Purine-Phosphoribosyltransferase Activities in Rat and Mouse Tissues and in Ehrlich Ascites-Tumour Cells

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1. The activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase in extracts of rat and mouse liver, brain, spleen, heart and kidney, of rat bone marrow and of Ehrlich ascites-tumour cells have been measured. The specific activity of adenine phosphoribosyltransferase in tumour cells (3 m μ -moles of nucleotide formed/min./mg. of protein) was between 15 and 60 times the activity of any mouse tissue examined. Hypoxanthine-phosphoribosyltransferase activity was greater in extracts of tumour cells than in mouse tissues, but the difference was not great. 2. The specific activities of both adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase in ascites-tumour cells decreased respectively by 57% and 36% 2 days after the cells had been inoculated into mice. After 4 days of tumour growth the specific activities of both enzymes increased, reaching a maximum at 10 days. 3. The activity of adenine phosphoribosyltransferase in rat-liver extracts increased steadily up to 4 days after partial hepatectomy, and was still above the control value after 14 days. Hypoxanthine-phosphoribosyltransferase activity in extracts of regenerating rat liver did not increase until the second day after operation and had begun to decrease by the fourth day. 4. From the results it was concluded that adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase may be physiologically important enzymes in rapidly growing tissues, and that inhibitors of the former enzyme have potential value in chemotherapy.

The purine bases adenine, hypoxanthine and guanine are converted into their respective 5'-phosphoribosyl derivatives in the presence of PRPP,* Mg²⁺ ions and the corresponding purine phosphoribosyltransferase. Kornberg (1957) postulated that these phosphoribosyltransferases, together with enzymes catalysing nucleotide formation via nucleosides, act as 'salvage' enzymes by conserving the purine ring as metabolically available nucleotide. In most animal tissues the purine ring has been assumed to arise by new biosynthesis from non-cyclic precursors (for a review see Buchanan & Standish, 1959). There is some evidence, however, that bone-marrow cells and Ehrlich ascites-tumour cells can utilize purines, derived from other tissues in the animal, for biosynthesis of nucleotides and nucleic acids (Smellie, Thomson, Goutier & Davidson, 1956; Henderson & LePage, 1959;

Kimball & LePage, 1963). Further, Lajtha & Vane (1958) suggested that bone-marrow cells depend on a supply of purines from the liver. If preformed purine bases are utilized by some specialized tissues, the purine phosphoribosyltransferases may play an important role in the nucleotide metabolism of these tissues.

There is little quantitative information available on purine-phosphoribosyltransferase activities in animal tissues. The activities of AMP-pyrophosphate phosphoribosyltransferase (adenine phosphoribosyltransferase; EC 2.4.2.7) and of IMP-pyrophosphate phosphoribosyltransferase (hypoxanthine phosphoribosyltransferase; EC 2.4.2.8) in extracts of various rat and mouse tissues and in Ehrlich ascites-tumour cells have now been determined and the results are given in the present paper. The activities of both enzymes are considerably greater in rapidly growing ascites-tumour cells and in regenerating rat liver than in more slowly growing tissues. The results obtained indicate that in-

* Abbreviation: PRPP, 5'-phosphoribosyl pyrophosphate.

inhibitors of adenine phosphoribosyltransferase have potential value in chemotherapy.

EXPERIMENTAL

Substrates. [8-¹⁴C]Adenine, [8-¹⁴C]hypoxanthine and [8-¹⁴C]guanine were obtained from California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A. Unlabelled adenine, hypoxanthine, guanine, xanthine, adenosine, inosine, guanosine, AMP, IMP and GMP were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A. None of the compounds contained any contaminants that could be detected by spectrophotometry or radioautography after paper chromatography as described by Atkinson & Murray (1965a).

PRPP, obtained from the Pabst Laboratories, Milwaukee, Wis., U.S.A., was similar in purity to that described by Atkinson & Murray (1965a).

