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ANALYSIS OF THE PURINYL-6-HISTAMINE BINDING TO RIBONUCLEIC ACIDS*

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Abstract—Purinyl-6-histamine, which is selectively cytotoxic to tumour cells in tissue culture, does not inhibit the incorporation of precursors of macromolecular syntheses into acid insoluble material of ascites tumour cells *in vitro*, but it is bound to RNA within cells and to isolated RNA *in vitro*, as demonstrated by sedimentation studies, Sephadex G50 chromatography, polyacrylamide gel eclectrophoresis and Millipore filtration. The intrinsic binding constant for the binding to isolated RNA is in the order of $10^3 \times M^{-1}$ (0·1 M NaCl). There is some evidence ($P \leqq 0{\cdot}1$) that purinyl-6-histamine has a higher affinity in ascites tumour RNA and yeast sRNA than to liver RNA.

PURINYL-6-HISTAMINE has been described to be only cytotoxic against tumour cells *in vitro* in a concentration of $0{\cdot}6 \times 10^{-3}$ M but shows no effect on normal fibroblasts in tissue culture in the same or even in higher concentrations.[1–3] This outstanding behaviour of the compound has also been studied and demonstrated by cinematographic techniques. In contrast to this selective cytotoxic effect of purinyl-6-histamine, some purinyl-6-substituted amines closely related in structure to purinyl-6-histamine show general cytotoxicity on cells in tissue culture.[1] Earlier investigations on the metabolism of purinyl-6-substituted amines have proved that the compound is catabolized and detoxified by xanthine oxidase.[4]

This catabolism and the rapid excretion of the compound following the injection into tumour-bearing animals[5] were considered to be the reasons for the unsatisfactory curative effects of the substance observed *in vivo*. Nevertheless the growth of retothel sarcoma is inhibited to 40 per cent by purinyl-6-histamine in a dosage of 25 mg/kg/day,[6] and Ehrlich–Lettré ascites tumour cells which had been incubated *in vitro* for 4 hr together with purinyl-6-histamine were unable to produce tumours when they were reinjected into animals, thus indicating that the drug inhibits tumour cell proliferation after having been in close contact with the cells for at least 4 hr.[7] On the basis of these findings it seemed useful to expand the studies to include experiments concerning the effects of this compound on macromolecular syntheses and interactions with cellular macromolecules. Some results of these studies are presented in this work.

METHODS

Materials. Purinyl-6-derivatives were synthesized in this laboratory.

Purinyl-6-L-leucine was prepared from 6-chloropurine and L-leucine according to a general method given for the preparation of purinyl-6-substituted amino acids.[8]

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Fp.: 246° UV_{max} pH 7·0: 271 nm. Analysis: C 53·21 (53·00), H 6·31 (6·06), N 27·79 (28·10).

Purinyl-6-histamine was first synthesized from 6-chloropurine and free histamine base.[9] We modified the synthesis by using 6-chloropurine, histamine–dihydrochloride and 3 moles of sodium-butanolate. This substance is also commercially available at Fa. PWA, Mannheim, W. Germany. Labelled purinyl-6-histamine has been prepared in this Institute as described in a previous paper.[5] In this work we used labelled purinyl-6-histamine with the specific activities: following purinyl [8-^{14}C]-6-histamine (ring-[2-^{14}C]) 0·035 mCi/mmole; purinyl-6-histamine-T (U) 0·3 mCi/mmole.

Purinyl-6-(2-pyridyl-β-ethylene-amine) was prepared from 6-chloropurine and 2 moles of 2-pyridyl-β-ethylene-amine according to the general method for preparation of purinyl-6-substituted amines.[9] Fp.: 235°. UV_{max} pH 7·4: 268 nm. Analysis: C 59·96 (59·98), H 5·21 (5·04), N 35·05 (34·98).

Purinyl-6-tryptamine was first synthesized from 6-mercapto-purine and free tryptamine base.[10] We modified the synthesis by using 6-chloropurine, tryptamine-hydrochloride and 2 moles of sodium-butanolate.

Labelled compounds were bought from Radiochemical Centre Amersham, England, Unless otherwise stated in the text all other chemicals were products of Fa. Merck. Darmstadt, W. Germany.

Incorporation of labelled precursors into acid insoluble cellular material. Ehrlich–Lettré ascites tumour cells were harvested 7 days after inoculation. Incubation mixtures contained 0·1 ml of packed cells (10^7), 2 μCi of labelled precursor (thymidine-6-T, 20 Ci/mmole; uridine-5-T, 20 Ci/mmole; DL-lysine-4,5-T, 12·8 Ci/mmole; in a total volume of 0·5 ml Hanks's balanced salt solution.[11] The concentration of purinyl-6-histamine was as indicated in Table 1. Incubations were performed at 37° for 1 hr. Twenty μl aliquots of each incubation mixture were pipetted on glass filter discs (Whatman, GF/B 2·1 cm), the acid insoluble material was precipitated by cold TCA (10%) and submitted to several cold (4°) TCA (5%) washings under the general conditions described in the literature.[12] Mean values of the counts/min/disc detected by a liquid scintillation spectrometer (Packard, Tricarb, model 3380) and standard deviations of six identical incubation mixtures are listed in Table 1. The scintillation fluid used in our experiments consisted of 5 g 2,5-diphenyloxazole (PPO), 0·3 g 1,4-*bis*-2(5-phenyloxazole)-benzene (POPOP) and 1 litre of toluene.

