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METABOLIC TRANSFORMATIONS OF 5-AZAURACIL AND 5-AZAOROTIC ACID IN MOUSE LIVER AND *ESCHERICHIA COLI*

EFFECT ON THE SYNTHESIS OF PYRIMIDINES

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Abstract—5-Azauracil undergoes, in the cell-free extract of *Escherichia coli*, metabolic transformations giving rise to 5-azauridine and 5-azauridine 5′-phosphate. Analogous transformations of 5-azauracil have not been observed in the liver extract. Uridine phosphorylase, however, is inhibited by 5-azauracil both in bacterial and liver cell-free systems. The inhibition of uridine synthesis appeared to be of a competitive character, whereas uridine phosphorolysis appeared to be noncompetitive. 5-Azaorotic acid is transformed in the liver to 5-azaorotidine 5′-phosphate, which gives rise to 5-azauridine 5′-phosphate. The same reaction takes place in *E. coli*. In the presence of 5-azaorotic acid, the total anabolic conversion of orotic acid is strongly decreased both in liver and in bacterial extracts. After the application of 5-azaorotic acid to mice, the utilization of orotic acid for the synthesis of liver nucleic acids is decreased and is paralleled by an increased orotic aciduria. 5-Azauracil affects the anabolic transformation of orotic acid to a considerably lesser degree.

5-Azauracil and 5-azaorotic acid were tested as possible carcinostatics for the treatment of liver and kidney tumors.[1–3] 5-Azaorotic acid is also formed in the organism by oxidative degradation of uric acid.[4] The inhibitory effects of 5-azaorotic acid[5,6] have been utilized for the investigation of hereditary orotic aciduria,[7] a rare recessive disorder associated with a deficiency of orotate phosphoribosyltransferase and orotidylic acid decarboxylase.[8,9] During the past few years, 5-azaorotic acid has also been used in experimental studies on hyperuricosuria[10] because of its inhibitory effect on uricase.[11]

Both 5-azauracil and 5-azaorotic acid are subjected to metabolic transformations in the organism which result in the formation of new metabolites.[12,13] The biological effect depends on the degree of conversion of the applied compound, which is different in various target tissues. In this study the effect of 5-azauracil and 5-azaorotic acid in an animal and a bacterial system was investigated.

MATERIAL AND METHODS

Biological material

The cultivation of *Escherichia coli* was carried out on a synthetic medium containing glucose[12] at 37° for 16 hr. For the preparation of the cell-free extract, the bacteria were harvested (5000 *g*, 45 min, 2°) and washed with 0·9% NaCl. For the experiments,

female mice (strain H, weight 24–25 g) kept under standard conditions were used. The compounds or physiological saline (maximal volume, 0·4 ml) were administered intraperitoneally.

Chemicals

Adenosine 5′-triphosphate, disodium, pentahydrate, uridine, uridine 5′-monophosphate, disodium, trihydrate, uridine 5′-triphosphate, disodium, hexahydrate, orotic acid, orotidine, cyclohexylammonium salt, orotidine 5′-monophosphate, trisodium, trihydrate, α-D-ribose 1-phosphate, di-(cyclohexylammonium) salt, and 5-phosphorylribose 1-pyrophosphate, dimagnesium salt, were obtained from Calbiochem, Los Angeles. Reduced glutathione was a product of Lachema, Brno. 5-Azauracil and potassium 5-azaorotate were prepared[14] by Dr. A. Píškala of this Institute. Uracil-2-[^{14}C] (10 μc/μmole), uridine-U-^{14}C (24 μc/μmole), orotic-6-[^{14}C] acid (2·3 μc/μmole) and 5-azauracil-2,4-[^{14}C] (2·5 μc/μmole) were prepared at the Institute for Research, Production and Uses of Radioisotopes in Prague. 5-Azaorotate-2,4-[^{14}C] (0·86 μc/μmole) was synthesized[14] by Dr. J. Morávek and orotidine-6-[^{14}C] 5′-phosphate (2·3 μc/μmole) was prepared from labeled orotic acid as described earlier.[13]

Preparation of cell-free extracts

Liver extract. The animals were killed by cervical dislocation and the excised liver was homogenized under cooling in a Potter–Elvehjem homogenizer with 4 vol. of ice-cold 0·15 M KCl. The homogenate was centrifuged (5000 *g*, 20 min, 2°) and the supernatant fraction was used as a source of enzyme activity.

