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FORMATION OF NUCLEOTIDES OF [6-^{14}C]ALLOPURINOL AND [6-^{14}C]OXIPURINOL IN RAT TISSUES AND EFFECTS ON URIDINE NUCLEOTIDE POOLS

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Abstract—Allopurinol-1-ribonucleotide, oxipurinol-1-ribonucleotide and oxipurinol-7 ribonucleotide have been found in concentrations ranging from less than 10^{-9}–10^{-6}M in rat liver and kidney after administration of [6-^{14}C]allopurinol. The amounts and relative proportions of each nucleotide were dependent upon the dose, route of administration and time. Red cells contained about one-tenth the amount of allopurinol-1-ribonucleotide present in liver and no oxipurinol ribonucleotides were detectable. Brain contained no analog nucleotides. Both of the oxipurinol ribonucleotides were recovered after administration of [6-^{14}C]oxipurinol. In general, the biological half-lives of the nucleotides were related to the amount of the free bases present in the tissues. There was no evidence for the presence of ribonucleoside di- or triphosphates of allopurinol or oxipurinol. Allopurinol and oxipurinol ribonucleotides have been reported to be inhibitors of orotidylate decarboxylase *in vitro*. High doses of allopurinol and oxipurinol caused a transient decrease of UMP and UDP pools in rat liver, which returned to control levels after 3 hr, whereas UTP levels were actually elevated. In kidney, allopurinol caused no changes in UMP, UDP or UTP levels at any of the times examined, even after a 100 mg/kg, i.p., dose. These findings suggest that control mechanisms can maintain uridine nucleotide levels in a normal range, even in the presence of strong inhibitors of *de novo* UMP biosynthesis.

ALLOPURINOL [4-hydroxypyrazolo (3,4-d)pyrimidine],* an analog of hypoxanthine, is converted to its principal metabolite, oxipurinol [4,6-dihydroxypyrazolo (3,4-d)-pyrimidine], by xanthine oxidase.[1] Allopurinol and oxipurinol inhibit xanthine oxidase[2] and, for this reason, allopurinol has been used extensively for the clinical control of uric acid production in gout and other forms of hyperuricemia.[3–5]

In addition to the primary effect of allopurinol as an inhibitor of purine catabolism, several laboratories have reported secondary effects of allopurinol on *de novo* purine and pyrimidine metabolism. In some, but not all, hyperuricemic patients, *de novo* purine biosynthesis is reduced when the drug is given.[6] The enzyme, HGPRTase, is essential for this effect.[7] However, there are several alternative explanations for the

* Abbreviations used in the text: 1-Alo-5′-P, allopurinol-1-ribosyl-5′-phosphate; 1-Oxi-5′-P, oxipurinol-1-ribosyl-5′-phosphate; 7-Oxi-5′-P, oxipurinol-7-ribosyl-5′-phosphate; xanthine oxidase, xanthine:O_2 oxido-reductase, EC 1.2.3.2; HGPRTase, inosine monophosphate:pyrophosphate phosphoribosyltransferase, EC 2.4.2.8; OPRTase, orotidine-5′-phosphate:pyrophosphate phosphoribosyltransferase, EC 2.4.2.10; ODCase, orotidine-5′-phosphate carboxy-lyase, EC 4.1.1.23; IMP dehydrogenase: IMP:NAD^+ oxidoreductase, EC 1.2.1.14; uridine kinase, EC 2.7.1.48; pyrimidine nucleoside phosphorylase, uridine:orthophosphate ribosyltransferase, EC 2.4.2.3; PNPase, purine nucleoside:orthophosphate ribosyltransferase, EC 2.4.2.1. Other abbreviations for nucleotides conform to the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (CBN).

decrease in *de novo* purine biosynthesis which require that HGPRTase be present. One of these requires the formation of allopurinol-1-ribonucleotide, but although this metabolite can be synthesized enzymatically *in vitro*,[8] it has not been detected previously *in vivo*.[9]

An effect on pyrimidine metabolism is more easily interpretable. Both allopurinol and oxipurinol have been found to increase the urinary excretion of orotic acid and orotidine when administered to laboratory animals and to man.[10,11] Furthermore, allopurinol has been reported to produce orotidinuria in patients who exhibit hereditary enzyme deficiencies in HGPRTase (Lesch–Nyhan syndrome),[12] ODCase (hereditary orotic aciduria), or xanthine oxidase (xanthinuria).[13] An unidentified inhibitor of ODCase has been reported to be formed from allopurinol and oxipurinol in the presence of PRPP and red cell lysates derived from normal individuals, as well as from those with Lesch–Nyhan's syndrome or hereditary orotic aciduria.[14,15] In another study, ^{14}C-orotic acid incorporation into the RNA of skin fibroblast cells was decreased by oxipurinol.[16] From these results, it was postulated that allopurinol ribonucleotide, and possibly two different ribonucleotides of oxipurinol, were formed and may inhibit ODCase *in vivo*. Although the conversion of allopurinol to the ribonucleotide has been shown to be catalysed by HGPRTase *in vitro*,[8] there has hitherto been no direct evidence for its presence *in vivo*.

MATERIALS AND METHODS

Materials

[6-^{14}C]allopurinol (2·72 mCi/m-mole) was custom synthesized by New England Nuclear Corp., Boston, Mass., by the method described by Elion *et al.*[9] and [6-^{14}C]-oxipurinol was made in these laboratories as reported previously.[9] DEAE–Sephadex A-25 was a product of Pharmacia, Inc. BioRad AG-1-X8 (chloride) was obtained from Calbiochem, and converted to the formate form with 1 M sodium formate before use. Sodium allopurinol, oxipurinol, oxipurinol-1-ribosyl-5′-phosphate (1-Oxi-5′-P), oxipurinol-7-ribosyl-5′-phosphate (7-Oxi-5′-P) and 4-hydroxy-6-aminopyrazolo pyrimidine-1-ribosyl-5′-phosphate were prepared in these laboratories; the nucleotides were synthesized by specific enzymatic methods.* Allopurinol ribonucleoside, oxipurinol-1-ribonucleoside and oxipurinol-7-ribonucleoside were obtained from T. Krenitsky.[15] 5′-Cytidylic acid was obtained from Sigma Chemical Company, St. Louis, Mo. Allopurinol-1-ribosyl-5′-phosphate (1-Alo-5′-P) was a product of Kyowa Hakko Kogyo Company, Tokyo, Japan. Triethylamine was obtained from Eastman Chemical Company and redistilled over 2,4-diaminophenol dihydrochloride before use. Other chemicals were reagent grade obtained from commercial suppliers.

Administration of [6-^{14}*C*]*allopurinol and extraction procedure*

Male Sprague–Dawley rats weighing 200–250 g were used in all the experiments reported. Animals were given the [6-^{14}C]allopurinol, dissolved with one molecular equivalent of sodium hydroxide, diluted with sodium chloride, 0·9%, and adjusted to pH 8. Solutions were administered orally, intraperitoneally or intravenously in the femoral vein, which was exposed while the animals were under ether anesthesia. The animals which were given the i.v. injections were allowed to regain consciousness.

* R. Miller and J. Fyfe, manuscript in preparation.

Two min before the tissues were removed, the animals were anesthetized with ether. In rapid succession, a blood sample, 2 ml, was removed from the abdominal aorta into a heparinized syringe. The renal blood supply was interrupted by clamping with a hemostat; then immediately the kidney was excised, squashed and frozen between silver blocks, mounted on tongs which were previously cooled with liquid nitrogen. Lastly, the major lobes of the liver were excised and freeze-clamped with the liquid nitrogen-cooled tongs. This sequence caused a minimum of anoxia and hemorrhage, which are known to cause nucleotide levels to change. The time between excision of the tissue and freezing was 3–4 sec. Tissues were wrapped in aluminium foil and held in liquid nitrogen or at −70° until extraction.

