MODULATION OF OROTATE PHOSPHORIBOSYLTRANSFERASE AND OROTIDINE 5'-PHOSPHATE DECARBOXYLASE ACTIVITIES IN REGENERATING RAT LIVER BY 5-AZACYTIDINE*

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5-Azacytidine administered immediately after partial hepatectomy depresses the enhanced conversion of orotic acid into uridine 5'-monophosphate in cell-free extracts from regenerating rat livers. Both the activity of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase are significantly lowered following 5-azacytidine. The activity of orotidine 5'-phosphate decarboxylase is inhibited by the newly formed 5-azacytidine 5'-phosphate while the activity of orotate phosphoribosyltransferase is inhibited by orotidine 5'-phosphate accumulating on account of the depressed orotidine 5'-phosphate decarboxylase during 5-azacytidine *in vivo* treatment. Uridine 5'-monophosphate is less effective as an inhibitor of hepatic orotate phosphoribosyltransferase as compared to orotidine 5'-phosphate (K_i $4{\cdot}9 \cdot 10^{-3}$ M and $6{\cdot}7 \cdot 10^{-5}$ M, respectively). Besides inhibition of the activity of both enzymes a depression of the enhanced enzyme synthesis in regenerating rat liver following 5-azacytidine administration was observed.

5-Azacytidine has been used successfully for the treatment of acute leukemia of childhood[1,2] and in patients with various solid tumors[3]. In leukemic mice the drug strongly affects the growth of leukemic cells[4-6], possibly due to its incorporation into nucleic acids[5,7]. However, after the administration of 5-azacytidine further changes were observed in various biological systems: depression of polyamine synthesis in leukemic mice[8,9], inhibition of stimulated DNA synthesis in mouse salivary glands[10], block of pyrimidine[11] and purine[12] synthesis *de novo*, degradation of liver polyribosomes[13-15], inhibition of ribosomal RNA maturation in HeLa[16] and Novikoff hepatoma cells[17], *etc.* Thus it is difficult to explain the biological activity of 5-azacytidine by a single inhibitory mechanism.

Using regenerating rat liver as a model of a rapidly growing tissue we found[18,19] 5-azacytidine to cause pronounced alterations in the synthesis of RNA and DNA. The aim of the present study is to add to the understanding of changes in the synthesis of RNA occurring in regenerating rat livers after the administration of this drug.

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Abbreviations used: PRPP, 5-phosphoribosyl-1-pyrophosphate; UMP, uridine 5'-monophosphate; OMP, orotidine 5'-phosphate; 6-AzUMP, 6-azauridine 5'-monophosphate; 5-AzCR, 5-azacytidine; OA, orotic acid; TdR, thymidine; UR, uridine and U, uracil.

The regenerating liver is characterized by the controlled synthesis of various enzymes culminating in the enhancement of DNA synthesis and cell division[20]. According to recent findings ribonucleic acids play an important role as regulators of the regenerative process[21,22] and their synthesis is accomplished by a set of enzymes in which orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase are especially active[23-26]. In this paper the results of experiments with regenerating rat livers revealing the inhibition of enhanced orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase activities following 5-azacytidine administration are presented.

MATERIAL AND METHODS

Chemicals. 5-Azacytidine and 6-azauridine 5'-monophosphate were prepared in this Institute. Orotidine and uridine 5'-monophosphates, and 5-phosphoribosyl-1-pyrophosphate were obtained from Calbiochem, Luzern. Orotic-[2-^{14}C]acid (44 μCi/μmol) was provided by the Institute for Research, Production and Uses of Radioisotopes, Prague.

Animals. For the experiments albino female rats (170—180 g) kept under standard conditions were used. Partial hepatectomy (66 per cent) and sham-operation were performed according to Higgins and Anderson[27] under light ether narcosis. 5-Azacytidine was administered intraperitoneally; the controls received the same volume of saline. The experiments were always started between 8—9 a.m. After operation the animals were killed by decapitation at selected time intervals and bled. Excised livers were homogenized under cooling in a glass homogenizer in 4 or 9 vol. of 0·025M-Tris-HCl buffer (pH 7·5) containing $5 \cdot 10^{-3}$M-$MgCl_2$, and $2{\cdot}5 \cdot 10^{-2}$M--KCl. Homogenates were centrifuged (12 000 *g*, 20 min, 2°C) and the defatted supernatant fractions were used for the determination of enzyme activity.