E. coli extract. The washed bacteria were suspended in 0·1 M tris–HCl buffer (pH 7·4) and disintegrated (MSE sonic oscillator, 1·2 kc, 2 min, 2°). The sonicate was centrifuged (5000 *g*, 20 min, 2°) and stored at −20° for 2 weeks without any loss of enzyme activity. The protein content was determined by the method of Lowry *et al.*[15]

Assay of enzyme activity

Uridine phosphorylase. Incubation was carried out in 2×10^{-2} M tris–HCl or $1{\cdot}5 \times 10^{-2}$ M phosphate buffer (pH 7·4) at 37° in a total volume of 1 ml after addition of 0·2 ml of enzyme fraction. Synthesis of uridine: 2×10^{-4} M uracil-2-[^{14}C] and 10^{-3} M ribose 1-phosphate with equimolar Mg^{2+} ions; phosphorolysis of uridine: 2×10^{-4} M uridine-U-[^{14}C]. Analysis of aliquots of the incubation mixture was performed chromatographically on Whatman No. 1 paper in a solvent system composed of 1-butanol–acetic acid–water (10:1:3).

Orotate phosphoribosyltransferase. Incubation was carried out in 2×10^{-2} M tris–HCl buffer (pH 7·4) at 37° with 2×10^{-4} M orotic-6-[^{14}C] acid and 8×10^{-4} M 5-phosphorylribose 1-pyrophosphate with equimolar Mg^{2+} ions. The analysis of the incubation mixture was performed chromatographically on Whatman No. 1 paper in a solvent system composed of isobutyric acid–ammonium hydroxide–water (66:1·5:33), after drying in isopropyl alcohol–ammonium hydroxide–water (7:2:1).

Orotidylic acid decarboxylase. The conditions of incubation and analysis of the reaction mixture were the same as described above, except that the 5-min incubation was carried out at 30°. Orotidine-6-[^{14}C] 5′-phosphate, 2×10^{-5} M, equimolar Mg^{+} ions and 2×10^{-4} M reduced glutathione were used. Products were located on the chroma-

tograms with respect to standards and by a radioactivity scanner. The radioactivity was measured with a Packard liquid scintillation spectrometer.

Utilization of orotic acid for RNA synthesis in liver

The incorporation was studied in a group of four to six mice. Orotic-6-[^{14}C] acid was applied intraperitoneally (1 μc/μmole/mouse) simultaneously with 5-azauracil or 5-azaorotic acid. The animals were killed after 2 hr by decapitation, bled, and each individual liver was homogenized with 4 vol. of 0·2 M $HClO_4$ under cooling. The sediments remaining after the subsequent 3-fold washing to remove the acid-soluble pool were subjected to alkaline hydrolysis (1 N KOH, 20°, 18 hr). Uridine 2′ (3′)-phosphate and cytidine 2′ (3′)-phosphate were isolated and purified as described elsewhere.[16] The incorporation of orotic acid was expressed as a specific radioactivity of both nucleotides in counts per minute per micromole.

Urinary excretion of orotic acid and orotidine in mice

Analogs were applied intraperitoneally between 8 and 9 a.m. and the animals were starved for 24 hr (with water *ad lib.*) in metabolic cages. The urine was collected on a strip of Whatman No. 3 paper and the level of orotic acid and orotidine was determined after chromatographic analysis as described.[16]

RESULTS

Metabolic transformations of 5-azauracil and 5-azaorotic acid

In earlier studies, we have observed that 5-azauracil is used as a substrate by bacterial phosphorylase and phosphoribosyltransferase.[12] A comparison of uracil and

