Hereditary Xanthinuria

Evidence for Enhanced Hypoxanthine Salvage

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Abstract

We tested the hypothesis that there is an enhanced rate of hypoxanthine salvage in two siblings with hereditary xanthinuria. We radiolabeled the adenine nucleotide pool with [8-^{14}C]adenine and examined purine nucleotide degradation after intravenous fructose. The cumulative excretion of radioactivity during a 5-d period was 9.7% and 9.1% of infused radioactivity in the enzyme-deficient patients and 6.0±0.7% (mean±SE) in four normal subjects. Fructose infusion increased urinary radioactivity to 7.96 and 9.16 × 10^6 cpm/g creatinine in both patients and to 4.73±0.69 × 10^6 cpm/g creatinine in controls. The infusion of fructose increased total urinary purine excretion to a mean of 487% from low-normal baseline values in the patients and to 398±86% in control subjects. In the enzyme-deficient patients, the infusion of fructose elicited an increase of plasma guanosine from undetectable values to 0.7 and 0.9 μM. With adjustments made for intestinal purine loss, these data support the hypothesis that there is enhanced hypoxanthine salvage in hereditary xanthinuria. Degradation of guanine nucleotides to xanthine bypasses the hypoxanthine salvage pathway and may explain the predominance of this urinary purine compound in xanthinuria.

Introduction

Xanthinuria is a rare hereditary disorder of purine metabolism resulting from a marked deficiency of the enzyme xanthine oxidase (1) (Fig. 1). As a consequence, hypouricemia and decreased urinary uric acid excretion have been recognized as the biochemical hallmarks of this disease (2). Urinary purine excretion, calculated by the sum of hypoxanthine, xanthine, and uric acid, is not increased (3), and hence, purine production is considered to be normal in hereditary xanthinuria.

Pharmacologic inhibition of xanthine oxidase by allopurinol is associated with decreased urinary purine excretion (4–7). Increased reutilization of hypoxanthine during allopurinol therapy has been shown to contribute substantially to decreased purine excretion (7). Patients with hereditary xanthinuria appear to be resistant to this pharmacologic effect of allopurinol (8–13). This has been explained by saturation of hypoxanthine phosphori-

bosyltransferase (HPRT)[1] by enhanced reutilization of hypoxanthine in hereditary xanthinuria (2).

In an effort to directly examine this issue, we have tested the hypothesis that there is an enhanced rate of hypoxanthine salvage in hereditary xanthinuria. Our approach has involved radioactively labeling the adenine nucleotide pool and stimulating ATP breakdown to observe whether the metabolic changes can be explained by this hypothesis.

Methods

Materials

Uricase, creatininase, purine nucleoside phosphorylase, xanthine oxidase, AMP, IMP, adenine, inosine, guanosine, hypoxanthine, xanthine, xanthosine, and guanine were purchased from Calbiochem-Behring Corp., American Hoechst Corp. (San Diego, CA). From Amersham Corp. (Arlington Heights, IL), we purchased [8-^{14}C]hypoxanthine (50 mCi/mmol) for hypoxanthine-guanine phosphoribosyltransferase assay. From New England Nuclear (Boston, MA), we purchased [8-^{14}C]adenine (52 mCi/mmol). Fructose was provided as a 20% solution for intravenous injection from Baxter Co., Madrid, Spain. All other reagents were of the highest commercial quality.

Case reports

Two siblings of a first cousin marriage were admitted for study of hypouricemia to the Metabolic Unit of "La Paz" University Hospital.

Case 1. A.S., a 13-yr-old boy, passed multiple brownish yellow stones during his first year of life. At 2 yr of age he had right renal colic, and an intravenous pyelogram disclosed ureteral lithiasis which required surgical extraction. Infrared spectrophotometric analysis of the calculi showed xanthine composition. At 6 yr of age he was reoperated on because of multiple pelvic and ureteral calculi composed of xanthine. He was advised to drink water (1,000 ml/24 h) with a sodium bicarbonate content of 0.5% (wt/vol) and since then he has been asymptomatic. Physical examination and routine blood and urine analysis gave normal results except for a serum urate of 0.3 mg/dl and a 24-h uric acid excretion of 6.2 mg while he was on a self-selected diet. Urinary oxypurine excretion was 2.14 mmol/24 h of which 79% was xanthine.