Enzyme assay. Orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase were measured using a highly sensitive radioactive method as described earlier[11]. Incubation was carried out for 2—15 min at 37°C in $6 \cdot 10^{-2}$M Tris-HCl buffer (pH 7·5) in a total volume 0·5 ml in the presence of cell-free liver extracts corresponding to 10—25 mg of the liver wet weight. For orotate phosphoribosyltransferase orotic-[2-^{14}C]acid ($1 \cdot 10^{-4}$M) and 5-phosphoribosyl-1-pyrophosphate ($5 \cdot 10^{-4}$M) with equimolar $MgCl_2$ were used. Orotidine 5'-phosphate decarboxylase was assayed with orotidine-[2-^{14}C]5'-phosphate ($5 \cdot 10^{-5}$ or $1 \cdot 10^{-4}$M) and reduced glutathione ($2 \cdot 10^{-4}$M). The newly formed radioactive compounds in an incubation mixture were separated chromatographically on Whatman No 1 paper as described[11]. The corresponding chromatographic spots were located according to standards and by radioactivity scanning. The radioactivity of individual spots was measured with a Packard liquid-scintillation spectrometer in 10 ml of scintillation fluid.

RESULTS

Modulation of Orotate Phosphoribosyltransferase and Orotidine 5'-Phosphate Decarboxylase in Regenerating Rat Liver by 5-Azacytidine

The enhancement of orotic acid utilization for the synthesis of RNA was detected very shortly after partial hepatectomy[19,21,22,28]. Orotate phosphoribosyltransferase and orotidine 5'-phos-

phate decarboxylase activities of liver supernatant fraction changed little during the first 12 h following partial hepatectomy[25]. The peak of activity was reached at about 36 h after the operation at the time when the rate of RNA synthesis was returning to its normal value. Orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase activities are thus not rate-limiting for RNA synthesis. In accordance herewith is the finding[29] that variations in the nutritional state of rats produce cyclic variations of RNA content in the liver in the absence of any alteration in the conversion of orotic acid to uridine 5'-monophosphate. Fausto suggested[30] that the decrease in liver size caused by partial hepatectomy may be in itself sufficient to account for an increase in the flow of metabolites along the pyrimidine pathway at the early stages of liver regeneration.

The administration of 5-azacytidine immediately after partial hepatectomy prevents the enhancement of orotic acid conversion in cell-free liver extracts catalyzed by orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase (Fig.1*A*). Repeated administration of lower doses of the drug does not prolong the time interval of maximal inhibition (Fig. 1*B*). To determine which enzyme in the pathway is inhibited the activity of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase and their alterations by 5-azacytidine were measured. The data presented in Table I show an increase in the activity of both enzymes in 24-

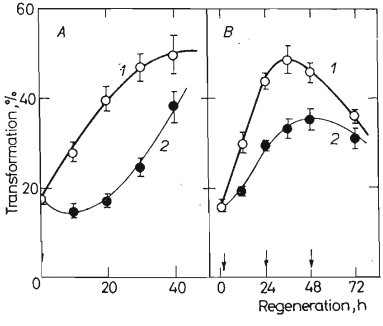

FIG. 1

Effect of 5-Azacytidine Treatment on the Transformation of Orotic Acid in Cell-Free Extracts from Regenerating Rat Livers

5-Azacytidine 2 was injected i.p. at the dose level of 10 μmol/100 g (*A*) or repeatedly at the dose level of 5 μmol/100 g (*B*) to groups of 4—5 female rats at different stages of liver regeneration (h) as indicated by arrows. The animals were killed 24 h later and cell-free liver extracts were prepared (2·22—2·31 mg of proteins per 0·1 ml). The controls *1* received saline. Metabolic transformation of $1 \cdot 10^{-4}$M orotic-[2-^{14}C] acid was measured during a 10 min incubation with 0·1 ml liver extract and is expressed as per cent of reacted substrate.

TABLE I

Activity of Various Enzymes in the Liver of Sham-Operated and Partially Hepatectomized Rats Following 5-Azacytidine Treatment

Groups of 6—8 female rats (170—175 g) subjected to sham-operation or partial hepatectomy were given i.p. saline or 5-azacytidine (10 μmol : : 100 g) immediately after the operation, 24 h before killing. Activity of enzymes was measured during a 5 min incubation period at 37°C in a total volume of 0·5 ml as described earlier[11,18].