Plasma and liver concentrations of allopurinol, oxipurinol and their ribosides

A 0·5-ml aliquot of plasma was deproteinized with 0·05 ml of 50% trichloroacetic acid (TCA) and 1·0 ml of 5% TCA at 0° followed by centrifugation at 10,000 rev/min for 10 min. The supernatant fraction was transferred to a clean tube and the TCA was extracted by shaking with 5 ml diethylether, four successive times, until the aqueous phase was above pH 4. A 0·1-ml aliquot of the extract was spotted on Whatman No. 3 MM paper, with appropriate marker compounds, and the chromatogram was developed with 5% Na_2HPO_4, isoamyl alcohol (two layers), 2:1, as described by Krenitsky *et al.*[17] The markers were located under an ultra-violet lamp, cut out, and placed in scintillation vials with 1·0 ml of 2% ammonium hydroxide and 10 ml of scintillation fluid. Counting efficiency was 80 per cent and recovery of radioactivity applied to the chromatogram was about 90 per cent. The per cent of each compound was based on the total recovered radioactivity and these percentages were applied to the radioactivity in the original plasma sample to derive the concentration of each compound. The concentrations reported for liver were determined by the same chromatographic procedure, using the radioactive material in the first large radioactive peak from DEAE–Sephadex A-25 columns, described below, or using the original PCA extract of liver.

Extraction of acid-soluble nucleotides

The frozen tissues were removed from liquid nitrogen, quickly weighed and ground to a powder with a mortar and pestle under liquid nitrogen. For each gram of tissue, 1 ml of 30% perchloric acid (PCA) and 1 ml of 5′-cytidylic acid, 1 mM, were added to the mortar, where they froze instantly. The mixture was powdered under liquid nitrogen, transferred to a centrifuge tube and allowed to thaw to −10° in an ice–ethanol bath. Three volumes of cold water per gram of tissue was added, and the acid-insoluble precipitate was removed by centrifugation at 10,000 *g* for 10 min. The precipitate was washed with 3 vol. of 0·3 N perchloric acid. After centrifugation, the wash was combined with the first extract and neutralized to pH 6 with freshly prepared 10 N KOH. The potassium perchlorate precipitate was discarded and the neutral extract was stored at −20° until analysis on columns of DEAE–Sephadex A-25, or on the LCS-1000 nucleotide analyzer.

Analysis of acid-soluble nucleotides on Sephadex A-25

The isolation of nucleotide metabolites of [6-^{14}C]allopurinol was accomplished by chromatography of tissue extracts on DEAE–Sephadex A-25 columns (2·5 × 100 cm)

using gradient elution with triethylammonium acetate buffers, similar to the method described by Caldwell.[18] TEA–acetate gradients were pumped at 90 ml/hr and 20 ml/fraction was collected. An ISCO model UA-2 ultra-violet monitor was used to record the elution profile at 254 nm. The acid-soluble extract from between 5 and 20 g of tissue was diluted with an equal volume of the first buffer, then applied to the column required for each analysis. The concentration of each allopurinol metabolite was calculated from the radioactivity recovered in each radioactive peak. The amount of each metabolite was too small to obtain an ultra-violet (u.v.) spectrum.

Nucleotide pools

PCA extracts were prepared from animals which had received unlabeled allopurinol or oxipurinol. Nucleotides in the PCA extracts were separated with a high-pressure liquid chromatograph, manufactured by Varian Aerograph, model LCS-1000, using an anion pellicular column, type PA-38, 1 mm × 3 m.[19–21] A linear gradient of ammonium formate, 0·03 M, pH 4·9, to 4 M, pH 3·9, with a 15-min gradient delay was employed for elution at a column flow rate of 24 ml/hr and a temperature of 70°. The gradient pump flow rate was 12 ml/hr. It was found that between each analysis the column had to be purged twice, with a complete change of the low concentration buffer in the mixing chambers, for 15 min each, in order to obtain reproducible retention times of the early peaks. A 20-μl sample of the tissue extract (representing 3–4 mg tissue) was applied to the chromatograph. The peak areas at 254 nm in the u.v. elution profile were used to calculate the concentration of each nucleotide with the internal standard, 5′-CMP, serving to normalize each analysis. The amount of endogenous 5′-CMP was less than 5 per cent of the added 5′-CMP marker and was not considered in the calculations.

Mixtures of known concentrations of nucleotides were analyzed in order to calculate a response factor, F_x, for each compound, x.

$$F_x = \frac{\text{area}/\mu\text{mole marker}}{\text{area}/\mu\text{mole of } x} \times \mu\text{moles of marker added/g of tissue.}$$

F_x is a constant for each nucleotide, x, provided that the same gradient elution scheme is used for each analysis. Nucleotide concentrations in tissue extracts, prepared with the addition of 5′-CMP as the marker, were then calculated from the following equation:

$$\text{Tissue concn of } x^* = \frac{\text{area of } x}{\text{area of marker}} \times F_x.$$

Nucleotide peak areas on the elution profiles were measured with a Hewlett-Packard model 9107A and 9125B Digitizer, Calculator Plotter system, which was coupled to the model 9100B programmable calculator to perform the calculations. With this system it was possible to convert 15 peak areas to tissue concentration in about 10 min with an error of less than 1 per cent.

The u.v. absorbancy at 280 nm was recorded simultaneously with a second u.v. flow monitor (supplied by Laboratory Data Control, model 1280) connected in series, immediately after the 254-nm flow cell, with low dead volume tubing. The

* Units are in micromoles/gram of tissue wet weight.

280/254 absorbancy ratio of a compound eluting from the LCS-1000 offered another means of identification of each u.v. peak, in addition to the elution time.

Liquid scintillation counting

Radioactivity was determined by liquid scintillation counting in a Nuclear Chicago Mark II instrument with a modified Bray's mixture,[22] which contained OmniFluor (New England Nuclear Corp.), 8 g/l., dissolved in a mixture of methanol, 10% and ethylene glycol monomethyl ether, 10%, to keep high salt samples in solution, naphthalene, 6%, and dioxane to 1000 ml. Up to 1·0 ml of sample, with as much as 1·4 M TEA–acetate buffer, was miscible in 10 ml of this scintillation fluid. Values were corrected for background and efficiency was estimated by the external standard method.

Characterization of nucleotides derived from [6-^{14}C]*allopurinol*

Fractions from DEAE–Sephadex A-25 columns which contained radioactivity were pooled and lyophilized under vacuum with a liquid nitrogen-cooled trap to remove TEA–acetate buffer. The residue was dissolved in a minimum volume of water and stored frozen at −20°. Since the amounts of each labeled compound isolated from tissue in this way were so small, it was necessary to rely entirely on co-chromatography of the radioactive metabolite with known markers as a means of identification.

BioRad AG-1-(formate). Aliquots from radioactive peaks were each mixed with 2 μmoles of authentic 1-Alo-5′-P, 7-Oxi-5′-P and 1-Oxi-5′-P, respectively, and these mixtures were analyzed on columns of BioRad AG-1 (formate), 1 × 20 cm, eluted with a concave gradient of water, 500 ml, in a straight-walled mixer, and 6 N formic acid, 500 ml, in a conical reservoir. These columns were eluted at 50 ml/hr, and fractions of 20 ml each were collected.

Phosphatase treatment. A 50-μl aliquot of each concentrated radioactive fraction, containing between 1000 and 5000 dis/min was mixed with 0·1 vol. of 1 M glycine buffer, pH 10·5, and 0·1 vol. of alkaline phosphatase (*Escherichia coli*), 40 mg/ml, and incubated at 37° for 1 hr. The reaction was stopped by heating for 2 min at 100°. The sample was diluted to 0·2 ml with water and centrifuged; the supernatant was spotted on Whatman 3 MM sheets. After ascending chromatography overnight in solvent A (*n*-butanol–H_2O, 84:16, v/v) the chromatograms were scanned for radioactivity with a Nuclear Chicago Mark I paper strip scanner, and the R_f values of the radioactive spots were compared with those of known compounds run in parallel strips.

