

URICOLYSIS IN NORMAL AND GOUTY INDIVIDUALS

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(RECEIVED FOR PUBLICATION JULY 6, 1955)

One of the many theories of gout has been that the hyperuricaemia in gout is due to a defect in uricolysis. Uricase has never been demonstrated in human tissue; thus this argument is on tenuous ground.

In the determination of the uric acid pool and turnover rate, Benedict and others (1949) consistently observed a discrepancy between turnover rate and excretion which they designated the uric acid excess. The excess could only be partly explained by sweating and loss through internal secretions such as the bile. In their experiments, the amount of N_{15} uric acid injected for the pool determination was low, and the extent of labelling of the nitrogenous products in the urine, other than uric acid, was not much greater than the natural abundance. Utilizing large doses of N_{15} uric acid in an effort to demonstrate significant enrichment of the urea and ammonia in urine, Wyngaarden and Stetten (1953) demonstrated significant uricolysis in normal man. It was determined that 18 per cent. of an intravenously administered dose of uric acid was degraded to other nitrogenous products that appeared in the urine, 6 per cent. was excreted in the faeces, and 78 per cent. was excreted as unchanged uric acid in a 2-week period. Repetition of the experiment in the same subject, with intestinal bacteriostasis accomplished with oral phthalylsulphathiazole, yielded essentially the same results. This was interpreted as an indication that degradation of urate by intestinal flora was not a major source of uricolysis.

It was important to demonstrate that uricolysis did not depend on the uric acid content of bile and the alkaline reaction of pancreatic juice. We have incubated juice with and without bile under sterile conditions (Seitz filtered) with dilute uric acid solutions. The rate of uric acid disappearance was measured by change of absorption at $292 \mu\text{u}$. in a Beckman du Quartz spectrophotometer, and by total chromogen determinations by the method of

Kern and Stransky, modified by Archibald (Forsham and others, 1948); 5 per cent. of the uric acid had been destroyed at the end of a 2-hour period, and 15 per cent. at the end of a 5-hour period. Since the pH of the duodenal secretions varied from 7.0 to 8.8, and would normally be much lower in man because of the acidity of the gastric contents, significant uricolysis cannot be accounted for by this route. In the above experiment, marked uricolysis occurred rapidly if there was bacterial contamination of the preparations.

Geren and others (1950) demonstrated the difference in fate of oral and intravenous urate, more than 91 per cent. being degraded after oral administration. The observations of Margules and Griffiths (1950) and Griffiths (1952) demonstrated the oxidation of uric acid at a physiological pH in the presence of a cytochrome-cytochrome oxidase system; Griffiths concluded that it is possible for urate to be oxidized in small quantities in organisms lacking uricase. Another uricolytic system at a physiological pH was reported by Tuttle and Cohen (1953); using leucoperoxidase coupled with a glucose-glucose oxidase system as a source of peroxide, and in the presence of C_{14} uric acid, these authors were able to demonstrate the degradation products of uric acid in this system as 33 per cent. allantoin and 20 per cent. urea. Urate incubated with uricase gave a 90 per cent. yield of allantoin. The nitrogen excretion as urea and ammonia in Wyngaarden's experiment could possibly be explained on the basis of peroxidase degradation. The uric acid degradation products involved in the cytochrome-cytochrome oxidase system have not yet been investigated. It is apparent, then, that although uricase is lacking, there exist in humans at least two enzymes capable of oxidase.

White blood cells contain approximately 2 per cent. verdo-peroxidase, and red blood cells have both peroxidase and cytochrome-oxidase activity. A fall in serum urate levels in blood from which the

formed elements have not been removed was observed by Yü (1953). Taking advantage of the above observations, an attempt was made to demonstrate uricolysis in whole blood plasma in the presence of both white and red cells.

Methods

50 ml. heparinized blood were obtained. A 5-ml. sample was removed and the remainder centrifuged. The plasma was removed and the buffy coat pipetted off. The buffy coat was centrifuged in a narrow tube in either the patient's own plasma or isotonic saline. After three to four separations, high concentrations of white blood cells, fairly free of red cells, were obtained. The red cell preparations were obtained by pipetting the red cells from the bottom of the tube and freeing them from white blood cells in the same manner as above. Each patient had the following samples incubated at 37° C. under 5 per cent. CO₂-95 per cent. O₂:

1. Whole blood.
2. Plasma.
3. White blood cells and the same patient's plasma.
4. Red blood cells and the same patient's plasma.