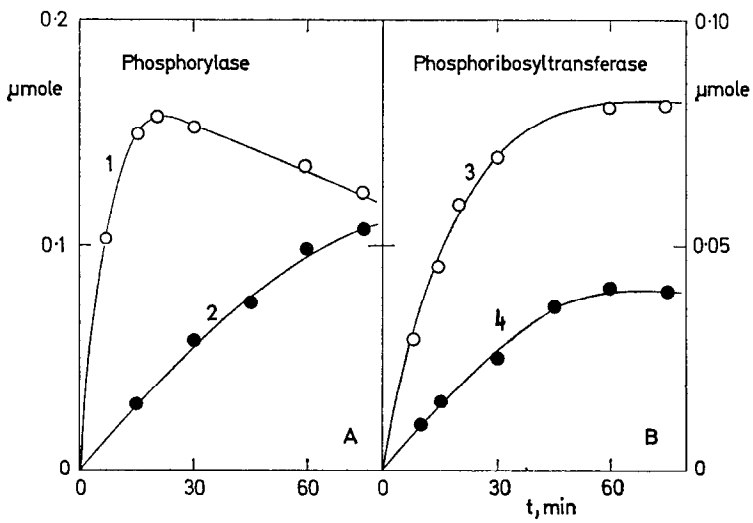

FIG. 1. Phosphorylase and phosphoribosyltransferase activity of cell-free extract of *E. coli* with uracil and 5-azauracil as substrates. 2 × 10^{-4} M uracil-2-[^{14}C] and 5-azauracil-2,4-[^{14}C] were incubated at 37° in 2 × 10^{-2} M tris–HCl buffer (pH 7·4) with 10^{-3} M ribose 1-phosphate or 10^{-3} M 5-phosphoryl-ribose 1-pyrophosphate, equimolar Mg^{2+} ions, adenosine 5′-triphosphate, and reduced glutathione. *E. coli* extract (0·2 ml) was added to a total volume of 1 ml. Duration of incubation in minutes, t; (a) uridine, 1; 5-azauridine (and its degradation products), 2; (b) uridine 5′-tri, -di and monophosphate, 3; and 5-azauridine 5′-phosphate, 4.

5-azauracil for the two enzymes in a cell-free extract of *E. coli* is shown in Fig. 1. The amount of reacted uracil is in both cases higher than the amount of reacted analog. While uridine is simultaneously degraded to uracil and its level in the incubation mixture decreases, the amount of reacted 5-azauracil constantly increases. An analogous formation of ribosylated derivatives of 5-azauracil has not been observed either in the liver extract during incubation *in vitro* or in the liver acid-soluble pool after the application of 5- azauracil-2,4-[^{14}C] to mice.

TABLE 1. TRANSFORMATION OF DIFFERENT PYRIMIDINES IN CELL-FREE EXTRACTS OF *E. coli* AND MOUSE LIVER*

Substrate ($2{\cdot}5 \times 10^{-4}$ M)	Reacted substrate (μmoles)			
	Ribose 1-phosphate		5-Phosphorylribose 1-pyrophosphate	
	E. coli	Liver	*E. coli*	Liver
Uracil-2-[^{14}C]	0·194	0·041	0·067	0
5-Azauracil-2,4-[^{14}C]	0·063	0	0·024	0
Orotic-6-[^{14}C] acid	0	0	0·059	0·112
5-Azaorotic-2,4-[^{14}C] acid	0	0	0·062	0·128

* Ribose 1-phosphate, 1×10^{-3} M, with equimolar Mg^{2+} ions or 1×10^{-3} M 5-phosphorylribose 1-pyrophosphate with Mg^{2+} ions, and reduced glutathione were incubated with $2{\cdot}5 \times 10^{-4}$ M substrate. The incubation was carried out for 30 min at 37° in a total volume of 1 ml with an *E. coli* extract (1·4 mg protein) or an extract of mouse liver (2·2 mg protein) in 2×10^{-2} M tris–HCl buffer, pH 7·4.

The comparison of uracil with 5-azauracil as substrates for liver and bacterial uridine phosphorylase and uracil phosphoribosyltransferase is shown in Table 1. (The metabolic transformations lower than 3% were considered as zero values.) The synthesis of uridine from uracil in the liver extract is approximately five times lower; 5-azauridine is not formed at all. Uridine 5′-phosphate and 5-azauridine 5′-phosphate are formed directly in bacterial extract; in the liver, the synthesis of both nucleotides from a corresponding base has not been demonstrated.

Orotic and 5-azaorotic acid react with 5-phosphorylribose 1-pyrophosphate; the synthesis of corresponding 5′-phosphates in liver is higher than in bacteria (Table 1).

