METABOLIC ALTERATIONS AFTER TOTAL BODY DOSES OF X-RADIATION

I. THE ROLE OF REGENERATING LIVER NUCLEI AND CYTOPLASM IN THE INHIBITION DUE TO X-RADIATION OF INCORPORATION OF TRITIUM-LABELED THYMIDINE INTO DNA

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SUMMARY

The effect of X-radiation on the incorporation of [^{3}H]TDR into the DNA of nuclei of reconstituted regenerating liver homogenates and into purified DNA incubated with irradiated and non-irradiated regenerating liver cytoplasm was studied. The results indicate that both the nuclei and the cytoplasm are affected by X-radiation but the effect is more marked on the cytoplasm than on the nucleus. These findings suggest that X-radiation interferes with the activity of the enzymes involved in the incorporation of nucleosides or nucleotides into the DNA.

INTRODUCTION

Inhibition of the incorporation of [^{3}H]TDR into the DNA of 30-h regenerating liver homogenates[1] obtained from rats irradiated with X-ray 6 h before sacrifice, suggests that the inhibitory effect occurs in the final steps of DNA biosynthesis, either through alteration of the primer or by interfering with the activity of the cytoplasmic enzymes involved in the synthesis of DNA. For all practical purposes, all the phosphorylating enzymes and polymerase activity can be recovered in the cytoplasmic fraction[2], and all the DNA can be found in the nuclear pellet[3] obtained after differential centrifugation of liver homogenate. Thus it is possible to study separately the effect of X-radiation on the activity of the cytoplasmic enzymes and on the nuclei, simply by recombining regenerating liver nuclei obtained from irradiated rats with regenerating liver cytoplasm obtained from non-irradiated animals, and *vice versa*. The purpose of this paper is to study the incorporation of [^{3}H]TDR in such reconstituted homogenates.

METHODS

Sprague-Dawley rats, weighing 180–200 g, were used in these experiments. The animals were hepatectomized according to the procedure of HIGGINS AND ANDERSON[4].

Abbreviations: [^{3}H]TDR, tritium labeled thymidine; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

The method of irradiation has already been described[1]. Tissue preparations were incubated according to the method of BOLLUM AND POTTER[3], slightly modified as described[1]. DNA was extracted, separated from RNA, and prepared for determination of specific activity[6]. Despite the fact that a self-absorption curve was determined for extracted [^{3}H]DNA, great care was taken to plate identical amounts of DNA in all experiments, and thus only minor corrections had to be applied. All incubations as well as all colorimetric and specific activity determinations were done in duplicate. In those experiments where nuclei and cytoplasm were recombined, 6 animals were hepatectomized for each experiment. 24 h later half the rats were irradiated with 1500 R and at 30 h or 48 h after hepatectomy all were sacrificed by decapitation. Livers from non-irradiated and from irradiated animals were pooled separately and prepared as 20 % homogenates in 0.25 *M* sucrose; the homogenates were then centrifuged at 600 × *g* for 10 min (4°). The nuclear pellet was washed 4 times with cold sucrose, but only the original cytoplasmic extract and the first washing were used to prepare the recombined homogenates or for incubation with purified DNA. Cytochrome oxidase, acid phosphatase and glucose-6-phosphatase activities and nitrogen were determined on the nuclear pellet by methods described previously[7] for the purpose of evaluating cytoplasmic contamination. The enzyme activities in the nuclear pellet never exceeded 5 % of the total activity in the whole homogenate. Conversely the DNA content of the cytoplasmic fraction[7] did not exceed 1 % of the total DNA. Purified DNA, to be used for comparison with whole nuclei, was prepared by the method for small scale preparation described by CHARGAFF[8] from the nuclear pellet (10 livers) washed 3 times with 0.25 *M* sucrose and twice with the following solution: Triton × 100, 0.001 %; sodium chloride 0.09 %; and trishexamethylene tetramine 0.05 *M*, pH 7.4.

REPORTS

The inhibitory effect of whole body irradiation on *in vitro* incorporation of [^{3}H]TDR into the DNA of 30 h regenerating livers is shown in Fig. 1. Inhibition was observed at all doses of X-radiation and the inhibition increased with the dose. While the individual variations in response to the administration of 350 R are considerable, variability is reduced at higher dose levels. It was therefore decided to employ higher dosages in the experiments with reconstituted homogenates. The data given in Fig. 1 are in good agreement with previous studies on incorporation of [^{14}C]orotic acid[6] and [^{3}H]TDR after X-radiation[1]. The incorporation of [^{3}H]TDR into nuclear DNA in the three types of reconstituted homogenates is presented in Table I for 30-h and 48-h regenerating livers respectively, after three different radiation doses. In Fig. 2 the same data are expressed as percentages of [^{3}H]TDR incorporation into DNA in homogenates reconstituted with non-irradiated regenerating liver nuclei and cytoplasm. It is clear that the incorporation of [^{3}H]TDR is inhibited whenever the reconstituted homogenate is made partly or entirely with constituents obtained from irradiated animals. Inhibition increases with the dose administered and it tends to be proportional to the logarithm of the dose. Inhibition is greater in those homogenates obtained from animals sacrificed 24 h after irradiation (48-h regenerating livers) than in those obtained from animals sacrificed 6 h after irradiation (30-h regenerating livers) when homogenates reconstituted under similar conditions are compared (Table I and Fig. 2). In both groups the incorporation of [^{3}H]TDR is

inhibited to a greater extent when the reconstituted homogenate is prepared with regenerating liver nuclei from non-irradiated and regenerating liver cytoplasm from irradiated animals than in homogenates reconstituted with nuclei obtained from irradiated animals and cytoplasm obtained from non-irradiated animals. The inhibitory effect of X-radiation on the activity of cytoplasmic enzymes concerned in DNA synthesis is confirmed by the results presented in Figs. 3 and 4. In these experiments the incorporation of [^{3}H]TDR into purified DNA was measured in presence of increasing amounts of regenerating liver cytoplasmic extract obtained from animals irradiated with 1500 R, 24 h after partial hepatectomy and sacrificed 6 or 24 h later.

