THE METABOLISM OF 6-CHLOROPURINE-8-C^{14} IN THE RAT

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The oxidation by purified xanthine oxidase of 6-chloropurine to 6-chlorouric acid (2,8-dihydroxy-6-chloropurine), and the strong competitive inhibitions, *in vitro*, of xanthine oxidase and uricase by 6-chloropurine and 6-chlorouric acid respectively have recently been reported (Duggan and Titus, 1959). Although previous reports (Clarke *et al.*, 1954; Murphy *et al.*, 1955; Bendich, 1957) have shown 6-chloropurine to be an effective carcinostatic agent with respect to various animal neoplasms and human leukemia, only a single abstract (Sartorelli, 1959) concerning the biochemical mechanisms associated with these properties is available. The present report describes those results relating to the metabolic fate of 6-chloropurine in the rat, a study which was undertaken as a preliminary survey of the biochemical systems involved in the mechanism of action of the drug. The metabolic effects of 6-chloropurine *in vivo* are to be described elsewhere.

METHODS. *Synthesis of C^{14}-labeled 6-chloropurine.* 6-Chloropurine-8-C^{14} was prepared in 35% overall yield from hypoxanthine-8-C^{14} of specific activity 1.65 mc/mmol by adaptation of the method of Bendich *et al.* (1954) to the micro scale. The once-recrystallized ether-extracted product, which was used without further purification, had a specific activity of 8.2×10^6 c/min/mg in a Robinson-type gas-flow counter (background, 2.5 c/min) used throughout these studies. Its radiochemical purity was established by radioautography in each of the paper chromatographic systems described below.

Administration of drugs to experimental animals. Male rats of the Sprague-Dawley strain weighing 120 to 150 g were given single intraperitoneal injections of 6-chloropurine-8-C^{14} or hypoxanthine-8-C^{14}, which, except where noted, were undiluted with carrier drug, and were of the specific activities stated above.

Radiation counting techniques. Determinations of total count in urine samples were made on aliquots of whole urine plated directly onto tared stainless steel planchets and corrected for self-absorption from a standard curve of percentage absorption *vs.* total weight of urinary residue; total counts in tissues were determined by direct plating of aliquots of whole 1:10 distilled water homogenates, and corrected for self-absorption by the use of internal standards in duplicate aliquots. Expired CO_2 was collected in sodium hydroxide according to MacKenzie (1949) and aliquots converted to barium carbonate, which was plated for counting onto fiberglass mats; self-absorption corrections were made from a standard curve of percentage absorption *vs.* weight of barium carbonate per unit area. Plasma, nucleic acid, and purine fractions were counted and corrected in the same manner as total tissue counts.

Radiochromatography. After preliminary experiments had indicated no loss of total plasma or urine count from the supernatant following precipitation with 90% ethanol, these fractions were prepared for paper chromatography by the addition of nine volumes of cold ethanol, evaporation of the supernatant to dryness, and redissolution of the residue in a volume of water equal to one-third that of the original plasma or urine aliquot. These samples were spotted on Whatman #1 filter paper and both one- and two-dimensional chromatograms developed by descent at room temperature. The systems employed were (A) isoamyl alcohol layered over 5% Na_2HPO_4 (Carter, 1950) and (B) a modification of the four-component system of Radhakrishnamurty and Sarma (1953) prepared from n-butanol:benzene:water (2:1:1) by the addition of sufficient methanol (*ca.* 1.25 parts) to achieve a single phase at room temperature. Radioautograms were prepared by contact with Eastman Kodak type KK X-ray film for periods of time calculated to be necessary to observe a spot corresponding to 5% of the total count applied. All one-dimensional chromatograms used for the characterization of metabolites included appropriate labeled reference compounds where available (6-chloropurine, 6-chlorouric acid, uric acid, allantoin and urea), applied in admixture

with reconstituted supernatants of unlabeled plasma or urine. Quantitation of individual labeled components was achieved by direct gas-flow counting of each spot (as located by radioautogram and cut out), and values for each were calculated as percentage of the summation of all the individual spots, each corrected for background.