Case 2. R.S., a 22-yr-old woman, was the only sister of A.S. and enjoyed good health from birth. Her physical examination, routine blood tests, urinalysis, and intravenous pyelogram gave normal findings. The diagnosis of hereditary xanthinuria was suspected upon the finding of a serum urate of 0.5 mg/dl and a uric acid excretion of 16 mg/24 h on a self-selected diet. Urine oxypurine excretion was 1.74 mmol/24 h of which 71% was xanthine.

Both patients display plasma and urinary purine changes that correspond exactly to the clinical and biochemical features of classical xanthinuria (1). These patients did not have neurologic abnormalities and showed normal I.Q. They did not suffer severe or recurrent infections; total lymphocyte counts, serum immunoglobulin levels, skin tests for delayed hypersensitivity, response to phytohemagglutinin, and differential

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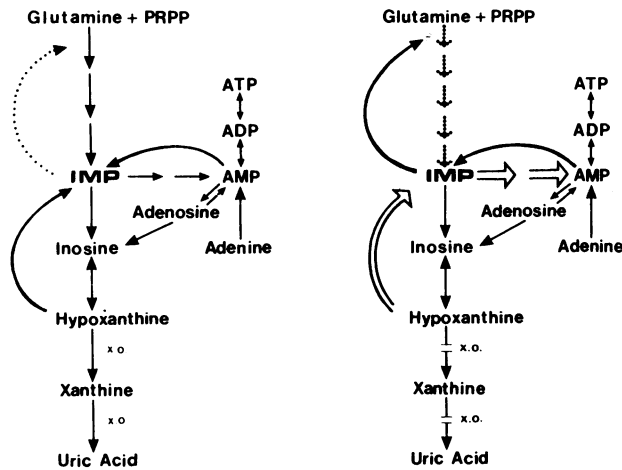

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1. *Abbreviations used in this paper:* HPRT, hypoxanthine phosphoribosyltransferase.

Normal Conditions   X.O. Deficiency

Figure 1. Hypothesis of enhanced reutilization of hypoxanthine in hereditary xanthinuria. A block of xanthine oxidase (x.o.) enhances reutilization of hypoxanthine. This increases IMP formation from hypoxanthine and inhibits de novo synthesis of purines. This change may lead to decreased total purine excretion. The IMP from de novo purine synthesis is not labeled by [8-^{14}C]adenine. Under normal circumstances IMP formed by this pathway dilutes the ATP formed from adenine or from hypoxanthine salvage. Inhibition of de novo purine synthesis reduces the formation of IMP. IMP is predominantly synthesized by the salvage pathway and is highly labeled by hypoxanthine formed from labeled ATP degradation. The ATP resynthesized from this higher specific activity IMP ultimately has a higher specific activity. Under these circumstances urinary purines will have a higher specific activity in xanthine oxidase-deficient patients. The stimulation of ATP degradation by fructose should lead to the excretion of increased radioactivity with purines having a higher specific activity. [8-^{14}C]adenine is used to radiolabel the adenine nucleotide pool (*AMP, ADP, ATP*). PRPP, 5'-phosphoribosyl-1-pyrophosphate.

B cell and T cell peripheral counts were normal. Their parents had normal serum urate concentrations and 24-h uric acid excretion.

Clinical studies

We have used two approaches to examine the hypothesis (14). One method involves the administration of [8-^{14}C]adenine to label the adenine nucleotide pool. Alterations in hypoxanthine reutilization are reflected by changes in urinary radioactivity excretion (7, 15). Intravenous fructose was given to impose a large substrate load on the hypoxanthine reutilization pathway, because this method stimulates the sudden degradation of ATP (14–17). The labeling of urinary purines by this technique in the basal state and after fructose infusion has been previously documented (7, 15).

