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METABOLIC STUDIES OF ALLOPURINOL, AN INHIBITOR OF XANTHINE OXIDASE

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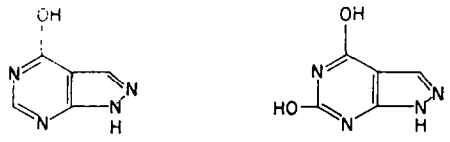
Abstract—The metabolic disposition of allopurinol [4-hydroxypyrazolo(3,4-d)-pyrimidine) was determined in mice, dogs, and human subjects. The drug is a substrate for, as well as an inhibitor of, xanthine oxidase and is converted in all species to the corresponding xanthine analog, alloxanthine, which is its major metabolite. Neither is bound to human plasma proteins, and both are distributed more or less equally in total body water in the mouse. Both analogs are cleared rapidly by the mouse and dog kidney. In the human subject allopurinol is cleared rapidly, but alloxanthine resembles uric acid in having a slow clearance responsive to probenecid. The accumulation of alloxanthine during prolonged therapy with allopurinol may contribute significantly to the therapeutic effects of the drug in the control of hyperuricemias.

ALLOPURINOL, 4-hydroxypyrazolo(3,4-d)pyrimidine (Fig. 1, I) is an inhibitor of xanthine oxidase[1, 2] which has been applied to the control of metabolic inactivation of 6-mercaptopurine and other purine analogs,[2–4] and the oxidation of hypoxanthine and xanthine to uric acid.[5, 6] The inhibition of normal purine catabolism serves to control both primary[6–9] and secondary[10] hyperuricemias. The drug is particularly useful in reducing the renal uric acid-filtered load in patients with recurrent or threatened renal deposition of urates which occurs in gouty over-excretors of uric acid, in patients with myeloproliferative disease, and in patients undergoing therapy for malignancies, which entails the rapid resolution of large tissue masses.

Allopurinol is both an inhibitor of, and a substrate for, the enzyme; and the product of its xanthine oxidase-catalyzed oxidation, alloxanthine [4,6-dihydroxy-pyrazolo(3,4-d)pyrimidine] (Fig. I, II), also is an inhibitor of the enzyme.[1]



I II

FIG. 1. Structural formulas of allopurinol [4-hydroxypyrazolo(3,4-d)pyrimidine] and alloxanthine [4,6-dihydroxypyrazolo(3,4-d)pyrimidine].

The present report is concerned with the biochemical pharmacology of allopurinol—its metabolism and the pharmacokinetics of both allopurinol and alloxanthine in the dog and in human subjects.

MATERIALS AND METHODS

Synthesis of 6-^{14}C-*allopurinol*

To 1 g (5·72 m/moles) 3-amino-4-carboxamidopyrazole·1/2 H_2SO_4[11] were added 63·2 mg ^{14}C-sodium formate (≅2·38 mc) and 1·2 g nonradioactive 98% formic acid. The mixture was heated under reflux conditions for 10 hr, and the excess formic acid was then removed by distillation under reduced pressure. Water (5-ml portions) was added to the residue twice and distilled to remove the last traces of formic acid. The product was dissolved in 6 ml 2 N sodium hydroxide and 20 ml water, treated with Darco, filtered, and acidified with 2 N hydrochloric acid. The ^{14}C-allopurinol was collected by filtration, washed with water, and dried at 100° *in vacuo* (550 mg, 71% yield). Its specific activity was 1220 dis/min/μg when measured in the scintillation counter. The ultraviolet absorption spectrum at pH 1 showed a $\lambda_{max} = 250$ mμ, $E_m = 7750$; at pH 11, $\lambda_{max} = 252$ (sh), 261 mμ, $E_m = 7600, 7800$.

Synthesis of 6-^{14}C-*alloxanthine*

An intimate mixture of 438 mg (2·5 m-moles) 3-amino-4-carboxamidopyrazole·1/2 H_2SO_4 and 150 mg ^{14}C-urea (≅2 mc) was heated in a small flask in an oil bath at 165–175° for 1·5 hr, followed by 0·5 hr at 190°. The residue was cooled, dissolved in the minimal amount of hot 2 N sodium hydroxide (about 6 ml), diluted with water to 20 ml, treated with Darco, filtered, and acidified (pH 1) with 2 N hydrochloric acid. The precipitate of 6-^{14}C-alloxanthine hemihydrate (275 mg, 69% yield) was collected, washed with water, and dried at 100°. It had a specific activity of 9450 dis/min/μg. The ultraviolet absorption spectrum at pH 1 showed a $\lambda_{max} = 252$ mμ, $E_m = 6050$; at pH 10, $\lambda_{max} = 242{\cdot}5, 267{\cdot}5$ mμ, $E_m = 9650, 6380$.

Plasma binding of allopurinol and alloxanthine

The method of equilibrium dialysis described by Anton[12] was employed, with ^{14}C-labeled drugs. The radioactivities of the two compartments were determined by counting 0·1-ml aliquots in a scintillation counter.

Metabolic experiments in mice

Fate of ^{14}*C-allopurinol.* Eleven white mice, each weighing 26 g, were dosed intraperitoneally per mouse with 0·8 mg ^{14}C-allopurinol (specific activity = 600 counts/min/μg of the flow counter). The mice were kept in a metabolism cage with water *ad lib.* but no food for the first 24 hr. The pooled urine was collected and infinitely thin samples were counted in a flow counter. The feces also were collected and extracted with dilute sodium hydroxide (0·1 N) for counting. The pooled urine sample (0–18 hr) was put through a Dowex-50 (H^+) column (11 × 50 mm) for the separation of allopurinol and alloxanthine (see below).

Carbon dioxide formation from allopurinol and alloxanthine in vivo

Three mice were injected i.p. with a solution containing 0·66 mg ^{14}C-allopurinol/mouse. The total radioactivity of the injected material for the group was $2{\cdot}34 \times 10^6$

cpm. The animals were kept for 2 hr in a metabolism chamber. A stream of carbon dioxide-free air was continuously passed through the chamber, and the exhaled gases were collected in a series of two traps, each containing 40 ml of a 1:2 mixture of ethanolamine and ethylene glycol monomethyl ether ("methyl Cellosolve"). At the end of 2 hr, aliquots of the solution in each trap were counted in a scintillation counter as described in the section on radioactivity measurements.

