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Research Article

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Study of Purine Metabolism in a Xanthinuric Female

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ABSTRACT A case of xanthinuria is briefly described, and the results of *in vivo* studies with ^{14}C -labeled oxypurines are discussed. The data demonstrate that the rate of the turnover of uric acid is normal, despite an extremely small uric acid pool. Xanthine and hypoxanthine pools were measured and their metabolism evaluated. The bulk of the daily pool of 276 mg of xanthine, but only 6% of the 960 mg of hypoxanthine, is excreted. Thus, xanthine appears to be a metabolic end product, whereas hypoxanthine is an active intermediate. Biochemical implications of this finding are discussed.

INTRODUCTION

Xanthinuria is a rare metabolic disorder previously described in only three patients in the English literature (1-3). It is characterized by a loss of xanthine oxidase activity with consequent decrease in serum and urine uric acid and increased urine (and serum) levels of xanthine and hypoxanthine as the main excretory products of purine catabolism. Thus it provides an unique opportunity to study purine metabolism. This paper presents the results of *in vivo* studies with ^{14}C -labeled xanthine, hypoxanthine, and uric acid in a fourth such patient with xanthinuria.

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Case report in brief. The patient is a 62 yr old Puerto Rican grandmother, whose illness is described in detail elsewhere.¹ Except for mild psoriasis present for 30 yr, she has been in good health. On 26 June 1966, the patient was admitted to the Second (Cornell) Medical Service, Bellevue Hospital, with a 3 day history of pain in the right foot and fever. The admission physical examination confirmed the presence of monoarticular arthritis and mild psoriasis. The patient's course in the hospital was characterized by recurrent fevers to 104°F and migratory polyarthritis, affecting both ankles, knees, elbows, wrists, and hands over a 6 wk period. Laboratory investigation of the arthritis was exhaustive and included serologic studies, study of joint fluid, and x-rays of all joints. The etiology of the arthritis was not established; the serum uric acid was 1.1 mg/100 ml and 0.8 mg/100 ml. The excretion of uric acid was 50 mg and nonuric acid oxypurines 280 mg in 24 hr. These results prompted her transfer to a metabolic ward at Bellevue, and later to James Ewing Hospital for further studies, including those with the ^{14}C -labeled purines.

METHODS

The study consisted of the investigation of body pools, turnover rates, and interconversions of xanthine, hypoxanthine, and uric acid. The patient was placed on a low purine diet containing approximately 35-50 mg of purine nitrogen and 40 g of protein per day. After an initial control period, ^{14}C -labeled xanthine, hypoxanthine, and uric acid were administered intravenously. Continuous

¹ Bradford, M. J., and V. Hrehorovich. Xanthinuria, psoriasis, and arthritis. In press.

24-hr urine collections were made except for the 1st "day" which was divided into three periods of 4, 8, and 7 hr respectively, after isotope administration. Measurements of urinary total oxypurines, xanthine, hypoxanthine, uric acid, and creatinine were determined as well as radioactivity in the different specimens.

After equilibration of the ^{14}C -label in the patient (2 wk after the isotope was given), the subject was given 16 g of yeast RNA over a 5 day period while the above measurements were continued.

Uricase,² xanthine oxidase,³ yeast RNA,⁴ uric acid-6- ^{14}C ,⁵ hypoxanthine-8- ^{14}C ,⁶ and guanine-6- ^{14}C ⁵ were obtained from commercial sources.

The guanine-6- ^{14}C was converted to xanthine-6- ^{14}C by deamination with nitrous acid followed by column chromatography on Dowex-50 (200-400 mesh). The specific activities were uric acid-6- ^{14}C , 2.48 mc/mole, hypoxanthine-8- ^{14}C , 4.52 mc/mole, and xanthine-6- ^{14}C , 1.15 mc/mole. For intravenous use ^{14}C isotopes were dissolved in sterile normal saline and infused together over a 20 min period. The patient received 0.43 mg of uric acid, 0.34 mg of hypoxanthine, and 1.31 mg of xanthine.