Acid hydrolysis. Another aliquot was made acidic by adding 0·1 vol. of 70 per cent perchloric acid, and was heated for 20 min at 100°. These samples were cooled, neutralized and subjected to paper chromatography as described above. One metabolite (see Fig. 1, peak 5) was only slightly hydrolyzed under these conditions and, therefore, it was dried and subjected to hydrolysis with 70% perchloric acid at 100° for 1 hr.

High-pressure liquid chromatography of bases, ribonucleosides and ribonucleotides. Each pooled radioactive peak was analyzed on the LCS-1000 high-pressure liquid chromatograph using the ammonium formate gradient elution system described above. In addition, the ribonucleosides and bases, which resulted from the hydrolysis by phosphatase and perchloric acid, were characterized by their retention times on the

LCS-1000. For analysis of bases and ribonucleosides, the LCS-1000, with the pellicular anion column, PA-38, was operated at 40°. Separations were achieved with a single buffer, potassium phosphate, 0·015 M, pH 5·7, and a flow rate of 15 ml/hr.

RESULTS

Separation and identification of metabolites of [6-^{14}C]allopurinol in tissues

In order to determine whether any radioactive nucleotides were present in the tissues of rats after administration of [6-^{14}C]allopurinol, material of high specific activity was employed. The perchloric acid extracts of tissue samples (weighing between 5 and 20 g) were analyzed by chromatography on DEAE Sephadex A-25 columns. A typical elution profile for a rat liver extract is shown in Fig. 1. Six major radioactive peaks were observed. Peak 1 contained most of the radioactivity applied to the column, and was shown by chromatography on the LCS-1000 to consist of allopurinol and oxipurinol and their nucleosides. Peak 2 has not been characterized. Peak 3 was found to be a trace contaminant (0·075 per cent) in the [6-^{14}C]allopurinol. There were no radioactive peaks beyond peak 6, and the recovery of the radioactivity applied to the column was completely accounted for in peaks 1–6. There was, therefore, no indication of the presence of di- or triphosphates of allopurinol or oxipurinol, which would have appeared after ADP.

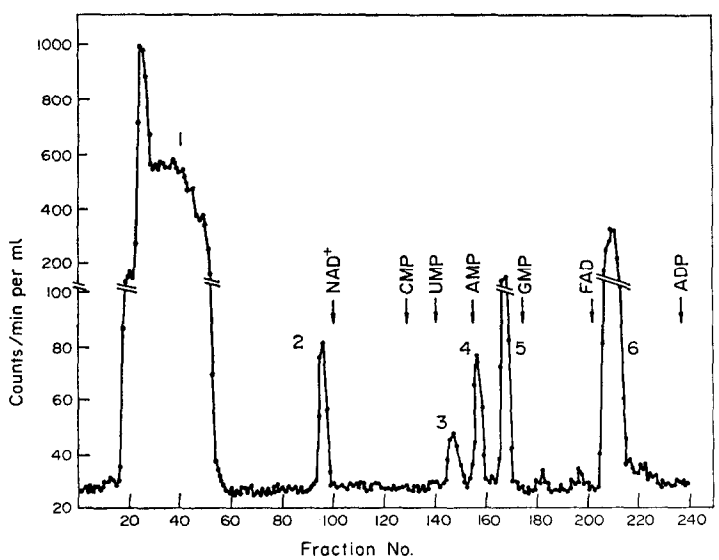

FIG. 1. Chromatography on DEAE-Sephadex-A 25 of the perchloric acid extract of liver from rats 6 hr after they had received [6-^{14}C]allopurinol 5 mg/kg, i.p. The extract was obtained from 16·9 g liver and contained a total of 565,000 dis/min; 20 ml/fraction was collected and aliquots of 1·0 ml were counted 40 min each.

In most of the elution profiles, peak 6 was a single peak, although the unusually large half-width of the peak suggested that more than one radioactive species was present. In 4 out of 31 DEAE–Sephadex A-25 analyses, peak 6 was resolved as two peaks of approximately equal radioactivity which were designated 6A and 6B. These

two components of peak 6 could be clearly separated into 6 A and 6B by subsequent chromatography on columns of BioRad AG-1 (formate), as shown in Fig. 2. Since the total amount of analog nucleotides obtained from each DEAE–Sephadex A-25 analysis was so small, it was not possible to obtain an ultra-violet absorption spectrum of the radioactive components. The identification of the radioactive metabolites present in peaks 4, 5 and 6B was accomplished by co-chromatography with authentic samples of allopurinol-1-ribonucleotide (1-Alo-5′-P), oxipurinol-7-ribonucleotide (7-Oxi-5′-P) and oxipurinol-1-ribonucleotide (1-Oxi-5′-P), before and after conversion to nucleosides by phosphatase and conversion to free bases by acid hydrolysis. The nucleotides were chromatographed on DEAE–Sephadex A-25, the LCS-1000, and BioRad AG-1 (formate); the nucleosides and bases were identified by paper chromatography and on the LCS-1000. The data are summarized in Table 1. The compound in peak 4 behaved like 1-Alo-5′-P; it was transformed by phosphatase to the corresponding nucleoside; acid hydrolysis in 1 N PCA at 100° for 20 min converted it to allopurinol. Radioactivity in peak 6B corresponded to 1-Oxi-5′-P and was converted to oxipurinol-1-ribonucleoside and to oxipurinol by phosphatase and the above described acid hydrolysis respectively. The metabolite in peak 5 co-chromatographed with 7-Oxi-5′-P, and gave oxipurinol-7-ribonucleoside after phosphatase treatment. The mild acid treatment, which was sufficient to hydrolyze the compounds in peaks 4 and 6B, resulted in only a 10 per cent conversion of the radioactive metabolite in peak 5 to oxipurinol; however, hydrolysis of peak 5 with 70% PCA at 100° for 1 hr converted the material to oxipurinol. This stability of 7-Oxi-5′-P to acid hydrolysis is analogous to that of pyrimidine nucleotides and 3-ribosylpurines.

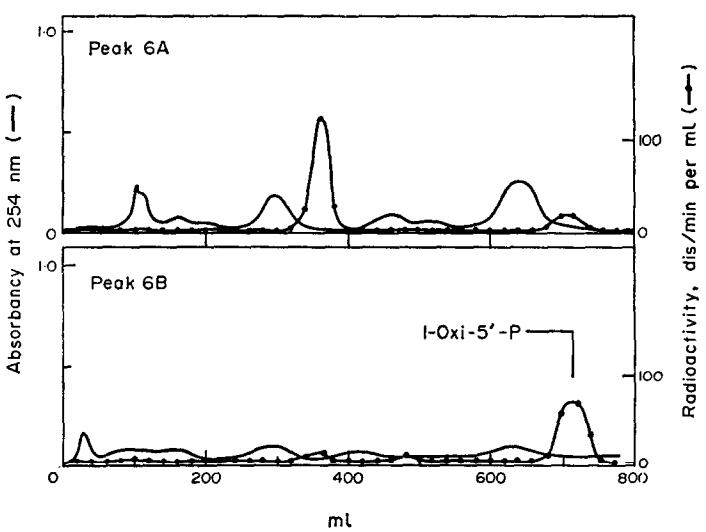

FIG. 2. Separation of peak 6A and 6B by chromatography on a BioRad AG-1 (formate) column, 1 × 20 cm. The radioactive samples were from peak 6A (6000 dis/min) and peak 6B (5100 dis/min) obtained from pooled fractions from a DEAE–Sephadex-A 25 column similar to that shown in Fig. 1. The BioRad AG-1 columns were eluted with concave gradients of water, 500 ml, and 6 N formic acid, 500 ml; 20-ml fractions were collected and aliquots of 1·0 ml were counted for radioactivity. The column effluent was continuously monitored at 254 nm.