Both patients and four normal male subjects 18–32 yr of age (mean, 28 yr) were admitted to the University Hospital. Informed consent was obtained from controls, patient R.S., and the parents of A.S. All medication was discontinued for at least 2 wk before the inclusion in the study. The subjects were placed on a weight-maintenance, isocaloric, purine-free diet with a protein content of 10%–15%. 3 d after hospital admission, 25 μCi of [8-^{14}C]adenine contained in 1.6 μmol of adenine was administered intravenously through a 0.22-μm cathivex filter. The filter was flushed with 40 ml of 0.9% sodium chloride to ensure delivery of the entire isotope dose. Urine samples were collected daily over the ensuing 5 d. An aliquot of 0.5 ml of each urine sample was added to 5 ml of aqueous counting scintillant, mixed and counted in an Isocap model 300 liquid scintillation spectrometer (Searle & Co., Nuclear Chicago, Chicago IL) (7, 15).

Upon completion of the fifth 24-h urine collection, fructose (0.5 g/kg of body weight) was infused as a 20% solution over a 15-min period according to the protocol described by Fox and Kelley (16). Urine was collected by voluntary voiding at hourly intervals for 1 h before and 3 h after the fructose infusion. Blood was drawn at 0, 15, 90, and 150 min after the start of the intravenous fructose. Blood samples for nucleosides and purine bases were drawn into prechilled heparinized tubes, cooled in ice, and immediately spun at 1,900 g for 10 min (18). Plasma was separated into prechilled tubes and stored at $-20°$C until assayed.

Plasma and urinary creatinine and uric acid concentrations were determined enzymatically by means of creatininase and uricase, respectively (19, 20). Plasma and urinary nucleosides and purine bases were determined by high pressure liquid chromatography (21). Purine compounds were identified by their specific retention times and the enzymatic peak-shift technique (22, 23). Hypoxanthine and adenine phosphoribosyltransferases were determined by radiochemical methods (24). Protein concentration was estimated by the method of Lowry et al. (25).

Results

Baseline studies. Plasma urate concentrations, 24-h urinary uric acid excretion, uric acid/creatinine ratio, and the fractional excretion of uric acid were very low in both xanthinuric patients (Table I). Conversely, plasma xanthine was markedly increased and plasma hypoxanthine was in the high-normal range. Plasma guanosine and urinary inosine, guanine, xanthosine, and guanosine were undetectable in controls and both patients. The lower limit of detectability of these purine compounds in plasma and urine was 0.1 μM and 0.0001 mmol/g of creatinine, re-

Table I. Clinical Data and Plasma and Urinary Purines in Two Siblings with Xanthine Oxidase Deficiency

	Normal subjects ($n = 4$)	Patients (no.) 1	2
Age (*yr*)	28±2[‡]	13	22
Sex	M	M	F
Plasma			
Urate (*mg/dl*)	4.7±0.4	0.4	0.6
Inosine (*μM*)	0.9±0.2	0.6	0.5
Hypoxanthine (*μM*)	1.7±0.4	3.6	1.6
Xanthine (*μM*)	0.9±0.1	12.4	6.8
Urine			
Uric acid (*mg/24 h*)	445±64	3	5
Urate/creatinine (*mg/mg*)	0.30±0.06	0.004	0.007
Hypoxanthine (*mmol/g creatinine per 24 h*)	0.05±0.01	0.5	0.5
Xanthine (*mmol/g creatinine per 24 h*)	0.04±0.01	1.7	1.3
Total purines* (*mmol/g creatinine per 24 h*)	2.51±0.11	2.14	1.83
Creatinine clearance (*ml/min per 1.73 m²*)	121±17	116	122
Fractional excretion of uric acid (%)	6.5±0.5	0.5	0.7

* Total purines are calculated by the sum of hypoxanthine, xanthine, and uric acid.
‡ Data is expressed as mean±SE.

spectively. Total 24-h urinary purine excretion, calculated by the sum of hypoxanthine, xanthine, and uric acid was in the low-normal range. Uric acid, hypoxanthine, and xanthine comprised 98%, 1.2%, and 0.8% of the normal control urinary purine excretion, respectively. The mean percentages in the two xanthinuric patients were 0.8%, 25%, and 74%, respectively. The marked elevation of plasma and urinary xanthine and the low uric acid values in both patients led to the diagnosis of xanthine oxidase deficiency. Erythrocyte hypoxanthine and adenine phosphoribosyltransferases in both xanthinuric patients were within normal limits.