Enzyme	Substrate (mol/l)		Sham-operated, m μmol ± S.E.			Partially hepatectomized, m μmol ± S.E.		
			without	with 5-azacytidine	%	without	with 5-azacytidine	%
Orotate phosphoribosyltransferase	OA	(0·1)	36·1 ± 4·2	34·0 ± 3·8	94·1	53·6 ± 4·2	37·6 ± 3·2	69·5
Orotidine 5′-phosphate decarboxylase	OMP	(0·05)	22·1 ± 2·1	14·6 ± 1·8	66·1	33·2 ± 3·1	12·8 ± 1·1	38·6
Thymidine kinase	TdR	(0·05)	1·42 ± 0·16	1·80 ± 0·10	126	29·32 ± 2·41	1·94 ± 0·10	6·5
Uridine kinase	6-AzUR	(0·02)	0·86 ± 0·09	2·21 ± 0·20	256	1·57 ± 0·14	3·04 ± 0·23	194

TABLE II

In vitro Effect of 6-Azauridine 5′-Monophosphate on Hepatic Orotate Phosphoribosyltransferase and Orotidine 5′-Phosphate Decarboxylase Activities

Groups of 4—5 female rats (170—175 g) were given i.p. 5-azacytidine (10 μmol/100 g) or saline immediately after partial hepatectomy, 24 h before killing. Incubation with and without $1 \cdot 10^{-3}$ M 6-azauridine 5′-monophosphate at 37°C for 5 min, and the activity of enzymes is expressed as mμmol ± S.E. of the reacted substrate.

Administered		OA Phosphoribosyltransferase		OMP Decarboxylase	
In vivo	*In vitro*	m μmol ± S.E.	%	m μmol ± S.E.	%
0	0	59·8 ± 3·3	100	44·8 ± 2·5	100
5-AzCR	0	40·2 ± 2·4	67·2	18·4 ± 1·6	41·1
0	6-AzUMP	42·6 ± 1·5	71·0	0·8 ± 0·1	1·8
5-AzCR	6-AzUMP	38·7 ± 2·6	64·7	0·6 ± 0·0	1·3

-hour regenerating liver (sham-operated untreated animals were used as corresponding controls). Simultaneously an increase of thymidine and uridine kinase activities is presented. 5-Azacytidine administration results in a differential decrease of the activity of individual enzymes with the exception of uridine kinase which was elevated both in intact and regenerating livers[31,32]. The inhibition of the enzymes in regenerating liver was significantly higher than in the liver of sham-operated animals. Although the initial activities of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase were different in intact and regenerating rat livers they dropped down to the same level following 5-azacytidine. Hence it seems that in addition to inhibiting the activity of enzymes 5-azacytidine depresses their enhanced synthesis which takes place during liver regeneration.

Orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase were measured under conditions optimal for their assay. Fig. 2 demonstrates linearity between the amount of orotidine 5'-phosphate decarboxylase present in cell-free liver extracts, and the activity measured, as well as a linear time course of decarboxylation. A similar linearity was observed with orotate phosphoribosyltransferase during a 2–6 min incubation period in the presence of 0·4–1·6 mg of proteins in cell-free

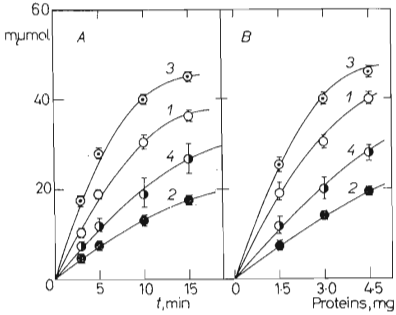

FIG. 2

Alteration of Orotidine 5'-Phosphate Decarboxylase Activity in Sham-Operated (*1*, *2*) and 48 h Regenerating Rat Livers (*3*, *4*) by 5-Azacytidine

The drug (10 μmol/100 g) or saline were given to groups of 4 female rats immediately after operation and 24 h later. The activity of enzyme (m μmol of decarboxylated substrate ±S.E.) was assayed at 37°C during a 3–15 min incubation (*A*) with liver extracts (3·02–3·15 mg of proteins), and during a 10 min incubation period (*B*) with increasing protein content in enzyme extracts used. *1* and *3*, without 5-azacytidine; *2* and *4*, following 5-azacytidine.

liver extracts used for the estimation of enzyme. Since no differences in the rate of PRPP synthesis could be found between normal and regenerating liver supernatants[25] the same concentrations of the compound with equimolar Mg^{2+}-ions were used.