24-hr urines were collected in glass bottles and refrigerated during collection. Specimens were either analyzed immediately or stored frozen. During the first 8 days, daily 24-hr stool collections were obtained. These were homogenized with the addition of known amounts of water. Aliquots (by volume) were dissolved in hyamine and assayed for total radioactivity appearing in the stool.

Urinary uric acid was measured by an enzymatic spectrophotometric method (4). Urinary oxypurines were determined by a modification of the method used by Ayzavian and Skupp (5, 6). Xanthine and hypoxanthine were first partially purified by passing the urine through a column of Dowex-50 to remove uric acid and urea. Elution with 1 M ammonium hydroxide resulted in a solution containing xanthine and hypoxanthine (as well as other purines). An aliquot was evaporated to dryness and assayed with xanthine oxidase to give a value for total oxypurines (expressed as xanthine equivalents). The oxypurines were separated on a second column of Dowex-50 that had been calibrated with standard solutions of xanthine and hypoxanthine. Xanthine was eluted first with 0.15 N HCl, and hypoxanthine was then eluted with 0.6 N HCl. Aliquots were concentrated and assayed with xanthine oxidase.

Urinary creatinine was determined by the alkaline picrate method.

Optical densities were determined with a Beckman DU spectrophotometer.

Measurement of radioactivity. Radioactivity measurements were made with a liquid scintillation counter (Beckman LS-200B). A toluene- ^{14}C internal standard⁶ was

² Henley and Company, New York.

³ Worthington Biochemical Corp., Freehold, N. J.

⁴ Mann Research Labs. Inc., New York.

⁵ Volk Radiochemical Company, Chicago, Ill.

⁶ New England Nuclear Corp., Boston, Mass.

used with 5.26×10^5 dpm/ml. Efficiency of counting was 70-80%. Sufficient disintegrations were obtained to be within 95% confidence limits. Activities are expressed as disintegrations per minute per millimole of purine except for uric acid-6- ^{14}C . The quantity of uric acid was so small that total dpm/24 hr is used instead.

Methods for studying metabolic fate of ^{14}C isotopes. Aliquots (20%) of xanthine and hypoxanthine fractions of the Dowex column separation were evaporated to dryness.

The residue (washed twice) was dissolved in 8 ml of 1/90 M glycine buffer, pH 9.2. Excess xanthine oxidase (0.1 ml of 1:10 dilution) and uricase (25 U) were added, the pH confirmed, and the reaction allowed to go to completion (more than twice the maximum time required for controls to reach completion). Uricase degrades uric acid to allantoin with the 6- ^{14}C being oxidized to $^{14}\text{CO}_2$ and the 8- ^{14}C remaining as part of the allantoin molecule which is not volatile. The residual counts were determined by acidifying the solution, evaporating to dryness, dissolving the residue in 1 ml of 1 M ammonium hydroxide, and adding diitol (7). This residual count is derived from the 8- ^{14}C originally given as hypoxanthine-8- ^{14}C . The $^{14}\text{CO}_2$, derived from the original xanthine-6- ^{14}C , was driven off, and its value was calculated from the difference between total and residual counts. Controls were analyzed with a closed system to trap the $^{14}\text{CO}_2$ in a hyamine-filled well suspended over the solution. Direct measurements of $^{14}\text{CO}_2$ corresponded very closely to the difference between total counts and 8- ^{14}C counts remaining in solution.

Some of the crude urine samples were subjected to enzymatic action directly in a manner similar to the xanthine and hypoxanthine fractions. An additional step was required in which the urines were first incubated with uricase to remove any of the ^{14}C activity in the form of uric acid-6- ^{14}C , which was driven off as $^{14}\text{CO}_2$. The 8- ^{14}C was then measured in the final solution, and the 6- ^{14}C was collected as $^{14}\text{CO}_2$ in hyamine. The results correlated well with those obtained by enzyme action on the column fractions.

