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ENHANCEMENT OF RAT LIVER URIDINE KINASE ACTIVITY BY VARIOUS METABOLIC INHIBITORS

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Abstract—Of 25 compounds tested, eight—namely, 5-azacytidine, gougerotin, cycloheximide, pactamycin, streptovitacin A, adriamycin, daunorubicin and thioacetamide—resulted in the enhancement of liver uridine kinase activity *in vivo*. Puromycin and actinomycin D produced a slight decrease in enzyme activity. With both 5-azacytidine and cycloheximide, the enhancement observed was independent of adrenal secretion. Some of the compounds which enhanced hepatic uridine kinase activity (5-azacytidine, cycloheximide and related glutarimide antibiotics) concomitantly suppressed the activity of the enzyme in the thymus, while daunorubicin, adriamycin and thioacetamide were much less effective in this respect. No relation has been found between the effect of the tested compounds on the incorporation of orotic acid into RNA and the enhanced activity of hepatic uridine kinase.

URIDINE kinase is important in the treatment of experimental neoplasias with pyrimidine analogues, since it catalyzes the phosphorylation of the latter to their corresponding 5′-monophosphates.[1,2] The deletion of uridine kinase in tumor cells is accompanied by the development of resistance to uridine and cytidine analogues.[3–5] Recently we have found[6,7] that the administration of 5-azacytidine results in the enhancement of liver uridine kinase activity by a process which is independent of adrenal secretion, and which is unaffected by a number of compounds interfering with DNA, RNA and protein synthesis. Cycloheximide also enhances the activity of uridine kinase in the liver.[8] The ability to enhance selectively the activities of enzymes responsible for the activation of oncolytic agents would be of considerable consequence in cancer chemotherapy, and the present report discusses the effects of a broad spectrum of drugs on the activity of hepatic uridine kinase.

MATERIALS AND METHODS

Chemicals. Adenosine 5′-triphosphate, 5-phosphorylribose-1-pyrophosphate and cycloheximide were obtained from Calbiochem, Luzern. 5-Azacytidine, 6-azauridine 5′-monophosphate and other tested drugs were synthesized in the Institute of Organic Chemistry and Biochemistry, Prague. Glucagon was provided by Lilly Laboratories, Indianapolis, Ill., and hydrocortisone by Spofa, Prague. Thioacetamide was obtained from Lachema, Brno, daunorubicin from May & Baker and adriamycin from Pharmitalia. Actinomycin D was purchased from Merck, Sharp & Dohme, West Point, N.Y., and puromycin aminonucleoside from Sigma, St. Louis, Mo. Orotic acid-2-^{14}C(48 μCi/μmole) and 6-azauridine-4,5-^{14}C(80 μCi/μmole) were provided by the Institute for Research, Production and Uses of Radioisotopes, Prague.

Animals and cell-free tissue extracts. Male Wistar rats (170–180 g) were kept under standard conditions with food and water *ad lib.* In general, experiments were started between 8 and 9 a.m. and drugs were administered intraperitoneally in a maximal volume of 0·3 ml. The animals were killed by decapitation and bled. The excized livers or tissues were homogenized in three vol. of 25 mM Tris-HCl buffer (pH 7·5) containing 25 mM KCl and 5 mM $MgCl_2$ in a cooled glass homogenizer with a tight-fitting Teflon pestle. Homogenates were centrifuged (10,000 rev/min, 20 min, 2°) and the fat-free supernatant fractions used for determination of enzyme activity.