The excretion of urinary radioactivity after the intravenous administration of tracer doses of [8-^{14}C]adenine allows quantitation of adenine nucleotide degradation separate from de novo purine synthesis (7, 15). The mean cumulative excretion 5 d after [8-^{14}C]adenine infusion was (mean±SE) 6.0±0.7% of the administered radioactive dose in normal subjects. Patients with xanthine oxidase deficiency showed an increased cumulative excretion of 9.7% and 9.1% of the administered radioactive dose.

Response to intravenous fructose. To assess further for reutilization of hypoxanthine, we studied this pathway under the conditions of acute substrate load caused by the rapid infusion of fructose (7, 14, 15). Dramatic changes in plasma purines were observed in the xanthinuric patients after intravenous fructose infusion and were markedly different from normal. Serum urate levels increased in control subjects 145% within the first 90 min after the infusion of fructose (Fig. 2). The enzyme-deficient subjects showed no substantial elevation in serum urate over their baseline value of 0.4 and 0.6 mg/dl. Plasma inosine levels increased to a mean of 2,063% in the patients from baseline values of 0.6 and 0.5 μM, and to 2,693±698% in controls from baseline values of 0.9±0.2 μM. Plasma hypoxanthine increased to 46.6 and 39.1 μM as compared with a normal response value of 21.2±4.9 μM. Fructose infusion increased plasma xanthine to an average value of 17.0 μM in the two xanthinuric patients. The normal plasma xanthine response was an increase in mean values to 4.0±0.9 μM. Plasma guanosine increased in control subjects from undetectable levels to 1.5±0.6 μM. Fructose infusion promoted a similar elevation of plasma guanosine in the enzyme-deficient patients (0.7 and 0.9 μM). Plasma xanthosine levels remained undetectable (< 0.1 μM) in controls and patients.

Profound differences in urinary purines were observed between xanthinuric patients and control subjects in response to fructose infusion. In the control group, urinary uric acid increased to 246% from a mean baseline value of 2.72±0.18 mmol/ g of creatinine within 60 min after fructose infusion. Uric acid excretion remained below 0.15 mmol/g of creatinine in the patients throughout the study. Fructose infusion promoted an elevation of urinary hypoxanthine and xanthine in the control group to 1.19±0.09 and to 0.22±0.08 mmol/g of creatinine, respectively. In both xanthinuric patients fructose elicited a marked elevation of urinary hypoxanthine (6.6 and 2.5 mmol/g of creatinine) and xanthine (2.7 and 2.1 mmol/g of creatinine). Urinary xanthosine was undetectable (< 0.0001 mmol/g creatinine) in the enzyme-deficient patients. Total purine excretion increased within 60 min after fructose infusion to 13.8 and 8.4 mmol/g of creatinine in the xanthinuric patients and to 11.2±2.4 mmol/ g of creatinine in the normal subjects. A stoichiometric replacement of uric acid by hypoxanthine and xanthine, and by inosine and purine bases was observed in the xanthinuric patients in the basal state and after fructose infusion, respectively. After fructose infusion, urinary uric acid, inosine, hypoxanthine, and xanthine
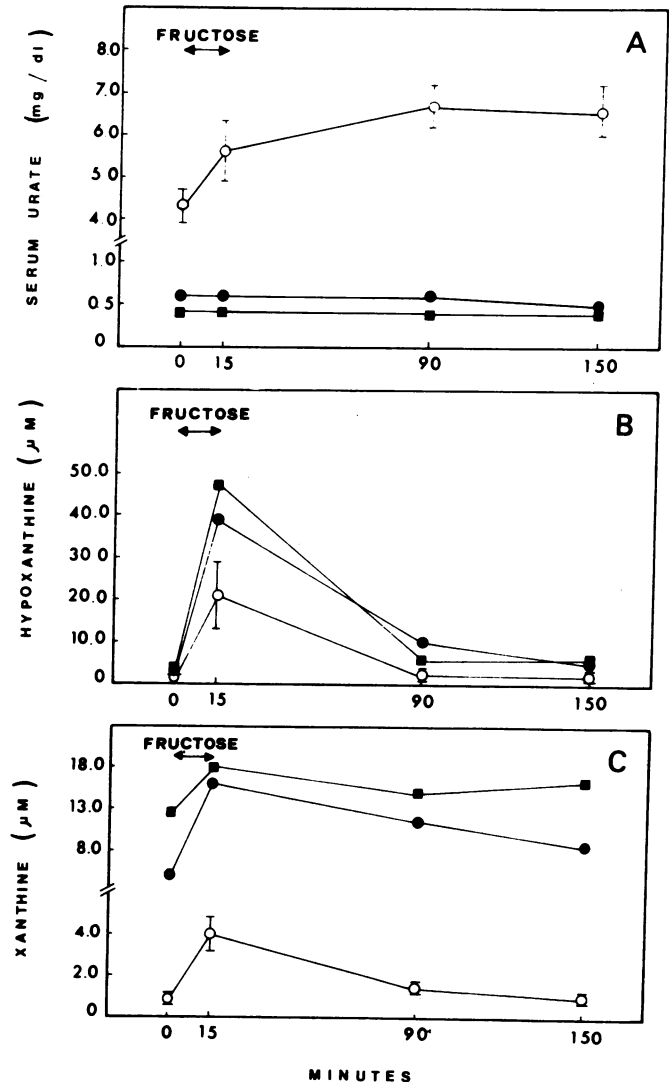