RESULTS

Measurement of urinary uric acid and oxypurines. Urinary uric acid, total oxypurines, xanthine, and hypoxanthine values while the patient was receiving a low purine diet are summarized in Table I and Fig. 1. The concentration of urine uric acid averaged 6.5 mg/24 hr, whereas the total oxypurines (expressed as equivalents of xanthine) averaged 287 mg/24 hr. Xanthine and hypoxanthine contributed 80 and 20 % of this total respectively (on a molar basis 78 and 22 %). These findings are very similar to those of the previously reported cases of xanthinuria (1-3, 5).

The results of the above determination during the 5 days that the patient received oral yeast

TABLE I
Daily Urine Uric Acid, Total Oxypurine, Xanthine,
and Hypoxanthine While Patient Received
Control Diet

	No. of determinations	Range	Mean
Urine uric acid	26	0-38.5	6.5
Urine total oxypurines (xan equivalents)	27	212-399	287 (1.88)
Urine xanthine	21	125-325	219 (1.44)
Urine hypoxanthine	21	27-76	54.4 (0.40)

Values in parenthesis are millimoles. Normal values are 270-580 mg of uric acid, 13-45 mg of xanthine, and 0.4-3.5 mg of hypoxanthine per day.

RNA (16 g) can be seen in Table II and Fig. 1. Here the urine uric acid averaged 19.3 mg, whereas the total oxypurine output increased to 478 mg (expressed as xanthine equivalent) with xanthine and hypoxanthine again contributing 80 and 20 % respectively (or 78 and 22 % on a molar basis), which is the baseline value. This increase in output of both xanthine and hypoxanthine on dietary RNA supplement contrasts with the finding of Ayvazian and Skupp that small amounts of RNA cause an increase in xanthine output but no significant change in hypoxanthine. The present increase in both fractions is confirmed by the dilution in specific activity of xanthine and hypoxanthine (Figs. 2 and 3). From the dilutions of specific activity and average values of xanthine and hypoxanthine during the control period, it can be calculated that the patient excreted 2.48

TABLE II
Daily Urine Uric Acid, Total Oxypurine, Xanthine,
and Hypoxanthine during 5 Days While
Patient Received 16 g RNA

	No. of determinations	Range	Mean
Urine uric acid	5	0-39.6	19.3
Urine total oxypurines (xan equivalents)	5	368-589	478 (3.14)
Urine xanthine	5	268-455	350.6 (2.30)
Urine hypoxanthine	5	54-110	89.0 (0.65)

Values in parenthesis are millimoles.

mmoles of xanthine and 0.61 mmoles of hypoxanthine while she received RNA. These values compare with the direct measurement of 2.30 and 0.65 mmoles, respectively. The 16 g of yeast RNA represent approximately 10.3 mmoles of adenine and 11.0 mmoles of guanine (8). During the 5 day period in which the patient received the dietary supplement, the purine output increased (above the previous control mean values) by 0.86 mmoles of xanthine per day and 0.15 mmoles of hypoxanthine per day. This increase represents a total increase of 5.05 mmoles of the two purines and accounts for 24 % of the added oral load. The rest apparently enters body pools, though some may be lost via the gastrointestinal tract.

Studies of ¹⁴C-labeled uric acid, xanthine, and hypoxanthine recovered in the urine and stool. The recovery of urine uric acid-6-¹⁴C is seen graphically in Fig. 4. The values are expressed as total disintegrations per minute per day because