Uridine kinase activity. The activity of uridine kinase was determined during a 10-min incubation period with 0·1 mM 6-azauridine-4,5-^{14}C as substrate[9] and 4 mM adenosine 5′-triphosphate with 2 mM $MgCl_2$. The incubation was carried out in 60 mM Tris-HCl buffer (pH 7·5) at 37° in a total volume of 0·3 ml with 0·1 ml of the postmitochondrial supernatant fraction (corresponding to 25 mg wet wt of tissue). Aliquots of incubation mixtures withdrawn during the linear course of the enzyme reaction were separated chromatographically on Whatman paper No. 1 in a solvent system composed of isobutyric acid–ammonium hydroxide–water (66:1·5:33). Chromatographic spots corresponding to 6-azauridine and 6-azauridine 5′-monophosphate were cut out, and their radioactivity was measured in a Packard liquid scintillation spectrometer in 10 ml of scintillation fluid (4 g 2,5-diphenyloxazole, 0·15 g *p*-bis[2-(4-methyl-5-phenyloxazolyl)]-benzene in 1 liter toluene). The activity of uridine kinase was expressed as μmoles of 6-azauridine phosphorylated during 1 hr of incubation at 37° in the presence of postmitochondrial extract corresponding to 1 g of liver. No significant differences in the content of liver proteins after the administration of the tested drugs have been found.

Pyrimidine synthesis in vitro. This was measured in the liver postmitochondrial supernatant fractions by incubating 0·1 mM orotic acid-2-^{14}C and 0·4 mM 5-phosphorylribose-1-pyrosphosphate with equimolar Mg^{2+} ions. Analysis of the reaction mixture and the level of newly formed uridine 5′-monophosphate were determined during a 5-min incubation period at 37°, as described previously.[10]

RESULTS

Uridine kinase activity was measured with 6-azauridine as substrate,[7,9] since this modification allows one to assay activity of the enzyme in unpurified cell extracts with high sensitivity.[6] Moreover, the newly formed 6-azauridine 5′-monophosphate is stable and can be separated easily from unreacted 6-azauridine by paper chromatography. Data presented in Fig. 1 show that the phosphorylation of 6-azauridine is linear over a 10-min incubation period and is almost proportional to the amount of tissue extract added. Conditions for optimal phosphorylation were the same for control and stimulated enzyme using uridine or 6-azauridine as substrates.

Only a few of the compounds tested resulted in the enhancement of liver uridine kinase (Table 1), while actinomycin D and puromycin were slightly inhibitory. The most potent drugs besides 5-azacytidine and cycloheximide were streptovitacin A, pactamycin and, at higher dose levels, thioacetamide. It is notable that those compounds which increase liver uridine kinase activity exert their cytotoxic effects by a different mechanism. Of the tested antimetabolites, 5-azacytidine is the only pyrimidine analogue which enhances the activity of the enzyme. The increase of uridine kinase in the liver in relation to the dose level of administered drugs is shown

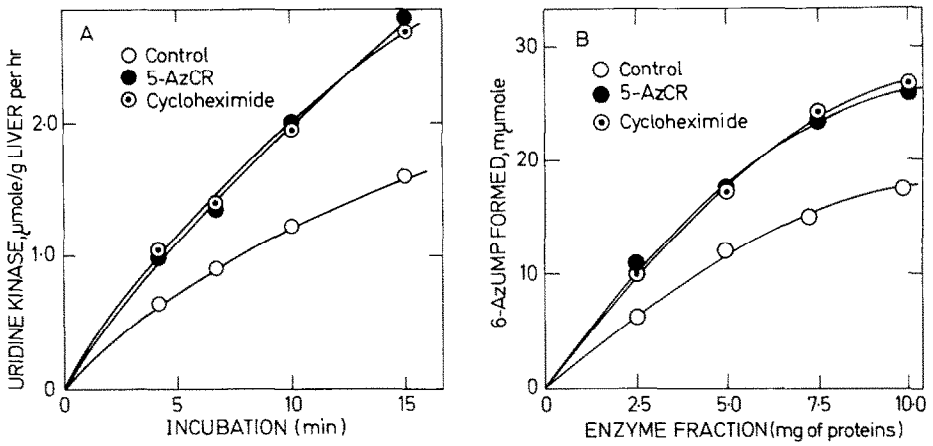

FIG. 1. Uridine kinase activity in cell-free liver extracts after 5-azacytidine (25 mg/kg) and cycloheximide (2·5 mg/kg) administration *in vivo* 24 hr before killing. Incubation was carried out at 37° in 60 mM Tris-HCl buffer (pH 7·5) in a total volume of 1 ml with 0·1 mM 6-azauridine, 4 mM adenosine 5′-triphosphate and 2 mM $MgCl_2$. (A) Incubation was carried out with 0·25 ml of the enzyme fraction (6·2 mg protein); (B) 10-min incubation period.

in Fig. 2. The maximal effect of 5-azacytidine was achieved at a dose of 25–40 mg/kg; cycloheximide at 2·5–3·0 mg/kg; daunorubicin at 8–10 mg/kg; thioacetamide at 150–250 mg/kg.

