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Study of the Utilization and Excretion of Dietary Purines in a Xanthinuric Man *

JOHN H. AYVAZIAN † AND SOLOMON SKUPP

(From the Medical and Research Services, Veterans Administration Hospital, New York, N. Y.)

In a previous study of this xanthinuric subject's purine metabolism, we observed that a tracer dose of adenine-8-^{14}C given intravenously was excreted predominately as hypoxanthine and a tracer dose of guanine-8-^{14}C predominately as xanthine. In addition, only 1% of the adenine-8-^{14}C label was excreted on the first day after administration, as compared to 63% of the guanine-8-^{14}C label (1).

This study was designed to determine whether similar differences in the incorporation and excretion of these purines could be observed if they were taken orally. The form and amounts of purine given were selected to approximate physiologic conditions and yet allow accurate quantitation. Ribonucleic acid and ribonucleotides were selected, since dietary purines would ordinarily appear in these forms in the gastrointestinal tract rather than as free bases. The quantity given was selected to be large enough to show significant differences in purine excretion without exceeding the subject's daily purine requirements.

Methods

The methods used in this study were reported in detail in prior publications (1, 2). These include methods of urine collection and storage; enzymatic and spectrophotometric analysis of uric acid, xanthine, and hypoxanthine in urine and plasma; chemical analysis of creatinine by the alkaline picrate method; column chromatographic and gradient elution methods for the separation and quantitation of the urinary purines; spectrophotometric methods for the identification and quantitation of individual purines; and methods for counting ^{14}C activity in the whole urine and purine fractions by liquid scintillation techniques.

Additional details and modifications include the following: A purine-free diet was ingested throughout the study; the diet was meatless and contained 40 g of protein, 40 g of fat, and 387 g of carbohydrates, affording the subject 2,100 calories each day. Yeast RNA,[1] 3′-adenylic acid,[2] 2′- and 3′-guanylic acid [2] (sodium salt, mixed isomers), hypoxanthine-8-^{14}C,[3] and 3′-adenylic acid-8-^{14}C [3] were obtained from commercial sources. Their purity and specific activities were checked and agreed with the manufacturer's specifications. The specific activity of hypoxanthine-8-^{14}C was 39.3 mc per mmole, and the specific activity of 3′-adenylic acid-8-^{14}C was 34.6 mc per mmole. The hypoxanthine-8-^{14}C was administered intravenously in 10 ml of sterile aqueous $\frac{1}{6}$ M lactate, and 3′-adenylic acid-8-^{14}C was given orally diluted in a sugar solution. The enzymatic and spectrophotometric analyses were modified by changing the concentration of the Tris buffer to 0.08 mole per L, and the plasma oxypurine analyses were done with whole plasma and protein-free ultrafiltrates.

Results

In each of the dietary studies the subject was first fed the purine-free diet for a minimum of 4 days to allow his oxypurine excretion to fall to control levels. These control levels remained constant throughout the study. The data obtained from the urine samples collected during the first few days of all collection periods have not been included in this report, as it took approximately 48 hours for the subject's oxypurine excretion to stabilize when there was a change in his diet.

In the first study the subject was fed the purine-free diet for 10 days and his urine collected. During the latter 7 days of this control period his oxypurine excretion, determined enzymatically, averaged 2.42 mmoles per 24 hours. In the second study 900 mg of yeast RNA was added to his daily diet in three divided doses given with meals

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† Address requests for reprints to Dr. John H. Ayvazian, Veterans Administration Hospital, 1st Ave. at E. 24th St., New York, N. Y. 10010.

[1] Worthington Biochemical Corp., Freehold, N. J.

[2] Sigma Chemical Co., St. Louis, Mo.

[3] Schwarz Bioresearch, Orangeburg, N. Y.

TABLE I

*Oxypurine recovered from urine**

Purine-free diet		RNA†		2′- and 3′-GMP‡		3′-AMP§	
Xanthine	Hypo-xanthine	Xanthine	Hypo-xanthine	Xanthine	Hypo-xanthine	Xanthine	Hypo-xanthine
			mmoles/day				
2.04	0.37	2.36	0.35	2.50	0.50	1.93	0.52
2.04	0.40	2.48	0.49	2.35	0.43	1.77	0.44
1.77	0.33	2.14	0.34	2.40	0.36	1.93	0.46
1.61	0.28	2.45	0.51	2.25	0.30	1.87	0.29
2.21	0.43	2.42	0.36	2.65	0.47	1.89	0.37
1.91	0.35	2.78	0.39	2.51	0.28	1.98	0.44
1.82	0.34	2.72	0.34				
1.91‖		2.48		2.44		1.90	
	0.36‖		0.40		0.39		0.42

