STIMULATION OF THYMIDYLATE KINASE ACTIVITY IN RAT TISSUES BY THYMIDINE ADMINISTRATION*

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Regenerating rat liver is known to contain systems which convert thymidylic acid (TMP) (Hecht et al, 1954), as well as other deoxynucleotides to their corresponding triphosphates, whereas normal adult liver is reported to be capable of phosphorylating the deoxynucleotides of adenine, guanine, and cytosine, but not of thymine (Canellakis and Mantsavinos, 1958). It has been postulated (Canellakis et al, 1959) that thymidylate kinase may be the site which controls DNA synthesis in regenerating liver, in contrast to normal liver in which little or no DNA is formed. We have demonstrated changes in thymidylate kinase activity in rat tissues during neonatal life. We have also observed marked alterations in activity in rat liver during the regenerative process, as previously reported by Bollum and Potter (1959). A several-fold stimulation of kinase activity has been found in resting rat liver and kidney following thymidine injection.

Male Wistar rats weighing 250-300 grams were used for the studies of liver regeneration and of the effects of thymidine injection; no attempt was made to ascertain the sex of the fetal and neonatal rats. Animals were fasted for 18 hours prior to sacrifice by decapitation.

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Tissues were disrupted in a glass homogenizer in 5 volumes of .05 M Tris buffer, pH 7.5, and centrifuged at 14,000 x g for 10 minutes. (Enzyme activity was found to be similar in extracts centrifuged at 100,000 x g for 1 hour.) TMP^{32} was prepared by the chemical procedure of Hurwitz (1959) for the synthesis of CMP^{32}. Further purification of the 5'nucleotide, which was used in the assay, was achieved by chromatography on Dowex-1-formate. The assay was that described by Lehman et al (1958), except that 40 mμmoles of TMP^{32} were used in the incubation with tissue extract, and the charcoal was collected, washed, and counted on membrane filters. A unit of enzyme is defined as that amount which renders 100 mμmoles of TMP^{32} resistant to semen phosphomonoesterase in 1 hour under our assay conditions. Specific activity is expressed in units per gram of protein, determined by the biuret procedure (Gornall et al, 1949). Paper chromatography (descending) in isopropanol:20% TCA: concentrated NH_4OH (75:25:0.25) followed by radioautography provided evidence that the phosphomonesterase-resistant, charcoal adsorbable radioactive material produced during incubation with liver extracts from normal, thymidine-treated, and partially hepatectomized rats was thymidine di-and triphosphates. The latter was present in several times the amount of the former.

The data in Table I indicate that high levels of thymidylate kinase activity are present in liver and kidney during fetal and neonatal life. In marked con-

Table I

THYMIDYLATE KINASE ACTIVITY IN FETAL AND NEONATAL RAT TISSUES

Age	Liver	Kidney
	(Units per gram protein)	
Fetal	200-400	
1-2 days	96;128;152;159	276
5-11 days	128;140;181;243	159;166;393
14-17 days	30;45;75	34;200
21 days	23;45	
Adult	7-13	1-10

trast, very low (but detectable) levels are present in adult liver. As is apparent from Figure 1, kinase activity rises sharply beginning about 18 hours following partial hepatectomy, remains elevated for several hours, and by 120 hours returns to resting levels. Similar observations have been reported by Bollum and Potter (1959), and Hecht and Potter (1956) have shown that this is also the period of maximal DNA synthesis. Our finding (Hiatt and Bojarski, 1960) that the rise in kinase activity in regenerating liver can be prevented