TABLE 1. CHARACTERIZATION OF NUCLEOTIDE METABOLITES OF [6-^{14}C]ALLOPURINOL—A COMPARISON WITH AUTHENTIC COMPOUNDS

Analytical method*	Peak 4†	1-Alo-5′-P	Peak 5†	7-Oxi-5′-P	Peak 6B†	1-Oxi-5′-P
A Nucleotides						
(1) Sephadex-A 25, fraction number	156	156	167	167	212	212
(2) LCS-1000, retention time	26 min	26 min	30 min	30 min	41 min	41 min
(3) Biorad AG-1-formate, elution vol.	700 ml	700 ml	860 ml	860 ml	740 ml	740 ml
B Product after phosphatase						
(1) Paper chromatography, R_f in solvent A	0·25	0·25	0·20	0·19	0·05	0·04
(2) LCS-1000, retention time	8·1 min	8·1 min	9·3 min	9·3 min	20 min	20 min
C. Product of acid hydrolysis						
(1) Paper chromatography, R_f in solvent A	0·44	0·44	0·25	0·26	0·25	0·26
(2) LCS-1000, retention time	10·0 min	10·0 min	20 min	20 min	20 min	20 min

* See Methods for respective procedures.

† The radioactive allopurinol metabolite peaks 4, 5 and 6B from several DEAE–Sephadex-A 25 columns were pooled in order to obtain a sufficient amount of material for comparison with authentic samples.

The compound present in peak 6A has not been identified. It was present in both liver and kidney extracts and was found also in experiments in which rats were given [6-^{14}C]oxipurinol. It is, therefore, presumably derived from oxipurinol. Peak 6A did not co-chromatograph with authentic 4-hydroxy-6-aminopyrazolopyrimidine-1-ribosyl-5′-phosphate, a GMP analog, synthesized by R. Miller of these laboratories.

Chromatography on the LCS-1000

The LCS-1000 was used to separate the ribonucleosides and the bases of oxipurinol and allopurinol from the phosphatase and acid hydrolysis reactions. The anion pellicular column retained oxypurines very well at pH 5·7, and a sample could be analyzed in 25 min. The retention times of a series of compounds are shown in Table 2. The cation pellicular column, which has been used in other studies to separate purine and pyrimidine bases,[23] could not be used, since it did not adsorb or resolve the oxypurines and allopurinol and oxipurinol derivatives over a pH range of 3–10. The use of the anion pellicular column in the LCS-1000 to achieve the separations of bases and ribosides is reported here for the first time. Anion-exchange resins have been used in conventional column chromatography to separate purine and pyrimidine bases and nucleosides,[24,25] so that the usefulness of the pellicular anion column was not unexpected. It is interesting to note that oxipurinol-7-ribonucleoside eluted much sooner than oxipurinol-1-ribonucleoside, as did the respective ribonucleotides, from the anion column and probably reflects the much lower p*K*a of the 1-substituted compound.[17]

Intracellular concentrations of allopurinol and oxipurinol ribonucleotides

The intracellular concentrations of 1-Alo-5′-P, 7-Oxi-5′-P and 1-Oxi-5′-P were determined in several rat tissues at various times after the administration of [6-^{14}C]-

allopurinol by different routes. DEAE–Sephadex A-25 columns were run for each sample, and the concentrations of each nucleotide were calculated from the amounts of radioactivity present in the various peaks. The bulk of the determinations were done with liver and kidney extracts, since these tissues are known to be high in phosphoribosyltransferases.[26] Several samples of red blood cells and one of brain were also examined. The results are given in Table 3.

The liver and kidney had about equal amounts of 1-Alo-5′-P under a given set of conditions. After a single 50 mg/kg, i.v., dose of [6-^{14}C]allopurinol, 1-Alo-5′-P reached a maximum in 1–3 hr and then declined rapidly until none was detectable at 18 hr in either kidney or liver. The highest levels of 1-Alo-5′-P found after a high i.v. dose of allopurinol were 5·06 nmoles/g in kidney and 4·18 nmoles/g in liver. The half-life of 1-Alo-5′-P in liver following the peak concentration (at 3 hr) was about 1 hr.

TABLE 2. RETENTION TIMES OF KNOWN COMPOUNDS FROM LCS-1000*

Compound	Retention time (min)	Compound	Retention time (min)
Cytidine	6·0	Allopurinol	10·0
Thymidine	6·1	Guanosine	12·4
Uridine	6·2	Guanine	13·0
Adenosine	7·5	Xanthosine	15·6
Inosine	7·9	Xanthine	16·0
Hypoxanthine	8·1	Oxipurinol-1-riboside	20·0
Allopurinol-1-riboside	8·1	Oxipurinol	20·0
Oxipurinol-7-riboside	9·3		

* A pellicular anion column, PA-38, was used in the LCS-1000 liquid chromatograph and the sample was eluted with potassium phosphate, 0·015 M, pH 5·7, at 15 ml/hr and 40°.

When the same high dose (50 mg/kg) of allopurinol was given orally, the level of 1-Alo-5′-P in liver and kidney 4 hr after dosing was approximately one-tenth of the level after i.v. administration.

The concentration of 1-Alo-5′-P was consistently lower in red cells than in either liver or kidney, and was absent in brain in the one case where it was examined (5 mg/kg, i.p.).

The concentrations of both oxipurinol ribonucleotides in liver and kidney were consistently lower than that of 1-Alo-5′-P at the early times after allopurinol administration. The relative levels of 1-Oxi-5′-P and 7-Oxi-5′P in these tissues were different with high doses (20 and 50 mg/kg) of allopurinol. The liver generally contained more 1-Oxi-5′-P than 7-Oxi-5′-P, whereas in kidney the reverse was true. This was particularly noticeable at the later time intervals, and was partly due to the more rapid disappearance of 7-Oxi-5′-P than 1-Oxi-5′-P from the liver. The highest levels of 1-Oxi-5′-P and 7-Oxi-5′-P in liver were seen 1 hr after a high (50 mg/kg) intravenous dose of allopurinol; in kidney, the maximum levels occurred at 3 hr (Fig. 3).

Neither 1-Oxi-5′-P nor 7-Oxi-5′-P was detectable in red blood cells 3 hr after the high i.v. dose or 4 hr after the high oral dose of [6-^{14}C]allopurinol, nor was there any

TABLE 3. TISSUE LEVELS OF NUCLEOTIDES DERIVED FROM [6-^{14}C]ALLOPURINOL IN RAT TISSUES

Dose (mg/kg)	Route	Time (hr)	Concn in plasma* (nmoles/ml)	Tissue	Concn in tissue† (nmoles/g)	1-Alo-5′-P (nmoles/g)	7-Oxi-5′-P (nmoles/g)	1-Oxi-5′-P (nmoles/g)
50	p.o.	4	16·6	Liver	11·3	0·28	0·09	0·22
			29·5	Kidney	34·9	0·21	0·12	<0·03
				RBC	13·5	0·12	<0·01	<0·01
50‡	p.o.	21·5	0·88	Liver	0·34	<0·001	0·002	0·026
				Kidney	18·2	<0·001	0·027	0·007
50	i.v.	0·5	290	Liver	194	1·23	0·25	0·22
				Kidney	276	0·98	<0·01	<0·01
50	i.v.	1	290	Liver	208	2·35	0·34	0·53
				Kidney	779	1·07	0·25	0·20
50	i.v.	3	79	Liver	89	4·18	0·076	0·73
			103	Kidney	600	5·06	2·51	0·56
			141	RBC	26	0·27	<0·01	<0·01
50	i.v.	3	57	Liver	52	2·36	0·07	0·24
			95	Kidney	290	0·84	1·21	0·40
50	i.v.	6	10·3	Liver	4·1	0·20	<0·01	0·09
			9·1	Kidney	60·5	<0·01	0·39	0·17
70	i.v.	18	0·88	Liver	0·79	0·03	<0·01	<0·01
20	i.v.	1	81	Liver	53·6	2·65	0·04	0·509
				Kidney	200	2·26	0·10	<0·01
				RBC	67	0·94	<0·01	<0·01
5‡	i.p.	6	1·6	Liver	1·56	0·010	0·022	0·110
5‡	i.p.	6	1·5	Liver	1·23	0·011	0·022	0·149
			1·6	Kidney	3·26	0·032	0·044	0·086
			1·6	RBC	0·62	0·008	<0·001	<0·001
			1·9	Brain	0·87	<0·001	<0·001	<0·001
2·5‡	i.v.	3	3·2	Liver	2·8	0·031	0·068	0·179
			3·3	Kidney	2·7	0·017	0·023	0·019
			3·4	RBC	2·24	0·012	<0·001	<0·001
			3·7					
			3·9					
			3·9					
70	i.p.	22	2·6	Liver	2·21	0·08	<0·03	<0·03
				Kidney	84	<0·01	<0·1	0·26