Blood counts were done of Preparations 3 and 4, and plasma samples from all tubes were taken at 1, 2, 4, and 24 hours. Uric acid determinations were done by Buchanan, Block and Christman method, as modified by Yü (1953). Repeat determinations on the same individual on three different occasions yielded the results given in Table I.

The final pH of the samples, after 24 hrs' incubation, was within 0.3 pH units of the original samples. The average normal sample, usually from a patient with an elevated leucocyte count, contained 52,200 white blood cells and 0.59M red blood cells per c.mm. in the white cell preparation; the red cell preparations contained an average of 4.0M red blood cells and 1,070 white cells

per c.mm. The values for the preparations made on gouty individuals were of the same order. 3 ml. plasma were incubated with 0.5 ml. cell concentrate, but no attempt was made to incubate any specific amount of urate with any specific number of cells. All white blood cell preparations contained some red cells, and almost all red blood cell preparations contained some white cells.

The results obtained in a group of 25 normal subjects without hyperuricaemia (12 males and 13 females), are listed in Line 1, Table II. There was no sex difference (with regard to uricolysis) and the results for the normal subjects are combined. The results of eight determinations in seven patients in whom the diagnosis of gout was established are listed on Line 2 of Table II.

The rate of uricolysis is not dependent on the level of urate in the plasma of normal individuals and was demonstrated by the following experiment:

With uric acid added to the plasma of normal subjects, so as to bring it to hyperuricaemic levels, the rate of uricolysis was unchanged. The mean fall in urate level is obviously less in the patients with gout, but little could be told about any particular individual from the rate of uricolysis because individual variation was so great. In our limited series, there is certainly no correlation between the severity of the disease and the rate of uricolysis. In addition, all our uric acid determinations are total chromogen determinations; and it is not inconceivable that there might be liberation or equilibration with the plasma of other substances that would reduce arsenophosphotungstic acid. Uricolysis was very slight in 6 hrs, but almost complete in all in 20 hrs, in plasma incubated with:

- (a) crude horse-radish peroxidase,
- (b) peroxidase and peroxide,
- (c) glucose-glucose oxidase and peroxidase.

Highly purified peroxidase preparations required the

TABLE I
PERCENTAGE DECREASE IN PLASMA URATE IN PATIENT WITH LYMPHATIC LEUKAEMIA
(Plasma Urate Level 7.4 mg./100 ml. Range 7.0-7.9)

Incubation	Whole Blood	Plasma	White Cells	Red Cells
Mean	11	+1	13	18
Range	9 to 13	-2 to +2	12 to 13	13 to 22

TABLE II
MEAN PERCENTAGE DECREASE IN PLASMA URATE AFTER INCUBATION

Subjects	No. of Determinations	Whole Blood	Plasma	White Cells	Red Cells
Normal (Standard Deviation from the Mean)	25	34.4 (3.2)	2.3 (3.6)	39.0 (4.8)	29.8 (4.6)
Gouty (Standard Deviation from the Mean)	8	23.2 (4.2)	3.5 (3.5)	26.5 (0.5)	19.2 (2.9)

presence of either peroxide or glucose and glucose oxidase before uricolysis occurred.

Summary

Uricolysis occurs in whole blood as well as in plasma incubated with white or red blood cells. It is conceivable that the diminished rate of uricolysis in gouty patients could contribute to the hyperuricaemia. The rate of uricolysis does not apparently depend on hyperuricaemia, as there is a marked difference between the rate of uricolysis found after raising normal plasma to a hyperuricaemic level by the addition of urate, and that found in the hyperuricaemia of gout.

We wish to express our thanks to Mrs. Phyllis Faber for her technical assistance.

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Uricolyse chez sujets normaux et gouteux

RÉSUMÉ

L'uricolyse a lieu aussi bien dans le sang complet que dans le plasma incubé avec des globules blancs. Il est concevable que le taux bas d'uricolyse chez les gouteux contribuerait à, ou serait responsable de l'hyperuricémie. Le taux d'uricolyse ne dépend pas apparemment de l'hyperuricémie, parce qu'il y a une différence marquée entre le taux d'uricolyse du plasma normal rendu hyperuricémique par l'addition d'urate et celui du plasma hyperuricémique gouteux.

Uricolisis en sujetos normales y gotosos

SUMARIO

Uricolisis ocurre en la sangre completa así como en el plasma incubado con globulos blancos. Se puede concebir que las cifras bajas de uricolisis en los gotosos contribuyen a, o sean responsables de la hiperuricemia. Aparentemente, la tasa de uricolisis no depende de la hiperuricemia, ya que hay una diferencia marcada entre las cifras de uricolisis del plasma normal hecho hiperuricémico con adición de urato y las del plasma hiperuricémico gotoso.