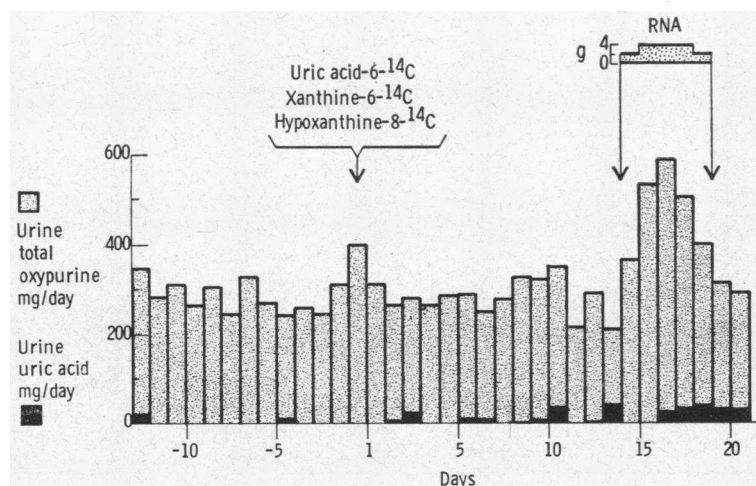


FIGURE 1 Daily excretion of urinary total oxypurines and uric acid. The values for total oxypurines are expressed as xanthine equivalents. The days are numbered in relation to the ¹⁴C isotopes which were administered on day 1.

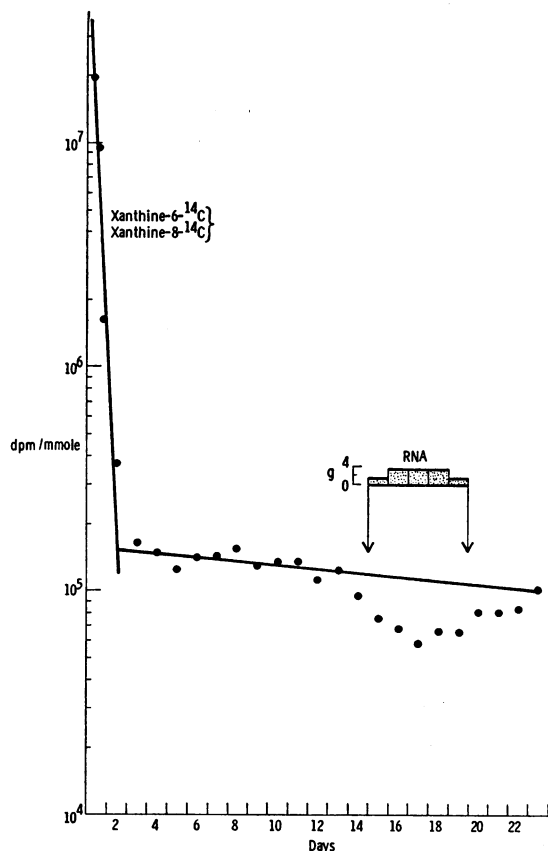


FIGURE 2 Xanthine specific activity. The closed circles represent the ^{14}C appearing in the xanthine fraction of the Dowex column chromatography separation, and thus include the sum of xanthine-6- ^{14}C and xanthine-8- ^{14}C .

the uric acid output per day was too low to be measured accurately. The overall recovery of uric acid-6- ^{14}C during the first 7 days was 61 % of the amount given, and excretion after day 8 was negligible.

Since the total amount of uric acid excreted daily is essentially constant on the control diet, one can substitute disintegrations per minute per day for specific activity in the calculation of uric acid turnover (9). The semilog plot is a straight line which represents a turnover of 0.57 pools/day, and a $t_{1/2}$ of 29 hr. The rate of disappearance of uric acid indicates that the compound is an end product not subject to reutilization. The fraction of uric acid-6- ^{14}C ultimately recovered is equal to the fraction of daily turnover which appears as urinary urate. Thus, on the basis of 61 % recovery and an average of 6.5 mg of urine uric acid per 24 hr, one can calculate that the uric acid turn-

over is approximately 11 mg/day, and the pool size is approximately 19 mg.

When subjected to uricase, essentially all of the ^{14}C is volatilized as $^{14}\text{CO}_2$. Only during the 1st day were there counts remaining, about 5 % of the total.

