COMPARISON OF THE EFFECTS OF X-RADIATION ON THE ELEVATION OF THYMIDINE KINASE AND THYMIDYLATE SYNTHETASE DURING LIVER REGENERATION[1]

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Received July 27, 1962

Increases in the activities of DNA[2] polymerase and of several enzymes that function in the biosynthesis and conversion of pyrimidine deoxyribotides to thymidine triphosphate are prevented in regenerating liver by prior exposure of partially hepatectomized rats to x-radiation (Stevens and Stocken, 1962; Myers et al., 1961; Bollum et al., 1960; van Lancker, 1960; Beltz and Applegate, 1959). Radiation administered up to 13 hr. postoperatively prevents the subsequent appearance of DNA polymerase and of kinases that phosphorylate thymidine and thymidylate, whereas irradiation between 14 and 17.5 hr. has been reported to be uninhibitory (Bollum et al., 1960). In either case the initiation of DNA synthesis is blocked (Beltz et al., 1957).

These results suggested that exposure to x-radiation between 14 and 17.5 hr. after surgery might prevent the onset of DNA synthesis in regenerating liver by interfering with the accumulation of one or more additional enzymes required to initiate and sustain DNA synthesis. Thymidylate synthetase was

[1]This investigation was supported by a Senior Research Fellowship (SF 191) from the Public Health Service and by a research grant (C-5066) from the National Cancer Institute, Public Health Service.

[2]Abbreviations: DNA, deoxyribonucleic acid; TMP, (deoxy) thymidine 5'-monophosphate; dCMP, deoxycytidine 5'-monophosphate; Tris, tris(hydroxymethyl)aminomethane; NAD, nicotinamide adenine dinucleotide.

chosen as a likely possibility because its content is elevated approximately 4-fold in regenerating liver by 24 hr. after partial hepatectomy (Maley and Maley, 1960; Beltz, 1962), and because it has been shown that inhibition of TMP synthetase by 5-fluoro-2'-deoxyuridine monophosphate effectively blocks DNA synthesis (Hartmann and Heidelberger, 1961; Cohen et al., 1958; Bosch et al., 1958; Taylor et al., 1962). A further incentive for determining whether processes leading to the elevation of TMP synthetase are susceptible to x-ray inhibition has been provided by the recent work of Myers (1962), which demonstrates clearly that the effects of x-radiation are highly selective. Out of twenty-four enzymes assayed at various times after partial hepatectomy, the accumulation of only three, i.e., dCMP deaminase, thymidine phosphorylase and NAD pyrophosphorylase, was inhibited by exposure of the animals to x-radiation (1,500 r) just before surgery.

Methods - Male albino rats[3] (180 - 220 g.) were partially hepatectomized in groups of three, fasted and sacrificed, and a 60,000 x g liver supernatant fraction ($S^{50\%}$) was prepared in isotonic KCl from the combined regenerating liver lobes of each group as described previously (Beltz, 1961). Under the conditions of an earlier experiment (Beltz and Applegate, 1959) two groups of rats were exposed to x-radiation (1,500 r; 24 r/min.) 6 - 7 hr. after partial hepatectomy, and another two groups were similarly exposed 15 - 16 hr. postoperatively. Each supernatant fraction was assayed for thymidine kinase, as well as TMP synthetase, in order to obtain an independent measurement of the effectiveness of the radiation, and to provide a basis for comparing the results of these experiments with the data reported by Bollum et al. (1960). The kinase assay was carried out using tritiated thymidine as previously described (Beltz, 1961), except that self-absorption corrections with internal

[3] Holtzman Company, Madison, Wisconsin.

standards were omitted, inasmuch as semi-quantitative measurements were considered adequate to distinguish between the complete inhibition of kinase elevation expected after early (6 - 7 hr.) x-irradiation, and the total absence of inhibition expected after late (15 - 16 hr.) irradiation on the basis of the results of Bollum et al. (1960).

Thymidylate synthetase was assayed by measuring the total yield of C^{14} in thymine after incubating deoxyuridylate and formaldehyde-C^{14} with supernatant fraction for 2 hr., under argon, in the presence of tetrahydrofolate, sodium fluoride and Tris buffer pH 7.5. Reactions were terminated by addition of 12 N HCl, carrier TMP was added, and the samples were hydrolyzed 1 hr. at 100°. Thymine was isolated by tandem ion-exchange and paper chromatography, plated at infinite thinness, and counted to a 0.95 error of ±5% in a windowless flow counter. The specific activity of each sample of radioactive thymine was determined, and the total yield of C^{14} in thymine was calculated from the total number of μmoles of thymine added as carrier TMP. Details of the procedure will be reported elsewhere (Beltz, 1962).