In contrast to various hepatic amino acid-metabolizing enzymes,[11,12] the increase of uridine kinase activity observed in both male and female rats is independent of the dietary regimen. Further, the enhancement of uridine kinase activity is independent of hormonal administration, which is known to induce other enzymes.[13,14] It has been reported that the effect of cycloheximide is influenced by the adrenal secretion.[15] Consequently, we undertook to investigate the effect of this drug on hepatic uridine kinase in adrenalectomized animals, and we found that under these conditions the

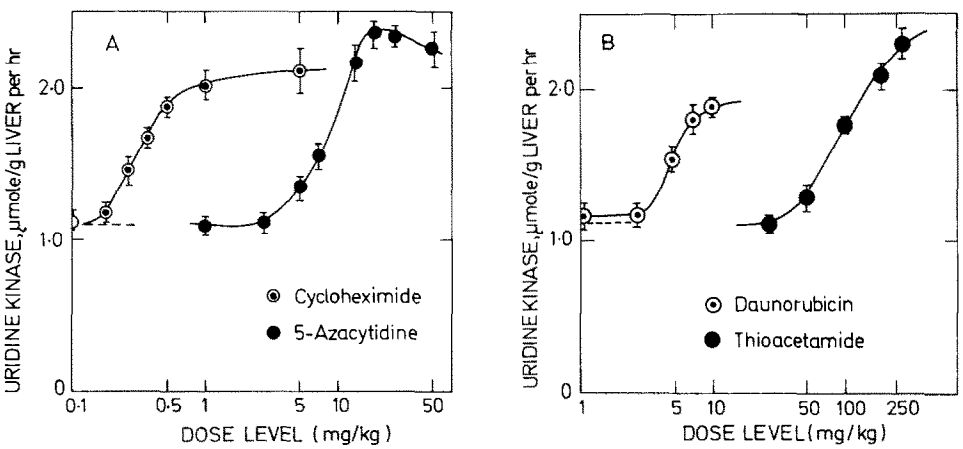

FIG. 2. Uridine kinase enhancement in the liver in relation to the dose of administered drugs. Compounds were injected i.p. into groups of five to eight male rats 24 hr before killing and cell-free liver extracts were immediately prepared. The assay of uridine kinase activity (μmoles/g of liver per hr ± S. E.) was carried out for 10 min at 37° in a total volume of 0·3 ml in the presence of 0·1 ml of enzyme fraction (2·5 mg protein).

TABLE 1. EFFECT OF VARIOUS INHIBITORS ON LIVER URIDINE KINASE*

Changed activity				No effects		
		Uridine kinase				
Compound	Dose level (mg/kg)	(μmoles/g per hr ± S.E.)	(%)	Compound	Dose level (mg/kg)	Uridine kinase (μmoles g per hr ± S.E.)
Control		1·12 ± 0·12	100	Cytosine arabinoside	200	1·16 ± 0·11
5-Azacytidine	15	2·45 ± 0·18	219	Hydroxyurea	200	1·03 ± 0·07
Streptimidon	2·5	1·31 ± 0·14	117	Mitomycin C	10	1·06 ± 0·08
Gougerotin	2·5	1·46 ± 0·17	130	5-Bromo-2′-deoxyuridine	60	1·17 ± 0·16
Cycloheximide	2·5	2·37 ± 0·18	212	5-Iodo-2′-deoxyuridine	60	1·09 ± 0·14
Streptovitacin A	0·2	2·35 ± 0·14	210	5-Fluoro-2′-deoxyuridine	20	1·13 ± 0·07
Pactamycin	2·5	2·49 ± 0·28	222	5-Aza-2′-deoxycytidine	25	1·10 ± 0·10
Adriamycin	5	1·64 ± 0·13	147	5-Fluorouridine	25	1·07 ± 0·10
Daunorubicin	5	1·87 ± 0·20	167	5-Azaorotate	80	1·03 ± 0·10
Thioacetamide	200	2·66 ± 0·17	238	5-Fluoroorotate	60	1·10 ± 0·07
Actinomycin D	0·5	0·84 ± 0·09	75	6-Azauridine	100	1·05 ± 0·16
Puromycin	25	0·80 ± 0·09	71	6-Azacytidine	100	1·12 ± 0·12