* Recovery by chromatographic fractionation and spectrophotometric quantitation.
† Diet supplemented with yeast RNA containing 1.2 mmoles of purine base.
‡ Diet supplemented with 0.6 mmole of 2′- and 3′-GMP (guanylic acid).
§ Diet supplemented with 0.6 mmole of 3′-AMP (adenylic acid).
‖ Mean values.

and his urine collected for an additional 9 days. The yeast RNA contained 1.2 mmoles of purine, 0.58 mmole of adenine, and 0.62 mmole of guanine (3). During the latter 7 days of RNA supplementation his total oxypurine excretion averaged 2.96 mmoles per 24 hours, an average increase of 0.54 mmole per day or slightly less than half the amount of purine ingested daily.

In the third study the subject, after 4 days of his purine-free diet, received 0.6 mmole of 2′- and 3′-guanylic acid (GMP) given in three divided doses each day for 9 days. His oxypurine excretions for the last 7 days of this study averaged 3.02 mmoles per 24 hours, an increase of 0.60 mmole above the average purine-free value. The fourth study was similar to the third except that his diet was supplemented with 0.6 mmole of 3′-adenylic acid (AMP) daily. The last 7 days' excretion of this study averaged 2.47 mmoles per 24 hours, approximately the same as that of the purine-free study.

After enzymatic analysis of the oxypurine content of each urine sample, the 24-hour samples of the purine-free period and the three dietary studies were processed and the individual purines separated and quantitated by two-stage column chromatography, gradient elution, and spectrophotometry. Table I lists the results of the fractional analysis of the oxypurines of these urine samples. The total amount of oxypurines obtained was slightly less than that determined by the enzymatic-spectrophotometric analysis because of the incomplete recovery during the extraction procedure. The recoveries ranged from 84% to 99%, averaging 94%, and there were no significant differences in the recoveries obtained from the urine samples of each of the four studies. Laboratory accidents resulted in the loss of one sample each from the AMP and GMP series. The data confirm the differences observed by enzymatic analysis, and the fractionation indicates that the difference is limited to an increase in xanthine excretion during the RNA and GMP studies. The mean values for xanthine excretion in these four studies were 1.91 mmoles per 24 hours for the purine-free period, 1.90 mmoles per 24 hours for the AMP period, 2.48 mmoles per 24 hours for the RNA period, and 2.44 mmoles per 24 hours for the GMP period. Statistical analysis by the two-sample rank test of Wilcoxan and White (4) confirms the significance of the increased xanthine excretion found in the RNA and GMP studies as compared to those obtained in the purine-free and AMP studies with p values of less than 0.01. The lesser differences observed in the hypoxanthine fractions of the four studies are not statistically significant although there is a suggestion that some ingested adenine is excreted as hypoxanthine, since the higher mean values were obtained from the urine samples collected while the subject was ingesting AMP and RNA.

At the beginning of the purine-free and RNA studies the subject was given 8 μc of hypoxanthine-8-^{14}C. Eighteen per cent of the radioiso-

tope label was excreted in the urine on the first day and a total of 39% by the end of 2 weeks. The slope of the 10-day cumulative excretion curve (Figure 1) calculated from days 3 to 13 is 1.6% per 24 hours. The respective values obtained from a previous study on the same subject when 25 μc of hypoxanthine-8-^{14}C was given were 16%, 36%, and 1.6% per day (1). This curve is also shown in Figure 1. The specific activities of xanthine and hypoxanthine were determined each day throughout the study and show an abrupt decrease with the onset of RNA supplementary feedings, indicating dilution by an unlabeled source of purine. These data are plotted in Figure 2. Projections of the specific activity curves were made starting at the dilution point. These projections were based on the previous study with hypoxanthine-8-^{14}C in which the diet was kept constant (1). Utilizing the differences between the observed and projected daily specific activities and the average values for the individual oxypurines, obtained while the subject was on the purine-free diet, one can calculate the increase in excretion of each oxypurine during the RNA feeding. It was determined that 0.02 mmole per 24 hours of unlabeled hypoxanthine would produce the decrease in specific activity of hypoxanthine observed from days 13 to 19. Similar calculations for xanthine revealed values that progressively increased from 0.42 mmole on the thirteenth day to 0.55 mmole on the nineteenth day. These estimated figures agree closely with the differences observed in the quantitative studies shown in Table I.