Preparation of extracts for measurement of purine-phosphoribosyltransferase activities. Female Swiss mice (49–56 days old) and female white rats (70–80 days old) were used. Animals were killed by decapitation. The liver, brain, spleen, heart and kidneys were rapidly removed, rinsed in cold (1°) 0.9% NaCl, blotted dry and weighed. Rat bone marrow was obtained from the long bones and weighed directly. After mice containing Ehrlich ascites-tumour cells were killed, 2 ml. of cold 0.9% NaCl was injected intraperitoneally. Tumour cells were collected by centrifuging the peritoneal contents at 2000 g for 5 min. at 2° and were washed once with cold 0.9% NaCl. The tissues were suspended in approx. 10 vol. of 50 mM-tris-chloride buffer, pH 7.8, and homogenized for 5 sec. with an Ultra-Turrax homogenizer (Janke und Kunkel Kg., Stauffer i. Br., Germany) at 1°. Longer periods of homogenization did not extract any additional purine-phosphoribosyltransferase activity.

The supernatants obtained after centrifuging at 20000 g (Serrall Superspeed type SS-4, rotor type SS-34) for 20 min. at 2° were used directly for determinations of phosphoribosyltransferase activity. Homogenates of rat and mouse liver did not centrifuge well and the supernatants were carefully removed with a Pasteur pipette. Assays were carried out within 6 hr. of preparation of the extracts. The adenine- and hypoxanthine-phosphoribosyltransferase activities of extracts remained unchanged during this period. Protein determinations on the extracts of all tissues except bone marrow were made as a routine by the biuret method (Gornall, Bardawill & David, 1949). Extracts from bone marrow contained material that interfered with the biuret reaction and in these extracts protein was measured by the method of Lowry, Bessey & Crawford (1949).

Partial hepatectomy of rats. Female white rats, 70–80 days old and weighing approx. 150 g., were used. Partial hepatectomy was carried out under ether anaesthesia as described by Higgins & Anderson (1931). The animals were fed on a standard laboratory diet and water *ad libitum*. To exclude the possibility of an effect of glucose on liver enzyme activities this substance was not added to the water supply [Higgins & Anderson (1931) replaced water with a 20% glucose solution for the first 24 hr. after surgery]. Cell-free extracts of the liver were made as described above at various times after surgery; extracts at zero time were made after discarding the lobes normally removed in partial hepatectomy. Samples of liver were taken before homogeniza-

tion for dry-weight determination (by heating at 110° for 48 hr.).

Counting of Ehrlich ascites-tumour cells. Samples of ascitic fluid containing ascites-tumour cells were suitably diluted (usually 1:100) in 0.9% NaCl. The cells were counted microscopically with a clinical haemocytometer (Levey-type counting chamber). Duplicate samples were taken of the ascitic suspension from each mouse and at least 500 tumour cells were counted in each sample. Throughout the work all counts were made by the one operator using the one haemocytometer.

When total numbers of tumour cells per mouse were required the bulk of the ascitic fluid was collected and the peritoneal cavity was then washed twice with 4 ml. portions of 0.9% NaCl to remove residual tumour cells. The washings and the original ascitic fluid were combined and the tumour cells in a diluted sample were counted as described above.

Measurement of purine-phosphoribosyltransferase activities. Assays were carried out essentially as described by Atkinson & Murray (1965a). Solutions containing 0.1 μ mole of PRPP, 2 μ moles of MgCl₂ and 20 μ moles of tris, adjusted to pH 7.8 with HCl, were mixed with the tissue extract (in a volume of 0.025–0.1 ml. and containing 0.2–1.5 mg. of protein). After 4 min. at 25° the reaction was started by the addition of 0.1 ml. of [8-¹⁴C]adenine or [8-¹⁴C]hypoxanthine (specific activities 2.72 and 0.91 μ C/ μ mole respectively). The concentrations of adenine and hypoxanthine were 61 μ M and 138 μ M respectively in a final volume of 0.4 ml. Nucleotide formation was measured after adsorption on to DEAE-cellulose disks as described by Atkinson & Murray (1965a). The radioactivity associated with the disks was determined by direct immersion in a scintillation fluid containing 6 g. of 2,5-diphenyloxazole and 0.1 g. of 1,4-bis-(5-phenyloxazol-2-yl)benzene in 1 l. of ethanol-toluene (1:2, v/v) and counting with automatic Nuclear-Chicago liquid-scintillation equipment.

Duplicate assays were carried out on each tissue extract at two time-intervals (2 min. and 4 min.) both in the presence and absence of PRPP.