The fate of the xanthine-6- ^{14}C and hypoxanthine-8- ^{14}C can be seen in Figs. 2, 3, and 5. Figs. 2 and 3 represent the total ^{14}C counts appearing as xanthine and hypoxanthine respectively, whereas Fig. 5 breaks these curves down according to their 6 and 8 carbon labels. It is immediately evident that there are rapid interconversions occurring even in the first few hours. During this time 5 % of the counts in xanthine are 8- ^{14}C and 19 % of the activity in hypoxanthine is 6- ^{14}C .

Since 78 % of the total oxypurine output is xanthine much more of the xanthine-6- ^{14}C label than hypoxanthine-8- ^{14}C is initially cleared. The actual recovery of the labels reveals that by day 21, 75 % of the original xanthine-6- ^{14}C but only 28 % of the hypoxanthine-8- ^{14}C has been recovered in the

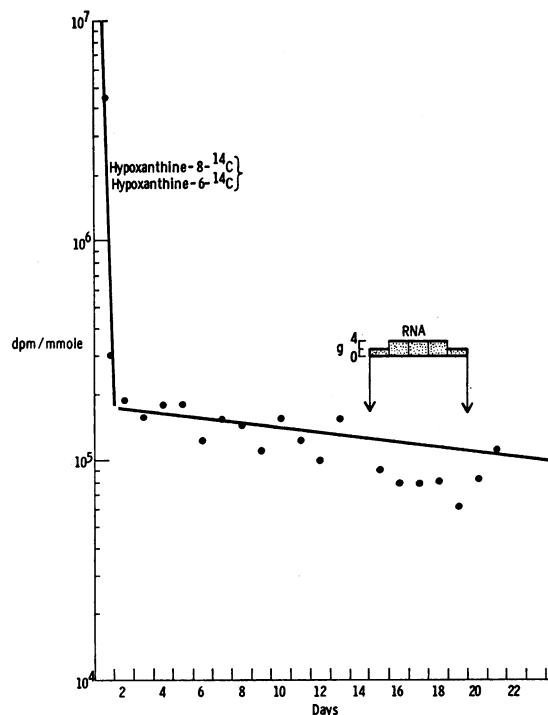


FIGURE 3 Hypoxanthine specific activity. The closed circles represent the ^{14}C appearing in the hypoxanthine fraction of the Dowex column chromatography separation, and thus include the sum of hypoxanthine-8- ^{14}C and hypoxanthine-6- ^{14}C .

urine. These values are of the same order of magnitude as those reported by Ayvazian and Skupp (5) for guanine and hypoxanthine (74 and 36 % respectively in the first 2 wk).

Sizes of body pools were determined from the first points, which were linear (Figs. 6 and 7). The xanthine pool was 72.5 mg with a $t_{1/2}$ of 4.4 hr and a turnover rate of 11.5 mg/hr or 276 mg/day. These data should be compared with the data presented by Engelman, Watts, Klinenberg, Sjoerdsma, and Seegmiller (2). They propose two pools for xanthine on the basis of its specific activity in urine. The first is a pool of 225 mg with a turnover of 62.3 mg/hr and a $t_{1/2}$ of 2.5 hr. The second is a pool of 586 mg with a turnover of 25 mg/hr and a $t_{1/2}$ of 15 hr. They have also calculated a pool of 144 mg of xanthine from its specific activity in plasma, with a turnover of 11 mg/hr and a $t_{1/2}$ of 9 hr.