From Fig. 2 it is apparent that in 48-hour regenerating livers even two doses of 5-azacytidine administered at 24-h intervals do not depress the enzyme activity to the level observed in livers of sham-operated 5-azacytidine-treated rats. This is different from the decline of the activity of both enzymes observed in 24-h regenerating livers (Table I) and is in agreement with the lower inhibition of orotic acid metabolism depicted in Fig. 1*B*. Inhibition of orotidine 5'-phosphate decarboxylase in cell-free extracts prepared from the liver of rats treated 24 h with increasing dose levels of 5-azacytidine is apparent from the data shown in Fig. 3. 5-Azacytidine is known to be metabolized to 5'-monophosphate[4,7] and this compound or product of its deamination[33,34] is present in cell-free liver extracts strongly blocking the decarboxylation of orotidine 5'-phosphate. The inhibition is higher at shorter time intervals after the administration of 5-azacytidine[11] and depends on the excretion of the drug from the organism.

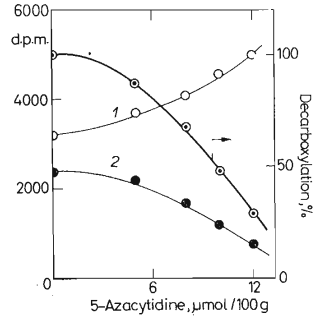

FIG. 3

Orotidine 5'-Phosphate Decarboxylase Activity in Cell-Free Extracts of 24 h Regenerating Livers of 5-Azacytidine Treated Rats

The analogue (μmol/100 g) was given i.p. immediately after partial hepatectomy to groups of 5—6 female rats. Enzyme activity was measured 5 min with $5 . 10^{-5}$M orotidine-[2-^{14}C]5'-phosphate and is expressed as d.p.m. of the newly formed products, and per cent of the decarboxylase activity of controls (43% decarboxylation). *1* Unreacted OMP; *2* newly formed UMP + + UR + uracil.

Inhibition of Hepatic Orotate Phosphoribosyltransferase by Orotidine 5'-Phosphate

Inhibition of orotidine 5'-phosphate decarboxylase[11] by 5-azacytidine 5'-monophosphate or a product of its deamination results in an accumulation of orotidine 5'-phosphate in the liver and in an increased urinary excretion of orotic acid and orotidine[35]. The data given in Table II indicate that the activity of orotate phosphoribosyltransferase is also depressed. 6-Azauridine 5'-monophosphate, a specific inhibitor or orotidine 5'-phosphate decarboxylase[36], similarly affects the activity of orotate phosphoribosyltransferase. In extracts from the liver of 5-azacytidine-treated rats 6-azauridine 5'-monophosphate does not cause any further inhibition of the transferase reaction (Table II).

To explain this phenomenon we tested orotidine 5'-phosphate as an inhibitor of orotate phosphoribosyltransferase (Fig. 4). The enzyme catalyzes a reversible reaction[37] with an equilibrium constant of about 0·1 and is known to be inhibited by orotidine 5'-phosphate[38,39]. The inhibition does not seem to be due to simple mass action since equilibrium of the reaction could not be shifted by the addition of inorganic pyrophosphate. Uridine 5'-monophosphate also inhibits orotate phosphoribosyltransferase though its effect is less pronounced. The lower effect of this

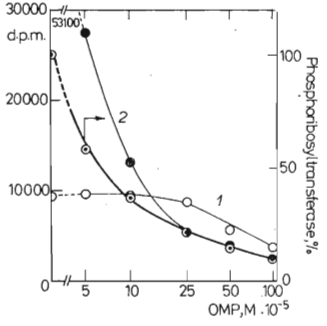

FIG. 4

Orotate Phosphoribosyltransferase Activity in an Extract of 24 h Regenerating Rat Livers Assayed in the Presence of Increasing Concentrations of Orotidine 5'-Phosphate

The activity of the enzyme (1·46 mg of proteins per 0·1 ml) was measured during a 5-min incubation period and is given as d.p.m. of the newly formed products (total $1{\cdot}2 \cdot 10^5$ d.p.m.), and as per cent of phosphoribosyltransferase activity of the control (52% conversion of orotic acid without added OMP). *1*, OMP; *2*, UMP + UR + uracil.

nucleotide cannot be explained by its preferential degradation since more than 70 per cent of the compound is still present in an incubation mixture ($1 . 10^{-3}$M, 20 min).