A similar experiment was conducted with ^{14}C-alloxanthine (total dose = 1·33 × 10^6 counts/min for 3 mice).

Examination for incorporation into nucleic acids. The mice used in the above CO_2-trapping experiments were held for 24 hr before sacrifice. The livers (group wet wt. = 3·5 g) were excised, homogenized in 25 ml of 7·5% cold trichloroacetic acid, washed twice more with 25-ml portions of cold trichloroacetic acid, then successively with 25 ml of 70% ethanol, 95% ethanol, ethanol:ether (1:2), and ether. The dried tissue was heated with 25 ml 7·5% trichloroacetic acid for 20 min in a boiling water bath to solubilize the nucleic acid. Aliquots of 0·1 ml of all washes ($\cong$0·5% of the total fraction) and of the hot trichloroacetic acid extract were counted in the scintillation counter for 100 min.

Similarly, the livers of mice from the distribution studies described below were examined for the possible incorporation of allopurinol and alloxanthine into the nucleic acids.

Distribution of allopurinol. Groups of five mice were injected i.p. with 6-^{14}C-allopurinol or 6-^{14}C-alloxanthine, and the tissues were homogenized with 10 volumes of cold 5% trichloroacetic acid 1 hr and 2 hr, respectively, after drug administration. Aliquots of 0·1 ml of these extracts were counted in the scintillation counter. The liver residues from the 2-hr experiments were further extracted for the determination of possible incorporation into nucleic acids, as described above.

Determination of nonradioactive allopurinol and alloxanthine in urine in man

The amounts of both compounds were determined by the isotope dilution technique. Measured quantities of 6-^{14}C-allopurinol or 6-^{14}C-alloxanthine were added to separate aliquots of urine. The urine was made slightly alkaline (pH 8–8·5) with sodium hydroxide, filtered, and put through a Dowex-1 × 8 (200–400 mesh) (formate) column for preliminary purification. Both allopurinol and alloxanthine were eluted with 0·02 N formic acid. For purification to constant specific activity, the radioactive eluates from the Dowex-1 columns were put through Dowex-50 × 12 (200–400 mesh) (H^+) columns. This permitted alloxanthine to pass through in the effluent, together with uric acid and xanthine; hypoxanthine and allopurinol were retained on the resin. The allopurinol was eluted with 1 N hydrochloric acid.* The radioactive solutions were chromatographed on paper in the butanol–ammonia system (solvent A, Table 1) until the alloxanthine and allopurinol showed satisfactory ultraviolet absorption spectra and constant specific activities. The compounds were eluted from paper with water; concentrations were determined by u.v. absorption spectroscopy at pH 1, and aliquots were counted in a scintillation counter. Later experiments have shown that the concentration of alloxanthine is best determined by

* Samples from Patients E. and W. were chromatogrammed in the opposite order, i.e. Dowex-50 prior to Dowex-1. However, this method was considered to be less satisfactory than that described.

measuring the u.v. absorption at pH 10 because of the higher extinction values and sharper maxima. Illustrative examples of the procedure are given below.

Allopurinol determination. To a 25-ml aliquot of a 24-hr urine collection (total volume = 1160 ml) was added 94·5 μg 6-^{14}C-allopurinol (specific activity = 1070 dis/min/μg). The urine was made alkaline (pH 8) with sodium hydroxide and put

TABLE 1. CHROMATOGRAPHIC DATA

	A	B (R_f values)	C
Hypoxanthine	0·18	0·63	0·55
Xanthine	0·08	0·48	0·48
Uric acid	0	0·44	0·40
Allopurinol	0·36	0·63	0·54
Alloxanthine	0·18	0·49	0·44
Allopurinol 'riboside'	0·18		0·68

A—*n*-butanol saturated with water, ammonia atmosphere.
B—5% ammonium sulfate–5% isopropanol.
C—5% disodium hydrogen phosphate–isoamyl alcohol (2 layers).

through a Dowex-1 (formate) column (19 mm in diameter × 30 mm in height). The column was washed with 20 ml water and eluted with 0·02 N formic acid in 10-ml fractions. The radioactive allopurinol began to come off after 20 ml acid had passed through the column. The fractions with the highest radioactivity were combined (total volume = 30 ml) and passed through a Dowex-50 (H^+) column (11 mm × 30 mm). The column was washed with 10 ml water, and the allopurinol was eluted with 1 N hydrochloric acid; approximately 20 ml acid had passed through the column before the radioactive material appeared. The fractions with the highest radioactivity (ca. 5000 dis/min/ml) were dried down in an air stream and chromatographed in duplicate on paper in ascending fashion in solvent A. The spots corresponding to allopurinol (R_f = 0·36) were eluted with 2 ml water. Enough 2 N hydrochloric acid was added to the eluate to make the solution 0·1 N with respect to acid, and the u.v. absorption spectrum was determined. A 0·1-ml aliquot of each solution was counted in the scintillation counter. The eluates from the paper chromatograms were dried down and rechromatographed in solvent A until the specific activity was constant. In this case the specific activity was found to be 121 dis/min/μg, representing a dilution of 8·85 from the original radioactive material added. This, therefore, corresponded to the presence of 827 μg unlabeled alloxanthine in the 25-ml aliquot of urine used.

Alloxanthine determination. To 20 ml urine (of a total daily output of 1790 ml) was added 127 μg 6-^{14}C-alloxanthine (specific activity = 970 dis/min/μg). This urine was made alkaline (pH 8), and put through a Dowex-1 (formate) column (11 × 45 mm). The column was washed with 20 ml water followed by elution with 0·02 N formic acid. The radioactive alloxanthine was eluted in four 6-ml fractions after about 25 ml acid had passed through the column. The radioactive eluates were evaporated to dryness in an air stream. The residues were dissolved in dilute ammonium hydroxide, chromatographed, and rechromatographed in solvent A three times, until the material eluted from paper showed a constant specific activity. The

specific activity of the reisolated alloxanthine was 30·4 dis/min/μg, representing a dilution of 31·9 and a total of 3·94 mg unlabeled alloxanthine in 20 ml urine.