Analysis of products formed in the presence of the purine-phosphoribosyltransferase preparation. (a) Tests for cleavage of AMP, IMP and GMP. Assays were carried out as described for the measurement of phosphoribosyltransferase activity, but with 4 μ moles of either AMP, IMP or GMP replacing PRPP in the reaction mixture. After 4 min. incubation at 25°, 0.05 ml. samples were transferred to Whatman 3MM chromatography paper and chromatographed in 80% (v/v) formic acid–butan-1-ol–propan-2-ol–acetone–30% (w/v) trichloroacetic acid (25:40:20:25:12, by vol.) for 6 hr. (Gerlach, Dreisbach & Deuticke, 1965). Compounds were detected on the paper by viewing under ultraviolet light. Standard AMP (R_F 0.40), IMP (R_F 0.26), GMP (R_F 0.26), adenosine (R_F 0.60), inosine (R_F 0.38), guanosine (R_F 0.40), adenine (R_F 0.78), hypoxanthine (R_F 0.55) and guanine (R_F 0.46) were chromatographed as marker compounds.

(b) Identification of products formed from radioactive purines. To analyse the products formed with adenine or hypoxanthine and PRPP the mixture was chromatographed as described by Atkinson & Murray (1965a). When guanine was used the products were chromatographed in the formic acid–butanol–propanol–acetone–trichloroacetic acid solvent described above. In this, xanthine had R_F 0.38 and uric acid R_F 0.20.

RESULTS

Validity of purine-phosphoribosyltransferase assays. Cell-free extracts from Ehrlich ascites-tumour cells, rat bone marrow and rat or mouse liver, brain, spleen, heart or kidney were incubated with 10mM-AMP, -IMP or -GMP as described in the Experimental section. No corresponding nucleotides or bases could be detected under ultraviolet light after chromatography, indicating the absence of significant activities of 5'-nucleotidases or other nucleotide-degrading enzymes in the extracts studied. Similarly when assay mixtures containing soluble tissue extracts, PRPP and either [8-¹⁴C]-adenine or [8-¹⁴C]hypoxanthine were incubated at 25° for 4 min. and chromatographed as described by Atkinson & Murray (1965a), the radioactivity not associated with residual purine base was found to be mainly associated with AMP or IMP respectively. With each tissue extract the activity associated with adenosine or inosine was less than 5% of that associated with AMP.

Similar assays carried out with [8-¹⁴C]guanine indicated that all tissue extracts except those from ascites-tumour cells contained high levels of guanase activity, as more than 50% of the radioactivity was associated with xanthine after the incubation. With extracts of the ascites-tumour cells less than 6% of the guanine was converted into xanthine, in agreement with previous observations (Atkinson & Murray, 1965a). As in all extracts the radioactivity associated with uric acid was less than 3%, it was concluded that xanthine oxidase did not significantly interfere with the assays. After adsorption of the products on to DEAE-cellulose disks xanthine could not be reproducibly eluted with ammonium hydrogen carbonate gradients without eluting some nucleotide. For these reasons it was

decided not to assay the activity of guanine phosphoribosyltransferase but to confine the work to a study of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase.

With each tissue extract the rate of nucleotide formation in the absence of PRPP was always less than 6% of the rate in the presence of this compound; the rates given have been corrected for this control activity. The small control rates observed were presumably due to PRPP extracted from the tissues. In agreement with the results of Henderson & Khoo (1965), I found no evidence of nucleotide formation by extracts from ascites-tumour cells in the absence of PRPP.

The above results indicate that assays carried out as described with extracts from each of the tissues examined gave a true measure of adenine- and hypoxanthine-phosphoribosyltransferase activities.

Adenine- and hypoxanthine-phosphoribosyltransferase activities in rat and mouse tissues. Values for the phosphoribosyltransferase activities in various mouse tissue extracts are shown in Table 1 and in rat tissue extracts in Table 2. Table 1 also lists the activities found in extracts of Ehrlich ascites-tumour cells 10 days after inoculation. Extracts of the packed erythrocytes of mouse or rat blood contained no hypoxanthine phosphoribosyltransferase and only traces of adenine-phosphoribosyltransferase activity. Negligible activity of both enzymes was detected in mouse or rat plasma. The activity of adenine phosphoribosyltransferase in tumour cells (3mμmoles of AMP formed/min./mg. of protein) was about 15 times the activity found in mouse liver, spleen and kidney, about 37 times the activity in mouse brain and 60 times the activity in mouse heart. Hypoxanthine-phosphoribosyltransferase activity in ascites-tumour cells (0.98mμmole of IMP formed/min./mg. of protein) was higher

Table 1. *Purine-phosphoribosyltransferase activities in mouse tissue extracts*

Values represent the means ± s.e.m. of determinations carried out on six animals. [8-¹⁴C]Purine bases were used in the assays of phosphoribosyltransferase activities. Details of the assays are given in the Experimental section.