On the basis of the initial decay value, a daily turnover of 276 mg of xanthine per day can be calculated. The average urinary output was 219 mg/day. Thus 79 % of the daily turnover appears in the urine. The second phase of the decay curve is undoubtedly due to release of xanthine which

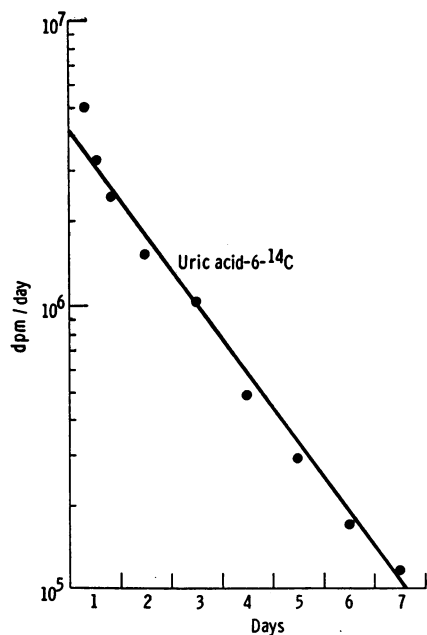


FIGURE 4 Uric acid- $6\text{-}^{14}\text{C}$ activity. The data is expressed as disintegrations per minute per day since urine uric acid was often too low to be measured. This represents a body pool of approximately 19 mg of uric acid with a turnover of 0.57 pools/day.

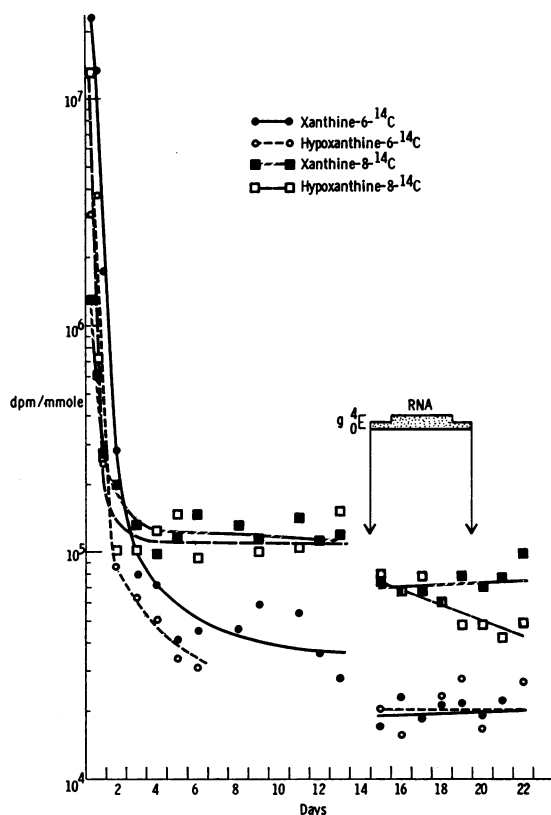


FIGURE 5 Xanthine and hypoxanthine specific activities separated into $6\text{-}^{14}\text{C}$ and $8\text{-}^{14}\text{C}$ components. After the xanthine and hypoxanthine fractions are separated by column chromatography, each is subjected to the action of xanthine oxidase and uricase. The $6\text{-}^{14}\text{C}$ is volatilized as $^{14}\text{CO}_2$ and the $8\text{-}^{14}\text{C}$ remains in solution. (See Results.)

had been converted to cellular purine derivatives including nucleic acids.

The hypoxanthine- $8\text{-}^{14}\text{C}$ data present a somewhat similar picture (Fig. 7), although the decrease in specific activity and interconversion is so rapid that only three points were obtained before the curve changes slope. These three points, however, can be represented by a straight line with a $t_{1/2}$ of 21 hr. This represents a pool of 118 mg with a turnover of 40 mg/hr. Engelman et al. (2) apparently found a similar rapid fall off in the hypoxanthine specific activity but were unable to plot any reliable curve.

The hypoxanthine turnover is 960 mg/day, of which 54.5 mg appears in the urine. Thus, hypoxanthine is extremely active metabolically. Only 5.7 % appears in the urine. The rest must be converted to metabolically stable products.