Determination of ^{14}C-*allopurinol and* ^{14}C-*alloxanthine in urine*

When metabolism experiments were conducted with radioactivity labeled material, paper chromatography of the urine samples in butanol–ammonia revealed the presence of only two radioactive components, at the R_f values corresponding to allopurinol and alloxanthine. In order to determine quantitatively the relative amounts of these two radioactive components, a 25-ml aliquot of urine (made alkaline, pH 8·5) was subjected to a preliminary purification on Dowex-1 (formate) column (19 mm in diameter $\times$ 45 mm high) to remove some of the salts (e.g sulfates, phosphates) The column was washed with 25 ml water, and the radioactive components were then eluted with 0·1 N formic acid, with a recovery of 95–100% of the radioactivity. The radioactive fractions (usually fractions 4–8 of 7-ml fractions) were pooled, and an aliquot was dried down in an air stream and chromatographed on paper in butanol–ammonia. The paper chromatogram was scanned in an Atomic Associates *4II* scanner (model RSC160), and the relative radioactivities of the two spots were determined by the areas under the curves.

In the later experiments with human urine, in the case of Patient L.M. (see Results), radioactive eluates from the Dowex-1 (formate) column were passed through a Dowex-50 (H^+) (11 $\times$ 25 mm) column to separate the alloxanthine from the allopurinol. Alloxanthine was present in the effluent and water washes, whereas allopurinol was retained on the resin. During subsequent purification of the alloxanthine fraction by paper chromatography in butanol–ammonia it was discovered that the alloxanthine spot was contaminated with another radioactive material, tentatively identified as allopurinol riboside, which had the same R_f as alloxanthine in this solvent system. It is not known to what extent this "riboside" contaminated the ^{14}C-alloxanthine fractions in the dog urines or in the clearance studies with Patients M.K. and K.C.

Determination of ^{14}C-*allopurinol and* ^{14}C-*alloxanthine in plasma*

This was attempted only where the ^{14}C-allopurinol was given intravenously, since in the cases where the radioactive drug was given orally, the concentration in the plasma was never high enough to make the fractionation practicable.

The plasma samples (ca. 4 ml) were deproteinized with 3% perchloric acid in the cold, and the perchloric acid was then precipitated as the potassium salt by neutralization of the perchloric acid extract with 5 N potassium hydroxide. The protein-free plasma filtrate was put through a Dowex-50 (H^+) column (11 mm $\times$ 30 mm). The alloxanthine appeared in the effluent and water washes; the allopurinol was retained on the resin. The difference between the total amount put through the column and the amount recovered in the effluent plus washes was considered to be due to the ^{14}C-allopurinol. Experiments with model columns with known mixtures of allopurinol and alloxanthine showed that the alloxanthine could be recovered quantitatively from Dowex-50 (H^+) columns and that none of the allopurinol was present in the effluent and water washes. It is not yet known whether detectable amounts of allopurinol "riboside" are present in plasma.

Radioactivity measurements

Radioactivity was measured in a Nuclear-Chicago liquid scintillation counter, model 8260, at ambient temperature. Samples of 0·1 ml were counted in 10 ml of a scintillation mixture composed of 14 g 2,5-diphenyloxazole (PPO), 140 g naphthalene, and 98 mg 2-*p*-phenylenebis(4-methyl,5-phenyloxazole) in 2 l. of 1,4-dioxane. Counts were corrected for counting efficiency on the basis of a quenching correction curve obtained by the channel ratio procedure (according to the Nuclear-Chicago model 180060 ^{14}C-quenched standards set). Corrected values are reported as disintegrations per minute (dis/min). For counting the ethanolamine–"methyl Cellosolve" solutions used as the trapping agent for respiratory carbon dioxide, 2 ml of the solution was added to 12 ml of a 1:2 mixture of methyl Cellosolve in toluene containing 5 g PPO/l.

In several of the earlier experiments, counting was carried out on infinitely thin samples in an internal flow counter (Nuclear Chicago D-47) for a sufficient time to give 10% accuracy or better. Such results are reported as counts per minute (counts/min), since the exact counting efficiency is not known.

Paper chromatography

Chromatograms were run in ascending fashion (S and S paper 597). The R_f values for the pertinent compounds are given in Table 1. For solvents A and C the chromatograms were run for 18–24 hr; in solvent B for 4–5 hr.

RESULTS

Plasma binding of allopurinol and alloxanthine

When samples of human plasma were subjected to equilibrium dialysis with ^{14}C-allopurinol and ^{14}C-alloxanthine the results in Table 2 were obtained. Since there was no significant difference between the concentrations of the drugs in the two compartments, there was no binding of the compounds to plasma proteins.

TABLE 2. EQUILIBRIUM DIALYSIS OF ^{14}C-ALLOPURINOL AND ^{14}C-ALLOXANTHINE WITH HUMAN PLASMA*

	Inside dialysis bag (dis/min/ml)	Dialysate
^{14}C-Allopurinol	11,344	11,104
^{14}C-Alloxanthine	4377	4222

* The dialysis bag contained 3 ml human plasma, and the outer solution contained the radioactive compound (10 μg/ml) in 10 ml buffered saline prior to equilibrium overnight in a shaking machine at 37°.

Metabolic studies in mice

The urines and feces from mice given ^{14}C-allopurinol were examined for total ^{14}C content (Table 3). The urine contained 57·2% of the radioactive dose after 18 hr, but only 1·3% was excreted in the next 6 hr. The 0- to 18-hr urine sample was subjected to Dowex-50 (H^+) chromatography. The effluent and water washes contained 56% of the radioactivity applied to the column. After paper chromatography in butanol–ammonia, the effluent gave a single radioactive spot corresponding to alloxanthine. Thus, 32% of the dose of allopurinol had been excreted as alloxanthine by the end of 18 hr.

Groups of three mice were injected with ^{14}C-allopurinol and ^{14}C-alloxanthine respectively, and their respiratory carbon dioxide was collected for 2 hr thereafter. Those animals receiving ^{14}C-allopurinol excreted radioactive carbon dioxide equivalent to 14,400 dis/min (0·06% of the dose) in 2 hr. During this time these animals excreted 44% of the dose of ^{14}C in the urine. Animals receiving ^{14}C-alloxanthine

TABLE 3. RECOVERY OF RADIOACTIVITY IN THE URINE AND FECES OF MICE AFTER AN INTRAPERITONEAL DOSE OF ^{14}C-ALLOPURINOL*

Fraction	Time (hr)	(Total dis/min)	(% of dose)
Urine	0–18	$2{\cdot}98 \times 10^6$	57·2
Urine	18–24	$6{\cdot}55 \times 10^4$	1·3
Feces	0–24	$1{\cdot}21 \times 10^5$	2·3
Urine + feces	24–48	$2{\cdot}04 \times 10^5$	3·9
Total	0–48	$3{\cdot}37 \times 10^6$	74·7

* Total dose = 8·67 mg $\cong 5{\cdot}2 \times 10^6$ dis/min.

showed no trace of radioactivity in their expired carbon dioxide in the 2-hr period; their urines contained 43% of the administered radioactivity. It therefore appears that, whereas there is some ring opening of allopurinol *in vivo* in mice, there is no similar ring opening of alloxanthine.