* These values were calculated from the radioactivity in a plasma sample and represent the sum of the allopurinol, oxipurinol and ribosides present. Values from plasma of individual animals in the groups are shown in the brackets.

† These values were calculated from the breakthrough from DEAE–Sephadex A-25 and represent the sum of the allopurinol, oxipurinol and ribosides present.

‡ The specific activity of [6-^{14}C]allopurinol was 2·72 mCi/m-mole in these experiments and about ten-times less in the others.

in brain 6 hr after 5 mg/kg, i.p. This means that intracellular concentrations in these tissues were less than 10^{-9} M, which was the minimum detectable level.

In one experiment, [6-^{14}C]oxipurinol was administered to rats at 5·2 mg/kg, i.p. Both 1-Oxi-5′-P and 7-Oxi-5′-P were found in liver and kidney 4 hr later, but none was detectable in red cells or in brain (Table 4).

Concentrations of radioactive bases and nucleosides in rat plasma and tissues

Since the breakthrough fractions of the Sephadex A-25 columns contained the bulk of the radioactive material present in the tissue extracts, the relative concentrations of

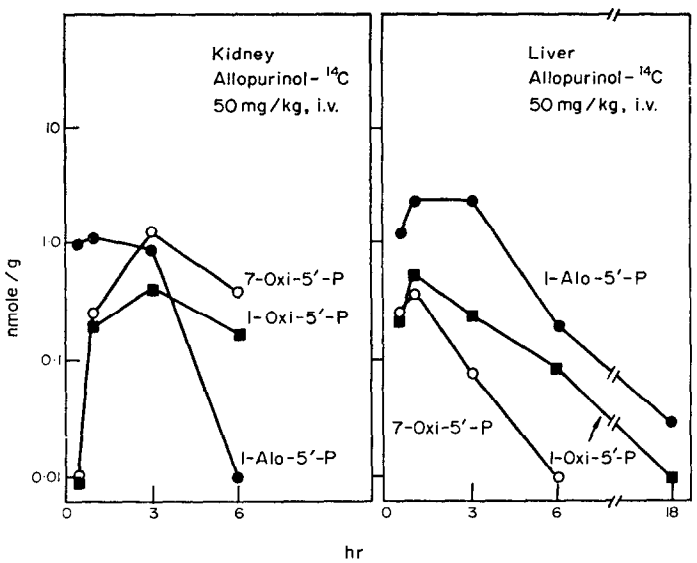

FIG. 3. Time course for the formation of 1-Alo-5′-P, 1-Oxi-5′-P and 7-Oxi-5′-P in rat liver and kidney after a single dose of [6-^{14}C]allopurinol, 50 mg/kg, i.v. Individual animals were used for each time point. The specific activity of [6-^{14}C]allopurinol was 0·27 mCi/m-mole and the minimum detectable level of the nucleotide metabolites was 0·01 nmole/g of tissue.

allopurinol, oxipurinol and their ribonucleosides were determined in representative samples to see how these correlated with dose, time and the concentrations of the respective ribonucleotides.

Table 5 shows the relative distribution of the radioactive bases and ribonucleosides in plasma and tissue extracts from some of the individual rats described in Table 3.

TABLE 4. TISSUE LEVELS OF OXIPURINOL NUCLEOTIDES AFTER [6-^{14}C]-OXIPURINOL ADMINISTRATION*

	Tissue level	
Tissue	7-Oxi-5′-P (nmoles/g)	1-Oxi-5′-P (nmoles/g)
Liver	0·073	0·041
Kidney	0·079	0·117
RBC	<0·005	<0·005
Brain	<0·005	<0·005

* The dose of [6-^{14}C]oxipurinol (sp. act., 0·60 mCi/m-mole) was 5·2 mg/kg, i.p., and the time of sacrifice was 4 hr. The plasma concentration of [6-^{14}C]oxipurinol at the time of sacrifice averaged 3·0 nmoles/ml (0·46 μg/ml) for the six animals used in the experiment. The values of <0·005 shown above indicate that there was no radioactivity detected in the fractions from the Sephadex A-25 columns where these compounds were expected. The limit of detection in these cases was about 5×10^{-9} M.

TABLE 5. RELATIVE AMOUNTS OF RADIOACTIVE BASES AND NUCLEOSIDES DERIVED FROM [6-^{14}C]ALLOPURINOL IN RAT PLASMA AND TISSUES

Dose (mg/kg)	Route	Time (hr)	Tissue	% ^{14}C in the form of			
				Allopurinol	Oxipurinol	Allopurinol-1-riboside*	Oxipurinol ribosides
50	i.v.	0·5	Plasma	71·9	19·8	6·3	2·0
		1	Plasma	61·5	27·0	9·3	2·2
		0·75	Liver	51·5	15·5	29·1	3·9
		3	Plasma	47·3	36·6	14·4	1·7
		3	Liver	23·0	25·0	49·9	2·1
50	i.v.	0·5	Plasma†	70·7	14·7	5·4	9·2
		1	Plasma†	65·0	21·9	10·7	2·4
		3	Plasma†	40·3	36·5	20·5	2·7
		6	Plasma†	19·1	51·5	26·7	2·6
		6	Liver†	17·2	37·1	42·9	2·8
4·6	i.p.	6	Plasma	2·7	83·9	9·6	3·8
		6	Liver	2·0	88·3	9·7	
		6	Kidney	1·8	78·5	19·7	
2·5	i.v.	3	Plasma	1·0	89·5	7·2	2·4
	i.v.	3	Liver	1·3	90·6	8·1	
		3	Liver	2·4	92·5	5·1	

* Allopurinol-1-riboside was identified by conversion to the free base by 7% perchloric acid 100°, for 20 min.

† All of these plasma samples were taken by serial bleeding from the tail of one individual rat; the liver of this animal was taken for analysis at the last time point.

In addition, serial samples of plasma from single rats were analyzed, after an intravenous administration of [6-^{14}C]allopurinol. The livers of these animals were also examined after 6 hr.

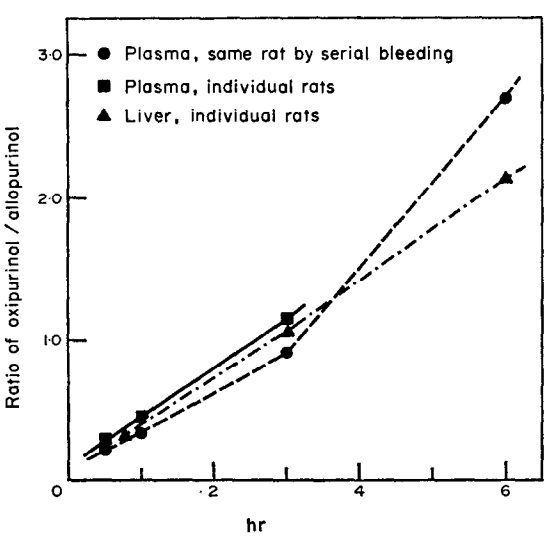

FIG. 4. Change of the ratio of oxipurinol/allopurinol in plasma and liver of the rat with time after single doses of [6-^{14}C]allopurinol, 50 mg/kg, i.v.