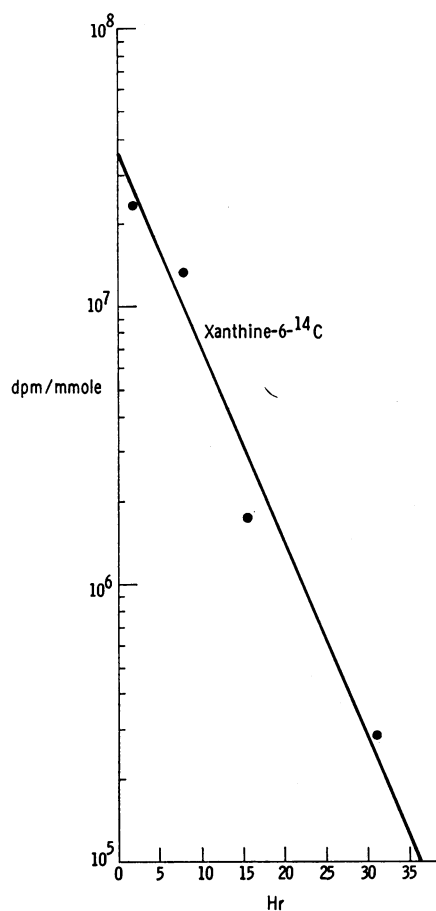


FIGURE 6 Xanthine-6-¹⁴C specific activity. This graph is a magnification of the first four points of the xanthine-6-¹⁴C curve of Fig. 5. It represents a xanthine miscible pool of 72.5 mg with a turnover of 276 mg/day.

During the first 8 days only 2,000,000 dpm are recovered in the stool (after appropriate quench correction). This value represents only 3% of the total counts given, and no attempt was made to separate this relatively small amount into the different 6- and 8-¹⁴C labels.

DISCUSSION

The studies reported above confirm the virtual absence of xanthine oxidase activity in this patient. The turnover of uric acid is 0.57 pools/day, which is quite similar to the values reported for normal individuals (9-13).

The small uric acid pool (approximately 19 mg) may be due to small residual xanthine oxidase activity. That this is the case is suggested by the nonvolatile ¹⁴C in the urinary urate in the 1st day

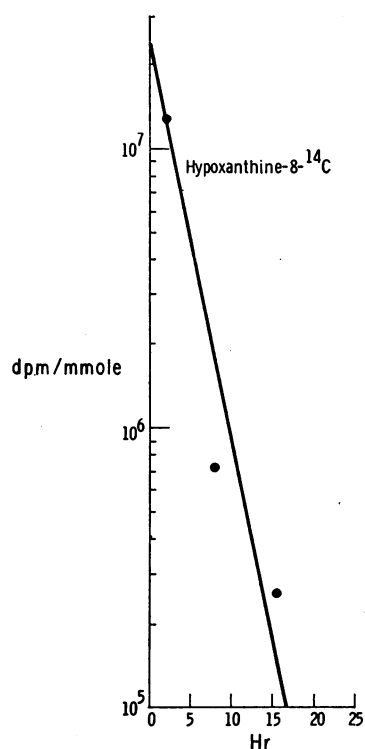


FIGURE 7 Hypoxanthine-8-¹⁴C specific activity. This graph is a magnification of the first three points of the hypoxanthine-8-¹⁴C curve of Fig. 5. It represents a hypoxanthine miscible pool of 118/mg with a turnover of 960 mg/day.

and the increase in the urate concentration that follows the ingestion of RNA.