The mice used for the above carbon dioxide-trapping experiment were held for 24 hr. During that time the animals which had received allopurinol had excreted 84·2% of the radioactive dose; those on alloxanthine had excreted 95·5% of the dose. The mice were then examined for possible incorporation of ^{14}C into the nucleic acid fraction of their livers. In each case no trace of ^{14}C was found in the acid-soluble fraction, the various washes, or in the hot trichloroacetic acid (nucleic acid) fraction.

Distribution studies with both allopurinol and alloxanthine (Table 4) revealed no marked variations from equality of distribution in body water in all tissues except

TABLE 4. TISSUE DISTRIBUTIONS OF PYRAZOLOPYRIMIDINES

Tissue	Allopurinol* (μg/g)		Alloxanthine† (μg/g)	
	1 hr	2 hr	1 hr	2 hr
Blood	8·65	6·19	10·04	13·79
Liver	11·90	6·67	9·78	12·94
Spleen	8·86	5·82	8·98	15·10
Heart	8·45	6·47	11·80	13·25
Lung	6·20	3·51	9·92	13·62
Intestine	7·88	6·53	8·29	10·64
Brain	4·52	2·78	3·16	4·26
Urine (total, % of dose)	42·5	51·5	17·9	43·4

* Each mouse received 1 mg containing $1{\cdot}3 \times 10^6$ dis/min i.p. Distribution calculated as allopurinol, μg/g wet weight of tissue.

† Each animal received 1 mg containing $1{\cdot}03 \times 10^6$ dis/min. Excretion values are based on total excretion for each group of 5 mice.

brain where about half the blood concentrations were found. Examination of the livers in both experiments 2 hr after drug administration revealed no incorporation into the nucleic acids (hot trichloroacetic acid extracts), although the acid-soluble fractions (cold trichloroacetic acid extracts) still contained appreciable radioactivity (Table 4). With alloxanthine, equilibration was slow (higher values at 2 hr than at 1 hr), possibly owing to precipitation in the peritoneal cavity; in a separate experiment where 0·111 mg/mouse was given, the 1-hr and 2-hr excretion values were 71·4% and 97·8% of the dose, respectively, and the tissue values at 2 hr were approximately one-fifth those at 1 hr.

Metabolic studies in dogs

A dog was given 44·3 mg (equivalent to 5 mg/kg) of 4-hydroxy-6-^{14}C-pyrazolo-(3,4-d)pyrimidine (22 × 10^6 counts/min) i.v. as a solution of the monosodium salt. The radioactivities of the plasma and the blood cells were measured at various time intervals, and the ^{14}C was found to be equally distributed between the plasma and cells

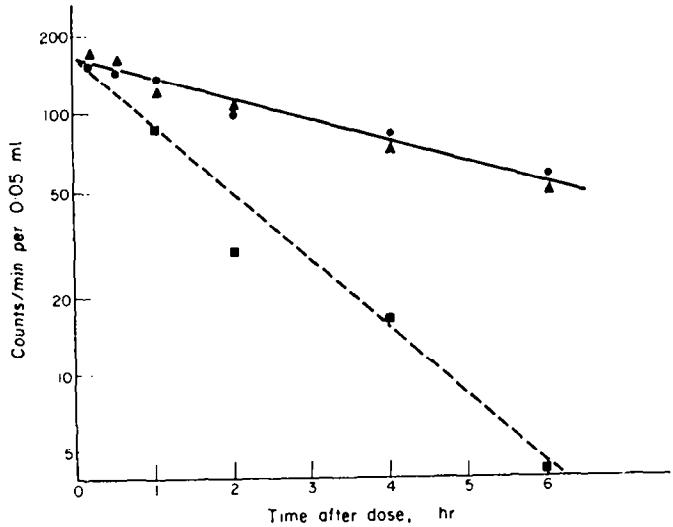


FIG. 2. Clearance of total ^{14}C and of ^{14}C-allopurinol from the plasma of a dog after a single i.v. injection of ^{14}C-allopurinol (44·3 mg ≅ 22 × 10^6 counts/min); ●, total ^{14}C in plasma; ▲, total ^{14}C in plasma filtrates, corrected for dilution; ■, ^{14}C-allopurinol in plasma (calculated).

in each instance. The decrease in the radioactivity of the plasma with time is shown in Fig. 2. The initial plasma concentration of allopurinol (at 10 min) corresponded to 6·3 μg/ml. The time for half-clearance of the radioactivity was 4 hr, and there was no detectable radioactivity in the plasma at 24 hr. When the relative amounts of ^{14}C-allopurinol and ^{14}C-alloxanthine in the plasma filtrates were determined by Dowex-50 chromatography (Table 5), the time for half-clearance of allopurinol from the plasma could be calculated to be 75 min (cf. Fig. 2). This disappearance is due partly to urinary excretion and partly to oxidation to alloxanthine (Table 6).

Urine specimens collected from the dog showed the presence of both ^{14}C-allopurinol and ^{14}C-alloxanthine during the first 5-hr collection period, but only ^{14}C-alloxanthine thereafter (Table 6).

TABLE 5. RADIOACTIVE COMPONENTS OF PLASMA AFTER INTRAVENOUS ADMINISTRATION OF ^{14}C-ALLOPURINOL TO A DOG

Time (hr)	Allopurinol* (% of ^{14}C in plasma)	Alloxanthine†
1	67·3	32·7
2	26	74
4	21·3	79·7
6	0	100

* Calculated by difference.

† Per cent of counts in Dowex-50 effluent and water washes.