* Compounds were administered i.p. to groups of four to nine male rats (175–185 g) 24 hr before killing. Uridine kinase was assayed in cell-free liver extracts as described in Methods.

process of uridine kinase enhancement remained unaffected (Table 2). In agreement with this finding, not only glucagon but also hydrocortisone (20 mg/kg), administered 24 and 3 hr before killing, alone or in combination with cycloheximide, were without effect on hepatic uridine kinase in intact or adrenalectomized rats. The daily administration of hydrocortisone (4 days, 10 mg/kg daily) was also without effect. Also L-tryptophan,[16,17] another enzyme inducer, given orally or intraperitoneally (1 g/kg) failed to influence the activity of uridine kinase.

TABLE 2. CYCLOHEXIMIDE-MEDIATED INCREASE OF URIDINE KINASE ACTIVITY IN THE LIVER OF INTACT AND ADRENALECTOMIZED RATS*

Conditions	Uridine kinase (μmoles/g liver per hr ± S.E.)			
	Intact	(%)	Adrenalectomized	(%)
Control	1·28 ± 0·16	100	1·37 ± 0·13	100
Cycloheximide	2·42 ± 0·21	189	2·34 ± 0·19	171
Glucagon	1·25 ± 0·18	98	1·30 ± 0·16	95
Cycloheximide + glucagon	2·63 ± 0·26	206	2·60 ± 0·20	190

* Groups of four to six male intact and adrenalectomized rats (175 g) 3 days after operation were given cycloheximide (1·2 and 0·6 mg/kg respectively) 24 hr before killing. Glucagon (1·5 mg/kg) or saline was injected i.p. 3 and 24 hr before killing. Uridine kinase activity was assayed in cell-free liver extracts as described in Methods.

Different tissues and organisms[18–22] have been used for the studies on uridine kinase. The activity in kidney, spleen and especially thymus, calculated per g of tissue wet wt, is higher than that in the liver (Table 3). We were interested to know to what extent drugs active in the liver would modify the activity of uridine kinase in other tissues. From our earlier work it is known that 5-azacytidine depresses the activity of the thymus enzyme.[7] Data presented in Table 3 show that cycloheximide also leads to the impairment of uridine kinase in the thymus, while daunorubicin and thioacetamide were much less effective. The activity of uridine kinase in kidney and spleen is practically unchanged. A similar pattern of uridine kinase modification has been observed in mouse tissues (unpublished).

Uridine kinase activity is generally considered to reflect the relative efficiency of the cell or tissue in utilizing preformed pyrimidine precursors by the salvage pathway. Metabolic transformation of orotic acid is of primary importance during the synthesis of pyrimidines *de novo*. For this reason we have studied the effect *in vivo* of the drugs

TABLE 3. TISSUE SPECIFICITY OF THE STIMULATORY EFFECT OF VARIOUS DRUGS ON URIDINE KINASE*

Administered dose (mg/kg)	Uridine kinase (μmoles/g tissue per hr)			
	Liver	Kidney	Thymus	Spleen
Control	0·88 ± 0·10	3·17 ± 0·30	4·02 ± 0·36	3·57 ± 0·21
5-Azacytidine (25)	1·86 ± 0·16	3·22 ± 0·23	2·16 ± 0·13	2·76 ± 0·13
Cycloheximide (2·5)	1·83 ± 0·14	3·52 ± 0·26	2·32 ± 0·17	3·51 ± 0·25
Daunorubicin (5·0)	1·52 ± 0·18	3·31 ± 0·33	3·44 ± 0·20	2·83 ± 0·19
Thioacetamide (200)	1·92 ± 0·17	3·28 ± 0·27	3·43 ± 0·27	3·48 ± 0·08

* Compounds were administered i.p. to groups of four male rats (175 g) 24 hr before killing. Uridine kinase was assayed in cell-free tissue extracts during a 10-min incubation period as described in Methods.