Determination of drug levels in plasma. Catheterization of the jugular veins of larger rats (240 to 260 g) was accomplished under light ether anesthesia, and the animals injected intraperitoneally or by tail vein 2 hours later. Each blood sample removed was replaced by an approximately equal volume of saline, and this dilution was taken into account in subsequent quantitation of plasma aliquots by the estimation of total protein (Weichselbaum, 1946). The corrected radioactivity of separate aliquots was determined as described above.

Isolation of nucleic acid fractions. The animals were sacrificed by cervical dislocation and the liver, spleen, and washed section of small intestine immediately removed to dry ice where they were stored until used. Total sodium nucleate was isolated by the method of Hurlbert and Potter (1952) through the hot sodium chloride extraction stage, and twice reprecipitated from cold 5% trichloroacetic acid. Following overnight incubation of the total nucleic acid fraction in 0.2 N NaOH at 38°, DNA was precipitated by chilling and acidification with hydrochloric acid, and further purified by an additional 2-hour incubation in 0.5 N NaOH followed by precipitation with HCl. RNA and DNA were determined by the orcinol (Dische and Schwarz, 1937) and diphenylamine (Seibert, 1940) colorimetric procedures, respectively, using as standards ribose nucleic acid and sperm desoxyribonucleic acid purchased from the Nutritional Biochemicals Co. The contribution of DNA to orcinol color was calculated on the basis of these standards and found to vary between 10 and 15% of the color given by an equivalent amount of RNA under the experimental conditions employed. The routine application of such a correction factor to each determination showed the DNA fractions to be virtually free of RNA. This had been previously confirmed chromatographically by the absence of a uracil spot on paper chromatograms of 60% perchloric acid hydrolysates of the DNA fraction.

Ion-exchange chromatography of nucleic-acid purines. The purified ribonucleotide fraction was hydrolyzed in 1 N hydrochloric acid at 100° for 1 hour, and an aliquot of hydrolysate containing the equivalent of 5.0 mg of nucleic acid plus 0.4 mg of carrier hypoxanthine in 5.0 ml of 0.5 N HCl acid was placed on a 1 x 12 cm column of Dowex-50 (H^+). Gradient elution with hydrochloric acid was accomplished by addition of 5.0 N HCl to a reservoir containing 100 ml of 0.5 N HCl. Pure fractions of hypoxanthine (100–120 ml), guanine (220–260 ml) and adenine (310–380 ml) were routinely located and identified spectrophotometrically. For the determination of the specific activities of purines, the two peak tubes (20 ml total) of each fraction were pooled, evaporated to dryness *in vacuo*, and taken up in a small volume of dilute sodium hydroxide. Individual aliquots of each were plated for counting, and quantitated spectrophotometrically.

TABLE 1

Overall fate of tracer dosage of 6-chloropurine-8-C^{14}
Dose: 45 μM of sp. act., 0.299 μc/μM (10 μc).

	% Total Counts				
	0–24 hr	24–48 hr	48–72 hr	72–96 hr	Total
Urine	49	23.5	8.7	1.6	82.8
Expired CO_2	5.1	3.2	0.9	0.2	9.4
Tissues	(Same animals at 96 hr)				3.7
					95.9

RESULTS. A. *Distribution and excretion of total radioactivity.* Following single intraperitoneal injections of 6-chloropurine-8-C^{14} in both tracer and therapeutic dosages, the total elimination of radioactivity in urine and expired air was measured at daily intervals for 96 hours (table 1), and the distribution in tissues of the total radioactivity still unaccounted for was determined. The weight for each organ or tissue was calculated from the values cited by Caster (1956) for percentages of total body weight of each tissue in the rat. From this figure and observed specific activities, the total count associated with each tissue was determined (table 2). The summation of total tissue counts and of count excreted in urine and expired air accounted, within experimental error, for all of the injected dosage. The dilution of labeled drug with carrier 6-chloropurine to give a total dosage of 80 mg/kg resulted in an increase in the initial rate of urinary excretion to 75% for 24 hours, and a concomitant decrease in the rate of CO_2 labeling to 3.0%.