Incorporation of labelled precursors into acid insoluble material of rat liver slices under the influence of various drugs was performed as described in a previous paper.[13]

RNA preparations. Unlabelled total cellular RNA was prepared from packed ascites tumour cells and livers of albino rats by the hot phenol method with minor modifications according to Schültz *et al.*[14] sRNA (yeast) was bought from Fa. Boehringer, Mannheim, W. Germany. Uridine-5-T labelled total cellular RNA was prepared from ascites tumour cells as follows; incubation mixtures contained 0·5 ml of packed cells (5×10^7 cells), 10 μCi of uridine-5-T (15–25 Ci/mmole) in a total volume of 5 ml Hank's balanced salt solution. Following the incubation for 1 hr at 37°, ten of these mixtures were combined, the cells collected by centrifugation, and the RNA was prepared by the hot phenol method.[14] The specific activities of these RNAs (counts/min/OD_{260} unit) were compared with the specific activities of RNA

samples received under the same conditions but in presence of purinyl-6-histamine ($0{\cdot}5 \times 10^{-3}$ M) in the incubation mixture. The complete procedure was repeated seven times. Incubation of Ehrlich–Lettré ascites tumour cells with purinyl-6-histamine-T (U) and isolation of RNA from these cells was performed under the same conditions except that purinyl-6-histamine was replaced by the same amount of the labelled derivative.

Sedimentation profiles. RNA samples were dissolved in 1 ml of water and carefully layered on 30 ml of sucrose density gradients containing 0·1 M sodium chloride, 0·02 M sodium acetate, 0·001 M EDTA, pH 5·0. The gradients were formed by a Hölzl gradient former (Fa. Hölzl Technik, Dorfen, W. Germany). Following the centrifugation (Beckman ultracentrifuge, model Spinco L-40, SW 25·1 rotor, for 40 hr at 20,000 *g*) the gradients were fractionated by a gradient fractionator (ISCO, model 182) connected with a spectrophotometer (Beckman DK 2, equipped with time drive attachment, a Helma flow cuvette of 1 cm path (Fa. Helma, Mühlheim, W. Germany, type 176 QS)) and a fraction collector (LKB Ultrorac 7000). Gradients were fractionated into 1 ml fractions, and the OD profile was recorded at 260 nm.

Detection of radioactivity: 1 mg of unlabelled RNA in 1 ml of 0·1 M sodium chloride solution was added to each tube. The RNA was precipitated by addition of 2 vol. of ethanol and collected by centrifugation. The RNA was then redissolved in 1 ml of Protosol (NEN-Chemicals, Dreieichenhain, W. Germany) and the radioactivity (counts/min/tube) detected as described above. In one experiment (Fig. 2) the values were taken by a Packard Scintillation Spectrometer (Tricarb 3380) attached to an analyser for absolute activity counting (model 544).

Polyacrylamide gel electrophoresis. Acrylamide and *N,N'*-methylene-*bis*-acrylamide were products of Fa. Serva, Heidelberg, W. Germany. Gels were prepared under the general conditions described by Maurer.[15] 2·375 g (recrystallized) acrylamide and 0·125 g *N,N'*methylene-*bis*-acrylamide were dissolved in 100 ml buffer (0·04 M Tris, 0·02 M sodium acetate, 0·002 M EDTA, adjusted to pH 7·2). 25 ml of this solution were degassed under vacuum, 0·2 ml of 10% ammonium-peroxo-disulphate solution and 20 μl of tetramethylene-ethylene-diamine added. Following the preparation of the gel columns a pre-run of 30 min was carried out in the same buffer as described above (5 mA/tube constant current). RNA samples (2 OD_{260} units) were dissolved in electrophoresis buffer (0·1 ml) containing 10% of sucrose and then layered on the tops of the gels. Electrophoresis was performed within 60 min (buffer as described above, 5 mA/tube constant current). The gels were then stained by methylene blue and destained by distilled water.[15]

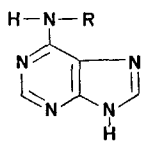
Sephadex G-50 chromatography. Ten g Sephadex G-50 (Fa. Pharmacia, Frankfurt, W. Germany) were swollen for 3 days in 200 ml of Tris–HCl buffer (0·05 M, pH 7·0) and poured into a column ($1{\cdot}5 \times 30$ cm). The column was loaded with a mixture containing 50 OD_{260} units of total cellular RNA from ascites tumour cells and 0·65 μmole of purinyl-6-histamine-T (U) in 2 ml of the buffer described above. Elution was performed by the same buffer. Twenty-three fractions (each 2·3 ml) were taken by using a monitored fraction collector (Linear II, Serva, Heidelberg, W. Germany). Aliquots of the individual fractions were diluted and used for detection of the exact optical densities (260 nm) (Beckman DK 2 spectrophotometer). Fifty μl aliquots were filtered through Millipore membrane filters and the radioactivity detected on the filters as described above. The radioactivity contained in the total fraction was calculated

from the values obtained by the counting of the aliquots. Fractions 4–9 were brought to 0·1 M with sodium chloride solution, and the RNA contained in these fractions precipitated by addition of 2 vol of ethanol. The precipitate was collected by centrifugation, redissolved in water and submitted to sucrose density gradient centrifugation as described above.