Results - The activities of thymidine kinase and TMP synthetase in supernatant fractions prepared from 24.5 hr. regenerating livers of irradiated and unirradiated rats are compared in Table I. Kinase activity in the livers of rats irradiated 6 - 7 hr. postoperatively was less than 3 per cent of the kinase activity in the livers of unirradiated controls. In the livers of rats exposed to x-radiation 15 - 16 hr. postoperatively, kinase activity approached 15 per cent of that of unirradiated controls. In contrast to these results, the activity of TMP synthetase in livers of irradiated rats averaged 75 per cent of that in livers of unirradiated controls, and the effect of early irradiation appeared to be somewhat less than the effect of late irradiation.

TABLE I

EFFECTS OF X-RADIATION ON THE ELEVATION OF THYMIDYLATE SYNTHETASE AND THYMIDINE KINASE DURING LIVER REGENERATION

Experiment	Sacrificed[a] (hr. after surgery)	Synthetase activity: C^{14} into thymine, c.p.m.[b]	Kinase activity: H^3 into thymidine phosphates c.p.m./plate[c]
Control	24.5 (3)	5,736	696
	24.5 (3)	5,012	639
Irradiated 15 - 16 hr. (1,500 r.)	24.5 (3)	-----	59
	24.5 (3)	3,638	97
Irradiated 6 - 7 hr. (1,500 r.)	24.5 (3)	3,889	16
	24.5 (3)	4,532	18
Control	16 (3)	1,433	32
	6 (3)	1,396	27

[a] Numbers in parentheses refer to the number of animals sacrificed and the number of regenerating livers pooled.

[b] Reaction mixture: 1.93 μmoles formaldehyde-C^{14} (0.27 μcurie); 1.5 μmoles deoxyuridylate; 0.15 ml. of liver supernatant fraction ($S^{50\%}$); 2.0 μmoles tetrahydrofolate; 50 μmoles Tris buffer pH 7.5; 4.0 μmoles NaF; final volume 0.6 ml. Tubes were incubated at 37° for 2 hr. under argon. The uptake of C^{14} into thymine was proportional to the volume of $S^{50\%}$ added up to 0.2 ml. (unpublished data).

[c] Reaction mixture: 0.1 μmole thymidine-H^3 (0.82 μcurie); 0.15 ml. of liver supernatant fraction ($S^{50\%}$) diluted with 1.5 vol. isotonic KCl; 100 μmoles Tris buffer pH 8.0; 3.0 μmoles 3-phosphoglycerate; 2.5 μmoles $MgSO_4 \cdot 7H_2O$; 2.5 μmoles ATP; final volume 0.5 ml. Tubes were incubated at 37° for 30 min. Thymidine phosphates were isolated by paper chromatography in a single band at the origin, eluted, and aliquots of the eluate were plated and counted (see text).

In order to compare the 6 - 24.5 hr. and 16 - 24.5 hr. transitional increases of enzyme activity in irradiated and control groups, livers of partially hepatectomized rats were excised 6 hr., and 16 hr. after surgery, and supernatant fractions were assayed for kinase and synthetase activity (Table I).

The data of Table I show that elevation of thymidine kinase is prevented completely by early irradiation, whereas the activity of TMP synthetase is elevated approximately 3-fold in spite of early exposure to x-radiation.

Discussion - The failure of early or late x-irradiation to prevent the elevation of TMP synthetase rules out the possibility that prevention of the initiation of DNA synthesis by prior x-irradiation could be attributed to interference with the activity of TMP synthetase. The sizable increase of synthetase activity in irradiated animals is also significant in view of the proposal by Maley and Maley (1960) that the elevation of TMP synthetase activity during liver regeneration may depend upon prior elevation of the level of dCMP deaminase. The present experiments appear to rule out such a possibility, inasmuch as the activity of dCMP deaminase was found to be the same in supernatant fractions prepared from 6 hr., 16 hr. and 24.5 hr. regenerating livers of rats irradiated 6 - 7 hr. after partial hepatectomy (unpublished data). Furthermore, Stevens and Stocken (1962) and Myers et al. (1961; 1962) have recently demonstrated that exposure of partially hepatectomized rats to x-radiation shortly before or after surgery blocks the elevation of dCMP deaminase in regenerating liver.

Interference with the accumulation of thymidine kinase by late x-irradiation (15 - 16 hr. postop.) was unexpected, because of the report by Bollum et al. (1960) that x-radiation administered after 14 hr. does not affect the appearance of thymidine and thymidylate kinases. However, if a radiosensitive process in the over-all mechanism leading to the elevation of thymidine kinase is triggered and completed prior to elevation of the kinase activity, as the data of Bollum et al. seem to indicate, variation in the timing of such an event under differing experimental conditions could account for the conflicting results.

The striking difference in the radiosensitivity of the kinase-elevating system and the synthetase-elevating system suggest that they may differ in a fundamental manner. Whether or not the postoperative elevation of either, or both of these enzymes is due to net protein synthesis would be of interest to determine.

Acknowledgement: The author is indebted to Dr. Paul H. Deeb, Dept. of Radiology, for making an X-ray machine available for these experiments, and to Mr. Ivar C. Woodward, Radiation Physicist, for the calibration.

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