As might be expected, the conversion of allopurinol to oxipurinol was much higher at the low doses of allopurinol (e.g. 2·5 and 5 mg/kg) than at the high dose (50 mg/kg). Allopurinol-1-ribonucleoside formation was favored at the high dose of allopurinol and was higher in liver than in plasma. No appreciable amounts of oxipurinol ribonucleosides were detectable. The ratio of oxipurinol to allopurinol was similar in plasma and in liver, and increased with time (Fig. 4). At 3 hr after an intravenous dose of 50 mg/kg, the amounts of allopurinol and oxipurinol were equal; at 6 hr, the oxipurinol/allopurinol ratio was 2 to 2·5.

The half-life of total ^{14}C in rat plasma after a 50 mg/kg i.v. dose of [6-^{14}C]allopurinol was about 5 hr, essentially glomerular filtration rate. However, allopurinol itself disappeared much faster than this ($T_{1/2}$ = 1·75 hr), while the oxipurinol and allopurinol-1-ribonucleoside concentrations remained elevated for 6 hr (Fig. 5), due to the continued conversion of allopurinol to these metabolites.

Effect of allopurinol and oxipurinol on nucleotide pool sizes

The demonstration that low levels of allopurinol and oxipurinol ribonucleotides occur in rat tissues led to an examination of the effects which these might have on the endogenous acid-soluble nucleotide concentrations in rat liver and kidney. The tissues were freeze-clamped and extracts were analyzed for nucleotides with the LCS-1000 high-pressure liquid chromatograph. A typical chromatogram of a normal rat liver extract is shown in Fig. 6. The sloping baseline is caused by the ammonium formate gradient. Profiles of this kind are reproducible to within about 3 per cent, and the biological variation in the concentration of nucleotides was on the order of ±10

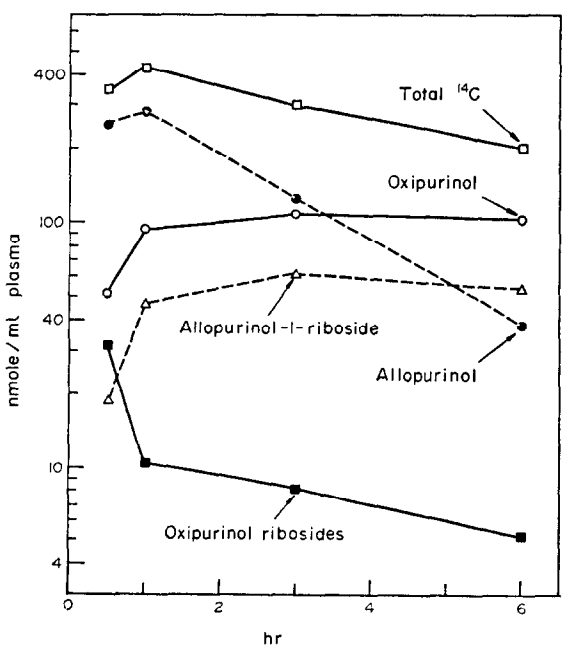

FIG. 5. Plasma concentrations of allopurinol, oxipurinol and the respective ribosides after a 50 mg/kg, i.v., dose of [6-^{14}C]allopurinol. The animal was bled periodically from the tail and the amount of each compound was determined as described in Methods. The specific activity of the [6-^{14}C]allopurinol used in this experiment was 0·34 mCi/m-mole.

per cent, so that a change in the intracellular concentration of the compound had to exceed this variation in order to be considered significant. A single i.v. dose of allopurinol, 20 mg/kg, resulted in a 47 per cent decline of UMP in the liver after 1 hr, as shown in Table 6. By 3 hr, the UMP level had returned to a normal value and remained there at 24 hr. UDP levels showed a similar response to allopurinol. UTP levels, on the other hand, changed relatively little and, if anything, may have been slightly above normal after 3 and 24 hr. The same dose of oxipurinol had a more pronounced effect, and UMP dropped by 66 per cent at 1 hr. As in the case of allopurinol, normal levels of UMP had been restored at 3 and 24 hr. UTP decreased slightly and then, returned to control values. AMP at 1 hr was decreased 56 per cent; however, at later times it was normal. ATP and guanine nucleotide pool sizes were not significantly altered from those found in the control group.

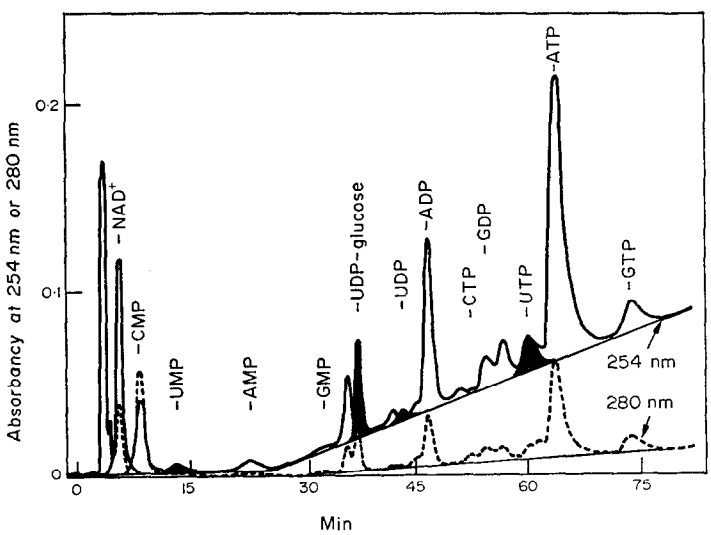

FIG. 6. Separation of acid soluble nucleotides from rat liver by high-pressure chromatography on the LCS-1000. A 15-μl sample, representing about 3 mg liver, was applied to the chromatograph and elution was performed as described in Methods. The elution position of known compounds is indicated above the u.v. profile. The large 5′-CMP peak is due to the 5′-CMP added as internal standard to the extract. The blackened areas were used to calculate the concentration of the respective uridine nucleotides. Orotidine and orotic acid when present after large doses of allopurinol had retention times of 18 and 33 min, respectively.

The effect of allopurinol on nucleotide pool sizes in rat kidney is shown in Table 6. Allopurinol, at a dose of 20 mg/kg, i.p., did not alter UMP or UDP, but did cause a temporary decrease in UTP, 38 per cent, after 1 hr. At 24 hr after this dose, or even after 7 days of treatment at 20 mg/kg/day, the UTP level was in the normal range. The 100 mg/kg, i.p., dose did not change the uridine nucleotide levels at any of the times tested. ATP was consistently elevated 20–30 per cent in kidney when a high dose of allopurinol was given. With both the 20 and 100 mg/kg dose of allopurinol, there was a marked elevation of the concentration of orotidine and orotic acid compared