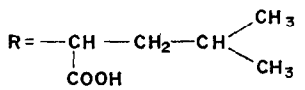
Millipore filtration. Mixtures of [^{14}C]labelled purinyl-6-histamine and RNAs of various sources (rat liver, ascites cells, yeast sRNA) were prepared in 0·05 M Tris–HCl buffer, pH 7·0, containing 0·1 M sodium chloride. Aliquots were filtered through membrane filters of 0·45 μm pore size (Fa. Millipore Filter GmbH., Neu-Isenburg, W. Germany, filtertype HAWGO 2400). The filters were then washed with 15 ml of the same buffer (containing the same molarity of sodium chloride as indicated above) to remove the unbound labelled compound. The radioactivity on the filters was detected by liquid scintillation spectrometry as described above. Efficiency counting showed that 65 counts/min were equivalent to 1 pmole of labelled purinyl-6-histamine.

RESULTS

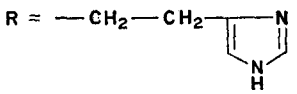
Inhibition of incorporation of labelled precursors into acid insoluble cellular material by purinyl-6-substituted amines. Numerous purinyl-6-substituted amines which are foreign to mammalian cells, show cytotoxic action[1] and can also inhibit the incorporation of labelled precursors of macromolecular syntheses into acid insoluble material of cells *in vitro*.[16] Disregarding the various causes by which drugs may inhibit the incorporation of labelled precursors (e.g. inhibition of macromolecular synthesis, change in precursor pool and membrane permeability) we came to the conclusion that some selected purinyl-6-substituted amines (formulas I–IV) exert an effect upon this



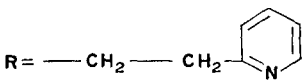
Formulas I – IV



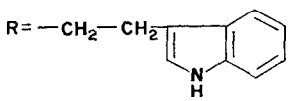
(I) Purinyl – 6 – L – leucine



(II) Purinyl – 6 – histamine



(III) Purinyl-6-(2-pyridyl-β-ethylene-amine)



(IV) Purinyl – 6 – tryptamine

incorporation of precursors into Ehrlich–Lettré ascites tumour cells and rat liver slices correlated to the cytotoxic action of the drugs.[16] The derivative I, for example, is not cytotoxic, nor does it show an effect on the incorporation of labelled precursors. At concentration of $0{\cdot}6 \times 10^{-3}$ M, the derivative III which is fairly cytotoxic, inhibits the incorporation of thymidine (40 per cent), of uridine (45 per cent) and of lysine (25 per cent) into ascites tumour cells and into rat liver slices [thymidine (60 per cent),

uridine (45 per cent), lysine (20 per cent)]. When applied in the same concentration, the derivative IV which is the most cytotoxic, produces an increased inhibition [ascites tumour cells: thymidine (80 per cent), uridine (70 per cent), lysine (50 per cent); rat liver slices: thymidine (95 per cent), uridine (90 per cent), lysine (60 per cent)].[16] The amount of inhibition produced by the cytotoxic substances III and IV and the lack of inhibition in the case of compound I is as expected. However, we could not detect any inhibition of incorporation of labelled precursors into ascites tumour cells by purinyl-6-histamine. Even at concentrations higher than $0{\cdot}6 \times 10^{-3}$ M, the incorporation of the precursors is not affected under the chosen experimental conditions (Table 1). These experiments demonstrate that the mechanism of action of this

TABLE 1. INCORPORATION OF THYMIDINE-6-T, URIDINE-5-T, DL-LYSINE-4,5-T INTO ACID INSOLUBLE MATERIAL OF ASCITES TUMOUR CELLS UNDER THE INFLUENCE OF VARIOUS CONCENTRATIONS OF PURINYL-6-HISTAMINE

Conc Purinyl-6-histamine	Incorporation of labelled:		
	Thymidine	Uridine	Lysine
Control	258 ± 86	412 ± 49	620 ± 80
$0{\cdot}6 \times 10^{-3}$ M	259 ± 63	486 ± 33	596 ± 56
$1{\cdot}3 \times 10^{-3}$ M	188 ± 27	441 ± 29	584 ± 123
$2{\cdot}5 \times 10^{-3}$ M	224 ± 21	445 ± 60	687 ± 56
F-value*	2·17	2·79	1·31

* Analysis of variance: No significant differences between the groups.

