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THE PATHWAY OF CATABOLISM OF CYTOTOXIC PURINYL-6-SUBSTITUTED AMINES *IN VIVO*

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Abstract—^{14}C-labelled purinyl-6-histamine is catabolized *in vivo* in the same way as *in vitro*. The main metabolites are 2,8-dihydroxy-purinyl-derivatives, carbon-dioxide and a product in which the imidazole ring of the purine ring system is opened. The speed of catabolism, the incorporation of produced carbon-dioxide into adenine and guanine, and the inhibition of the catabolism by allo-purinol has been measured.

PURINYL-6-histamine is of interest because of its selective cytotoxic action on tumor cells *in vitro*.[1] Its oxidation by xanthine oxidase and peroxidase has been described.[2] This pathway of catabolism is of significance for the selectivity of purinyl-6-histamine *in vitro* as well as for attempts to transform this selective activity observed *in vitro* into *in vivo* systems. The hypothesis that normal cells may catabolize the drug, whereas tumor cells do not, could explain the selective action *in vitro* and the absence of activity on tumor-bearing animals.[3] Provided that the catabolism *in vivo* is carried out by the same enzyme, which is effective *in vitro* (xanthine oxidase/peroxidase), it might be possible to reduce it by administration of xanthine oxidase inhibitors, such as allo-purinol.[4]

MATERIALS AND METHODS

Female Swiss mice were used for all animal experiments. Allo-purinol was a gift from Firma Zellstoff, Mannheim-Waldhof, whereby all other chemicals and solvents were purchased from Firma Merck, Darmstadt.

Radiochemicals were synthesized in this Institute[5]: (I) Purinyl (8-^{14}C)-6-histamine, activity 0·085 μC/mg; 19·5μC/mM (II) Purinyl (8-^{14}C)-6-histamine (ring^{14}C), activity 0·29 μC/mg 66·4 μC/mM.

Chromatography

Drinking water uptake was determined for 5 ml/day/mouse. It contained 1 mg II/5 ml, in the case of simultaneous allo-purinol treatment 0·2 mg II/5 ml and 0·4 mg allo-purinol/5 ml. Urines were collected every day, some drops of toluene were added in order to prevent bacterial growth. 10–100 μl of the collected urines were used for chromatography. We used ascending chromatography on Schleicher and Schüll paper Nr.: 2043 b, 18 cm length. Solvent was *n*-propanol/water 75:25. The chromatograms were cut into 18 strips of 1 cm width and the radioactivity in the strips was counted in a windowless Siemens gasflow counter (argon/methane 95:5).

Recovery experiments

Detection of radioactivity in the urine: Animals were treated with a single oral dosage of 1·3 mgII/0·5 ml of water. The urines were collected in intervals, and toluene was added and stored at −20°. At the end of the experiment all urine samples were brought to a uniform volume (10 ml), and aliquots were dropped on glass filter discs (Whatman GF/B 2·1 cm). The discs were dried and dipped into 10 ml of scintillation fluid (toluene) and counted in a Tri-Carb liquid scintillation counter. Total activities were calculated from the activities of the aliquots. Detection of radioactivity in the breath: Dry and carbon dioxide free air was sucked through the glasscage and through 10 ml of 2n KOH. The alkaline solutions were changed in intervals and the radioactivity was detected in the same way as described for the urine samples.

Incorporation of the C-atom 8 from I into the 6-position of adenine and guanine

Animals were treated for 50 and 75 days with drinking water containing 0·2 mg I/5 ml. Organs of these animals were homogenized and defatted along conventional lines. The residue was hydrolyzed with 5% trichloro acetic acid and H_2SO_4, and the free purine bases isolated via their silver salts.[6] The solution containing only the free bases was chromatographed according to Wyatt.[7] The adenine and guanine spots were located under the u.v.-lamp and identified after elution by 0·02n HCl by their E 250/260 and E 280/260 ratio, which proved to be nearly identical with theoretical values.[8]

TABLE 1. DATA FOR ISOLATED ADENINE AND GUANINE

Base	$\varepsilon \times 10^{-3}$	O.D. 250/260	O.D. 280/260	R_f
Adenine	12·6	0·79 (theor. 0·76)	0·35 (theor. 0·37)	0·4–5
Guanine	10·9	1·39 (theor. 1·37)	0·80 (theor. 0·84)	0·25–35

Specific activities were calculated from the O.D. after elution from the chromatogram (ε value see Table 1), and from the radioactivity counted by the windowless gasflow counter.