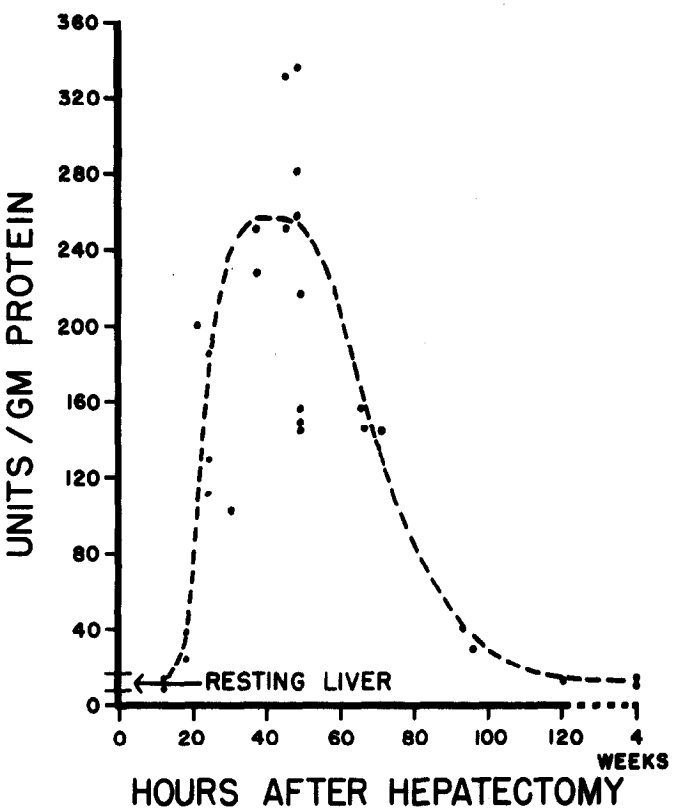

Figure 1. Thymidylate kinase activity in rat liver following partial hepatectomy.

by 5-fluorouracil (5-FU) together with the report of Cohen et al (1958) that this antimetabolite interferes with the conversion of deoxyuridylic acid to TMP suggested that TMP might be necessary for the appearance of TMP kinase. Subsequent studies demonstrated that the administration of thymidine, which presumably is converted to TMP by a mechanism not involving the 5 FU-sensitive

Table II

THYMIDYLATE KINASE ACTIVITY IN TISSUES OF NORMAL RATS FOLLOWING THYMIDINE ADMINISTRATION

Hours after first thymidine	Liver	Kidney
	(Units per gram protein)	
1/60	17	7
1	29	52
2	45	39
3	65	84
4	98	131
6	65	111

100 mg. of thymidine in 2 ml. distilled water was injected intraperitoneally at 0 time, and then hourly until 1 hour prior to sacrifice. The first rat was sacrificed 1 minute after thymidine administration. Uridine injections had no effect on kinase activity. Thymidine added to the assay mixture did not stimulate kinase activity.

system, results in the appearance of TMP kinase activity not only in the livers of 5 FU-treated hepatectomized rats (Hiatt and Bojarski, 1960), but in the livers and kidneys of untreated normal animals as well (Table II). If one assumes that the administered thymidine is converted to TMP within the cell, then the presence of the deoxynucleotide would appear to be a sufficient, as well as a necessary condition for the increase of kinase activity observed in rat liver and kidney. These data also suggest that a regulatory mechanism for DNA synthesis in regenerating rat liver exists at a reaction earlier than that involving phosphorylation of TMP. Studies of the process leading to increased thymidylate kinase activity, including efforts at enzyme purification are now in progress.

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References

Bollum, F. J., and Potter, V. R., Cancer Research, 19, 561 (1959).

Canellakis, E. S., Jaffe, J. J., Mantsavinos, R., and Krakow, J. S., J. Biol. Chem., 234, 2096 (1959).

Canellakis, E. S., and Mantsavinos, R., Biochim. et Biophys. Acta, 27, 643 (1958).

Cohen, S. S., Flaks, J. G., Barner, H. D., Loeb, M. R., and Lichtenstein, J., Proc. Nat. Acad. Sci., 44, 1004 (1958).

Gornall, A. G., Bardawill, C. S., and David, M. M., J. Biol. Chem., 177, 751 (1949)

Hecht, L. I., and Potter, V. R., Cancer Research, 16, 988 (1956).

Hecht, L. I., Potter, V. R., and Herbert, E., Biochim.et Biophys. Acta, 15, 134 (1954).

Hiatt, H. H., and Bojarski, T. B., Fed. Proc., 1960, submitted for publication.

Higgins, G. M , and Anderson, R. M., Arch. Path., 12, 186 (1931).

Hurwitz, J., J. Biol. Chem., 234, 2351 (1959).

Lehman, I. R., Bessman, M. J., Simms, E. S., and Kornberg, A., J. Biol. Chem., 233, 163 (1958).