TABLE 4. EFFECT OF VARIOUS DRUGS *in vivo* ON THE SYNTHESIS OF PYRIMIDINES *de novo* AND URIDINE KINASE ACTIVITY IN THE CELL-FREE LIVER EXTRACT*

Administered dose (mg/kg)	Transformation of orotic acid		Uridine kinase	
	(μmoles/g liver per hr ± S. E.)	(%)	(μmoles/g liver per hr ± S. E.)	(%)
Control	41·3 ± 3·8	100	1·22 ± 0·12	100
5-Azacytidine (25)	27·5 ± 1·9	66·5	2·69 ± 0·18	222
5-Azaorotate (25)	22·8 ± 2·4	55·1	1·27 ± 0·14	105
Cycloheximide (2·5)	43·0 ± 4·2	104	2·54 ± 0·22	208
Thioacetamide (200)	47·2 ± 3·6	114	2·84 ± 0·11	233
Daunorubicin (5·0)	41·3 ± 4·6	100	1·86 ± 0·15	153

* Compounds were administered i.p. to groups of four to six male rats (175–185 g) 24 hr before killing. Transformation of 1×10^{-4} M orotic acid and uridine kinase activity in cell-free liver extracts were assayed as described in Methods.

that increase hepatic uridine kinase on the pyrimidine synthesis in cell-free liver extract. The data presented in Table 4 show that the enhancement of uridine kinase is not related to the decreased pyrimidine biosynthesis *de novo*: (1) both 5-azacytidine and 5-azaorotic acid inhibit the transformation of orotic acid to uridine 5′-monophosphate,[23,24] while only 5-azacytidine leads to the increase of uridine kinase; (2) cycloheximide, thioacetamide and daunorubicin have no effect on the synthesis of pyrimidines *de novo* from orotic acid and result in the enhancement of uridine kinase in the liver.

DISCUSSION

The molecular mechanism responsible for the enhancement of hepatic uridine kinase activity after administration of various drugs, possessing differing modes of action, is at present unknown. It is possible that the degradation of uridine kinase is impaired.[7] This mechanism has been demonstrated in studies on liver tyrosine aminotransferase after the administration of various compounds including cycloheximide[25,26] and 5-azacytidine.[27,28] Uridine kinase, however, is not increased by many other compounds known to inhibit the degradation of liver enzymes (e.g. L-tryptophan[16,17]). Since cycloheximide and related glutarimide antibiotics also increase the activity of uridine kinase in the liver, it is permissible to suppose that there is no increase of enzyme synthesis *de novo*, although direct immunologic evidence is missing due to the difficulty of obtaining uridine kinase of sufficient purity. One can assume the preferential inhibition of the liver degradation system resulting in the increase of hepatic uridine kinase. The question remains, however, why another inhibitor of protein synthesis, puromycin, displays no stimulatory effect on this enzyme (Table 1). Experiments are in progress to define in more detail the molecular mechanism responsible for this phenomenon.

Cycloheximide, streptovitacin A and pactamycin inhibit protein synthesis, acting at various stages of polypeptide chain formation;[29–32] as a result, the synthesis of RNA is inhibited.[33] The 5-azacytidine-mediated inhibition of protein synthesis in rat liver seems to be a secondary effect, and is attributable to the enhanced degradation of liver polyribosomes.[34,35] Daunorubicin and adriamycin, antitumor antibiotics of the anthracycline group, produce only moderate increases of uridine kinase. Both

drugs inhibit DNA and RNA synthesis[36–40] by binding to DNA. Unlike the above compounds, thioacetamide is hepatotoxic and carcinogenic and does not affect liver protein synthesis. This drug increases the concentration of RNA in the nucleoli[41,42] and markedly enhances the rate of uridine 5′-triphosphate incorporation into nucleolar RNA.[43]