The data represent mean values of six individual experiments and the standard deviations. Counts/min/4×10^5 cells. The cells were incubated after addition of the labelled precursor and purinyl-6-histamine for 1 hr at 37° in Hanks's balanced salt solution.

selective cytotoxic substance must be different from that of the closely related compounds III and IV and that the first attack of purinyl-6-histamine on tumour cells seems to be irrelative to an inhibition of the macromolecular syntheses (DNA, RNA, protein).

TABLE 2. SPECIFIC ACTIVITIES (counts/min/OD_{260} UNIT) OF RNA SAMPLES PREPARED FROM ASCITES TUMOUR CELLS FOLLOWING THE INCUBATION WITH URIDINE-5-T WITHOUT PURINYL-6-HISTAMINE (CONTROL RNA) AND IN PRESENCE OF PURINYL-6-HISTAMINE ($0{\cdot}5 \times 10^{-3}$ M) (PH-RNA)

Experiment	Control RNA ($\times 10^{-3}$)	PH-RNA ($\times 10^{-3}$)	Control (%)
1	28·0	24·8	88·6
2	54·4	51·2	94·1
3	55·5	49·3	88·8
4	57·1	48·0	84·9
5	40·2	35·7	88·8
6	47·5	37·0	77·7
7	65·0	60·6	93·4
Mean values	49·7	43·8	88·3
P (Wilcoxon) $\leqq 0{\cdot}01$.			

Variations in specific activities which appear between the individual experiments (1–7) are the results of different specific activities of the uridine-5-T batches.

Specific radioactivities of RNAs prepared from ascites tumour cells following incubation with purinyl-6-histamine. Preparations of total cellular RNA from ascites tumour cells which were incubated with uridine-5-T and purinyl-6-histamine ($0{\cdot}5 \times 10^{-3}$ M) showed significantly lower specific radioactivities (counts/min/OD_{260} unit) than RNAs prepared from cells which were only incubated with uridine-5-T (Table 2). These lower specific activities of RNAs prepared from cells which were in contact with purinyl-6-histamine for 1 hr (PH-RNAs) could be due to inhibition of uridine-5-T incorporation. However, this would be in contradiction to the data given in Table 1. It was concluded therefore that the lower specific activities of these PH-RNAs were due to relatively higher optical densities resulting from binding of the compound to cellular RNA. As the effect is to be noticed at the level of isolated RNA, this binding must be stable enough to withstand the conditions of the hot (65°) phenol extraction.

This stable binding of purinyl-6-histamine to RNA can be proved by the results described below, and one has to take into account that both types of RNA, the control-RNA and the PH-RNA have the same specific activities, but that one OD_{260} unit of PH-RNA consists of 0·883 OD_{260} units of pure RNA and of an amount of purinyl-6-histamine which is equivalent to 0·117 OD_{260} units. From the molar absorption of purinyl-6-histamine ($\epsilon_{260} = 1{\cdot}0 \times 10^4$ M^{-1} cm^{-1}) it can be calculated that 11·2 pmole of the substance would exhibit an optical density of 0·117 OD_{260} units. Total RNA from ascites cells which were incubated with purinyl-6-histamine under the conditions described must therefore be charged with 11·2 pmole of purinyl-6-histamine per 0·883 OD_{260} units or with 1 molecule per 9 nucleotides. Sedimentation studies (Fig. 2) reveal that purinyl-6-histamine is not bound uniformly to all RNA species so that there must exist RNA species which show more or less affinity.

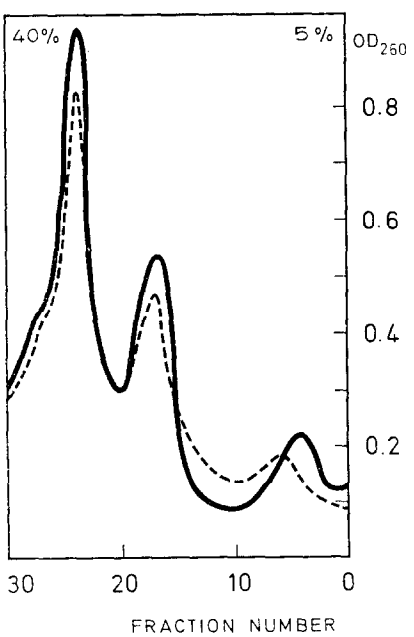


FIG. 1. Sedimentation profiles of RNAs from ascites tumour cells: ——— from untreated cells; - - - - from cells after incubation with purinyl-6-histamine. Sucrose density gradients 5–40 per cent. 11·5 OD_{260} units RNA per centrifuge tube.

Evidence for the binding of purinyl-6-histamine to cellular RNA. The u.v.-spectras of RNA preparations from ascites tumour cells which were incubated with purinyl-6-histamine for 1 hr *in vitro* show very small (1–2 nm) but significant shifts to higher wavelengths (OD_{max} of purinyl-6-histamine 273 nm at pH 7·0). Sedimentation profiles of control-RNA and RNA which was prepared from cells after incubation with purinyl-6-histamine (PH-RNA) reveal differences between these types of RNA. Especially the RNAs peak of PH-RNA is shifted to higher *S*-values (Fig. 1). After incubation of cells with purinyl-6-histamine-T (U) the radioactivity which can be detected in the RNA is preferentially associated with RNA species sedimenting between 5S and 18S and between 18S and 28S (Fig. 2).