The rates of appearance in expired carbon dioxide of the label from i.p. injections of tracer dosages of 6-chloropurine-8-C^{14} and hypoxanthine-8-C^{14} were followed throughout the 0- to

24-hour period in a number of animals, and the pattern for each precursor was found to be quite reproducible (fig. 1). The sharp peak in the appearance of the hypoxanthine label at 5–8 hours was barely observable in the case of 6-chloropurine, suggesting that the expected hydrolysis of 6-chloropurine to hypoxanthine proceeded to only a limited degree. By summation of areas under the curves, the total elimination of $C^{14}O_2$ during the 24 hours following 6-chloropurine-8-C^{14} was calculated to be only 12% that of an equivalent dosage of hypoxanthine-8-C^{14}.

TABLE 2

Tissue distribution of counts following tracer dosage of 6-chloropurine-8-C^{14}

Tissue	c/m/g × 10^3	Total Wt	Total c/m × 10^{-3}	% of Dosage
Spleen	10.9	0.32	3.5	0.035
Sm. intestine	9.60	2.9	27.8	0.278
Liver	8.04	6.25	50.4	0.504
Heart	7.60	0.43	3.27	0.033
Lung	7.26	0.84	6.1	0.061
Thymus	6.78	0.19	1.29	0.013
Kidney	6.78	1.15	7.80	0.078
Testes	4.04	1.40	5.65	0.056
Muscle	3.60	68.2	246.0	2.46
Blood	0.70	7.5	5.25	0.052

Rats (150 g) were given 10 μc of 6-chloropurine 8-C^{14} and sacrificed at 96 hours by bleeding. Each figure represents the mean for 3 animals.

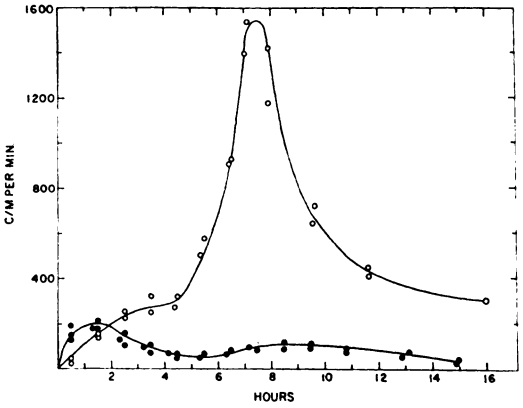

FIG. 1. Labeling of expired CO_2 following hypoxanthine-8-C^{14} (O——O) and 6-chloropurine-8-C^{14} (●——●).

Each 130 g rat given a carrier-free dosage of 2.0 μc intraperitoneally at zero time.

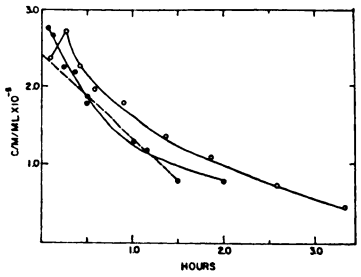

FIG. 2. Plasma levels of radioactivity following 6-chloropurine-8-C^{14} (O——O) and hypoxanthine-8-C^{14} (●——●).

Each 250 g rat given 5.0 μc of carrier-free compound intraperitoneally at zero time. ◑——◑ represents plasma levels of unchanged 6-chloropurine-8-C^{14}.

TABLE 3

Radioautography of rat plasma following injection of 6-chloropurine-8-C^{14}

Component	R_f	Percentage of Total at:		
		30 min	60 min	90 min
6-Chloropurine	0.72	82	76	61
6-Chlorouric acid	0.54	14	18	30
Hypoxanthine	0.39	0	0	0
Allantoin	0.14	3	6	9

Levels of total radioactivity in plasma following both intraperitoneal and intravenous injections of 6-chloropurine-8-C^{14} were followed in a number of catheterized animals for periods up to 5 hours. The results (fig. 2) for representative individuals indicate a rapid absorption from the peritoneum, and an initial circulating half-life of approximately 1 hour. An exact half-life of 55 minutes for unchanged drug was estimated by determining radioautographically the percentage of total circulating count corresponding to 6-chloropurine (table 3), and correcting the 30-, 60- and 90-minute points on the i.p. clearance curve by the values thus obtained.