Figure 2. Effect of fructose infusion on (*A*) serum urate and (*B*) plasma hypoxanthine, and (*C*) xanthine. Intravenous fructose (0.5 g/ kg of body weight) was administered in 15 min. The change in plasma purines in four normal subjects (*open circles*) (mean±SE) and two xanthinuric patients (*solid symbols*) was observed for 150 min.

comprised 58%, 28%, 12%, and 2%, respectively, of normal total purine excretion. In the xanthinuric patients the mean proportion of these compounds were 1%, 39%, 40%, and 20%, respectively.

The excretion of radioactively labeled purines after fructose infusion allows quantitation of the relative contribution of adenine nucleotide degradation to total purine excretion (15). Normal subjects showed a significant positive correlation between urinary radioactivity and total purine excretion (*r* = 0.813; *P* < 0.01). Baseline urinary radioactivity was 0.51 and 0.61 · 10^6 cpm/g of creatinine in the patients (controls, 0.31±0.05 · 10^6 cpm/g of creatinine). Urinary radioactivity increased markedly in the xanthinuric patients during the first hour after fructose infusion and remained elevated for the following 2 h. The cumulative radioactivity excretion 3 h after fructose infusion was 7.96 and 9.16 · 10^6 cpm/g of creatinine in the patients and 4.73±0.69 · 10^6 cpm/g of creatinine in the control group.

In an effort to assess the increase in radioactivity relative to the rate of purine excretion, an estimate of the specific activity of the urinary purines was made by calculating the ratio of the radioactivity excreted to the total urinary purines excreted. In the baseline period and after intravenous fructose infusion, both xanthinuric patients showed a substantial elevation of the apparent urinary specific activity as compared with normal subjects (Fig. 3).

Discussion

Our studies have revealed elevated urinary radioactivity after radiolabeling the adenine nucleotide pool with [8-^{14}C]adenine in hereditary xanthinuria. Four possible mechanisms can be formulated to account for the increased radioactivity excretion: (a) enhanced adenine nucleotide degradation, (b) diminished reutilization of hypoxanthine, (c) decreased intestinal loss of purines, and (d) increased ATP pool specific activity. Our data allow analysis of these potential mechanisms. Conditions associated with increased ATP turnover (21, 26) or decreased reutilization of hypoxanthine, such as HPRT deficiency (6), are characterized by increased urinary total purine excretion. Thus, the low-normal excretion of urinary purines in our xanthinuric patients makes the first two possibilities unlikely. However, the observations are consistent with either or both of the last two mechanisms.