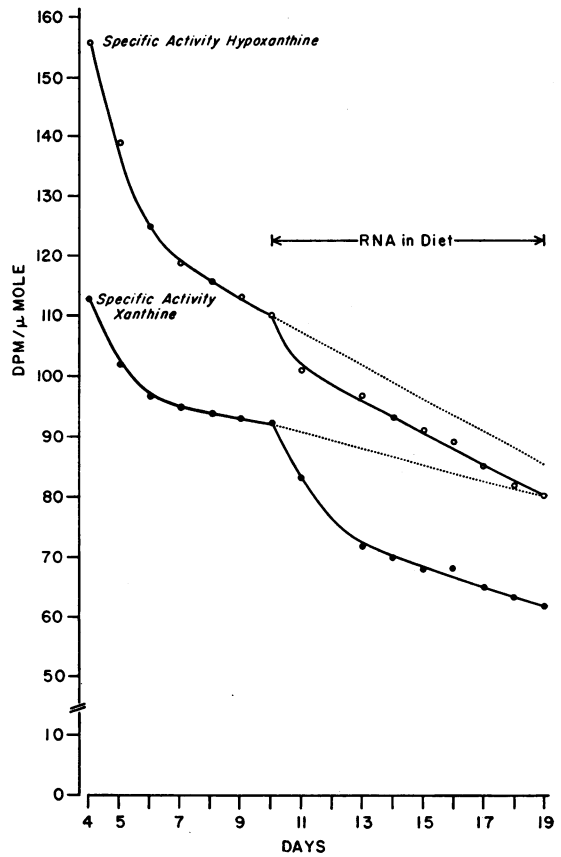

FIG. 2. PATTERN OF LABELING OF URINARY OXYPURINES AFTER IV ADMINISTRATION OF 8 μC OF HYPOXANTHINE-8-^{14}C. Dotted lines represent estimated decay of specific activities if RNA had not been added to the purine-free diet.

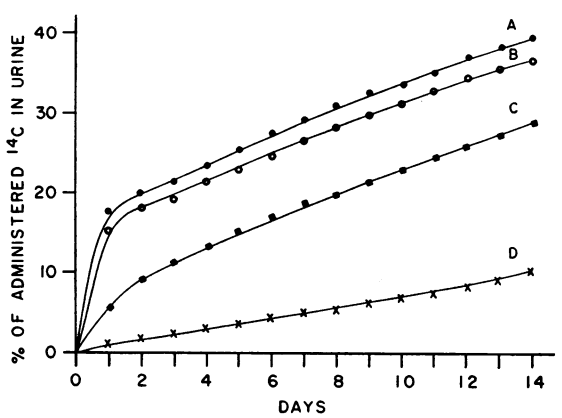

FIG. 1. CUMULATIVE EXCRETION OF ^{14}C IN URINE. Plotted as per cent of administered dose after administration of 8 μc of hypoxanthine-8-^{14}C intravenously (A), 25 μc of hypoxanthine-8-^{14}C intravenously (B), 8 μc of 3′-adenylic acid-8-^{14}C orally (C), and 100 μc of adenine-8-^{14}C intravenously (D).

In the fifth study the subject was stabilized on the purine-free diet and then given 0.6 mmole daily of unlabeled AMP supplements as before. On the third day of AMP supplementation 8 μc of 3′-AMP-8-^{14}C in a sugar solution was given by mouth. On the fourth day the AMP supplements were stopped, and the subject continued on his purine-free diet. Urine samples were collected for an additional 14 days. The cumulative excretion curve of the urinary ^{14}C is shown in Figure 1. On the first day after ingestion of the 3′-AMP-8-^{14}C 6.7% of the label was excreted in the urine and a total of 29% after 14 days. The 10-day slope calculated from days 3 to 13 was 1.6% per 24 hours (Figure 1). The per cent of ^{14}C label