Fig. 5 shows the reciprocal plot of orotate phosphoribosyltransferase inhibition by orotidine and uridine 5'-monophosphates. The K_m value of the reaction is $3{\cdot}0 . 10^{-4}$M and the K_i values for $2 . 10^{-4}$M orotidine 5'-phosphate $6{\cdot}7 . 10^{-5}$M and for $5 . 10^{-3}$M uridine 5'-monophosphate $4{\cdot}9 . 10^{-3}$M. Inhibition of orotate phosphoribosyltransferase caused by uridine 5'-monophosphate leads to the accumulation of orotidine 5'-phosphate as a result of inhibited decarboxylation by uridine 5'-monophosphate[40,41]. This complicates the estimation of the inhibitory effect of uridine 5'-monophosphate on hepatic orotate phosphoribosyltransferase. Uridine 5'-monophosphate strongly inhibits bacterial uracil phosphoribosyltransferase whereas orotidine 5'-phosphate is much less inhibitory in this case[42].

A time course of orotate phosphoribosyltransferase activity in a cell-free liver extract together with the inhibitory effect of orotidine 5'-phosphate added are shown in Fig. 6. The levels of the newly formed orotidine 5-'phosphate, uridine 5'-monophosphate, uridine and uracil are depressed almost to zero. Coordinated changes in the activity of liver orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase in various tissues and systems[26,43-48] led to the proposal that they

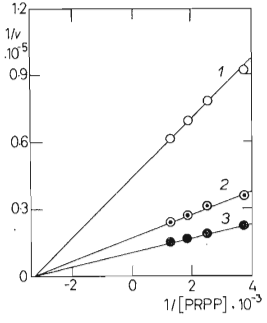

FIG. 5

Double Reciprocal Plot for Orotate Phosphoribosyltransferase in an Extract of 24 h Regenerating Rat Liver Inhibited *in Vitro* by $2 . 10^{-4}$M Orotidine 5'-phosphate (*1*) and $5 . 10^{-3}$M Uridine 5'-Monophosphate (*2*)

Incubation 5 min with $1 . 10^{-4}$M orotic-[2-^{14}C] acid and varying PRPP with equimolar $MgCl_2$ and 0·1 ml liver extract (1·38 mg of proteins). Control incubation *3*. *v*, The amount of reacted orotic acid in mol per liter.

form an enzyme complex[45]. The effect of 5-azacytidine on orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase activities, and the inhibition of orotate phosphoribosyltransferase by orotidine 5'-phosphate are not in contradiction with the above findings[45–48].

DISCUSSION

The biological conversion of orotic acid to uridine 5'-monophosphate is a two-step reaction catalyzed by orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase[37]. While these two enzymes were separated in yeast[39] and calf thymus[49], some difficulties in their separation were indicated in other animal tissues[50]. Two different enzymes have been detected in microorganisms and the corresponding genes are known to occupy different loci of linkage maps[38,51,52]. However, some orotidine 5'-phosphate decarboxylase mutants might simultaneously lack orotate phosphoribosyltransferase, a phenomenon observed in human cells[53]. On the other hand, hereditary orotic aciduria, established as an inborn error of pyrimidine biosynthesis, is associated with both the deficiency of the two sequential enzymes, and lack of orotidine 5'-phosphate decarboxylase along with a simultaneously markedly increased level of orotate phosphoribosyltransferase[54]. Two different enzymes are thus involved in the conversion of orotic acid to uridine 5'-monophosphate though during purification and heat denaturation studies orotate phosphoribosyltransferase appeared to parallel orotidine 5'-phosphate decarboxylase activity[44,50].