RESULTS

The radiochromatograms shown in Fig. 1 indicate that the metabolites of purinyl (8-^{14}C)-6-histamine (ring^{14}C) formed *in vivo* are quite similar to those formed *in vitro* by incubation of the drug with xanthine oxidase. Curve A demonstrates the distribution of radioactivity after chromatography of the original drug II. Incubation of II with xanthine oxidase for several hours[2] results in Curve B. With the exception of some activity near the starting line, all radioactivity is accumulated to a peak of R_f 0·1–0·3, characteristic for 2,8-dihydroxy-purinyl-6-histamine. The incubation mixture had not been deproteinized before chromatography, and the additional peak near the starting line may result from protein.

Curve C shows the distribution of radioactivity after chromatography of the urine of a mouse, which has been treated for three days with drinking water containing 1 mg II/5 ml. The main peak of activity has the R_f-value of purinyl-6-histamine, hydroxylated in 2- and 8-position. Near the starting line there is a second peak of activity

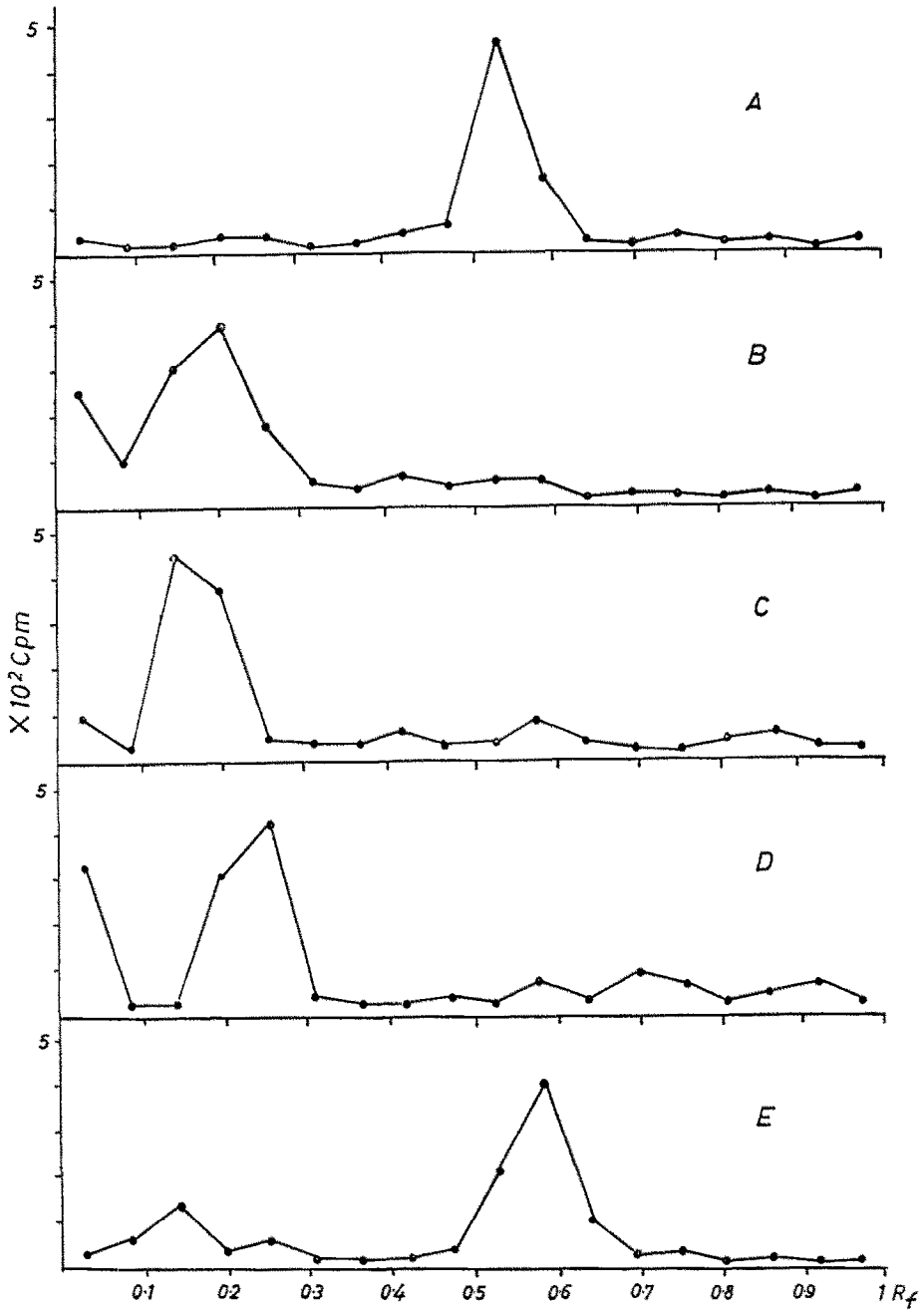


FIG. 1. Distribution of radioactivity on paper chromatograms after chromatography of urines. For details see text.