TABLE 6. NUCLEOTIDE POOLS IN RAT LIVER AND KIDNEY AFTER TREATMENT WITH ALLOPURINOL AND OXIPURINOL*

Tissue Treatment	Time (hr)	UMP (μmoles/g wet wt)	UDP (μmoles/g wet wt)	UTP (μmoles/g wet wt)	UDP-glucose (μmoles/g wet wt)	Orotidine (μmoles/g wet wt)
Liver						
Control		0·207 ± 0·013	0·136 ± 0·042	0·459 ± 0·022	0·324 ± 0·016	0
Allopurinol 20 mg/kg, i.v.	1	0·109 ± 0·014‡	0·063 ± 0·007‡	0·418 ± 0·024	0·240 ± 0·012‡	0
	3	0·201 ± 0·030	0·136 ± 0·022	0·584 ± 0·034‡	0·339 ± 0·023	0
	24	0·194 ± 0·011	0·119 ± 0·019	0·578 ± 0·040‡	0·483 ± 0·045‡	0
Oxipurinol 20 mg/kg, i.v.	1	0·071 ± 0·017‡	0·072 ± 0·012‡	0·361 ± 0·011‡	0·257 ± 0·019‡	0
	3	0·223 ± 0·029	0·113 ± 0·015	0·483 ± 0·034	0·371 ± 0·016‡	0
	24	0·149 ± 0·108	0·098 ± 0·007	0·517 ± 0·075	0·471 ± 0·036‡	0
Kidneys						
Control		0·140 ± 0·029	0·126 ± 0·036	0·183 ± 0·019	0·330 ± 0·040	0
Allopurinol 20 mg/kg, i.p.	1	0·152 ± 0·014	0·096 ± 0·007	0·114 ± 0·018‡	0·510 ± 0·060‡	0·040 ± 0·012
	24	0·121 ± 0·011	0·104 ± 0·014	0·140 ± 0·024	0·580 ± 0·060‡	0·071 ± 0·025
	7 days	0·138 ± 0·022	0·120 ± 0·021	0·147 ± 0·048	0·480 ± 0·030‡	0·031 ± 0·012
Allopurinol 100 mg/kg, i.p.	1	0·139 ± 0·020	0·105 ± 0·018	0·165 ± 0·028	0·460 ± 0·050‡	0·059 ± 0·009
	24	0·129 ± 0·019	0·132 ± 0·017	0·153 ± 0·028	0·470 ± 0·040‡	0·096 ± 0·040
	7 days‖	0·071 ± 0·019	0·113 ± 0·019	0·143 ± 0·029	0·300 ± 0·040	0·105 ± 0·034

* The values shown are the mean ± standard error for each group.

† Allopurinol and oxipurinol were given intravenously and the animals were sacrifiecd at the times shown. There were five animals in each group.

‡ Significantly different from controls ($P < 0·05$).

§ Allopurinol was administered intraperitoneally as a single dose, except for the 7-day experiment, in which the animals were dosed daily for 7 days and then sacrificed 24 hr after the last dose.

‖ After 7 days of treatment, the kidneys of this group were markedly enlarged and mottled in appearance.

with the control where their concentration was too low to measure. It is possible that orotidine and orotic acid were concentrated in the tubular lumens rather than in the intracellular space, since they are excreted in the urine in increased amounts when allopurinol was administered.

DISCUSSION

The enzymatic reactions responsible for the interconversions of allopurinol and oxipurinol to their respective ribonucleosides and ribonucleotides are summarized in Fig. 7. Allopurinol is converted to oxipurinol by xanthine oxidase.[1] Both allopurinol and oxipurinol can be converted to their respective 1-ribonucleosides by purine ribonucleoside phosphorylase.[17] Allopurinol-1-ribonucleoside has been found to be a urinary metabolite of allopurinol,[17] but no oxipurinol-1-ribonucleoside has been detected in urine, possibly because of chemical instability.

The relative concentrations of allopurinol ribonucleoside and oxipurinol-1-ribonucleoside found in rat liver after high doses of allopurinol are in agreement with the enzyme studies, which showed allopurinol to be a better substrate than oxipurinol for purine ribonucleoside phosphorylase.[8] Oxipurinol-7-ribonucleoside can be formed enzymatically by action of guinea pig uridine phosphrylase;[17] this nucleoside has also been found in the urine of patients taking allopurinol.[17] However, no oxipurinol-7-ribonucleoside has been detected in rat urine after [6-^{14}C]allopurinol administration.* This may reflect a difference in the uridine phosphorylases of man and rat. Species variations have been observed in the uridine-cleaving enzymes with respect to the

* D. J. Nelson, unpublished observation.

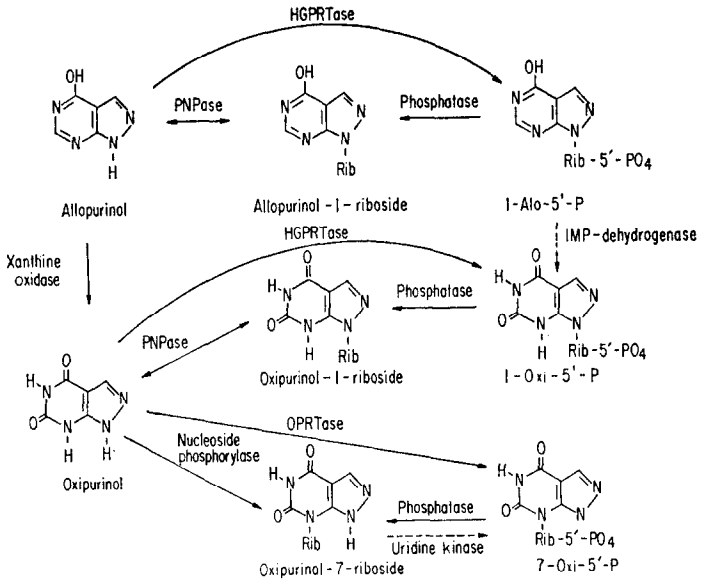

FIG. 7. Metabolic transformations of allopurinol.

specificities for both the pyrimidine and sugar moieties. Moreover, the rat enzyme has been shown to have a different pH optimum from the human and guinea pig enzymes.[27]

The formation of 1-Alo-5′-P undoubtedly takes place through the action of HGPRTase, with the utilization of PRPP.[8] Since there is only marginal inosine kinase activity in mammalian cells,[28–30] it is unlikely that allopurinol-1-ribonucleoside can be phosphorylated to yield 1-Alo-5′-P.

Two possible routes exist for the formation of 1-Oxi-5′-P. Although purified human erythrocyte HGPRTase did not form 1-Oxi-5′-P from oxipurinol at a measurable rate *in vitro*,[8] it appears likely that oxipurinol is a substrate for this enzyme but that the K_m is high and the V_{max} low. It is known, for example, that the velocity for the conversion of xanthine to XMP by HGPRTase is less than 0·5 per cent of the rate for hypoxanthine to IMP.[8,13] The other possible route for the formation of 1-Oxi-5′-P is by the action of inosinate dehydrogenase on 1-Alo-5′-P. Attempts to oxidize 1-Alo-5′-P with IMP dehydrogenase *in vitro* have thus far been unsuccessful using a substrate concentration of 7·7 mM and an enzyme derived from Sarcoma 180 ascites cells.*

The formation of 7-Oxi-5′-P from oxipurinol and PRPP probably occurs via the action of OPRTase. A beef erythrocyte enzyme has been described which can form 5′-ribonucleotides form xanthine, uric acid, orotic acid and uracil.[32,33] The position of the ribosyl group attachment is on the N-1 of the 2,4-dioxopyrimidine ring; this corresponds to the N-3 in purines, and the N-7 in pyrazolo(3,4-d)pyrimidines. This enzyme has been used to synthesize 7-Oxi-5′-P *in vitro*.† Another possibility for the

* R. Miller, personal communication.
† J. Fyfe, submitted for publication.

formation of 7-Oxi-5′-P which has not been excluded, but for which there is no evidence as yet, is that oxipurinol-7-ribonucleoside may act as a substrate for uridine kinase.

Allopurinol-1-ribonucleoside and both oxipurinol-1- and 7-ribonucleosides may be formed by the action of phosphatases on the corresponding ribonucleotides. However. this seems not to be a major route for oxipurinol-7-ribonucleoside, since none was found in rat urine, even though the corresponding ribonucleotide was formed. The fact that children with the Lesch–Nyhan syndrome, who lack HGPRTase, excrete very little allopurinol-1-ribonucleoside[34] has been interpreted as evidence that the ribonucleoside is probably formed via the ribonucleotide. However, there is an alternative explanation. Since these children lack the ability to reutilize hypoxanthine and xanthine for nucleotide synthesis, they excrete large amounts of these purine bases while on treatment with allopurinol. The binding constant of allopurinol for purine ribonucleoside phosphorylase is 100-times higher than that of hypoxanthine and 25 times higher than that of xanthine,[35] so that elevated levels of hypoxanthine might well interfere with binding of allopurinol to this enzyme.