The levels of total radioactivity in the liver, spleen, kidney and gut were determined at intervals during the 18 hours following a single i.p. injection of 6-chloropurine-8-C^{14} (fig. 3), duplicate animals being used only for the 6- and 18-hour values. Plasma radioactivity levels for each animal were determined at the time of sacrifice and compared to corresponding points on the standard plasma clearance curve (repre-

sented here as broken line) to insure that each individual was representative of the group. Each organ, with the exception of liver, was found to show only a transient accumulation of label during the first 2 hours, concomitant with the greatest rate of disappearance from the blood. Since the 30-minute values could not be reliably corrected for the contribution to total count of varying amounts of blood of higher specific activity, the actual rates of accumulation from 30 to 90 minutes are doubtless higher than those indicated.

B. *Characterization of urinary metabolites.* The identification of the various labeled components of the urines of animals given tracer dosages of 6-chloropurine-8-C^{14} was achieved on the basis of paper chromatographic data. For this purpose, urines accumulated during the first and second 24-hour periods following injection were used.

Preliminary experiments with solvent system B alone (butanol:benzene:methanol:water) succeeded in resolving six radioactive spots, none of which showed significant changes either qualitatively or quantitatively following incubation with β-glucuronidase, acid phosphatase, or takadiastase, indicating the absence of the common conjugating processes in the excretion of 6-chloropurine or its metabolites. Following hydrolysis in 0.5 N hydrochloric acid for 1 hour at 100°, the chromatographic pattern changed only quantitatively (table 4). Component VI, which moved with reference 6-chloropurine, was found to decrease in intensity by approximately 50%, while the spot corresponding to hypoxanthine showed a concomitant increase. Similarly, component V, whose R_f corresponded to that of 6-chlorouric acid, decreased one-third, while component III, assumed to be uric acid, increased by the same amount. Component I was tentatively identified as allantoin, while component II corresponded to none of the model compounds tested.

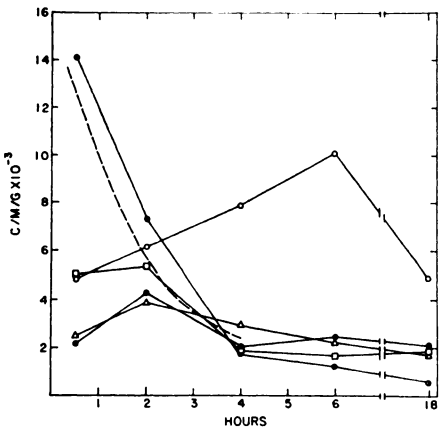

FIG. 3. Tissue distribution of radioactivity following 6-chloropurine-8-C^{14}.

Each 250 g rat given 0.5 μc of carrier-free drug at zero time. Total radioactivity, corrected for self-absorption, determined for liver (O——O), spleen (●——●), kidney (□——□) and gut (△——△) at times indicated. Total plasma radioactivity levels are expressed for each animal used here (◑——◑), and the standard plasma disappearance curve (——) included for comparison.

TABLE 4

Distribution of label in urinary components following injection of 6-chloropurine-8-C^{14}

Component	R_f	Percentage of Total Count			
		0–24 hr Urine		24–48 hr Urine	
		Original	Hydrolyzed	Original	Hydrolyzed
I	0.09	14	12	32	32
II	0.14	17	17	13	15
III	0.21	3	8	4	8
IV	0.39	8	28	14	24
V	0.55	19	15	16	11
VI	0.76	39	20	21	10

One-dimensional experiments with system A had revealed only three components. Comparison of R_f values with reference compounds, and of quantitative data with that obtained for the same urine samples in system B revealed that 6-chloropurine, 6-chlorouric acid and uric acid were traveling as a single ill-defined spot; that the allantoin spot, which showed a higher count than in system B, contained a second component, probably urea; and that the unknown component II was traveling alone.