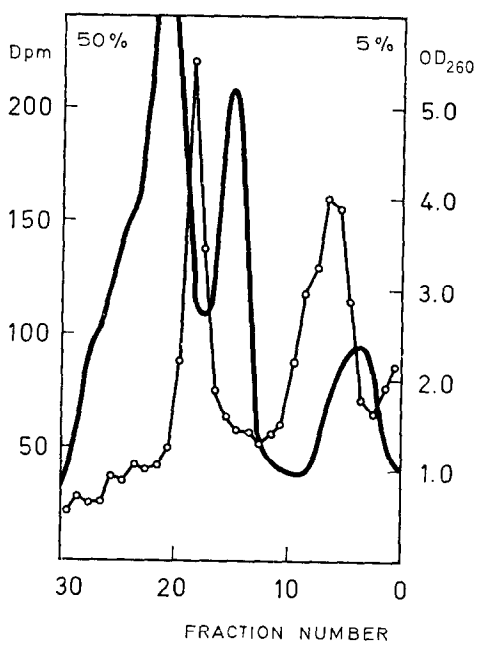


FIG. 2. Sedimentation profile of RNA from ascites tumour cells which had been incubated with purinyl-6-histamine-T (U) for 1 hr: ——— OD_{260}; ○ - - - - ○ radioactivity (dis/min/tube). Sucrose density gradient 5–50 per cent. 12·5 OD_{260} units per centrifuge tube.

Binding of purinyl-6-histamine to isolated RNA. It can be demonstrated that purinyl-6-histamine is also bound to isolated RNA *in vitro* indicating that this binding observed after incubation of cells with the compound is not an enzyme catalyzed process. This binding has been investigated by various techniques including sedimentation studies, polyacryl-amide gel electrophoresis, Sephadex G-50 chromatography and Millipore filtration:

(a) Sedimentation studies: Fig. 3 shows a sedimentation profile of a mixture containing 0·040 μmole of purinyl-6-histamine-T (U) per OD_{260} unit of total cellular RNA from ascites tumour cells. A small amount of RNA containing radioactivity is sedimented to the bottom of the centrifuge tube and does not appear in the profile. Figure 4 shows a sedimentation profile of a mixture containing

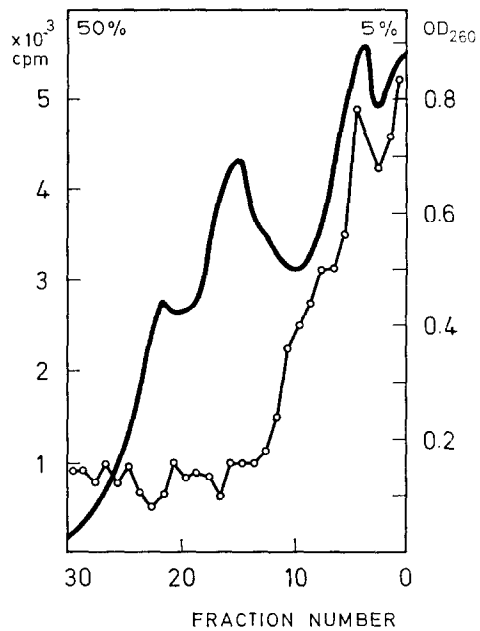


FIG. 3. Sedimentation profile of a mixture (1 ml) of 11·5 OD_{260} units of RNA from ascites tumour cells and 0·44 μmoles of ^{14}C-labelled purinyl-6-histamine: ——— OD_{260}; ○ - - - - ○ radioactivity (counts/min/tube). Sucrose density gradient 5–50 per cent.

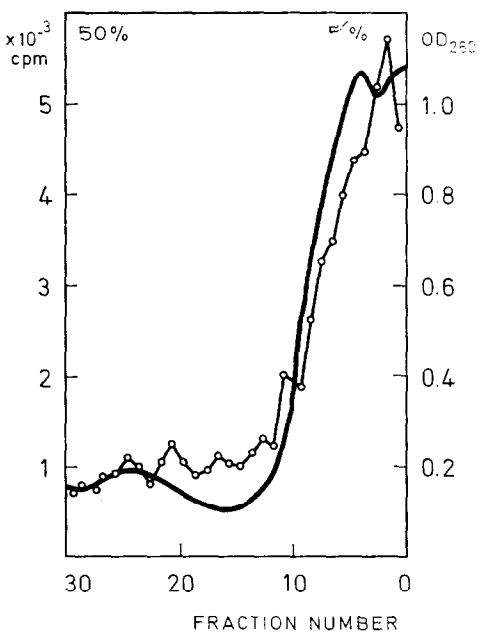


FIG. 4. Sedimentation profile of a mixture (1 ml) of 10·5 OD_{260} units of RNA from ascites tumour cells and 1·8 μmoles of ^{14}C-labelled purinyl-6-histamine: ——— OD_{260}; ○ - - - - ○ radioactivity (counts/min/tube). Sucrose density gradient 5–50 per cent.