The time course of the reaction of 5-azaorotic acid with 5-phosphorylribose 1-pyrophosphate in the cell-free liver extract is demonstrated in Fig. 2. The newly formed 5-azaorotidine 5′-phosphate is decarboxylated[17] and 5-azauridine 5′-phosphate appears in the incubation mixture. The formation of higher phosphates of 5-azauridine has not been observed either during incubation *in vitro* or in the liver acid-soluble pool after application of 5-azaorotic-2,4-[^{14}C] acid to mice *in vivo*.

Mechanism of inhibitory action

In our previous studies, we followed the effect of 5-azauracil on the phosphorolysis of uridine.[18] Figure 3 shows the time course of uridine synthesis in the presence of liver uridine phosphorylase. The reaction is markedly inhibited by 5-azauracil but not by 5-azaorotic acid. The inhibition by 5-azauracil is competitive and is charac-

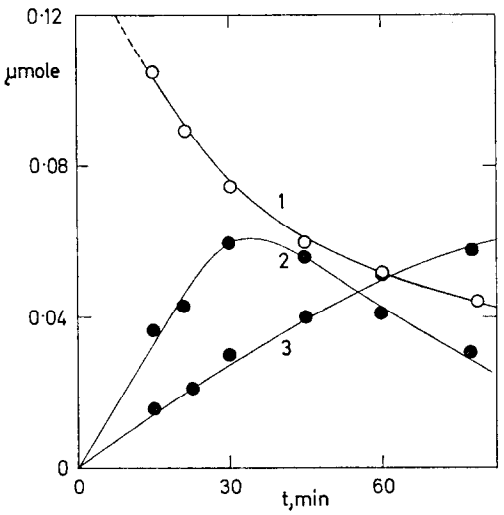

FIG. 2. Anabolic conversion of 5-azaorotic acid in a cell-free liver extract. 5-Azaorotic-2,4-[^{14}C] acid, $1{\cdot}5 \times 10^{-4}$ M, was incubated at 37° in 2×10^{-2} M tris–HCl buffer (pH 7·4) with 8×10^{-4} M 5-phosphorylribose 1-pyrophosphate and equimolar Mg^{2+} ions. Liver extract (0·2 ml) was added to a total volume of 1 ml. Duration of incubation in minutes, t; non-reacted 5-azaorotic acid, 1; newly synthetized 5-azaorotidine 5′-phosphate, 2; and 5-azauridine 5′-phosphate, 3.

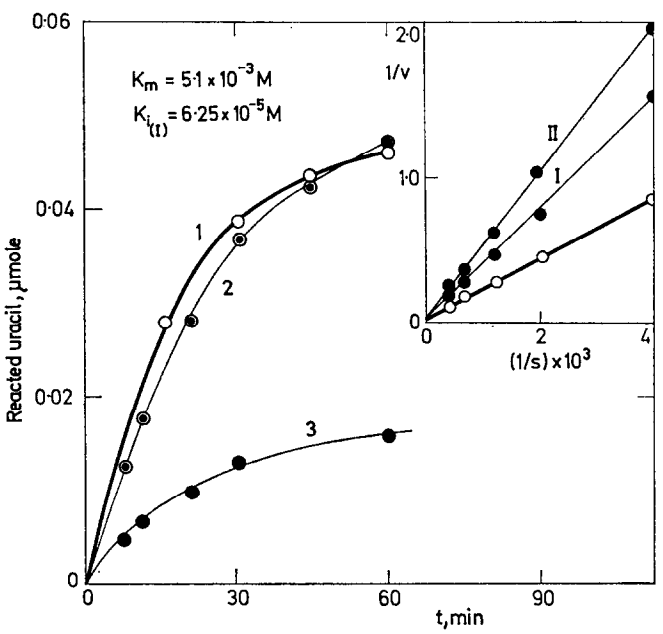

FIG. 3. Inhibition of uridine synthesis by 5-azauracil in cell-free liver extract. Uracil-2-[^{14}C], 2×10^{-4} M, was incubated at 37° with 8×10^{-4} M ribose 1-phosphate and equimolar Mg^{2+} ions in 0·1 M tris–HCl buffer (pH 7·4). Cell-free liver extract (0·2 ml; 4·6 mg protein) was added to a total volume of 1 ml. Control, 1; 10^{-3} M 5-azaorotic acid, 2; 10^{-3} M 5-azauracil, 3. For the determination of the K_i constant [5-azauracil, 5×10^{-4} M (I), and 10^{-3} M (II)] the incubation was carried out at a constant ratio of ribose 1-phosphate to uracil of 1:4 for 15 min. Concentration of uracil in moles per liter, s; amount of uridine formed in moles per liter per minute, v.