Recently it has been found that the activity of ornithine decarboxylase involved in polyamine synthesis is strongly increased in thioacetamide-treated animals.[44–46] The level of polyamines is known to be highest in rapidly growing tissues, as is the activity of uridine kinase. However, 5-azacytidine blocks the increase of ornithine decarboxylase observed during liver regeneration[47] and during the development of mouse leukemia.[48,49] It is presumptive to correlate the increase of uridine kinase with the possible enhancement of the proliferative activity of the liver after administration of the compounds mentioned above. Although the synthesis of polyamines that is characteristic of rapidly proliferating systems is inhibited by 5-azacytidine, the administration of the drug to rats prior to partial hepatectomy results in increased mitotic activity and enhanced DNA synthesis in regenerating liver.[50] We cannot thus exclude the possibility that the molecular mechanism responsible for the enhancement of uridine kinase is different depending on the particular compound used.

Uridine kinase induced following 5-azacytidine administration differs from the control enzyme preparation in its increased stability to heating.[7] It is not known whether a similar change in the enzyme stability also occurs after administration of the metabolic inhibitors used in this study. The finding of two different molecular forms of uridine kinase in rat liver and different hepatomas[22] led us to propose that the administration of 5-azacytidine changes the relation of the two uridine kinase species in favor of the more heat-stable low molecular isozyme normally prevailing in embryonic rat liver. However, comparing embryonic and adult rat livers, we were unable to confirm the existence of multiple forms of uridine kinase.[51] Moreover, the administration of 5-azacytidine depressed liver uridine kinase activity in embryos.

Tumors are characterized by high uridine kinase activity.[52,53] The deletion of this enzyme in tumor cells is associated with the development of resistance to uridine and cytidine analogues.[3–5] Unfortunately, the increase of uridine kinase activity described here has been detected only in the liver. If it were possible to elucidate the mechanism responsible for the enhancement of uridine kinase, and to produce this effect selectively in hepatoma cells, the drugs might then be utilized more effectively in combination schedules.

REFERENCES

1. C. A. Pasternak, G. A. Fischer and R. E. Handschumacher, *Cancer Res.* **21**, 110 (1961).
2. J. Veselý, A. Čihák and F. Šorm, *Int. J. Cancer* **2**, 639 (1967).
3. P. Reichard, O. Sköld, G. Klein, L. Révész and P. H. Magnusson, *Cancer Res.* **22**, 235 (1962).
4. G. B. Elion and G. H. Hitchings, *Adv. Chemother.* **2**, 91 (1965).
5. J. Veselý and A. Čihák, *Oncology* **28**, 204 (1973).
6. A. Čihák, M. Seifertová and J. Veselý, *Archs. Biochem. Biophys.* **148**, 400 (1972).
7. A. Čihák and J. Veselý, *J. biol. Chem.* **248**, 1307 (1973).
8. A. Čihák and J. Černá, *FEBS Lett.* **23**, 271 (1972).
9. J. Kára, F. Šorm and A. Winkler, *Neoplasma* **10**, 3 (1963).
10. A. Čihák and J. Brouček, *Biochem. Pharmac.* **21**, 2497 (1972).
11. C. Peraino, R. L. Blake and H. C. Pitot, *J. biol. Chem.* **240**, 3039 (1965).
12. H. Schmidinger and H. Kröger, Hoppe-Seyler's *Z. physiol. Chem.* **348**, 1367 (1967).