Two-dimensional chromatograms of urines were run, using systems A and B in either order. Six spots were reproducibly obtainable and consistent qualitatively and quantitatively with the various one-dimensional data. Component IV in system B was found to be not hypoxanthine, but urea. Acid hydrolysis gave rise to a seventh component, the hypoxanthine obtained from 6-chloropurine. All two-dimensional chromatographic data are summarized in table 5,

TABLE 5

Composition of urine 24 hours following injection of 6-chloropurine as determined by two-dimensional chromatography

Component	R_f in A	R_f in B	% of Total	
			Control	Hydrolyzed
Allantoin	0.75	0.09	14	12
Unknown	0.80	0.14	15	13
Uric acid	0.40	0.21	5	10
Urea	0.73	0.40	6	9
Hypoxanthine	0.55	0.39	trace	18
6-Chlorouric acid	0.40	0.53	18	14
6-Chloropurine	0.38	0.74	42	24

each figure representing the mean value for 24-hour urines of three rats, each of which had received a single carrier-free dosage.

C. *Incorporation of 6-chloropurine label into nucleic acids.* The qualitative relationship between the order of specific activities of various tissues (table 2) and that of their respective mitotic indices suggested that 6-chloropurine or a labeled metabolite was being utilized in nucleic acid synthesis. A study of the incorporation of 6-chloropurine label into the nucleic acids of the more rapidly proliferating tissues of the small intestine, spleen, and normal and regenerating liver was undertaken. All animals were sacrificed 24 hours following injection of a single tracer dosage of 6-chloropurine-8-C^{14} (2.0 μc), and the specific activities of whole RNA and DNA fractions and of the isolated purine components of RNA were determined. In the isolation of free purine bases, carrier hypoxanthine was added to the HCl hydrolysates prior to ion-exchange chromatography to trap any traces of labeled hypoxanthine which would have been formed during the hydrolysis step from 6-chloropurine present in the nucleic acid as such.

From each hydrolysate small and variable quantities of labeled hypoxanthine were recovered. The possibility that this may have been an artifact formed by the acid hydrolysis of labeled adenine was investigated. A synthetic mixture of 0.5 mg each of hypoxanthine, guanine and carbon-labeled adenine was carried through the hydrolysis and chromatography steps. Labeled hypoxanthine of a specific activity corresponding to conversion of 3.8% of the adenine originally present to hypoxanthine was found for the RNA procedure. This figure, which was found quite reproducible for a given set of experimental conditions, was used to correct the observed hypoxanthine specific activities, yielding in every case an insignificantly small value for incorporation of 6-chloropurine as such.

The ability of 6-chloropurine to serve as a precursor of nucleic acid adenine and guanine while not being incorporated itself, suggested that it was being first converted to a common intermediate, most likely hypoxanthine. Labeled hypoxanthine-8-C^{14} of the same specific activity as the 6-chloropurine used above was therefore administered to control animals under identical conditions. It was found to be a less efficient precursor of RNA purines than 6-chloropurine, and virtually ineffective as a precursor of DNA purine. A summation of all nucleic acid labeling data is presented in table 6.

DISCUSSION. The metabolism of 6-chloropurine is, in several respects, similar to that of other purine antimetabolites, particularly 6-mercaptopurine. The apparently rapid absorption of parenterally administered 6-chloropurine and its disappearance from the circulation with a half-time of 55 minutes are of the same order of magnitude as for 6-mercaptopurine in other species (Hamilton and Elion, 1954; Elion *et al.*, 1954; Loo *et al.*, 1958), though many times slower than for 6-thioguanine (Moore and LePage, 1958) and 8-azaguanine (Gellhorn, 1953; Mandel *et al.*, 1951). A more striking difference between 6-chloropurine and the latter compounds is seen in its prolonged retention in tissues. While only traces of 8-azaguanine and 6-thioguanine are still present in the animal at 6 hours, more than half of administered 6-chloropurine-8-C^{14} count is still unaccounted for in the urine and expired air at 24 hours, and considerable radioactivity is still present in tissues after 4 days (table 1). Tracer studies with 6-mercaptopurine using both sulfur-35 and carbon-14 labeling have demonstrated a similar retention of administered radioactivity, and utilization of the purine skeleton in the biosynthesis of nucleic acid adenine and guanine (Elion *et al.*, 1954).