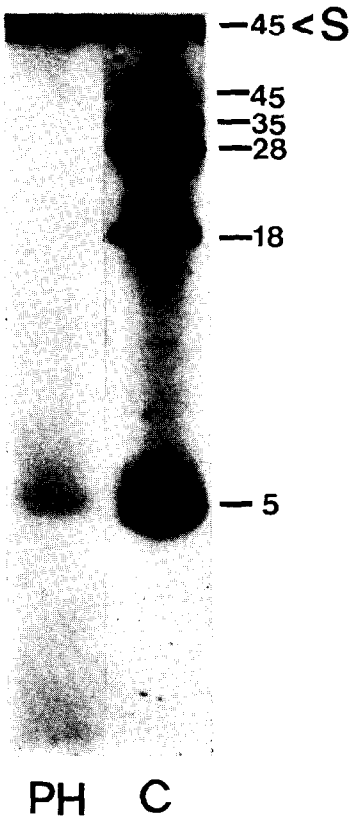


FIG. 5. Polyacrylamide gel electrophoresis of RNA (2 OD_{260} units) from ascites tumour cells (5 C) and of a mixture of the 2 OD_{260} units of the same RNA with 0·34 μmoles of purinyl-6-histamine (5 pH).

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0·17 μmole of purinyl-6-histamine-T (U) per OD_{260} unit of total cellular RNA from ascites tumour cells. The bulk of the RNA containing most of the radioactivity forms a pellet at the bottom of the centrifuge tube indicating that purinyl-6-histamine combines RNA molecules to aggregates of higher densities.

(b) Polyacrylamide gel electrophpresis: The aggregation of RNA molecules by purinyl-6-histamine could also be demonstrated by polyacrylamide gel electrophoresis. Total cellular RNA from ascites tumour cells can be separated by electrophoresis in large pore gels into the main RNA species which can also be obtained by sucrose density gradient centrifugation (Fig. 5C). Polyacrylamide gel electrophoresis of a mixture containing 0·17 μmole of purinyl-6-histamine per OD_{260} unit RNA (Fig. 5 (PH)) shows that the compound combines various RNA species to form aggregates which cannot penetrate the gel and which are not separated by electrophoretic forces.

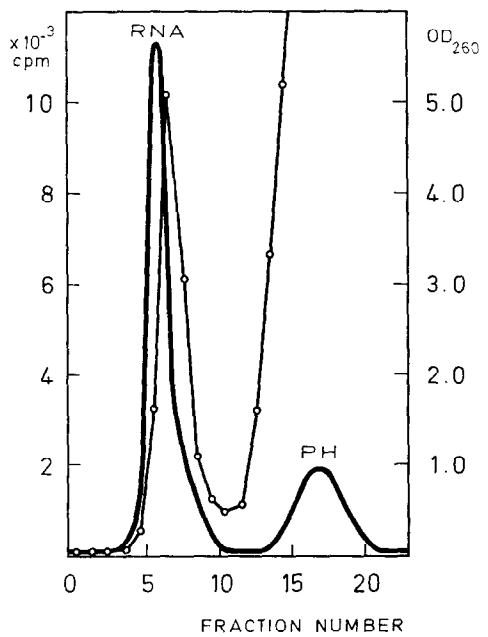

FIG. 6. Elution profile of a Sephadex G-50 column which was loaded with a mixture of 50 OD_{260} units RNA from ascites tumour cells and 0·65 μmoles of purinyl-6-histamine-T (U): ——— OD_{260}; O - - - - O radioactivity (counts/min/tube). Fractions 4–9 contain RNA.

(c) Sephadex G-50 chromatography: Fig. 6 shows an elution profile of a Sephadex G-50 column which was loaded with a mixture containing 0·013 μmole of purinyl-6-histamine-T (U) per OD_{260} unit of total cellular RNA from ascites cells. A part of the radioactivity is associated with the RNA-peak. The RNA contained in fractions 4–9 (Fig. 6) was collected by ethanol precipitation, redissolved in distilled water and submitted to density gradient centrifugation (Fig. 7). The distribution of the radioactivity differs from that in RNA received from cells which were incubated with purinyl-6-histamine-T (U) (Fig. 2), but again the sRNA species seem to be more charged with the compound.