Tissue	Purine nucleotide formed				Hypoxanthine formed Adenine formed
	(mμmoles/min./mg. of protein)		(mμmoles/min./g. of tissue)		
	Adenine	Hypoxanthine	Adenine	Hypoxanthine	
Liver	0.15 ± 0.014	0.25 ± 0.056	26 ± 2.8	43 ± 4	1.7
Brain	0.08 ± 0.012	0.95 ± 0.076	4.2 ± 0.3	49 ± 3	11.7
Spleen	0.18 ± 0.016	0.53 ± 0.060	30 ± 1.2	85 ± 5	2.9
Heart	0.05 ± 0.005	0.13 ± 0.036	7.4 ± 0.5	20 ± 2.5	2.7
Kidneys	0.14 ± 0.013	0.20 ± 0.026	16 ± 1.2	22 ± 2.6	1.4
Ascites-tumour cells (10 days)	3.00 ± 0.14	0.98 ± 0.076	106 ± 6*	42 ± 2*	0.39

* Activities expressed as mμmoles of nucleotide formed/min./g. of packed tumour cells.

Table 2. *Purine-phosphoribosyltransferase activities in rat tissue extracts*

Values represent the means $\pm$ S.E.M. of determinations carried out on six female rats (70–80 days old). [8-¹⁴C]-Purine bases were used in the assays of phosphoribosyltransferase activities. Details of the assays are given in the Experimental section.

		Purine nucleotide formed				
		(mμmoles/min./mg. of protein)		(mμmoles/min./g. of tissue)		Hypoxanthine formed
Tissue	Adenine	Hypoxanthine	Adenine	Hypoxanthine	Adenine formed	
Liver	0.38 ± 0.03	0.44 ± 0.04	57 ± 2.7	77 ± 6.3	1.16	
Brain	0.30 ± 0.04	0.52 ± 0.08	15.7 ± 1.5	28 ± 3.8	1.74	
Spleen	1.31 ± 0.14	0.47 ± 0.07	178 ± 7.3	64 ± 8.7	0.36	
Heart	0.55 ± 0.04	0.57 ± 0.08	65 ± 2.6	78 ± 11	1.04	
Kidneys	0.46 ± 0.04	0.36 ± 0.02	45 ± 4.9	36 ± 3.4	0.78	
Bone marrow	0.45 ± 0.11	0.41 ± 0.07	154 ± 27	140 ± 41	0.91	

than that found in any mouse tissue but the ratio was not as great as for adenine phosphoribosyltransferase. High adenine- and hypoxanthine-phosphoribosyltransferase activities have been reported previously in rapidly dividing mammalian tissues (Lieberman & Ove, 1960; Brockman, Kelley, Stutts & Copeland, 1961). Similar determinations were carried out with tissues taken from mice containing ascites-tumour cells after 10 days of tumour growth. The results obtained did not differ significantly from those given in Table 1. Thus the presence of the tumour has no effect on the enzyme activities of other tissues, at least at the stage of tumour growth examined. In mouse liver, kidney, spleen and heart the activity ratio of hypoxanthine phosphoribosyltransferase to adenine phosphoribosyltransferase varied from 1.4 to 2.9 and in brain the ratio was 11.9. However, in ascites-tumour cells adenine-phosphoribosyltransferase activity was considerably higher than that of hypoxanthine phosphoribosyltransferase and the ratio was 0.39. In general the specific activities of both phosphoribosyltransferases in extracts of rat tissue were higher than those of the corresponding tissues of mice. In addition, the activity ratios of hypoxanthine phosphoribosyltransferase to adenine phosphoribosyltransferase were lower.

Specific activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase in rat bone marrow were of the same order as those of other rat tissues. However, bone marrow contained a high proportion of soluble protein and when expressed on a wet-weight basis the activities were higher than those of all other rat tissues except for spleen adenine phosphoribosyltransferase. The activities of both enzymes in rat bone marrow showed considerable variation between animals.