TABLE 6. RADIOACTIVE COMPONENTS OF URINE AFTER INTRAVENOUS ADMINISTRATION OF ^{14}C-ALLOPURINOL TO A DOG

Time of collection (hr)	Per cent of dose excreted as	
	Allopurinol	Alloxanthine
0–5	22·2	21·3
5–24	0	38·3
24–48	0	2·1

Urinary excretion of allopurinol and alloxanthine in man

Prior to the clearance studies with ^{14}C-allopurinol to be described below, the urines of several patients receiving nonradioactive allopurinol daily were examined for the amounts of allopurinol and alloxanthine excreted in 24 hr. These were determined by the isotope dilution technique (see Methods), and the results are shown in Table 7. In all cases, except in the xanthinuric Patient P., the amount of unchanged allopurinol excreted was under 10% of the daily dose. Alloxanthine accounted for the major

TABLE 7. URINARY EXCRETION OF ALLOPURINOL AND ITS METABOLITE, ALLOXANTHINE, IN MAN

Patient	Allopurinol (mg/day)	Treatment duration	Urinary excretion in 24 hr			
			Allopurinol		Alloxanthine	
			(mg)	(% of dose)	(mg)	(% of dose)
E.	600	3 days	40	6·7	297	45
W.	600	3 days	16	2·7	428	64
K.	400	2 mo.*	38	9·6	701†	157
I.	600	1 yr‡	49	8·1	353	53
P.§	800	1 day	434	54	18·2	2·3

* Had taken 600 mg/day for 2 weeks, 2 weeks prior to the experiment (outpatient of Dr. Yü).

† This material was still chromatographically impure and probably contained xanthine. There was insufficient sample to repeat the determination.

‡ Had received sulfinpyrazone periodically in addition to allopurinol. Last dose of sulfinpyrazone was 3 weeks prior to the experiment (outpatient of Dr. Yü).

§ Xanthinuric patient (patient of Dr. Seegmiller[13]).

portion of the dose of allopurinol in these patients. In Patient E. the recovery may be low as the result of relatively poor absorption from the gastrointestinal tract. The xanthinuric Patient P., who had been shown to have a marked deficiency of xanthine oxidase,[13] showed a high excretion of unchanged allopurinol and only a very small amount of alloxanthine.

Clearance studies with ^{14}C-allopurinol in man

With the background of the clearance study in the dog, and a knowledge of the metabolic disposition of allopurinol in man, a more detailed investigation of the clearance in man was undertaken with the use of radioactively labeled drug. These studies were conducted in three patients. One received a single intravenous dose, one received a single oral dose, and one patient received a single oral dose of radioactively labeled drug while being maintained on a regimen of unlabeled drug for months and again after the day of the ^{14}C dose. Each of these cases will be described individually.

1. *Intravenous administration*

Patient M.K. received a single i.v. dose of 140 mg 6-^{14}C-allopurinol ($\cong 171 \times 10^6$ dis/min) as the solution of the sodium salt. The initial plasma level of allopurinol was 4·5 μg/ml. The decrease in the ^{14}C content of the plasma with time is shown in Fig. 3.

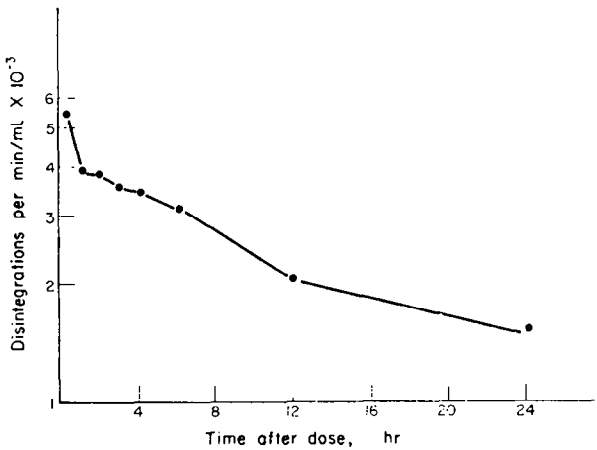


FIG. 3. Clearance of ^{14}C from plasma of Patient M.K. after a single intravenous dose (140 mg $\cong$ 171 $\times$ 10^6 dis/min) of ^{14}C-allopurinol.

The time for half-clearance of the radioactivity was 8 hr during the initial 8-hr period and increased to 16 hr thereafter. Fractionation of the radioactive components of the plasma filtrates on Dowex-50 revealed that, after 1 hr, 65% of the radioactivity in the plasma was in the alloxanthine fraction; after 2 hr, 85%; and after 6 hr, 100%. Thus, the clearance of allopurinol from the plasma by oxidation and excretion was complete in 6 hr.

The cumulative urinary excretion of ^{14}C was equal to 8·2% of the dose at the end of the first hour, 14% at 2 hr, 17·5% at 4 hr, 28% at 6 hr, 38% at 12 hr, and 47·5% by 24 hr. The excretion per hour for allopurinol and the alloxanthine fraction is

shown in Fig. 4. After 1 hr, allopurinol accounted for 43% of the radioactivity in the urine; after 2 hr, 15%; and after 12 hr all the radioactivity was in the form of alloxanthine. This patient showed an endogenous creatinine clearance of 136 ml/min, calculated on the basis of the 24-hr creatinine excretion and the average plasma creatinine value of 0·55 mg/100 ml. The approximate clearance of alloxanthine calculated at the 6- to 12-hr period after drug administration was 28 ml/min; between 12 and 24 hr it was 20 ml/min. The uric acid clearance was 6·2 ml/min over a 24-hr period, with a serum uric acid of 6·15 mg/100 ml.

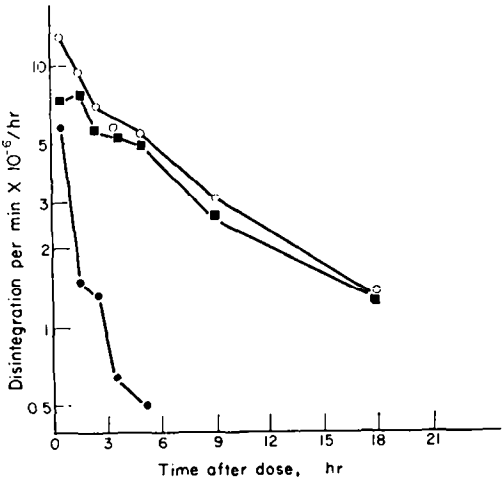


FIG. 4. Excretion of radioactive compounds in the urine of Patient M.K. after a single i.v. dose of ^{14}C-allopurinol (140 mg $\cong$ 171 $\times$ 10^6 dis/min); ○, total ^{14}C; ■, ^{14}C-alloxanthine; ●, ^{14}C-allopurinol. Urines were collected at 0–1, 1–2, 2–3, 3–4, 4–6, 6–12,and 12–24 hr after the drug administration. Values for excretion per hr are plotted in the middle of the time period of collection.