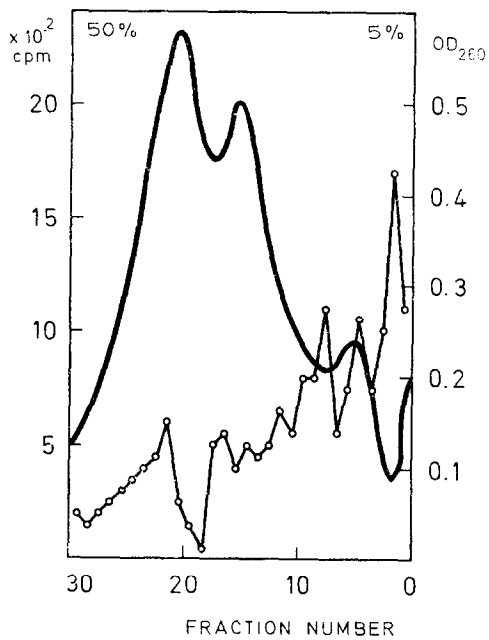

FIG. 7. Sedimentation profile of the RNA contained in fractions 4–9 (Fig 6): ——— OD_{260}; O - - - - O radioactivity (counts/min/tube). Sucrose density gradient 5–50 per cent.

(d) Millipore filtration: An accurate and quantitative study of the purinyl-6-histamine binding to RNA as proposed by Dourlent and Hélène[17] for the proflavine binding to RNA is not possible because the absorption spectras of RNA and purinyl-6-histamine are very similar. However, one can estimate the order of the intrinsic binding constant (k) from the data given in Table 3 according to the simplified equation:[18]

$$k_m = \frac{\theta}{1 - \theta}$$

where θ is the fraction of occupied sites (occupied nucleotides) and m is the molar concentration of the free absorbate. The equation implies that nearest neighbour interactions are negligible and that there exists only one single binding process. The k-values which were calculated under the assumption that two successive nucleotides form a potential binding site are in the order of $10^3 \times M^{-1}$. There exist no differences between the k-values for ascites RNA and the k-values for yeast sRNA. However, the compound seems to have less affinity for the binding to liver RNA. The significance of the differences between k-values of liver RNA and ascites RNA, and between liver RNA and yeast sRNA, are as low as $P \leqq 0{\cdot}1$ but cannot be neglected. The P-level of these differences was calculated by using Student's t-test for unpaired variates.

DISCUSSION

Purinyl-6-histamine has become a valuable tool for the study of differences between normal and malignant cells by its outstanding effect on cells in tissue culture. In view of this behaviour it seems important; (a) to elucidate the reasons for the resistance of

TABLE 3. RNAs OF VARIOUS SOURCES WERE MIXED WITH TWO DIFFERENT CONCENTRATIONS OF [^{14}C]PURINYL-6-HISTAMINE IN TRIS–HCl BUFFER (0·05 M pH 7·0 0·1 M NaCl). 0·1 ml ALIQUOTS WERE PASSED THROUGH MEMBRANE FILTERS AND WASHED WITH 15 ml OF THE SAME BUFFER

	RNA (rat liver)		RNA (ascites)		sRNA (yeast)	
nMoles purinyl-6-histamine per 0·1 ml	43	96	43	96	43	96
OD_{260} units of RNA per 0·1 ml	3·44	3·24	2·72	3·56	2·48	2·64
Equivalent to nucleotides $\times 10^{-17}$	2·4	2·3	1·9	2·5	1·7	1·8
nMoles purinyl-6-histamine bound to RNA per 0·1 ml	2·1 ± 0·8	4·1 ± 1·0	2·7 ± 1·0	5·3 ± 1·7	2·2 ± 1·2	4·5 ± 2·0
Equivalent to molecules bound to RNA per 0·1 ml $\times 10^{-14}$	12·6 ± 4·7	24·7 ± 6·0	16·0 ± 6·2	31·8 ± 10·3	17·4 ± 7·2	27·1 ± 6·4
Fraction of occupied nucleotides (θ) $\times 10^{3}$	5·3 ± 1·9	10·7 ± 2·6	8·4 ± 3·3	12·8 ± 4·1	7·7 ± 4·1	14·7 ± 6·4
$k \times 10^{-3}$ (M^{-1})	1·70 ± 0·66	1·69 ± 0·46	2·76 ± 1·13	2·11 ± 0·73	2·53 ± 1·40	2·38 ± 1·09

Mean values and standard deviations of determinations.

normal cells against the cytotoxic effect exerted by purinyl-6-histamine and; (b) to learn more about the molecular mechanism of this cytotoxic effect.

A former hypothesis which was the first attempt to explain this selective effect and which was based on the theory that normal cells should be able to catabolize the drug by xanthine oxidase whereas malignant cells (on account of low xanthine oxidase level) should not,[4] could be rejected since it had become possible to demonstrate that the xanthine oxidase levels of normal and malignant cells in culture are not significantly different.[19] Since the resistance of normal cells is not based on such a catabolic difference between normal and malignant cells, one should rather think of an increased membrane permeability or a higher number of binding sites in tumour cells.