13. F. Rosen and R. J. Milholland, *Enzyme Synthesis and Degradation in Mammalian Systems*. Karger, Basel (1971).
14. T. D. Gelehrter, *Metabolism* **22**, 85 (1973).
15. S. Fiala and E. Fiala, *Biochim. biophys. Acta* **103**, 699 (1965).
16. R. T. Schimke, E. W. Sweeney and C. M. Berlin, *J. biol. Chem.* **240**, 4609 (1965).
17. A. Čihák, C. Lamar and H. C. Pitot, *Archs Biochem. Biophys.* **156**, 188 (1973).
18. O. Sköld, *J. biol. Chem.* **235**, 3273 (1960).
19. E. P. Anderson and R. W. Brockman, *Biochim. biophys. Acta* **91**, 380 (1964).
20. J. Seifert and J. Riman, *Neoplasma* **13**, 391 (1966).
21. A. Orengo, *J. biol. Chem.* **224**, 2204 (1969).
22. G. Krystal and T. E. Webb, *Biochem. J.* **124**, 943 (1971).
23. A. Čihák and F. Šorm, *Biochem. Pharmac.* **21**, 607 (1972).
24. A. Čihák, C. Garret and H. C. Pitot, *Eur. J. Biochem.* **34**, 68 (1973).
25. F. T. Kenney, *Science, N.Y.* **156**, 525 (1967).
26. B. Szepesi and R. A. Freedland, *J. Nutr.* **97**, 255 (1969).
27. I. B. Levitan, H. P. Morris and T. E. Webb, *Biochim. biophys. Acta* **240**, 287 (1971).
28. A. Čihák, D. Wilkinson and H. C. Pitot, *Adv. Enzyme Regulat.* **9**, 267 (1971).
29. A. C. Trakatellis, M. Montjar and A. E. Axelrod, *Biochemistry, N.Y.* **4**, 2065 (1965).
30. L. Felicetti, B. Colombo and C. Baglioni, *Biochim. biophys. Acta* **119**, 120 (1966).
31. T. G. Obrig, W. J. Culp, W. L. McKeehan and B. Hardesty, *J. biol. Chem.* **246**, 174 (1971).
32. L. S. Kappen, H. Suzuki and I. H. Goldberg, *Proc. natn. Acad. Sci. U.S.A.* **70**, 22 (1973).
33. M. Muramatsu, N. Shimada and T. Higashinakagawa, *J. molec. Biol.* **53**, 91 (1970).
34. A. Čihák and J. Veselý, *Biochem. Pharmac.* **21**, 3257 (1972).
35. A. Čihák, L. M. Narurkar and H. C. Pitot, *Colln Czech. chem. Commun.* **38**, 948 (1973).
36. A. DiMarco, R. Silvestrini, S. DiMarco and T. Dadia, *J. Cell Biol.* **27**, 545 (1965).
37. A. Rusconi and A. DiMarco, *Cancer Res.* **29**, 1507 (1969).
38. W. D. Meriwether and N. R. Bachur, *Cancer Res.* **32**, 1137 (1972).
39. K. Dano, S. Frederiksen and P. Hellung-Larsen, *Cancer Res.* **32**, 1307 (1972).
40. F. Zunino, R. Gambetta, A. DiMarco and A. Zaccara, *Biochim. biophys. Acta* **277**, 489 (1972).
41. H. R. Adams and H. Busch, *Cancer Res.* **23**, 576 (1963).
42. W. J. Steele, N. Okamura and H. Busch, *J. biol. Chem.* **240**, 1742 (1965).
43. J. G. Villalobos, W. J. Steele and H. Busch, *Biochim. Biophys. Acta* **91**, 233 (1964).
44. N. Fausto, *Biochim. biophys. Acta* **190**, 193 (1969).
45. N. Fausto, *Cancer Res.* **30**, 1947 (1970).
46. M. Ono, H. Inoue and Y. Takeda, *Biochim. biophys. Acta* **304**, 495 (1973).
47. E. Cavia and T. E. Webb, *Biochim. biophys. Acta* **262**, 546 (1972).
48. O. Heby and D. H. Russell, *Cancer Res.* **33**, 159 (1973).
49. O. Heby and D. H. Russell, *Polyamines in Normal and Neoplastic Growth*, p. 221. Raven Press, New York (1973).
50. A. Čihák, M. Seifertová, J. Veselý and F. Šorm, *Int. J. Cancer* **10**, 20 (1972).
51. J. Veselý and A. Čihák, *Biochem. J.* **133**, 609 (1973).
52. R. J. Rutman, A. Cantarow and K. S. Paschkis, *Cancer Res.* **14**, 119 (1954).
53. C. Heidelberger, K. C. Liebman, E. Habers and P. M. Bhargava, *Cancer Res.* **17**, 399 (1957).