There is evidence for decreased intestinal loss of purines in hereditary xanthinuria as compared with normal subjects. Two thirds of uric acid normally turned over each day is excreted through the kidney, while one third is secreted into the gut and undergoes uricolysis (27). This proportion leads to the estimate that over 200 mg/24 h of uric acid was excreted into the gut by our normal subjects. The recalculated mean purine excretion in our normal subjects is estimated to be 3.77 mmol/24 h. The virtual absence of uric acid in xanthine oxidase-deficient patients assures that these patients do not excrete a substantial amount

of uric acid via the gut. In fact, only 3% of the total radioactivity administered as labeled purine compounds could be recovered in the stools of a xanthinuric patient (28), suggesting minimal intestinal purine loss. Therefore, the addition of the intestinal loss of uric acid to the total purine excretion in the normal subjects creates a total purine excretion considerably in excess of the xanthinuric patients (Table I).

Increased ATP pool specific activity in xanthine oxidase deficiency is suggested by the elevated apparent specific activity of urinary purines in both xanthinuric patients compared with control subjects. This occurs as well after intravenous fructose where the increased urinary purines acutely result from ATP degradation (29).

These two abnormalities, decreased total purine excretion and increased ATP pool specific activity, are in accord with the hypothesis of enhanced reutilization of hypoxanthine in hereditary xanthinuria. During pharmacologic inhibition of xanthine oxidase by allopurinol there is decreased total purine excretion, an associated reduced rate of de novo purine synthesis, and enhanced reutilization of hypoxanthine (15, 30–32). In hereditary xanthinuria a decrease of de novo purine synthesis, secondary to enhanced reutilization of hypoxanthine (33), could account for increased ATP pool specific activity by decreasing the contribution of nonlabeled purine nucleotides to the ATP pool (Fig. 1). This would decrease the dilution of the radiolabeled adenine nucleotide pool after [8-^{14}C]adenine infusion.

Active hypoxanthine salvage in hereditary xanthinuria is suggested by the finding of an extremely rapid disappearance of labeled hypoxanthine (34) and the small proportion of urinary hypoxanthine excretion (54 mg/24 h) compared with its daily turnover (980 mg) (28). Furthermore, enhancement of hypoxanthine salvage has been demonstrated in allopurinol-mediated xanthine oxidase inhibition (7) and by the increased incorporation of hypoxanthine into purine nucleotides of rat hepatocytes when stimulated with fructose (35).

It is possible to estimate roughly the proportion of ATP degradation products that are salvaged by comparing radioactive purine excretion products in control subjects, xanthinurics, and HPRT-deficient patients. Normally, 75% of hypoxanthine is reutilized via the salvage pathway (15). There is 63% hypoxanthine reutilization if intestinal loss of uric acid is considered (7). Ayvazian and Skupp (36) reported an excretion rate of 0.11% of infused [8-^{14}C]hypoxanthine per hour in a xanthinuric subject. Patients with the Lesch-Nyhan syndrome and an inability to reutilize hypoxanthine excreted 2.07% of infused [8-^{14}C]inosine per hour (15). This difference (1.96%) in radioactivity excretion between xanthine oxidase and HPRT-deficient patients estimates a 95% reutilization of hypoxanthine in hereditary xanthinuria [(1.96/2.07) · 100]. This is a substantially higher rate of hypoxanthine reutilization than the rate estimated for control subjects.

