Disturbance of RNA turnover in the cell nucleus by X-irradiation in the early phase of rat-liver regeneration

Several investigators[1-3] have shown that a rather low dose of total-body X-radiation could give a delay of DNA synthesis and mitosis in regenerating liver, if that dose was administered before DNA synthesis had started, *i.e.* in the so-called presynthetic period. In case of regeneration after partial hepatectomy this presynthetic period ends 15–18 h after operation[4,5] and is followed by a period of accelerated DNA synthesis with a maximum at about 24–30 h after operation. The first mitoses are seen at about 22–24 h after operation. The same low radiation dose given in the period of DNA synthesis had no immediate effect on the incorporation of precursors in DNA but could delay the onset of mitotic activity[2].

Preliminary results[6] of investigations on the effect of total-body X-irradiation on the metabolism of nuclear RNA in resting liver prompted us to study the sensitivity of nuclear RNA metabolism in the presynthetic period of liver regeneration after partial hepatectomy.

RNA turnover in the nucleus was studied by following the incorporation rate of ^{32}P between 0 and 28 h after operation. Seven groups of 4 male WAG-rats (about 200 g) were hepatectomized as described by HIGGINS AND ANDERSON[7]; at 0, 4, 8, 12, 16, 20 or 24 h after operation 200 μC ^{32}P (in 0.5 ml physiological saline containing 0.1 % Na_2HPO_4) were injected intravenously into each animal and 4 h later the remaining liver lobes of each group were pooled and analyzed by standard methods for the determination of the specific activity of DNA and of the mononucleotides of nuclear RNA.

The results of this experiment are shown in Fig. 1. The start of DNA synthesis

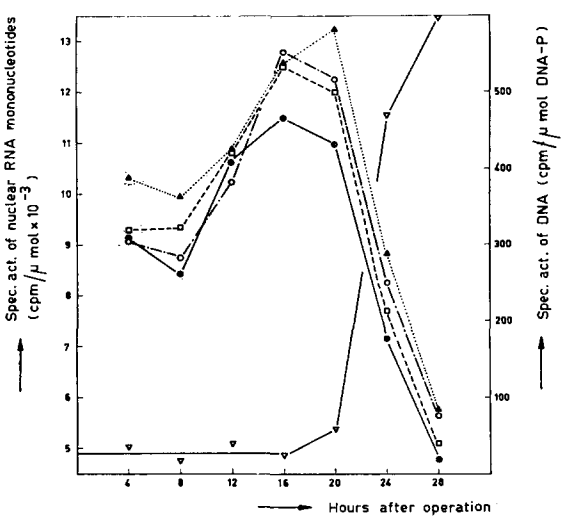

Fig. 1. Incorporation of ^{32}P in DNA and the monopnucleotides of nuclear RNA at various intervals after partial hepatectomy. ●—●, GMP; ○–·–·–○, UMP; □----□, CMP; ▲·····▲, AMP; ▽—▽, DNA.

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; CMP, cytidine monophosphate; AMP, adenosine monophosphate; GMP, guanosine monophosphate; UMP, uridine monophosphate.

coincides with the maximum of RNA turnover and it seems that synthesis of RNA must precede that of DNA.

It was found that irradiation with 700 R 12 h after partial hepatectomy resulted in a slight increase of the specific activity of nuclear RNA 4 h later. In subsequent experiments the irradiation was therefore carried out at shorter time intervals after operation, *i.e.* at 2, 4 and 6 h; moreover, nuclear RNA was fractionated into a fraction extractable from the nuclei with 1 *M* NaCl (n-RNA I) and a fraction not extractable with 1 *M* NaCl (n-RNA II).

The results of the irradiation experiments at short time intervals after operation are summarized in Table I. They show clearly that incorporation of ^{32}P into nuclear RNA is inhibited by ionizing radiation in the very early phase of liver regeneration.

TABLE I

DEPRESSION OF INCORPORATION OF ^{32}P INTO RNA OF RAT LIVER NUCLEI BY X-IRRADIATION IN THE EARLY PHASE OF REGENERATION AFTER PARTIAL HEPATECTOMY

Time of irradiation (h after operation)	*RNA fraction*	*Depression of specific activity in percent of the control for*			
		CMP	*AMP*	*GMP*	*UMP*
2	n-RNA I	40	29	40	41
2	n-RNA II	43	63	38	39
4	n-RNA I	50	58	54	57
4	n-RNA II	47	42	33	—
6	n-RNA I	24	20	17	9
6	n-RNA II	no depression			

6 groups of 4 animals were partially hepatectomized; 3 groups were irradiated with 700 R at respectively 2, 4 and 6 h after operation. Control and irradiated animals received at respectively 4, 6 and 8 h after operation 200 μC ^{32}P and were killed 2 h later. In one of these experiments the specific activities of cytoplasmic inorganic phosphate of irradiated and control animals were compared and found to be the same.

This observation combined with the fact that synthesis of RNA precedes that of DNA suggests that the inhibition of the latter by irradiation may be due to disturbance of the metabolism of nuclear RNA. The same interpretation is borne out by the results of BOLLUM *et al.*[8], who showed that radiation in the early stages of liver regeneration interferes with the production of enzymes required for DNA synthesis.

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