($R_f < 0{\cdot}1$) which seems to result from a further metabolite. This spot on the chromatogram produces with diazo reagent (Pauly-reagent) a red dye, similar to those produced by I and hydroxylated I. On a urine chromatogram of a mouse, which has not been treated with this drug, the identical spot is diazo negative. Histamine which produces a very sensitive reaction with Pauly-reagent would have an R_f-value of about 0·4–0·5 in this solvent system. The metabolite seems to be, therefore, a histamine derivative (see scheme). The breath of a mouse treated with II contains in addition $^{14}CO_2$ resulting, as it is demonstrated later, from the C-atom 8 of the purine ring. It would appear that the original drug is oxidized first in 2- and 8-position of the purine ring system. Part of the 2,8-dihydroxy-compound formed is excreted, and another part undergoes further oxidative decomposition. As the C-8 of the purine ring is split off to form $^{14}CO_2$, the resulting metabolite is expected to have the structure of a pyrimidine-histamine. After treatment over a longer period of time (50 days), the urines contain some other unidentified metabolites (see Curve D). Curve E shows the distribution of radioactivity after chromatography of a urine of a mouse which has been treated simultaneously with allo-purinol and II. In this case, the main peak of activity has an R_f-value of about 0·6, which is the exact R_f-value of the unchanged drug. Only a small amount of activity can be detected near an R_f-value which is characteristic of 2,8-dihydroxy-purinyl-6-histamine. This inhibition of the catabolism by allo-purinol demonstrates that II is first attacked by xanthine oxidase and that all other metabolites are the result of the oxidative decomposition of the 2,8-dihydroxy-purinyl-6-histamine.

As a contrast, purinyl-(8-^{14}C)-6-histamine was used to be sure that the activity exhaled in the form of CO_2 originated from the purine moiety of the molecule. The drug was given orally (0·11 μC). This activity is the equivalent (yield 38 per cent) to 92 796 cpm.

After 74 hr 16·2 per cent of the total radioactivity was excreted in either the urine (Fig. 2) or exhaled (Fig. 3), and only a small amount of activity is incorporated into adenine and guanine (Table 3).

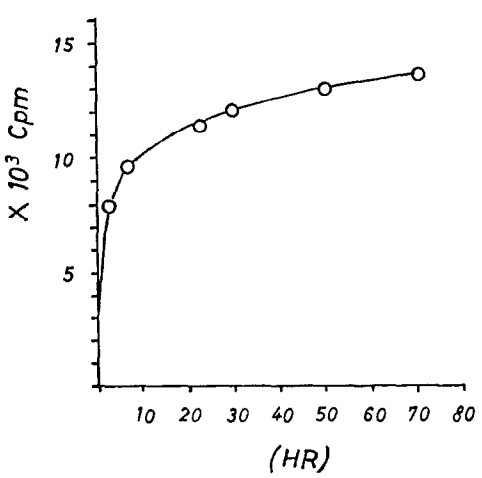


FIG. 2. Excretion of 2,8-dihydroxy-purinyl-6-histamine after treatment of a mouse with a single dosage of purinyl (8-^{14}C)-6-histamine.

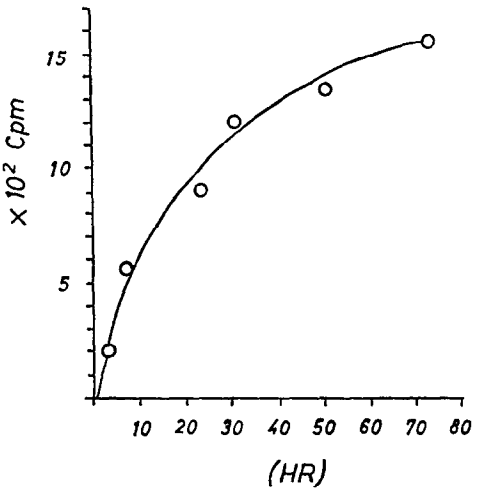


FIG. 3. Excretion of $^{14}CO_2$ after treatment of a mouse with a single dosage of purinyl (8-^{14}C)-6-histamine.