The hypothesis of enhanced hypoxanthine reutilization in hereditary xanthinuria has specific implications. Firstly, it suggests that de novo purine synthesis should be decreased when xanthine oxidase is deficient. Studies will need to be performed to examine this possibility. Secondly, enhanced reutilization of hypoxanthine in xanthinuria may explain in part the failure to decrease total purine excretion during allopurinol therapy (8–13). Because allopurinol mediates inhibition of de novo purine synthesis through increased hypoxanthine reutilization (7) and the salvage pathway is already operating at a near-maximal rate in hereditary xanthinuria, no substantial change might be expected to occur in response to allopurinol administration (2).
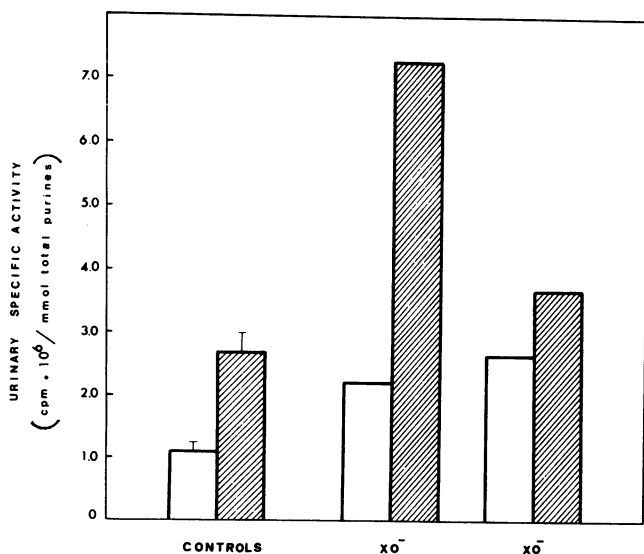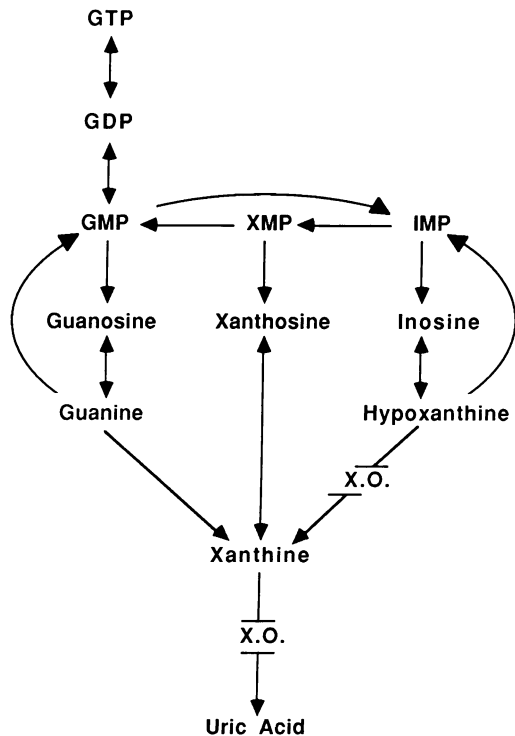

Figure 3. Apparent specific activity of urinary purines before and after fructose infusion. The urinary apparent specific activity was estimated for 1 h before and after (hatched area) the intravenous infusion of fructose. The data in controls are expressed as mean±SE. XO$^-$, xanthine oxidase-deficient patients.

Figure 4. Degradation of guanine nucleotides to xanthine in hereditary xanthinuria. With xanthine oxidase (*x.o.*) deficiency, xanthine can originate from xanthylic acid (*XMP*) or guanylic acid (*GMP*). Stimulation of purine nucleotide degradation by fructose increases guanine nucleotide degradation and leads to xanthine formation. This pathway is separate from the hypoxanthine salvage pathway.

Furthermore, the absence of xanthine oxidase prevents an allopurinol-mediated increase in hypoxanthine available for reutilization. Finally, our studies indicate a component of nucleotide degradation that bypasses the hypoxanthine salvage pathway. The data suggest that GTP degradation may also occur after fructose infusion. This is inferred from our observations in both xanthinuric patients of increased plasma and urinary xanthine concentrations in response to fructose infusion. In that xanthine oxidase is virtually absent, xanthine could originate from either xanthylic acid (XMP) or guanylic acid (GMP) (Fig. 4). Fructose infusion promoted a substantial increase of plasma guanosine whereas xanthosine remained undetectable. This is in accordance with recent studies demonstrating that fructose depletes GTP in the liver (37). Thus, our data most likely indicate that xanthine is mainly derived from GTP to GMP degradation in hereditary xanthinuria both in the basal state and after intravenous fructose. This bypass of the hypoxanthine salvage pathway may explain why xanthine is the predominant urinary purine excreted in xanthinuria.

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