Adenine- and hypoxanthine-phosphoribosyltransferase activities during the growth of Ehrlich ascites-tumour cells. Female Swiss mice were inoculated

with about 10^8 ascites-tumour cells (taken from mice after 10 days of tumour growth) and adenine- and hypoxanthine-phosphoribosyltransferase activities were measured on cell extracts at intervals of 2 days. Plots of the specific activities of the two phosphoribosyltransferases and of the ratio of their activities against time are shown in Fig. 1. The changes in total numbers of ascites-tumour cells and of enzyme activities/ 10^6 tumour cells are shown in Fig. 2.

During growth of the tumour cells the activity of both enzymes/mg. of protein decreased at first and then increased. Two days after inoculation the activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase had decreased to 57% and 36% respectively of their activities at inoculation. The activities decreased slightly more in the next 2 days of growth and then rose rapidly, reaching a maximum after 10 days of growth. Substantially the same results were obtained when the activities/ 10^6 tumour cells were plotted against time (Fig. 2). There is not a direct relationship between enzyme activity and rate of cell division as cell growth was rapid after 4 days but the enzyme activities showed no increase at this time.

Considered as a proportion of the original levels the activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase did not parallel each other during the growth period. Initially the activity of hypoxanthine phosphoribosyltransferase decreased more rapidly than that of adenine phosphoribosyltransferase. The specific activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase in the tumour cells after 7 days' growth (2.3 and 0.6 m μ -moles of nucleotide/min./mg. of protein) were similar to those previously obtained on tumour cells of this age (Atkinson & Murray, 1965a) with extracts that had been dialysed for 24 hr. against 10 mM-tris-chloride buffer, pH 7.8, before the

Fig. 1. Changes during growth in the specific activities of adenine phosphoribosyltransferase (○) and hypoxanthine phosphoribosyltransferase (●) in cell-free extracts of Ehrlich ascites-tumour cells. At zero time mice were injected with about 10^8 tumour cells. Preparation of the extracts and measurements of transferase activity were carried out as described in the Experimental section. The ratios of the specific activities of adenine phosphoribosyltransferase at each time-interval are also plotted (△). Values represent the means $\pm$ S.E.M. of separate determinations on tumour cells isolated from four mice.

Fig. 2. Activities/ 10^6 tumour cells of adenine phosphoribosyltransferase (○) and hypoxanthine phosphoribosyltransferase (●) during the growth of Ehrlich ascites-tumour cells. The mean number of tumour cells present at intervals during the experimental period are also plotted (△); for details of the procedure for determination of total tumour cells per mouse see the Experimental section. These results were derived from the same experiment shown in Fig. 1, and the values represent the means $\pm$ S.E.M. of separate determinations on tumour cells isolated from four mice.

assays were carried out (1.7 and 0.66 $m\mu$ moles of nucleotide formed/min./mg. of protein respectively).

Adenine- and hypoxanthine-phosphoribosyltransferase activities during rat-liver regeneration. The activities of both phosphoribosyltransferases at intervals after partial hepatectomy are shown in Fig. 3. Preliminary experiments showed that operations on rats in which no actual hepatectomy was carried out did not significantly alter the enzyme activities of liver extracts. Adenine-phosphoribosyltransferase activity had begun to rise rapidly 24 hr. after surgery, but hypoxanthine-phosphoribosyltransferase activity did not increase until the second day after partial hepatectomy. The activity of hypoxanthine phosphoribosyltransferase had begun to decrease rapidly by the fourth day whereas the activity of adenine phosphoribosyltransferase was still increasing at that stage. The activities of both enzymes were still significantly higher than their control levels 14 days after operation. Enzyme activities determined on a group of control rats at the end of the experimental

period did not differ significantly from the control values found at zero time. The same trends were apparent when the enzyme activities were expressed as nucleotide formed per unit wet wt. or per unit dry wt.

DISCUSSION

It is clear that each of the rat and mouse tissue extracts examined contained considerably lower specific activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase than extracts from Ehrlich ascites-tumour cells. The specific activity of the former enzyme in ascites-tumour cells (10 days after inoculation) was between 15- and 60-fold greater than that of any mouse tissue studied. The difference between hypoxanthine-phosphoribosyltransferase activity in extracts of ascites-tumour cells and that in other rat or mouse tissues was not so great. The differences were not so marked when the phosphoribosyltransferase activities/g. wet wt. of mouse tissues are compared with activities/ml. of ascites-tumour cells. One ml. of packed tumour cells weighs approx.