excreted in the urine was intermediate between that obtained when adenine-8-^{14}C (1) and hypoxanthine-8-^{14}C were given intravenously. However, the slope of the cumulative excretion curve is exactly similar to the slope obtained after administration of hypoxanthine-8-^{14}C. In addition, except for the first day, the specific activities of the individual purines isolated from the daily urine samples agreed closely with those obtained on comparable days after the iv administration of 8 μc of hypoxanthine-8-^{14}C (Table II), indicating almost complete absorption of the orally administered ^{14}C-labeled AMP. On the first day after 3'-AMP-8-^{14}C administration the specific activities of the urinary purines (in dpm per μmole) were as follows: hypoxanthine, 1,430; adenine, 240; xanthine, 150; and 7-methylguanine, 130. On the second day the specific activities were hypoxanthine, 360; adenine, 250; xanthine, 160; and 7-methylguanine, 140. After this the specific activities of these purines tended to equalize as they decayed. On the seventh day their specific activities varied from 90 to 130 dpm per μmole and at 14 days from 90 to 100 dpm per μmole. The plot of the ratio of the specific activities of xanthine to hypoxanthine over time parallels that originally reported in an earlier study after the administration of hypoxanthine-8-^{14}C rather than that obtained after the administration of adenine-8-^{14}C (1). This comparison can be seen in Figure 3. No significant amounts of uric acid were found in the urine, nor were there any significant changes in the quantity of excreted guanine, adenine, 7-methylguanine, 1-methylguanine, or N-2-methylguanine observed during the dietary studies. In addition, the specific activities of 7-methylguanine and adenine closely paralleled those of xanthine and hypoxanthine, respectively, as reported earlier (1).

TABLE II

Comparison of specific activities of oxypurines after oral and iv administration of ^{14}C-labeled purines

Day	Xanthine		Hypoxanthine	
	Hx-8-^{14}C*	AMP-8-^{14}C†	Hx-8-^{14}C*	AMP-8-^{14}C†
	dpm/μmole			
1	130	150	5,240	1,430
2	125	160	205	360
4	113	118	156	180
7	95	106	119	125
10	92	94	110	108

* Specific activities of xanthine and hypoxanthine after iv administration of 8 μc of hypoxanthine-8-^{14}C.
† Specific activities of xanthine and hypoxanthine after oral administration of 8 μc of AMP-8-^{14}C.

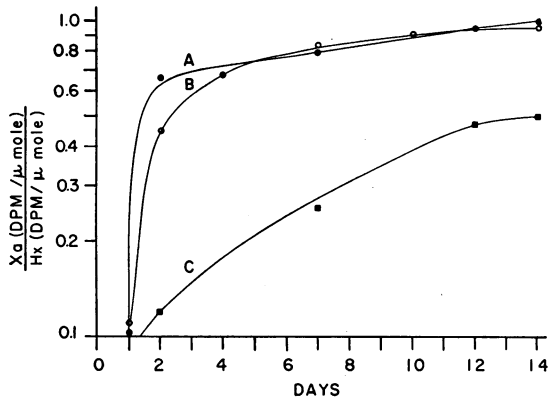

FIG. 3. SEMILOGARITHMIC PLOT OF RATIOS OF SPECIFIC ACTIVITIES OF XANTHINE (XA) TO HYPOXANTHINE (HX). Data obtained from the studies in which hypoxanthine-8-^{14}C was given intravenously (A), 3'-adenylic acid-8-^{14}C was given orally (B), and adenine-8-^{14}C was given intravenously (C). The data indicated by curves A and C were obtained during a previous study (1).

The plasma oxypurine concentrations varied from 0.2 to 0.7 mg per 100 ml throughout the studies with a mean value of 0.5 mg per 100 ml. There was no significant increase noted in the plasma oxypurine concentrations while the subject was ingesting the purine supplements.

Discussion

The work presented demonstrates that the increase in this subject's urinary excretion of oxypurines accounted for approximately half of the purines he had ingested in the form of yeast RNA. Quantitative analysis of the individual urinary purines demonstrated that this increase in excretion consisted predominately of xanthine, suggesting that from 90 to 100% of the guanine bases of RNA were being absorbed from the gastrointestinal tract, catabolized to xanthine, and excreted. This was confirmed when the subject was fed AMP and GMP separately in amounts equivalent to those he had ingested in the yeast RNA and his purine excretion during the GMP feedings qualitatively and quantitatively reproduced that observed during the RNA study.