terized by a high K_m/K_i ratio (81·6). The same character of inhibition of uridine synthesis was observed with an extract of *E. coli*. Although 5-azauracil is not utilized in the liver as a substrate of uridine phosphorylase (Table 1), it inhibits markedly the enzyme in both systems. A comparison of the effect of 5-azauracil in an extract of *E. coli* and in the liver (Table 2) shows that the inhibition of uridine phosphorylase is practically the same in both cases.

TABLE 2. EFFECT OF 5-AZAURACIL AND 5-AZAOROTIC ACID ON METABOLIC TRANSFORMATIONS OF URACIL*

Analogs (1×10^{-3} M)	*E. coli*		Liver	
	(μmoles)	(%)	(μmoles)	(%)
Uridine phosphorylase				
Control	0·156	100	0·037	100
5-Azauracil	0·078	50·0	0·017	46·2
5-Azaorotic acid	0·152	100	0·038	100
Uracil phosphoribosyltransferase				
Control	0·127	100	0	0
5-Azauracil	0·096	75·6	0	0
5-Azaorotic acid	0·073	57·5	0	0

* Ribose 1-phosphate, 8×10^{-4} M, with equimolar Mg^{2+} ions or 8×10^{-4} M 5-phosphorylribose 1-pyrophosphate with Mg^{2+} ions was incubated with 2×10^{-4} M uracil-2-[^{14}C] at 37° in a total volume of 1 ml *E. coli* with extract (1·92 mg protein) or a cell-free extract of mouse liver (2·05 mg protein). The incubation was carried out for 30 and 60 min, respectively, to measure uridine phosphorylase (in 2×10^{-2} M tris–HCl buffer, pH 7·4) and uracil phosphoribosyltransferase (in $1·5 \times 10^{-2}$ M phosphate buffer, pH 7·4) respectively. The activity of enzymes is expressed as micromoles of reacted uracil.

In the cell-free extract of *E. coli*, 5-azaorotic acid decreases the formation of uridine 5′-phosphate from uracil and 5-phosphorylribose 1-pyrophosphate.[6] According to our unpublished data, this inhibition is due to the competition of 5-azaorotic acid and uracil for 5-phosphorylribose 1-pyrophosphate, and the reaction involves both uracil and orotate phosphoribosyltransferase activities. Also, 5-azauracil affects (Table 2) the direct formation of uridine 5′-phosphate in the same system. In this case, however, there is a competition for 5-phosphorylribose 1-pyrophosphate only in the presence of uracil phosphoribosyltransferase, which catalyzes the formation of 5-azauridine 5′-phosphate.[12] Neither 5-azauracil nor 5-azaorotic acid affects the degradation of the arising uridine 5′-phosphate. The activity of uracil phosphoribosyltransferase in the liver was not observed.

5-Azaorotic acid has a pronounced effect on the synthesis of pyrimidines *de novo* by inhibiting orotate phosphoribosyltransferase and orotidylic acid decarboxylase.[5,13] A comparison of its effects with those of 5-azauracil on the anabolic transformation of orotic acid in bacterial and liver cell-free extracts is given in Table 3. The effect of 5-azauracil is less considerable than that of 5-azaorotic acid. The time course of the anabolic transformation of orotic acid in the liver extract and the effect of both compounds is shown in Fig. 4. The inhibition of the total anabolic conversion of orotic acid in the presence of 5-azaorotic acid is of a noncompetitive character with a ratio K_m/K_i of 56·5. The data of Rubin *et al.*[19] are similar, except that they have been interpreted as a competitive inhibition.