TABLE 2. TOTAL EXCRETION OF RESORBED RADIOACTIVITY IN TIME INTERVALS

Time (hr)	3	6·5	23·5	30	50	74
Excretion	51·5%	64%	80%	83·5%	92%	95%

Spectrally and chromatographically pure adenine and guanine was isolated from livers of animals fed daily with I over a long period of time. The adenine and guanine spots on chromatograms showed a specific radioactivity, increasing with the feeding time. As we could not detect further activities in organ fractions, it would appear that the rest is not resorbed. The amount of CO_2 also suggests the approximate amount of excreted metabolite with $R_f < 0{\cdot}1$.

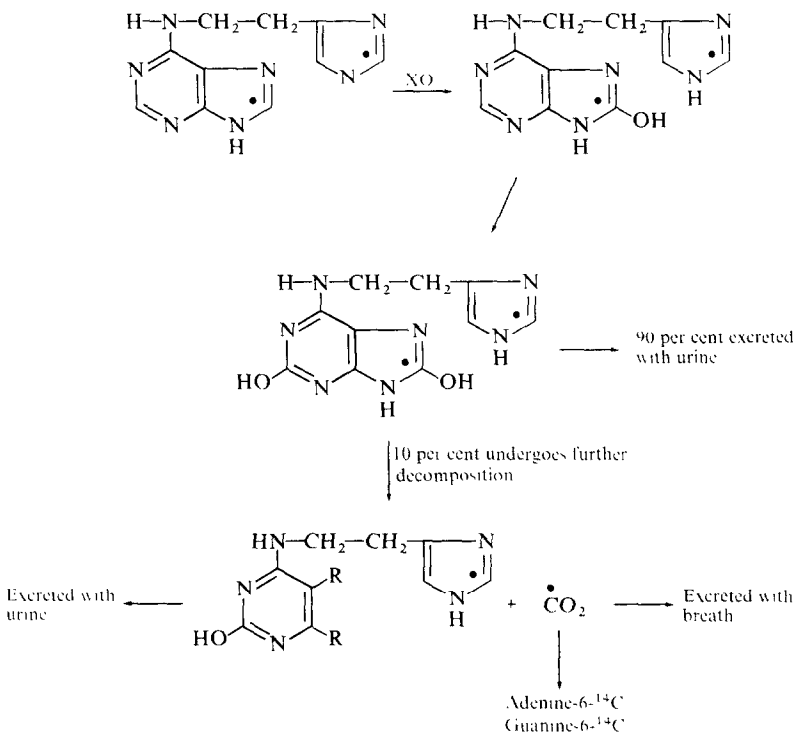
TABLE 3. SPECIFIC ACTIVITIES OF ADENINE AND GUANINE FROM MICE LIVERS AFTER TREATMENT OF THE ANIMALS WITH PURINYL-(8-^{14}C)-6-HISTAMINE OVER A LONG PERIOD OF TIME

Feeding time (days)	Base	μM	Counts/10 min	Sp. act. counts(10 min)/μM
50	adenine	0·72	391	543
75	adenine	0·46	353	767
50	guanine	0·82	135	165
75	guanine	0·54	199	369

DISCUSSION

The oxidative decomposition of purinyl-6-histamine by the xanthine oxidase/peroxidase system *in vitro*, the R_f-values of the metabolites *in vivo* (Fig. 1), the utilisation of produced carbon dioxide for *de novo* synthesis of purines, and the

inhibition of this catabolism by xanthine oxidase inhibitors, suggest that purinyl-6-histamine and all other purinyl-substituted amines which can serve as substrates for this enzyme[2] are catabolized as shown for purinyl-6-histamine. The labelled positions are marked by ●.



In view of these results, the earlier assumptions concerning the existence of a simple purinyl-6-histamine splitting enzyme[5] have to be revised. Though the experiments with allo-purinol and purinyl-6-histamine (similar experiments have been carried out by Elion and Hitchings *et al.*[9] with allo-purinol and 6-mercapto-purine) do not permit final conclusions on the therapeutic value of the drug, it seems that there is only a slight prolongation in survival of tumor-bearing animals. Apparently the rapid excretion of the unchanged drug is more significant for the biological test than the rapid catabolism without allo-purinol. We feel, therefore, that approaches to change the drug by chemical methods (for example, by attaching lipophilic groups which are hydrolysed *in vivo* nonenzymatically) might be useful.

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