Fig. 3. Activities/mg. of protein of adenine phosphoribosyltransferase (○) and hypoxanthine phosphoribosyltransferase (●) during rat-liver regeneration. The points at X and Y represent the mean activities of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase respectively in extracts obtained from control animals at the end of the experimental period. Preparation of the extracts and measurements of phosphoribosyltransferase activities were carried out as described in the Experimental section. The values represent the means $\pm$ S.E.M. of separate determinations on four animals.

1g. but a proportion of this weight would be contributed by extracellular fluid surrounding the packed cells. Of the tissues examined extracts of those with the most rapid cell division (rat and mouse spleen and rat bone marrow; see Lajtha, 1959; Leblond, 1959) contained the highest phosphoribosyltransferase activities. The high phosphoribosyltransferase activities found in rat bone marrow are consistent with the reported dependence of rabbit bone marrow on preformed purines (Lajtha & Vane, 1958). Aktinson & Murray (1965a,b) showed that the anti-tumour drug 6-mercaptopurine inhibited hypoxanthine phosphoribosyltransferase and guanine phosphoribosyltransferase from Ehrlich ascites-tumour cells, and it is possible that these inhibitions may explain the toxic effects observed on the bone marrow of experimental animals and of human patients after administration of this drug (see Galton, 1957).

The physiological significance of high activities of purine phosphoribosyltransferases in rapidly growing tissues could be either to conserve extracellular purines derived from other organs or to conserve intracellular purines formed by nucleotide degradation. The latter possibility implies that rapidly growing tissues have higher activities of enzymes catalysing nucleotide degradation, as free purine bases or nucleosides do not accumulate to any extent in most animal tissues (see Baddiley, 1955), including Ehrlich ascites-tumour cells (A. W.

Murray, unpublished work). In view of the low capacity of Ehrlich ascites-tumour cells for new purine biosynthesis (Smellie *et al.* 1956) it is more logical to postulate that these cells at least are making use of preformed purines obtained from other tissues in the host animal. If this is also true in regenerating rat liver then other organs besides the liver must be capable of providing intact purine bases for nucleotide biosynthesis.

The changes in activity of adenine phosphoribosyltransferase and hypoxanthine phosphoribosyltransferase during the growth of ascites-tumour cells and during rat-liver regeneration varied considerably in relation to each other. In ascites-tumour cells the activity of the latter enzyme initially decreased rapidly relative to that of adenine phosphoribosyltransferase; this was followed by a steady increase in the activity ratio of hypoxanthine phosphoribosyltransferase to adenine phosphoribosyltransferase between 4 and 10 days after inoculation. In regenerating rat liver the activity of adenine phosphoribosyltransferase began to increase about 24 hr. before that of hypoxanthine phosphoribosyltransferase. In addition, the activity of the latter enzyme had started to decrease after 4 days' regeneration whereas the activity of the former enzyme was still increasing after that time. It may be that there are separate mechanisms controlling the activity of each enzyme. There is no way of deciding from the results of this study if the increases in adenine- and hypoxanthine-phosphoribosyltransferase activities are due to increased protein synthesis. Bresnik (1965) concluded that the increase in activities of various enzymes involved in the new biosynthesis of pyrimidine nucleotides during rat-liver regeneration was brought about by a means not immediately involving an increase in enzyme synthesis. The increase in the activities of enzymes involved in pyrimidine nucleotide metabolism occurring after partial hepatectomy has been well documented (for recent references see Bresnick, 1965). Presumably biosynthesis of purine nucleotides also increases during regeneration and the increases observed in adenine- and hypoxanthine-phosphoribosyltransferase activities suggest a physiological importance of these enzymes in tissues undergoing rapid growth.

The high levels of adenine-phosphoribosyltransferase activity found in Ehrlich ascites-tumour cells in this study and reported in human leukaemic leucocytes (Davidson & Winter, 1964) suggest that more emphasis should be placed on the designing of effective inhibitors of this enzyme. If the problem of resistance could be overcome adenine phosphoribosyltransferase should represent a logical target for anti-tumour drugs. The widely used anti-tumour compound 6-mercaptopurine inhibits hypoxanthine phosphoribosyltransferase and guanine

phosphoribosyltransferase but not adenine phosphoribosyltransferase (Atkinson & Murray, 1965*a,b*).

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