TABLE 3. ANABOLIC TRANSFORMATION OF OROTIC ACID IN CELL-FREE EXTRACTS OF *E. coli* AND MOUSE LIVER IN THE PRESENCE OF 5-AZAURACIL AND 5-AZAOROTIC ACID*

Analogs (1×10^{-3} M)	OMP† (counts/min)	UMP (counts/min)	UR + U (counts/min)	Conversion (%)	Inhibition (%)
E. coli extract					
Control	12,430	80,630	7150	91·0	0
5-Azauracil	12,040	66,240	5870	74·3	18·4
5-Azaorotic acid	5370	32,470	2825	37·4	58·9
Liver extract					
Control	1356	6230	32,050	44·0	0
5-Azauracil	1820	6390	23,130	34·3	22·1
5-Azaorotic acid	3985	4450	760	10·5	76·1

* Orotic-6-[^{14}C] acid, 2×10^{-4}M, was incubated for 30 min with 8×10^{-4} M 5-phosphorylribose 1-pyrophosphate, equimolar Mg^{2+} ions and reduced glutathione. The incubation was carried out at 37° with the cell-free liver extract (2·35 mg protein, 2×10^{-2} M tris–HCl buffer, pH 7·4) or with the *E. coli* extract (2·85 mg protein, $1{\cdot}5 \times 10^{-2}$ M phosphate buffer, pH 7·4) respectively; total volume 1 ml.

† Abbreviations used: OMP, orotidine 5′-phosphate; UMP, uridine 5′-monophosphate; UR, uridine; U, uracil.

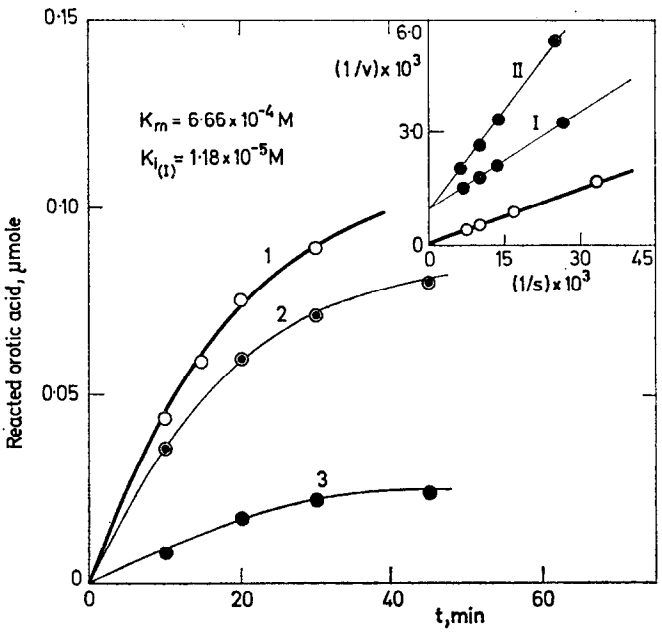

FIG. 4. Inhibition of metabolic conversion of orotic acid in liver cell-free extract by 5-azaorotic acid. Orotic-6-[^{14}C] acid, 2×10^{-4} M, was incubated at 37° with 8×10^{-4} M 5-phosphorylribose 1-pyrophosphate, Mg^{2+} ions and reduced glutathione in 0·05 M tris–HCl buffer (pH 7·4). Liver extract (0·2 ml; 6·44 mg protein) was added to a total volume of 1 ml. Control, 1; 10^{-3} M 5-azauracil, 2; 10^{-3} M 5-azaorotic acid, 3. For the determination of the K_i constant [5-azaorotic acid 10^{-4} M (I), and 2×10^{-4} M (II)] the incubation was carried out at a constant concentration of 8×10^{-4} M 5-phosphorylribose 1-pyrophosphate for 20 min. Concentration of orotic acid in moles per liter, s; reacted orotic acid, i.e. arising orotidine 5′-phosphate, uridine 5′-mono, di-, and triphosphates, uridine and uracil in moles per liter per minute, v.