The amounts of the ribonucleotides of allopurinol and oxipurinol are of an order of magnitude which have made their discovery and quantitation extremely difficult. It was necessary to give high doses intravenously to the rat to achieve tissue concentrations of allopurinol high enough to obtain micromolar concentrations of 1-Alo-5′-P in liver and kidney, tissues high in HGPRTase. The conversion of oxipurinol to its ribonucleotides was even poorer than that of allopurinol to 1-Alo-5′-P. This is consistent with the finding that allopurinol was a substrate for HGPRTase[8] when tested at 0·125 mM *in vitro*, whereas the conversion of oxipurinol to 1-Oxi-5′-P was not detectable under the same conditions. The relationship between the amount of ribonucleotide formed and the tissue concentration of the free base is not a linear one since, as the enzyme approaches saturation, increases in substrate concentration have relatively less effect on the amount of nucleotide formed.

It is clear that the amount of the ribonucleotides formed is also dependent on the levels of phosphoribosyltransferase and, probably, PRPP present in the tissue. Thus, red blood cells formed less 1-Alo-5′-P than liver, even when tissue concentrations of allopurinol were comparable, and no detectable amounts of 1-Oxi-5′-P or 7-Oxi-5′-P were present in red cells even after high i.v. doses of [6-^{14}C]allopurinol. The fact that ribonucleotides can be formed *in vivo*, even when the concentrations of the base are far below the K_m-values determined *in vitro*, is perhaps not surprising, if one considers that the relative enzyme concentration may be very much higher *in vivo*.

The absence of di- and triphosphates of the ribonucleosides of allopurinol and oxipurinol in the acid-soluble pools is consistent with their lack of incorporation into nucleic acids.[9,36]

In man, after therapeutic oral doses of allopurinol (3–10 mg/kg), allopurinol disappears rapidly from the plasma with a half-life of approximately 1·25 hr. Oxipurinol, on the other hand, has a long half-life (18–30 hr) because of its reabsorption by the kidney tubule.[37] Plasma concentrations of oxipurinol at the steady state level range from 5 to 40 μg/ml (30–240 nmoles/ml), depending upon dose and renal function. The formation of oxipurinol nucleotides would, therefore, be expected to be of the same order of magnitude as reported here for the rat, provided that levels of the phosphoribosyltransferases and PRPP are similar in the two species.

It has been reported that in patients who have normal HGPRTase levels, treatment with allopurinol for hyperuricemia results in a feedback inhibition of *de novo* purine biosynthesis.[3,6,7,38] Alternative explanations for this have been suggested: decreased levels of PRPP,[39] reutilization of hypoxanthine and xanthine for nucleic acid synthesis accompanied by feedback inhibition by natural purine nucleotides,[3,6] or pseudo-feedback inhibition by 1-Alo-5′-P.[40] The current studies indicate that the third explanation is highly unlikely. It is apparent that even the highest levels of 1-Alo-5′-P which were found in liver, e.g. 4×10^{-6} M, 3 hr after a high (50 mg/kg) intravenous dose of allopurinol, were 50-fold less than the amount of 1-Alo-5′-P necessary for 50 per cent inhibition of the glutamine phosphoribosylamidotransferase from pigeon liver.[40] In man, with oral doses of 3–10 mg/kg, it is doubtful that 1-Alo-5′-P would even approach 10^{-6} M. On the other hand, the K_i of AMP for this enzyme ($1–2 \times 10^{-3}$ M) lies close to the intracellular levels of AMP. Moreover, there is known to be a cooperative inhibition by AMP and GMP. By inhibiting xanthine oxidase, allopurinol promotes the conservation of purine bases[41,42] and substitutes the salvage pathway for the *de novo* synthesis of these nucleotides. Allopurinol at 10^{-3} M had no effect on the utilization of [^{14}C]hypoxanthine at 10^{-5} M for nucleic acid synthesis or on the growth rate of cultured skin fibroblast cells, which lack xanthine oxidase.[36]

The ribonucleotides of allopurinol and oxipurinol have been thought to be responsible for the increased urinary excretion of orotic acid and orotidine, breakdown products of OMP, in patients and animals treated with allopurinol.[10–12,15] The present studies indicate that the oxipurinol ribonucleotides can reach a sufficient concentration in tissues to account for ODCase inhibition, if it is assumed that the enzyme *in vivo* shows the same sensitivity as it does *in vitro*. Fyfe's studies* with partially purified yeast and rat liver ODCase have shown a marked inhibition of this enzyme by 1-Oxi-5′-P and 7-Oxi-5′-P at concentrations lower than 10^{-8} M, when OMP is 10^{-6} M. In the rat liver, the combined intracellular concentrations of 1-Oxi-5′-P and 7-Oxi-5′-P were high enough to inhibit ODCase for up to 6 hr after a single 50 mg/kg dose of allopurinol. 1-Alo-5′-P, on the other hand, is a much weaker inhibitor than the oxipurinol nucleotides,* and probably does not contribute very much to the inhibition of ODCase *in vivo*, especially when its concentration is below 10^{-6} M.

An important result of this study is the finding that the uridine nucleotide pools in rat liver and kidney were affected only transiently in liver after a 20 mg/kg, i.v., dose of allopurinol or oxipurinol. Several factors may be responsible for the compensatory restoration of uridine nucleotide pools to essentially normal levels in the presence of inhibitors of ODCase. It is possible that OMP levels increase when ODCase is inhibited by oxipurinol nucleotides. An increased level of OMP could compete more effectively with 1-Oxi-5′-P for binding to ODCase and reduce the inhibitory effect on the enzyme. Another factor in the normalization of the *de novo* pyrimidine pathway may be the induction or stabilization of OPRTase and ODCase. This has been found to occur with orotic acid in rat liver[43] and with allopurinol in human red cells,[14,43] and in rat liver.[44] Uridine kinase has been shown to be under feedback control by UTP[45] and, in other studies, uridine kinase activity was shown to be enhanced by 5-azacytidine, which is known to inhibit several enzymes in the *de novo* pyrimidine pathway, including ODCase.[46] Some suggestion of enhanced uridine salvage was also

* J. Fyfe, submitted for publication.

seen in the increased incorporation of uridine into the nucleic acids of human fibroblast cells in the presence of allopurinol and oxipurinol.[16]

The question might be raised concerning the long-term effects of allopurinol administration on uridine nucleotide pools in human tissue, particularly in patients with impaired renal function, in whom plasma and tissue levels of oxipurinol may become abnormally elevated. Ten years of clinical experience with patients taking therapeutic doses of allopurinol supports the view that a new metabolic steady state is achieved in which both purine and pyrimidine excretion remain constant during therapy, and that these revert to the original level when the drug is withdrawn. It has been estimated that the increased excretion of orotic acid and orotidine in patients taking allopurinol represents only 10 per cent of the approximately 600 mg/day of pyrimidines supplied by the *de novo* pathway.[47] Presumably, the remaining 90 per cent is still converted to uridine nucleotides, in which case the uridine nucleotide pools might be expected to remain virtually unchanged from normal. In addition, there has been no evidence for the hematological side effects, growth retardation, or crystalluria in allopurinol-treated patients which are found in some patients with hereditary orotic aciduria. The action of oxipurinol ribonucleotides on pyrimidine metabolism appears to be a benign biochemical side effect which does not produce symptoms of a nutritional deficiency, probably as a result of compensatory control mechanisms which act to maintain uridine nucleotide pools in a normal range.

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