intestinal obstruction, cirrhosis, prostatic enlargement, albuminuria, lupus vulgaris, Addison's disease, low renal threshold, hepatosplenomegaly, ulcerative colitis, chronic urinary infection, hypopituitarism, and gangrene of the foot.

Normals.—In 38 normal people the mean total uric acid value, irrespective of sex, was 4.14 ± 0.16 mg./100 ml. and the mean non-uric acid reacting substances value 0.072 ± 0.005 mg./100 ml. There was a significant difference (Table I) between the mean total uric acid values for men and women in this group ($t=2.836$, $n=38$, $P<0.01$).

Uraemia.—Of 14 uraemic patients the total uric acid value in eight was over 7 mg./100 ml., while in 11 the value of the non-uric acid reacting substances was over 0.20 mg./100 ml., the highest reading, from a case of polycystic renal disease, representing 2.00 mg. uric acid/100 ml.

Leukaemia.—Of eight cases, acute and chronic, seven showed normal values. The eighth, a child in the terminal stages of acute lymphatic leukaemia, had a total uric acid value of 26 mg./100 ml. plasma and the non-uric acid reacting substances value represented 8.2 mg. uric acid/100 ml. plasma.

Rheumatoid Arthritis, Diabetes Mellitus, and Carcinoma.—All values were within the normal ranges in seven cases of rheumatoid arthritis, 10 cases of diabetes mellitus, and four cases of carcinoma.

Gout.—In six of the eight cases the total uric acid value was over 7.00 mg./100 ml. All non-uric acid reacting substances values were normal.

Miscellaneous.—Only one, a patient with obstructive jaundice, had a raised total uric acid value. One patient with obstructive jaundice,

one with hepatic cirrhosis, and one with albuminuria of obscure origin had non-uric acid reacting substances with values greater than 0.20 mg./100 ml.

Discussion

The nature of the non-uric acid reacting substances is obscure. In this work it was found that dihydric phenols, such as resorcinol, metol, guaiacol, and pyrogallol, gave strong colours with the reagents used: 1 mg./100 ml. solutions of resorcinol and guaiacol, when treated as plasma samples, gave colours representing 3.6 and 3.08 mg. uric acid/100 ml. respectively. However, the phenolic extract of a sample of normal urine (10 minutes' hydrolysis with dilute acid and 12 hours' benzene extraction) accounted for only 0.08 mg. of the non-uric acid reacting substances value of 7.00 mg./100 ml. urine.

There is no correlation between the values for total uric acid and non-uric acid reacting substances. In the cases of gout all the raised uric acid values were accompanied by normal non-uric acid reacting substances values. On the other hand, in many of the uraemic patients there were raised values for both the non-uric acid reacting substances and true uric acid.

In routine laboratory practice determination of total plasma uric acid values by Brown's method (1945) is quite satisfactory. In the majority of cases it will give values not more than 0.20 mg./100 ml. greater than the true uric acid value. It is principally in cases of considerable renal damage that the error may be greater.

Summary

A slightly modified method for the determination of true uric acid values using uricase is described.

The mean total plasma uric acid value in 38 normal people was 4.14 ± 0.16 mg./100 ml.

The total uric acid value, estimated by the method of Brown (1945), is normally less than 0.20 mg./100 ml. greater than the true uric acid value.

I should like to express my gratitude to Professor E. C. Dodds for permission to do this work and to those who provided me with some of the blood samples for investigation.

REFERENCES

- Blauch, M. B., and Koch, F. C. (1939). *J. biol. Chem.*, **130**, 443.
 Block, W. D., and Geib, N. C. (1947). *Ibid.*, **168**, 747.
 Brown, H. (1945). *Ibid.*, **158**, 601.
 Buchanan, O. H., Block, W. D., and Christman, A. A. (1945). *Ibid.*, **157**, 181.
 Bulger, H. A., and Johns, H. E. (1941). *Ibid.*, **140**, 427.
 Folin, O. (1930). *Ibid.*, **86**, 179.
 — (1934). *Ibid.*, **106**, 311.