Increased activities of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase have been observed following the administration of orotic

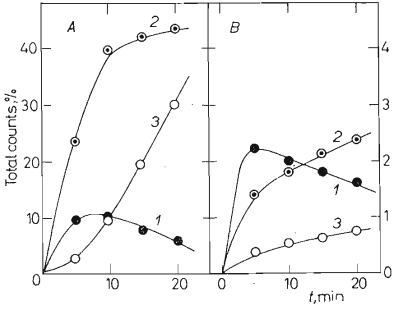

FIG. 6

Time Course of the Conversion of Orotic Acid in Extracts of 24 h Regenerating Rat Livers
A, Control; *B*, $5 \cdot 10^{-3}$M orotidine 5'-phosphate. The level of newly formed orotidine 5'-phosphate *1*, uridine 5'-monophosphate 2 and uridine + uracil *3* is expressed as per cent of total counts in mixture (total $6 \cdot 10^4$ d.p.m.).

acid[47], and under varying of conditions, including fast growing tissues[48], and changes during the postnatal development[26]. Studies of their activity in aging red cells[43] and Ehrlich ascites hepatoma cells[46] led to the postulation of their existence as an enzyme complex[45,46]. If such a complex exists (recently it was suggested that decarboxylase is composed of subunits which are in dynamic equilibrium with an aggregate[55]) there must be a special arrangement of both enzymes to allow the sequential inhibition of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase by their products, *i.e.* by orotidine and uridine 5'-monophosphates.

The pattern of increase of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase activities following partial hepatectomy suggest[19,24,25] that the enzymes are not rate-limiting for RNA synthesis, especially at early stages of liver regeneration. Perfused regenerating livers do not differ in their orotic acid uptake, and uridine 5'-monophosphate synthesis, from livers of comparable size in which regeneration does not take place[30]. It is not clear whether the increase of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase activities during liver regeneration (Table I) solely reflects an increased metabolic activity of rapidly proliferating tissue since neither of these two enzyme activities correlated with the growth rates of selected Morris hepatomas[48]. The effect of 5-azacytidine on the activity of both enzymes is in agreement with the general growth-inhibitory properties of this drug[56]. Besides inhibiting the enhanced synthesis of orotate phosphoribosyltransferase and orotidine 5'-phosphate decarboxylase the possible involvement of compounds accumulating on account of 5-azacytidine metabolism and interference with pyrimidine biosynthesis must be taken into consideration during 5-azacytidine-evoked inhibition of hepatic RNA synthesis.

REFERENCES

1. Hrodek O., Veselý J.: Neoplasma *18*, 493 (1971).
2. Karon M., Sieger L., Leimbrock S., Finklestein J. Z., Nesbit M. E., Swaney J. J.: Blood *42*, 359 (1973).
3. Weiss A. J., Stambaugh J. E., Mastrangelo M. J., Laucius J. F., Bellet E. R.: Cancer Chemoth. Rept. *56*, 413 (1972).
4. Veselý J., Čihák A., Šorm F.: Inter. J. Cancer *2*, 639 (1967).
5. Li L. H., Olin E. J., Buskirk H. H., Reineke L. M.: Cancer Res. *30*, 2760 (1970).
6. Vadlamudi E., Choudry J. N., Waravdekar V. S., Kline I., Goldin A.: Cancer Res. *30*, 362 (1970).
7. Čihák A., Veselý J., Šorm F.: Biochim. Biophys. Acta *108*, 516 (1965).
8. Heby O., Russell D. H.: Cancer Res. *33*, 159 (1973).
9. Heby O., Russell D. H.: *Polyamine Synthesis and Neoplastic Growth* (D. H. Russell, Ed.), p. 221. Raven Press, New York 1973.
10. Simson J. A. V., Baserga R.: Lab. Invest. *24*, 464 (1971).
11. Čihák A., Brouček J.: Biochem. Pharmacol. *21*, 2497 (1972).
12. Martin D. W., Owen N. T.: J. Biol. Chem. *247*, 5477 (1972).
13. Čihák A., Veselá H., Šorm F.: Biochim. Biophys. Acta *166*, 277 (1968).