2. *Oral administration*

Patient K.C. received a capsule containing 169 mg 6-^{14}C-allopurinol ($\cong$207 $\times$ 10^6 dis/min). The changes in the ^{14}C content of the plasma with time are shown in Fig. 5. There was no radioactivity in the plasma at 30 min; at 1 hr the radioactivity corresponded to a concentration of allopurinol of 2·2 μg/ml, reached a plateau between 2 and 6 hr, and fell slowly thereafter to a level corresponding to 1 μg/ml (alloxanthine) at 48 hr.

The rate of excretion in the urine reached a maximum after 2–3 hr (Fig. 6). After 6 hr all the radioactivity in the urine appeared to be in the form of alloxanthine, as in the case of M.K. above. The cumulative excretion in the urine amounted to 9·5% in 6 hr, 25% in 24 hr, and 38·5% in 48 hr. The stool specimens collected between 0 and 48 hr showed the presence of 19·9% of radioactive material, presumably unabsorbed drug.

The time for half-clearance of alloxanthine from the plasma (i.e. after 6 hr) was 28 hr (cf. Fig. 5). From the plasma levels and the urinary excretion of alloxanthine after 6 hr, the clearance was calculated to be 15 ml/min, approximately half that shown by Patient M.K., and the uric acid clearance was 5·3 ml/min at a serum uric acid level of 3·1 mg/100 ml.

3. *Prolonged administration*

This study was designed to determine the metabolic fate of an oral dose of ^{14}C-allopurinol in a patient who had been taking the nonradioactive drug over a long period of time. Patient L.M. has had severe gout for 5½ yrs and his symptoms have been well controlled for over 1 yr with 600 mg allopurinol and 0·5 mg colchicine daily.

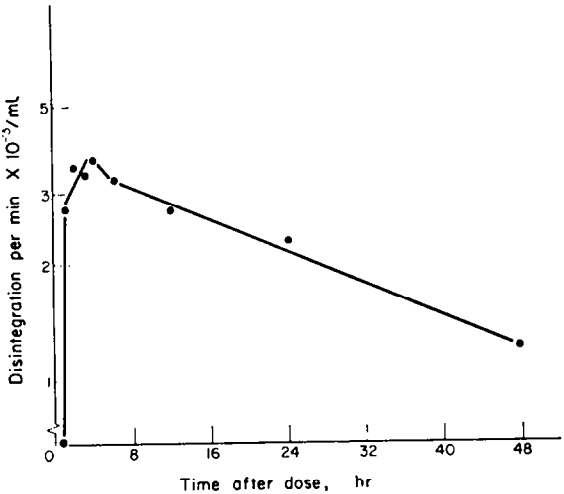


FIG. 5. Clearance of radioactivity from plasma of Patient K.C. after a single oral dose (169 mg ≅ 207 × 10^6 dis/min) of ^{14}C-allopurinol.

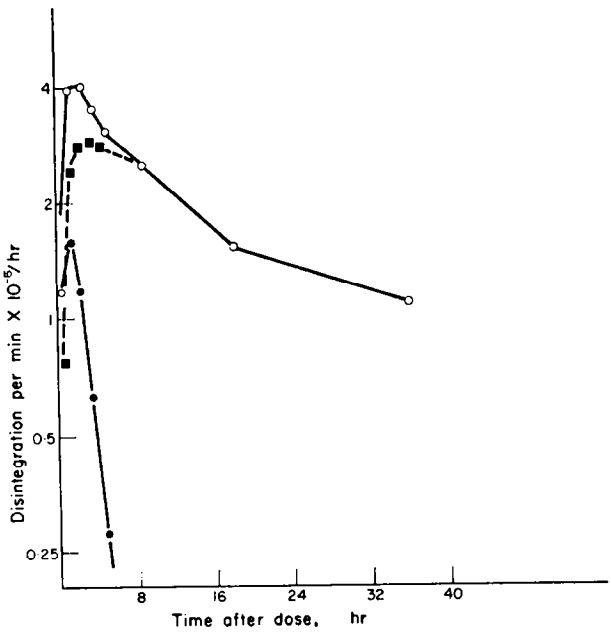


FIG. 6. Excretion of radioactive compounds in the urine of Patient K.C. after a single oral dose of ^{14}C-allopurinol (169 mg ≅ 207 × 10^6 dis/min); ○, total ^{14}C; ■, ^{14}C-alloxanthine; ●, ^{14}C-allopurinol. Urines were collected 0–1, 1–2, 2–3, 3–4, 4–6, 6–12, 12–24, and 24–48 hr after the dose, and the values are plotted at the middle of each time period.

On the day on which 70 mg ($\cong 85{\cdot}5 \times 10^6$ dis/min) 6-^{14}C-allopurinol was given, the regular doses of allopurinol were omitted. However, on the day after the radioactive dose, allopurinol therapy (600 mg/day in three divided doses at 7 a.m., noon, and 7 p.m.) was resumed. Seventy-two hr after the dose of radioactive allopurinol, probenecid was added to the dosage schedule (1 g at 7 a.m., 1 g at noon, and 0·5 g 6 hr thereafter) for the purpose of studying the effect of this uricosuric agent on the every excretion of alloxanthine.