The rapidly miscible body pools of xanthine (72.5 mg) and hypoxanthine (118 mg) are smaller than the pool of uric acid in normal individuals (9-13). A similar disparity is seen in normal and gouty subjects given allopurinol (14-19) in whom the molar increase of total oxypurines averages only about 65% of the decrease in uric acid. The major reason for this "deficit" appears to be the reutilization of xanthine and hypoxanthine by the body and the concurrent repression of de novo purine synthesis. The data presented here support the rapid interconversion of xanthine and hypoxanthine, presumably at the nucleotide and nucleic acid levels. Ayvazian and Skupp (5) have also shown evidence of such reutilization. After administration of ¹⁴C-labeled hypoxanthine (or adenine) they found labeling of 7-methylguanine as early as the first 4 hr. Since methylation of RNA occurs at the macromolecular

level (20), they conclude that these interconversions occur rapidly and at the nucleic acid level, whereas other interconversions occur at the nucleotide level. It has also been shown, in mice, that utilization of hypoxanthine (21) and xanthine (22) for nucleic acid synthesis is increased by allopurinol, which blocks their degradation to urate. Similarly, in a patient with chronic granulocytic leukemia allopurinol caused an increase in the incorporation of hypoxanthine-¹⁴C into the nucleic acids of leukocytes.⁷

In contrast to the study by Ayvazian and Skupp (23) an increase in hypoxanthine as well as xanthine excretion is seen after an oral RNA load was given. Ayvazian and Skupp (23) found that when they fed adenosine 5'-phosphate (AMP) and guanosine 5'-phosphate (GMP) separately, in amounts equivalent to those the patient had ingested in yeast RNA, the purine excretion during the GMP feedings qualitatively and quantitatively reproduced that observed during the RNA study. They concluded that in their patient from 90–100 % of the guanine bases of RNA were converted to xanthine and excreted, whereas the majority of the adenine appeared to enter into the metabolic pool. In the present study, the relative increase in both xanthine and hypoxanthine after ingestion of 16 g of yeast RNA is virtually identical (60 vs. 62.5 %). The dilution of specific activity is also quite similar (Figs. 2 and 3), and the total number of counts excreted per day does not change significantly.

One reason for these differences may be the size of the RNA load, only 0.9 g/day in the patient of Ayvazian and Skupp (23) and an average of 3.2 g/day in our patient. Alternatively, a possible difference between patients in endogenous levels of anabolic enzymes may also be a contributing factor. Recent studies have shown large differences in the levels of IMP: pyrophosphate phosphoribosyltransferase and AMP: pyrophosphate phosphoribosyltransferase, two enzymes primarily responsible for anabolism of exogenous purines, in a series of leukemic patients.⁸

These data suggest certain generalities regarding purine metabolism in man. As has been seen with other subjects, the rate of disappearance of

uric acid indicates that the compound is an end product not subject to reutilization. The turnover of uric acid seen in this patient is similar to that in patients with normal pools, a finding which supports the idea that turnover is not an absolute function, and the 61 % recovery of uric acid-6-¹⁴C is similar to the normal recovery of 50–90 % tracer dose. The complex curve for xanthine and hypoxanthine shows that these compounds are subject to anabolism, and they enter dynamic equilibria with more stable body pools.

Certain contrasts are apparent, however. In this study, the average daily output of xanthine is 79 % of the daily turnover, and 71 % of the administered xanthine is found (by day 21) as urinary oxypurines. On the other hand, only 5.7 % of the daily hypoxanthine turnover is found in the urine, and 28 % of the label is recovered in toto after 21 days. These facts show that xanthine, which leaks from cells, is only slightly reutilized. Hypoxanthine, on the other hand, is extremely active metabolically. The daily reutilization of hypoxanthine is of the same magnitude as the amount of hypoxanthine excreted in the urine of children with juvenile hyperuricemia during allopurinol therapy (6). These children lack IMP: pyrophosphate phosphoribosyltransferase (24) and cannot convert hypoxanthine to inosinic acid.

Since the total excretion of xanthine involves somewhat more than half the amount of purine normally found as urinary uric acid, it follows that less than half of the uric acid normally found comes from hypoxanthine. The maximal hypoxanthine contribution to uric acid, thus, represents less than 25 % of its daily turnover. The above data implies that a major recycling of hypoxanthine with continual synthesis of inosinic acid from hypoxanthine occurs in normal purine metabolism, whereas xanthine is not an anabolic intermediate.

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