Quantitation of the urinary metabolites of 6-chloropurine-8-C^{14} (table 5) reveals that allantoin and uric acid together comprise 28 and 45% of total labeled catabolite during the first and second 24-hour periods, respectively. Unknown

TABLE 6

6-Chloropurine as precursor for nucleic acid purines

Dosage	Tissue	No.	Sp. Act. (c/m/mg)				
			RNA	DNA	RNA Purines		
					Ad	Gu	H_2X
6-Chloropurine (2 μc)	Liver, normal	4	320	39	1820	1040	73
6-Chloropurine (2 μc)	Liver, hep'd	2	860	345	4450	2460	195
6-Chloropurine (2 μc)	Spleen, normal	4*	690	33	3660	1440	150
6-Chloropurine (2 μc)	Spleen, hep'd	2*	380	102	2130	1225	84
6-Chloropurine (2 μc)	Gut, normal	4*	335	49	—	—	—
6-Chloropurine (2 μc)	Gut, hep'd	2*	300	55	—	—	—
Hypoxanthine (2 μc)	Liver, normal	2	242	2	940	1130	43
Hypoxanthine (2 μc)	Spleen, normal	2*	131	3	—	—	—

* Number of animals indicated pooled for fractionation.

metabolite II shows no change quantitatively upon acid hydrolysis, suggesting that it too is a non-chlorinated product. The inclusion of this substance among the non-halogenated catabolites yields respective values of 55 and 62% for the same two periods, indicating that the major catabolic pathway for 6-chloropurine at some stage involves the loss of the 6-chloro substituent. In view of the high chemical lability of the 6-chloro group, such a dehalogenation was not unexpected. However, in terms of the best *in vivo* analogy, namely the metabolism of pyrimidines substituted in ring positions with fluoro-groups (Heidelberger *et al.*, 1958; Chaudhuri *et al.*, 1958) or chloro-groups (Barrett and West, 1956), an extensive dechlorination is unusual.

The degree of CO_2 labeling following 6-chloropurine-8-C^{14} is consistent quantitatively with the urinary excretion data. Had 6-chloropurine labeled at C6 been available, a direct estimation of the extent of dehalogenation of 6-chloropurine and its still-chlorinated metabolites to the corresponding oxypurines would have been possible by measurement of the CO_2 which is rapidly and almost quantitatively formed from 6-labeled hydroxypurines (Ultman and Feigelson, 1958). Since both hypoxanthine and uric acid labeled in C8 gave rise to the same, highly characteristic CO_2 labeling patterns (fig. 1), it was felt that the rate of appearance of the C8 label of 6-chloropurine in expired CO_2 should provide a valid criterion of the extent of dehalogenation of 6-chloropurine and 6-chlorouric acid to normal intermediates. Comparison of the respective rates of CO_2 labeling through the 6- to 10-hour interval (fig. 1) indicates that for this period, 5% of administered 6-chloropurine has entered the normal purine catabolic pathway. A corresponding figure of 28% for 24 hours is obtained from the urinary excretion data. Thus, on the basis of either criterion, an average conversion of approximately 1% of total 6-chloropurine to hypoxanthine takes place each hour. The initial peak in CO_2 labeling by 6-chloropurine at 1 to 2 hours probably reflects an independent catabolic pathway and is not included in the preceding calculation.

The utilization of the intact purine skeleton of 6-chloropurine for nucleic acid biosynthesis appears to be a phenomenon common to all purine antimetabolites. A similar utilization to a greater or lesser degree has been demonstrated for purine (Gordon *et al.*, 1957), diaminopurine (Bendich and Brown, 1948; Bendich *et al.*, 1950; Bennett *et al.*, 1955), 6-mercaptopurine (Elion *et al.*, 1954), 6-thioguanine (LePage, 1959) and 8-azaguanine (Mitchell *et al.*, 1950; Matthews, 1953), the last two compounds having been clearly demonstrated to be incorporated as such into RNA and DNA.

The incorporation of unchanged 6-chloropurine into polynucleotides would be indicated by the appearance of label in the acid hydrolysates of the nucleic acid as hypoxanthine, since a quantitative conversion of 6-chloropurine to hypoxanthine is to be expected under the hydrolytic conditions employed. From the specific

activity (table 6) of a known quantity of carrier hypoxanthine, the total hypoxanthine count per unit weight of nucleic acid was calculated by isotope dilution, and corrected for the contribution to the hypoxanthine fraction by the small but reproducible hydrolysis of nucleic acid adenine. In every sample, all hypoxanthine radioactivity could be accounted for as having arisen through adenine hydrolysis, clearly demonstrating that 6-chloropurine serves only as a precursor of the normal purine constituents of RNA.[1]