TABLE 4. UTILIZATION OF OROTIC ACID FOR THE SYNTHESIS OF LIVER RNA AND OROTIC ACIDURIA AFTER APPLICATION OF 5-AZAURACIL AND 5-AZAOROTIC ACID TO MICE*

Analogs (20 μmoles/ mouse)	Incorporation of orotic acid (counts/min/μmole)				Urinary excretion (μmoles/day)	
	2′(3′)-UMP	(%)	2′(3′)-CMP	(%)	Orotic acid	Orotidine
Control	2,267 ± 322	(100)	423 ± 26	(100)	0·29 ± 0·05	0·21 ± 0·03
5-Azauracil	1,918 ± 106	(84·6)	348 ± 42	(82·1)	0·27 ± 0·03	0·19 ± 0·02
5-Azaorotic acid	275 ± 28	(12·1)	42 ± 3	(10·0)	4·86 ± 0·91	2·64 ± 0·35

* Orotic-6-[^{14}C] acid (1 μc/μmole/mouse) was injected to groups of 4–6 mice 2 hr before killing simultaneously with saline or 5-azaanalogs.

Effects of 5-azauracil and 5-azaorotic acid on orotic acid metabolism in mice

A marked inhibition of the utilization of orotic acid for the synthesis of liver ribonucleic acids after the administration of 5-azaorotic acid was first observed by Handschumacher *et al.*[3,5,19] He found also that 5-azauracil, though practically inactive in liver slices, is effective in L 51784 leukemic cells.[5] Not even the application of high doses of 5-azauracil affects markedly the utilization *in vivo* of orotic acid in the liver (Table 4). In accordance with this observation, orotic aciduria remains unaltered after the application of 5-azauracil, whereas after 5-azaorotic acid it is strongly increased (Table 4). There is a close parallel between the inhibition of pyrimidine

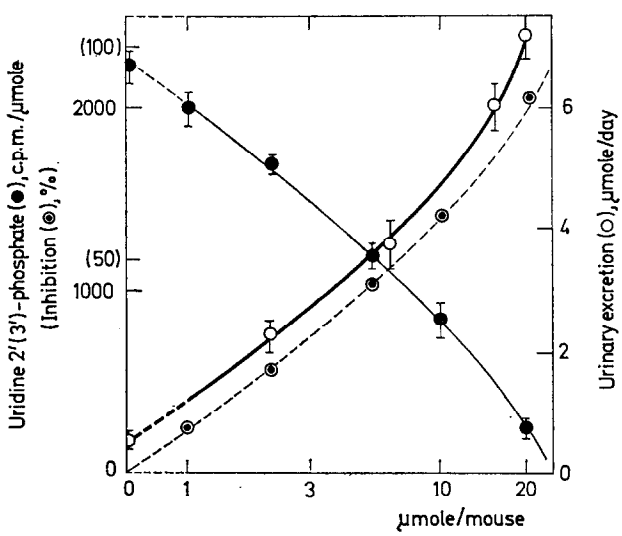

FIG. 5. Orotic acid utilization for liver RNA synthesis and orotic aciduria in mice after administration of 5-azaorotic acid. 5-Azaorotic acid (micromoles per mouse) was injected intraperitoneally to groups of four to six mice. Orotic-6-[^{14}C] acid (1 μc/μmole/mouse) was administered simultaneously with the analog 2 hr before killing the animals. The degree of inhibition (per cent) was calculated from the specific radioactivity of uridine 2′ (3′)-phosphate (counts per minute per micromole) isolated from an alkaline hydrolysate of liver RNA. Orotic aciduria (micromoles per day) represents the sum of orotic acid and orotidine in 24-hr urine after intraperitoneal application of the same doses of the analog.

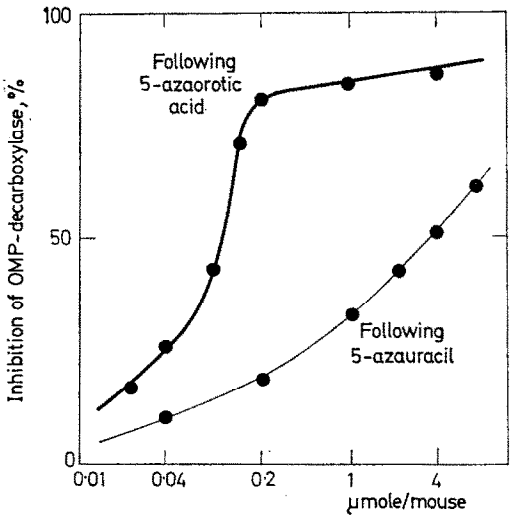

FIG. 6. Orotidylic acid decarboxylase in liver of mice after treatment with 5-azaorotic acid and 5-azauracil. Groups of four to six mice were injected intraperitoneally with the above compounds (micromoles per mouse) 1 hr before killing. Portions of cell-free liver extracts (0·1 ml) were used to measure orotidylic acid decarboxylase. The incubation was carried out at 30° for 5 min in 2×10^{-2} M tris–HCl buffer (pH 7·4) containing 2×10^{-5} M orotidine-6-[^{14}C] 5′-phosphate and equimolar Mg^{2+} ions.