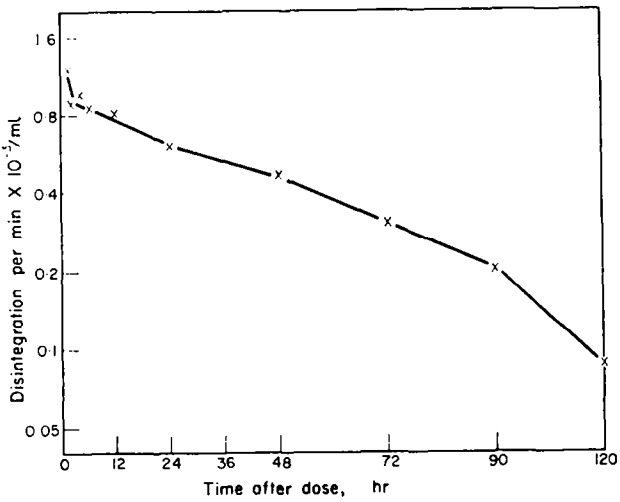


FIG. 7. Clearance of radioactivity from plasma of Patient L.M. after an oral dose of ^{14}C-allopurinol (70 mg $\cong 85{\cdot}5 \times 10^6$ dis/min). See text for details of subsequent doses of non-radioactive allopurinol and probenecid.

The clearance of ^{14}C from the plasma is shown in Fig. 7. There was no radioactivity in the plasma at 1 hr; the peak level of radioactivity occurred at 2 hr when it corresponded to an allopurinol concentration of 1·0 μg/ml.

The excretion of ^{14}C in the urine was equivalent to 40% of the dose at the end of 72 hr; 18·6% of the dose was found in the feces. An additional 13·2% of the ^{14}C (in the form of alloxanthine) was excreted in the urine between 72 and 120 hr, the period following the administration of probenecid.

Each urine sample was subjected to Dowex-50 (H^+) column chromatography. The effluents from the columns contain the alloxanthine fraction, whereas allopurinol remains adsorbed on the columns. This experiment afforded an opportunity to determine total alloxanthine as well as ^{14}C-alloxanthine since, by determining the specific activity of each fraction, one could estimate the dilution of the radioactive material and calculate the total amount of alloxanthine. During the course of purification of the alloxanthine fraction to constant specific activity by paper chromatography, it was observed that the specific activity of the alloxanthine decreased on repeated chromatography and that a new radioactive spot, at the R_f of allopurinol, appeared with each purification. This was first thought to represent co-chromatography of alloxanthine and allopurinol, but control experiments with mixtures of the two compounds showed that they separated well both on Dowex-50 and on paper in a

butanol–ammonia system. This suggested that the alloxanthine was contaminated with a derivative of allopurinol which was slowly breaking down to free allopurinol during the purification. This indeed proved to be the case. Acid hydrolysis (0·2 N HCl in a boiling water bath for 10 min) converted the contaminating substance to allopurinol, and alloxanthine of constant specific activity could then be obtained. To separate and identify the acid-labile derivative of allopurinol, various chromatographic solvents were investigated. The most satisfactory proved to be 5% Na_2HPO_4-isoamyl alcohol (Table 1). The acid-labile component, eluted from paper, was then subjected to tests for glucuronic acid,[14] hexoses,[15, 16] and pentoses.[16, 17] The orcinol[17] revealed the presence of a pentose with the characteristics of ribose. Moreover, the amount of ribose was approximately equivalent to the amount of allopurinol as determined spectrophotometrically. A more definitive identification of this "riboside" must await the isolation of larger amounts of material. In the meantime, its lability to acid hydrolysis made it possible to remove it from the alloxanthine fraction of the urine.

Hydrolysis of the urine (0·1 N HCl, boiling water bath, 10 min) prior to Dowex-50 chromatography led to conversion of the riboside to allopurinol which was retained on the column. The effluents from such solumns contained alloxanthine as the only radioactive component. Table 8 shows the difference in the percentages of radioactive material in the effluents of Dowex-50 column before and after hydrolysis of the urine.

TABLE 8. ALLOPURINOL RELEASED BY ACID HYDROLYSIS OF URINE (PATIENT L.M.)

Time of urine collection (hr)	Total dis/min $\times 10^{-6}$	Per cent of total ^{14}C in Dowex-50 effluents	
		Before hydrolysis	After hydrolysis*
0–2	0·70	34·9	19·5
2–4	4·15	56·4	7·0
4–6	3·74	74·2	47·0
6–12	5·71	91·4	46·7
12–24	5·34	96·5	66·7
24–48	9·47	97·9	84·9
48–72	5·02	98·8	98·8
72–96	6·24		
96–120	5·14		

* Aliquots of urine collections were made 0·1 N with respect to HCl and heated for 10 min in a boiling water bath.

From these data the apparent amount of riboside could be calculated. The urinary excretion of the three radioactive components per hour is plotted in Fig. 8. It will be noted that the excretion of allopurinol riboside reached its maximum at the same time as allopurinol itself (at 3 hr) and was then excreted at a somewhat slower rate than allopurinol, but much more rapidly than alloxanthine. As compared with the two previous cases (Figs. 4 and 6), the disappearance of ^{14}C-allopurinol appeared to be slower. In those two cases the patients had received only single doses of allopurinol, and no radioactive allopurinol was detectable in the urine in the samples collected 6 to 12 hr after the dose. In the present patient (L.M.), allopurinol was detected not only in the 6- to 12-hr specimen but even in the 12- to 24-hr sample. This suggests that the rate of oxidation of allopurinol may be slower in a patient who has been on allopurinol therapy for a prolonged period.

The slowness of the clearance of ^{14}C-alloxanthine which was observed in the other patients is likewise apparent here, both in the slow decline of the ^{14}C in the plasma after 24 hr (Fig. 7) and the low rate of excretion in the urine (Fig. 8). The plasma clearances for alloxanthine for L.M. ranged between 10 and 20 ml/min; the clearance for uric acid was 3·0 ml/min, with a serum urate of 4·3 mg/100 ml, and for endogenous creatinine 106 ml/min.