The eventual dehalogenation of 6-chloropurine in the course of its utilization in ribonucleic acid biosynthesis might either precede the sequence of anabolic steps to the nucleotide stage, or follow the formation of a chlorinated riboside or ribotide. The former possibility seems unlikely in light of the evidence at hand. The rapid catabolism of hypoxanthine-6-C^{14} in the rat suggests that the permeability of cells to hypoxanthine is at least as great as to 6-chloropurine. Assuming then, that intracellular hypoxanthine levels equal or exceed 6-chloropurine levels, and that a direct conversion of 6-chloropurine to hypoxanthine would subject 6-chloropurine radioactivity to isotope dilution by the same endogenous pool, the greater efficiency of 6-chloropurine as a precursor of RNA purines is best explained by the presence of a more efficient anabolic mechanism involving the still-halogenated purine. Furthermore, the presence of a chloropurine ribotide might reasonably be expected in view of the recent elucidation of the inhibitory and resistance mechanisms for 6-mercaptopurine, in which its ribotide has been implicated (Salser *et al.*, 1960; Davidson, 1960), and the cross-resistance between 6-mercaptopurine and 6-chloropurine. Finally, the different ratios of specific activity between the adenine and guanine derived from 6-chloropurine and hypoxanthine, respectively (table 6), indicate independent pathways for the utilization of each substance.

[1] The 2 μc injections of undiluted 6-chloropurine-8-C^{14} used in the nucleic acid experiments correspond to dosages of drug of approximately 2 mg/kg. Preliminary experiments had indicated that the addition of sufficient carrier drug to achieve the normal therapeutic levels of 80 mg/kg resulted in the dilution of isolated purine specific activities to impractically low levels, and attempts to demonstrate the nucleic acid incorporation of unchanged 6-chloropurine when administered in massive dosage were not undertaken.

In explaining the quantitative difference in the efficiency of 6-chloropurine and hypoxanthine as RNA purine precursors, however, it should be considered that the prolonged retention of 6-chloropurine in tissues might provide threshold amounts of intracellular hypoxanthine by a slow, direct hydrolysis, and that the accumulation of labeled purine in RNA at a slow rate, but over a prolonged period, could in fact exceed that resulting from a large, but transient pool of labeled hypoxanthine.

Hypoxanthine as a precursor for DNA purines has been variously reported as ineffective in the rat (Getler *et al.*, 1948), and approximately one-half to one-third as effective as for RNA purines in various tissues of the rat (Bennett and Skipper, 1955) and in rabbit bone marrow (Abrams and Goldinger, 1952). In the present work, DNA isolated in a high state of purity from both the liver and spleen of rats at various time intervals following tracer dosages of hypoxanthine-8-C^{14} was consistently found to be virtually unlabeled. This qualitative difference in the labeling of DNA by 6-chloropurine and hypoxanthine might relate either to an absolute inability of hypoxanthine to serve as a precursor for DNA purines, or to only negligible levels of intranuclear hypoxanthine when it is parenterally administered as such. The latter situation can conceivably arise if there is a selective permeability of the nuclear membrane unfavorable to hypoxanthine, or if the rate of nuclear penetration by hypoxanthine is so slow as to allow a virtually complete destruction of hypoxanthine by extranuclear xanthine oxidase.

SUMMARY

Carbon-labeled 6-chloropurine, when administered parenterally to adult rats is rapidly absorbed and disappears from the blood with a half-time of approximately 1 hour, accumulating to the greatest extent in the liver.

In 24 hours, 5% of administered radioactivity is excreted as carbon dioxide, and 50% in the urine, which contains principally unchanged drug, 6-chlorouric acid, an unknown dehalogenated metabolite, and the normal products of purine catabolism. The presence of the last indicates an appreciable degree of dechlorination *in vivo*.

The dehalogenated purine skeleton of 6-chloropurine is utilized in the biosynthesis of both RNA and DNA purines. The respective degrees of

labeling of nucleic acid adenine and guanine suggest that this utilization proceeds through an anabolic pathway which does not include hypoxanthine as an intermediate.

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