synthesis in the liver after the application of 5-azaorotic acid and the increased urinary excretion of orotic acid and orotidine (Fig. 5).

The administration of 5-azaorotic acid to mice leads to a marked inhibition of orotidylic acid decarboxylase measured *in vitro* in the liver cell-free extract. The effect of 5-azauracil is significantly lower. The inhibition of decarboxylation in relation to the dose level of both compounds, applied 1 hr prior to killing of animals, is shown in Fig. 6. The application of orotic acid simultaneously with analogs did not reverse the inhibition.

DISCUSSION

In comparison to 5-azaorotic acid, 5-azauracil is biologically less effective in the liver. The difference in the activity of both 5-azapyrimidines reflects differences in their metabolic transformations (Table 1). Phosphoribosyltransferases responsible for the necessary metabolic conversion of both compounds play an essential role in the synthesis of pyrimidines *de novo* in mammals and in a variety of microorganisms.[20,21] The difference between orotate phosphoribosyltransferase and uracil phosphoribosyltransferase has been shown independently several times.[22–24] However, the activity of uracil phosphoribosyltransferase indispensable for the transformation of 5-azauracil is absent in the liver (Table 1); it is present in Ehrlich ascites cells,[25] in various microorganisms,[6,21,26] and in *Trypanosoma equiperdum*.[27]

In analogy to the direct conversion of 6-azauracil to 6-azauridine 5′-phosphate,[27] the synthesis of 5-fluorouridine 5′-phosphate from 5-fluorouracil is catalyzed in mouse leukemic cells by mammalian pyrimidine phosphoribosyltransferase.[28] The formation of 5-azauridine 5′-phosphate from 5-azauracil in Ehrlich ascites cells is

catalyzed probably by pyrimidine phosphoribosyltransferase of a similar type.[6] The substrate specificity of these enzymes seems to be rather low, as has been observed also in beef erythrocytes.[29,30]

Unlike 5-azauracil and similarly to 5-fluoroorotic acid,[31] 5-azaorotic acid serves as a substrate for both liver and bacterial orotate phosphoribosyltransferase (Table 1). The lower antibacterial activity of 5-azaorotic acid[6] can be partly explained by lower uptake of the ionized molecule into bacterial cells. The marked inhibition of anabolic transformations of orotic acid in the presence of 5-azaorotic acid (Table 3) has been explained previously.[13] 5-Azaorotic acid reacts with 5-phosphorylribose l-pyrophosphate,[17] and this reaction leads to a decrease of orotate phosphoribosyltransferase;[5,19] the resulting 5-azaorotidine 5′-phosphate simultaneously inhibits orotidylic acid decarboxylase.[13] The inhibition is associated with a proportional increase of orotic aciduria (Fig. 6), as has been observed also after applications of other compounds which prevent the utilization of orotic acid (cf. references 32–34).

5-Azauracil inhibits liver and bacterial uridine phosphorylase (Table 2), both in the direction of uridine synthesis and its phosphorolysis. Thus in many respects 5-azauracil resembles 5-fluorouracil[35,36] and especially 2-thiouracil,[37] which also inhibits uridine synthesis in the liver. 5-Azauracil does not interfere significantly with the synthesis of liver pyrimidines *de novo* (Table 4); neither is orotic aciduria increased after its application. The conversion of 5-azauracil to 5-azauridine 5′-phosphate is a requisite of its biological activity, and the presence of uracil phosphoribosyltransferase in respective tissues or organisms is decisive for the degree of its inhibitory effects. The same contention regards different analogs of orotic acid[13,31,38] where the presence of nonspecific orotate phosphoribosyltransferase may be decisive for their action.

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