These hypotheses strongly suggested the study of interactions between purinyl-6-histamine and cellular macromolecules. A first hint at the binding of the compound to nucleic acids could be derived from autoradiographical studies.[20] Inspections of autoradiographies of cells which had been incubated with labelled purinyl-6-histamine show that the radioactivity is incorporated and after some hours of incubation appears associated with cellular structures which can morphologically be attributed to nucleic acids. The biochemical results presented in this paper support the aspect that RNA can serve as an intracellular receptor for purinyl-6-histamine. Though there is some evidence for a higher affinity of the substance to tumour-RNA (Table 3), it only refers to the RNA preparations used in these experiments while a general difference of the affinity between tumour-RNA and normal tissue-RNA can hardly be claimed by these results. The question is whether this differential binding to tumour and normal tissue RNA or the higher affinity to special RNA species (Fig. 2) stands in connection with the selective effect. Comparable differences in the binding proportions have shortly been described by Dourlent and Hélène[17] for the proflavine binding to poly(A), poly(U) and tRNA. These differences were explained by the authors by assigning an important role to the secondary structure in the ability of polynucleotides to bind cationic dyes and in the distribution of bound dyes between two classes of complexes.

These considerations, in connection with our results, lead us to the conclusion that purinyl-6-histamine may also form two classes of complexes with RNA. The first complex which is preferentially formed with isolated RNA (Figs. 3–6) and which might be relatively weak and irrelative to the cytotoxic action, originates from electrostatic interactions between the cationic purinyl-6-histamine and the negatively charged phosphate groups of the RNA. On the cellular level there exists a second type of complex which is based on Van Der Waals type interactions and a special secondary structure of the RNA. This second type of complex is stable against the hot phenol extraction (Table 2, Figs. 1 and 2). Under the assumption that the formation of this second complex is to be seen in relation to the cytotoxic mechanism of the compound one should predict two possibilities with respect to the selective action of purinyl-6-histamine: (a) The compound forms this stable complex with special RNA species only present or essential in sensitive (tumour) cells. (b) The intracellular concentration in sensitive (tumour) cells is high enough for complex formation whereas this concentration in resistant (normal) cells is too low for the complex formation.

Though these hypotheses for the selective action of the compound are rather tempting, it still remains unclear by which mechanism this type of complex formation should lead to the total lysis of sensitive (tumour) cells. It has been demonstrated by

the results given in Table 1 that the breakdown of DNA, RNA and protein syntheses is not an early event in the time course of the drug action, and autoradiographical studies had proved that the incorporation of labelled amino acids is only inhibited by the substance in a late phase, at a time when the cellular lysis of tumour cells is just about to begin.[20]

REFERENCES

1. H. LETTRÉ, in *Experimental Tumor Research* (Ed. F. HOMBURGER), Vol. 1, p. 229. S. Karger, Basel (1960).
2. H. LETTRÉ, H. BALLWEG, H. ENDO, A. SCHLEICH and W. SIEBS, *Biochem. Pharmac.* **1,** 137 (1958).
3. H. LETTRÉ and D. WERNER, *Naturwiss.* **55,** 43 (1968).
4. H. LETTRÉ, N. K. KAPOOR and D. WERNER, *Biochem. Pharmac.* **16,** 1747 (1967).
5. H. LETTRÉ and D. WERNER, *Biochem. Pharmac.* **17,** 1951 (1968).
6. C. NEUGEBAUER, Thesis Scientific Medical Faculty, University of Heidelberg (1972).
7. P. STASCH, Thesis Scientific Medical Faculty, University of Heidelberg (1971).
8. D. W. WARD, J. WADE, E. F. WALBORG JR. and T. S. OSDENE, *J. org. Chem.* **26,** 5000 (1961).
9. Ch. G. SKINNER and W. SHIVE, *J. Am. Chem. Soc.* **77,** 6692 (1955).
10. H. LETTRÉ and H. ENDO, *Naturwiss.* **43,** 84 (1956).
11. J. H. HANKS, *J. Cell Comp. Physiol.* **31,** 235 (1948).
12. R. J. MANS and G. D. NOVELLI, *Archs Biochem. Biophys.* **94,** 48 (1961).
13. H. LETTRÉ, H. IMMICH, D. MARQUETVAND and D. WERNER, *Naturwiss.* **56,** 517 (1969).
14. G. SCHÜLTZ, D. GALLWITZ and C. E. SEKERIS, *Eur. J. Biochem.* **4,** 149 (1968).
15. H. R. MAURER, *Disk Elektrophorese, Theorie und Praxis der diskontinuirlichen Polyacrylamid-Elektrophorese*, Walter de Gruyter, Berlin (1968).
16. D. WERNER, Habilitationsschrift, Scientific Medical Faculty, University of Heidelberg (1970).
17. M. DOURLENT and C. HÉLÈNE, *Eur. J. Biochem.* **23,** 86 (1971).
18. R. F. STEINER and R. F. BEERS, *Natural and Synthetic Nucleic Acids.* p. 291. Elsevier, Amsterdam (1961).
19. H. LETTRÉ, D. WERNER and A. SCHLEICH, *Z. Krebsforsch.* **74,** 368 (1970).
20. A. SCHLEICH and A. MAYER, in *Radionuklide in der klinischen und experimentellen Onkologie* (Ed. G. HOFFMAN), p. 17. F. K. Schattauer, Stuttgart (1965).