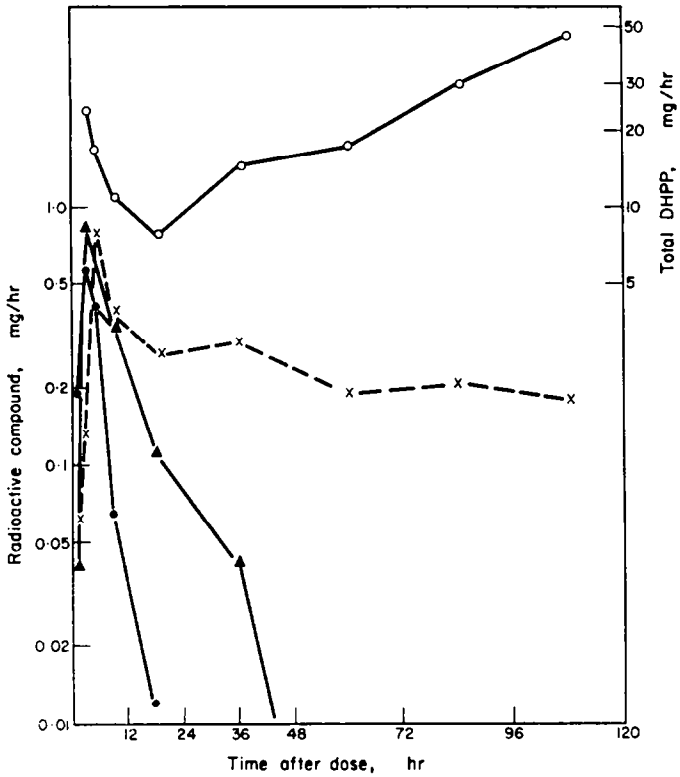


FIG. 8. Excretion of radioactive compounds and of total alloxanthine in the urine of Patient L.M. after a single oral dose (70 mg ≅ 85·5 × 10^5 dis/min) of ^{14}C-allopurinol, followed by 600 mg/day of nonradioactive allopurinol beginning 24 hr after the radioactive dose and probenecid beginning 72 hr after the radioactive dose (see text); ●—^{14}C-allopurinol; ▲—^{14}C-allopurinol, as riboside; × —^{14}C-alloxanthine; ○—total alloxanthine. Urines were collected at 0–2, 2–4, 4–6, 6–12, and 12–24 hr after the radioactive dose, and daily 24-hr collections were made thereafter. Values for excretion per hr are plotted in the middle of each time period.

The effect of the uricosuric agent, probenecid, on the clearance of alloxanthine is apparent in Fig. 8. The excretion of ^{14}C-alloxanthine increased slightly after 72 hr, the time when probenecid therapy was begun. Even more striking was the pronounced increase in total alloxanthine excretion to 732 mg (30·5 mg/hr) in the 72–96 hr period and 1130 mg (47 mg/hr) in the 96- to 120-hr period. Concomitant with this increase in alloxanthine clearance was the increased rate of ^{14}C disappearance from the plasma (Fig. 7).

DISCUSSION

Allopurinol is both an inhibitor of, and a substrate for, xanthine oxidase. *In vivo*, in all species examined, its oxidation to 4,6-dihydroxypyrazolo(3,4-d)pyrimidine (alloxanthine) was rapid. As a result, urinary alloxanthine accounted for a considerable, in many instances the major, part of administered allopurinol in the mouse, dog, and man. In a human subject allopurinol gave rise to a small amount of a metabolite, not yet fully characterized, which may be a riboside.

There was no detectable incorporation into the nucleic acids of mouse tissues after the administration of either allopurinol or alloxanthine. The failure of the xanthinuric patient to convert more than a minor fraction of administered allopurinol to alloxanthine is of considerable interest. This patient[13] and another xanthinuric[18] freely convert hypoxanthine and aminoimidazolecarboxamide to xanthine, although they lack xanthine oxidase. It seems probable, therefore, that this transformation takes place primarily at the nucleotide level[13, 18] Allopurinol, therefore, must fail to participate in one or more of the reactions involved in the transformation, and the principal route of its oxidation to alloxanthine must be via xanthine oxidase catalysis.

Therapy with allopurinol appears not to affect the intrinsic xanthine oxidase activity levels of the recipients. Thus a patient receiving long-term therapy showed a slower conversion of allopurinol to alloxanthine than did patients receiving single doses of the drug, and maintenance doses of drug appear to produce constant effects in hyperuricemic patients over long periods of treatment.[6, 7, 9] The synthesis of xanthine oxidase, therefore, seems neither to be induced by the abnormal substrate, nor derepressed as a consequence of the subnormal level of activity maintained in the presence of the inhibitor.

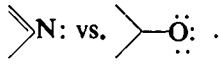
Neither allopurinol nor alloxanthine exhibits binding to human plasma proteins, and the tissue and organ concentrations in the mouse do not differ strikingly from equal distribution in total body water, with the exception of brain which exhibited about half the concentrations of other tissues.

Allopurinol is cleared by both the canine and human kidney at rates below but close to glomerular filtration rates. Its clearance is thus similar to those of the natural "oxypurines" hypoxanthine and xanthine (see Ref. 19). The clearance of alloxanthine provides a striking contrast. In the dog this substance also is cleared at a high rate, close to that of glomerular filtration. However, in man the rate of renal clearance is much less and, in each of the three subjects studied, was a small multiple of the uric acid clearance of the subject. Since it does not bind to plasma proteins, alloxanthine, like uric acid, may be subject to reabsorption in the human renal tubule. The increased excretion in one subject after the administration of probenecid is consistent with this view. The results justify the determination of the clearance of alloxanthine in a more precise manner, further studies of the effects of uricosuric drugs on its clearance, and efforts to establish the respective roles of tubular reabsorption and secretion in its clearance.

Alloxanthine obviously is not a member of the "organic acid system"[20–22] nor of the basic uricosuric group of substances,[22] since it has a pK_a value of 7·7.* Furthermore, it seems to have no significant effect on the clearance of uric acid.[23, 24] However, it appears to behave as an analogue of uric acid rather than xanthine, at least in regard

* Unpublished data of G. B. Elion.

to some aspects of its clearance by the human kidney. In addition to identical functional groups in the pyrimidine ring, it possesses in common with uric acid, and in distinction from xanthine, an unshared electron pair in the region of the purine-8 position



and it may be this structural feature that determines its behavior.

The accumulation of alloxanthine, as a consequence of its low clearance rate, suggests that it may play a significant role in the therapeutic effects of allopurinol. Clinical findings are consistent with this, since effectiveness increases over several days when therapy is initiated and persists for a like period when therapy is stopped.[6, 7, 9] Allopurinol is much more potent than alloxanthine as an inhibitor of xanthine oxidase *in vitro*,[1] *in vivo* in mice,[2] and when single doses are given to human subjects.* These measurements, however, do not reflect the accumulation of alloxanthine which occurs during prolonged administration of allopurinol to man. Trials of this substance, therefore, must be extended over periods sufficient for equilibrium levels to be established in order to assess its efficacy.

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