The metabolic fate of the ingested adenine is less clear. The failure to recover more than a small portion, approximately 5%, of the ingested

adenine bases as urinary hypoxanthine allows speculation without supporting evidence. For this reason the additional study, in which the subject ingested 3′-AMP-8-^{14}C, was done to confirm its absorption from the gastrointestinal tract and, if possible, to determine its metabolic fate. The tracer dose of 3′-AMP-8-^{14}C was given in conjunction with the usual dose of unlabeled AMP in order to approximate the conditions of the dietary study. The early labeling of the urinary purines, including the substituted guanines, after ingestion of the 3′-AMP-8-^{14}C and the levels of specific activity achieved proved that the major portion of the AMP was promptly absorbed, interconverted, and utilized, at least in part, for nucleic acid synthesis (1).

Ingested nucleic acids are depolymerized to polynucleotides and mononucleotides by the pancreatic nucleases and phosphodiesterases, and the nucleotides are then converted to nucleosides by intestinal nucleotidases and phosphatases after which absorption can occur (5). On the basis of the present knowledge of the catabolism of ingested nucleic acids it may be assumed that the bulk of the ingested AMP was absorbed into the intestinal epithelium as adenosine. This nucleoside can serve as a substrate for three possible metabolic conversions. It can be converted to adenine by the action of adenosine phosphorylase, 5′-AMP by the action of adenosine kinase, or inosine by the action of adenosine deaminase (5). Our data indicate that the latter pathway was preferred. The high specific activity of the hypoxanthine (six times that of adenine) observed in the first 24 hours after the ingestion of the 3′-AMP-8-^{14}C indicates that the principal conversion undergone by the ingested material involved deamination. The fact that approximately 3% of the ingested 3′-AMP-8-^{14}C was excreted as hypoxanthine during the first 24 hours indicates that a small portion of the newly formed inosine-8-^{14}C was degraded to the free base by the action of inosine phosphorylase. However, the major portion was probably converted to 5′-inosinic acid by the action of inosine kinase, then converted in equal amounts to 5′-adenine and guanine nucleotides and utilized. This is confirmed by the relatively high percentage of ^{14}C-labeled purines excreted, at a relatively constant rate, each day throughout the 2-week period of the study; by the prompt and approximately equal distribution of the ^{14}C label among the adenine and guanine derivatives in the urine; and by the marked similarity of these results to an earlier study in which hypoxanthine-8-^{14}C was given intravenously after which labeled purines were recovered from cellular nucleotides and nucleic acids (1).

These speculations have some confirmation in the recent work of Cook and Vibert, who found, using rabbit erythrocytes, that the major route for the utilization of adenosine in the formation of ATP and GTP is via deamination to inosine (6).

Although the 3′-AMP-8-^{14}C study confirms the absorption, conversion, and urinary excretion of the labeled purine, no net change in the urinary excretion of purines was noted while this subject was ingesting relatively large amounts of unlabeled AMP. Wyngaarden and Ashton have shown that purine 5′-ribonucleotides inhibit their *de novo* synthesis by regulating the activity of glutamine phosphoribosylpyrophosphate amidotransferase (7). This enzyme activates the first reaction of purine nucleotide synthesis in which 1-glutamine and 5-phosphoribosyl-1-pyrophosphate form 5-phosphoribosyl-1-amine. Theoretically once the preformed purines are salvaged from their dietary source and converted to 5′-nucleotides, this inhibition can occur. Studies indicate that AMP and GMP are more effective inhibitors than either alone (8, 9) and that 5′-inosinic acid probably is converted to 5′-AMP or 5′-GMP or both before it can act as an inhibitor (10). Our data would indicate that this inhibition occurred in equimolar amounts to the purines salvaged from the diet. Although the data and conclusions presented in this study apply only to this xanthinuric subject, a similar dietary study of normal and hyperuricemic subjects is now being conducted. Initial observations indicate that normal subjects and gouty subjects with normal excretion of uric acid do not increase their uric acid excretion while ingesting 3′-AMP, whereas gouty subjects with hyperexcretion of uric acid decrease their uric acid excretion.

Summary

The utilization and excretion of purines absorbed from the gastrointestinal tract of a xanthinuric man have been studied with purine supple-

ments to the diet as well as tracer doses of hypoxanthine-8-^{14}C and 3′-adenylic acid-8-^{14}C.

The data indicate that guanine ingested as yeast RNA or as 3′-guanylic acid is absorbed and the major portion excreted as xanthine. Only a small fraction of the adenine, ingested in the form of yeast RNA or 3′-adenylic acid, can be accounted for in the urinary purines. The data obtained after the ingestion of 3′-adenylic acid-8-^{14}C indicate that it is converted into 5′-nucleotides via a deamination pathway and probably inhibits the *de novo* synthesis of purine ribonucleotides.

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