14. Levitan I. B., Webb T. E.: Biochim. Biophys. Acta *182*, 491 (1969).
15. Čihák A., Narurkar L. M., Pitot H. C.: This Journal *38*, 948 (1973).
16. Reichman M., Karlan D., Penman S.: Biochim. Biophys. Acta *299*, 173 (1973).
17. Weiss J. W., Pitot H. C.: Arch. Biochem. Biophys. *160*, 119 (1974).
18. Čihák A., Veselý J.: Biochem. Pharmacol. *21*, 3257 (1972).
19. Čihák A., Veselý J., Šorm F.: This Journal *38*, 3944 (1973).
20. Bresnick E.: Methods Cancer Res. *6*, 347 (1971).
21. Church R., McCarthy B. J.: Proc. Natl. Acad. Sci. U.S. *58*, 1548 (1967).
22. Mayfield J. E., Bonner J.: Proc. Natl. Acad. Sci. U.S. *69*, 7 (1972).
23. Sköld O.: Biochim. Biophys. Acta *44*, 1 (1960).
24. Bresnick E.: J. Biol. Chem. *240*, 2550 (1965).
25. Fausto N.: Biochim. Biophys. Acta *182*, 66 (1969).
26. Pausch J., Keppler D., Decker K.: Biochim. Biophys. Acta *258*, 395 (1972).
27. Higgins G. M., Anderson R. M.: Arch. Pathol. *12*, 186 (1931).
28. Fujioka M., Koga M., Lieberman I.: J. Biol. Chem. *283*, 3401 (1963).
29. Whittle E., Potter V. R.: J. Nutrit. *95*, 228 (1968).
30. Fausto N.: Biochem. J. *129*, 811 (1972).
31. Čihák A., Seifertová M., Veselý J.: Arch. Biochem. Biophys. *148*, 400 (1972).
32. Čihák A., Veselý J.: J. Biol. Chem. *248*, 1307 (1973).
33. Čihák A., Šorm F.: This Journal *30*, 2091 (1965).
34. Chabner B. A., Drake J. C., Johns D. G.: Biochem. Pharmacol. *22*, 2763 (1973).
35. Veselý J., Čihák A., Šorm F.: Biochem. Pharmacol. *17*, 519 (1968).
36. Handschumacher R. E.: J. Biol. Chem. *235*, 2971 (1960).
37. Lieberman I., Kornberg A., Simms E. S.: J. Biol. Chem. *215*, 403 (1955).
38. O'Donovan G. A., Neuhard J.: Bacteriol. Rev. *34*, 278 (1970).
39. Umezu K., Amaya T., Yoshimoto A., Tomita K.: J. Biochem. *70*, 249 (1971).
40. Creasey W. A., Handschumacher R. E.: J. Biol. Chem. *236*, 2058 (1961).
41. Blair D. G. R., Potter V. R.: J. Biol. Chem. *236*, 2503 (1961).
42. Čihák A.: This Journal *39*, 221 (1974).
43. Fox R. M., Wood M. H., O'Sullivan W. J.: J. Clin. Invest. *50*, 1050 (1971).
44. Murray M. G., Ross C.: Phytochem. *10*, 2645 (1971).
45. Brown G. K., Fox R. M., O'Sullivan W. J.: Biochem. Pharmacol. *21*, 2469 (1972).
46. Shoaf W. T., Jones M. E.: Biochemistry *12*, 4039 (1973).
47. Pausch J., Keppler D., Decker K.: FEBS Letters *20*, 330 (1972).
48. Hoffman D. H., Sweeney M. J.: Cancer Res. *33*, 1109 (1973).
49. Kasbekar D. K., Nagabhushanam A., Greenberg D. M.: J. Biol. Chem. *239*, 4245 (1964).
50. Appel S. H.: J. Biol. Chem. *243*, 3924 (1968).
51. Pynadath T. I., Fink R. M.: Arch. Biochem. Biophys. *118*, 185 (1967).
52. Jund R., Lacrouts F.: J. Bacteriol. *109*, 196 (1972).
53. Krooth R. S.: Cold Spring Harbor Symp. Quant. Biol. *29*, 189 (1964).
54. Fox R. M., O'Sullivan W. J., Firkin B. G.: Am. J. Med. *47*, 332 (1969).
55. Krooth R. S., Pan Y. L., Pinsky L.: Biochem. Genet. *8*, 133 (1973).
56. Škoda J.: Handbook of Exptl. Pharmacol. *38*, 2 (1974).

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