X-RAY INHIBITION OF ENZYME CHANGES ASSOCIATED WITH THE DNA SYNTHESIS OF LIVER REGENERATION

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The DNA synthesis of liver regeneration in the rat is initiated approximately 18 hours after partial hepatectomy and is accelerated to a maximal rate by 24 hours after surgery (Hecht and Potter, 1956, 1958). The onset and acceleration of DNA synthesis in vivo is accompanied by an ultimately large, and apparently simultaneous increase in the activities of thymidine phosphorylating enzymes and deoxynucleoside triphosphate polymerizing enzyme, measured in high-speed supernatant fractions of homogenates of regenerating livers (Bollum and Potter, 1959). In supernatant fractions of normal livers the activities of these kinases and of polymerase are lower by factors of 10, and 25 or more, respectively, than in supernatant fractions of 23-hour regenerating livers (Bollum, 1958; Bollum and Potter, 1959). DNA synthesis measured as incorporation of tritiated thymidine into acceptor DNA in homogenates is very low in 0 and 15-hour rat liver homogenates, but high in 24-hour homogenates; thus, the time course of DNA synthesis in vivo is reflected in the capacity of liver cell fractions to incorporate thymidine into DNA in vitro (Bollum and Potter, 1957, 1958). These data could be taken to indicate that key enzymes required in biosynthetic pathways leading to the production and polymerization of the deoxynucleoside triphosphates are not present in significant amounts in normal liver, and that DNA synthesis begins in regenerating liver as stimulated cells synthesize these enzymes from amino acids and/or produce them by zymogen activation.

Abbreviations: DNA, deoxyribonucleic acid; DATP, DGTP, DCTP and TTP, 5'-triphosphates of deoxyadenosine, deoxyguanosine, deoxycytidine and thymidine; ATP, adenosine triphosphate; Tris, tris(hydroxymethyl)aminomethane.

It has been shown previously that exposure of rats to X-ray irradiation of the whole body (1500 r) at any time between 11 hours before, and 17.5 hours after partial hepatectomy, effectively prevents the initiation of DNA synthesis. The same dose administered 28 hours postoperatively, while the first wave of DNA synthesis accompanying liver regeneration is in progress, inhibits DNA synthesis only to a small degree (Beltz, van Lancker and Potter, 1957). One could interpret these data on the basis that the ionizing radiation inhibits some part of the mechanism involved in supplying key enzymes required for DNA synthesis, whereas the active enzymes, once formed, are relatively insensitive to radiation. Evidence has now been obtained that the production of enzymes specifically required for the incorporation of thymidine into DNA is blocked by X-irradiation.

Experimental. Eight male albino rats weighing 180 - 220 grams were partially hepatectomized and divided into two groups of four each. One group was exposed to 1500 roentgens of whole body X-irradiation* between 12 and 13.5 hours postoperatively, and all the animals of both groups were sacrificed 25 hours after surgery. The livers were quickly removed, chilled in ice-cold buffered sucrose solution (Bollum and Potter, 1958) and homogenized in pairs in 4 volumes of the buffered sucrose medium. Supernatant fractions were prepared from each homogenate by centrifuging for 60 minutes at 105,000 x g in the Spinco preparative ultracentrifuge, and incubations were carried out in 15 ml. centrifuge tubes. Reactions were started by placing the tubes in a 37° water bath and terminated by cooling to 0° and adding cold trichloroacetic acid to a final concentration of 5 per cent. Isolation, plating and analysis of the DNA from acid-washed precipitates were carried out essentially as described by Bollum (Bollum, 1958). Radioactivity was assayed in a windowless flow counter with an over-all counting efficiency of 30 per cent. Corrections for self-absorption were not made because approximately the same amount of DNA was placed on each plate, and only relative values were sought.

It is apparent that this experiment measures the capacity of the 25-hour liver supernatant fraction to incorporate thymidine into DNA under conditions requiring only the presence of polymerase, and the kinases which convert thymidine to thymidine-5'-triphosphate. The direct addition of DATP, DGTP and

* 175-kv. X-rays filtered through 0.5 mm. Cu and 1 mm. Al at a rate of 20 r/min. and a target distance of 72 cm.

TABLE I

INHIBITION OF INCORPORATION OF THYMIDINE-H^3 INTO DNA IN SUPERNATANT FRACTIONS OF HOMOGENATES OF 25-HOUR REGENERATING LIVERS OF PARTIALLY HEPATECTOMIZED RATS PREVIOUSLY EXPOSED TO 1500 ROENTGENS OF X-RADIATION

	Supernatant Fraction	Specific Activity of DNA (counts/min./mg.)*	
	(unirradiated controls)		(average)
2 rats	1	9,843	7,796
2 rats	2	5,750	
	(1500 roentgens)		
2 rats	1	354	551
2 rats	2	747	

Each tube contained in 1 ml. of reaction mixture: 0.05 μM each of DATP, DGTP and DCTP; 40 μM of Tris. HCl, pH 8; 6 μM of 3-P-glycerate; 5 μM each of ATP and $MgCl_2$; 0.5 mg. of highly polymerized DNA; 0.5 ml. of supernatant fraction; and 0.05 μM of tritiated thymidine (3.95 microcuries). All components were mixed at 0° and the tubes were incubated 1 hour at 37°.

*Specific activity minus the S. A. of DNA added to tubes which contained no DNA, but all other constituents, during the incubation, i.e., 128 c.p.m./mg.

DCTP rules out any requirement for the action of other kinases on corresponding deoxynucleosides and deoxynucleotides to prime the polymerization step of the reaction sequence. The results show that 25-hour supernatant fractions from livers of rats previously exposed to 1500 roentgens of X-radiation between 12 and 13.5 hours after partial hepatectomy possess less than 10 per cent of the capacity of the same tissue fractions from unirradiated controls for incorpororating thymidine-H^3 into DNA under these conditions. The effect of the X-radiation is to prevent the synthesis, or the production by zymogen activation, of one or more enzymes in the group of enzymes functioning to incorporate thymidine into DNA. When polymerase is assayed independently by measuring the incorporation of tritiated DCTP into DNA in the presence of TTP, DATP and DGTP, 1500 roentgens of X-irradiation administered as before inhibits polymerase activity less than 50 per cent (Beltz and Applegate, unpub. data).

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