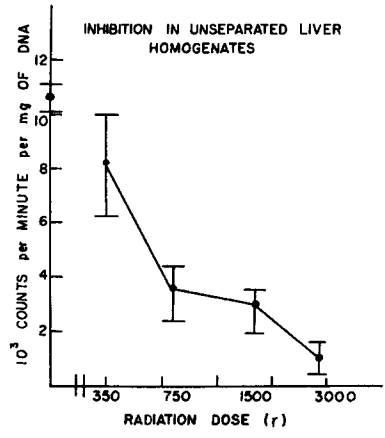

Fig. 1. The animals were irradiated 24 h after partial hepatectomy and sacrificed 6 h later. The homogenate of each liver was incubated in the presence of [^{3}H]TDR for 25 min at 37° (see ref. 1). Each point of the curve is the mean of DNA specific activity in 5 animals and the brackets correspond to the standard deviations.

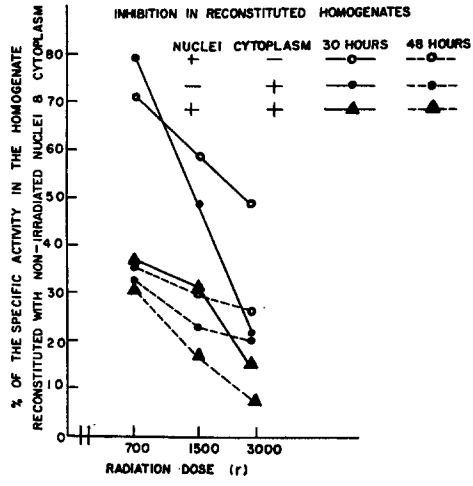

Fig. 2. Each point corresponds to the DNA specific activity in the recombined homogenate of which either the nuclei, the cytoplasm or both were obtained from animals irradiated *in vivo*. The data are expressed as the percentage of the specific activity of DNA in homogenates reconstituted with non-irradiated nuclei and cytoplasm. The (+) and (—) signs refer to the composition of the homogenate as in Table I.

TABLE I

INCORPORATION OF [^{3}H]TDR INTO DNA OF RECONSTITUTED HOMOGENATES

All animals were irradiated 24 h after partial hepatectomy and sacrificed either 30 or 48 h after the operation. The recombined homogenates were incubated for 24 min at 37°. The (+) sign, indicates that the cell fraction used in the reconstituted homogenates was prepared from livers of irradiated rats, the (—) sign indicates that it was prepared from livers of non-irradiated rats. Counts × 10^3/min/mg DNA.

Composition of the homogenate		*700 R*		*1500 R*		*3000 R*	
Nuclei	*Cytoplasm*	*30 h*	*48 h*	*30 h*	*48 h*	*30 h*	*48 h*
—	—	7,000	9,700	7,100	11,180	7,600	9,400
—	+	4,950	3,480	4,100	3,500	3,700	2,560
+	—	5,500	3,240	3,400	2,640	1,560	2,060
+	+	2,720	2,800	2,200	1,680	1,190	700

The incorporation of [^{3}H]TDR into purified DNA in presence of cytoplasm from livers of irradiated rats was compared with the incorporation of [^{3}H]TDR into purified DNA in presence of non-irradiated regenerating liver cytoplasm. The incorporation of [^{3}H]TDR was found to be markedly inhibited by X-radiation, both when the animals were sacrificed 6 h (30-h regenerating livers) after X-radiation and 24 h (48-h regenerating livers) after X-radiation.

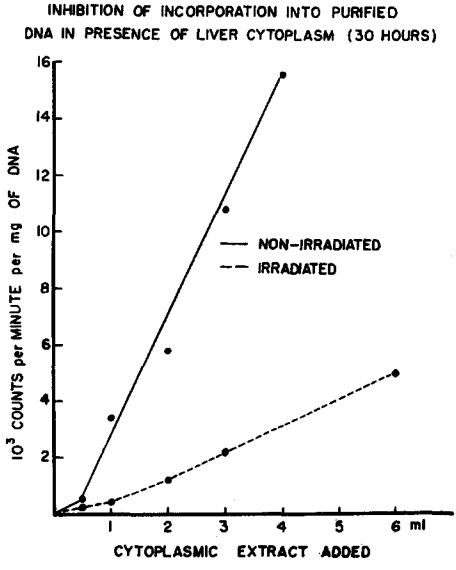

Fig. 3. A 30-h regenerating liver cytoplasmic extract of irradiated (5.6 mg of nitrogen/ml) or non-irradiated (5.65 mg of nitrogen/ml) animals was prepared by centrifuging the liver homogenate to sediment the nuclei. The cytoplasm was incubated for 25 min at 37° in the presence of [^{3}H]TDR (except that 0.5 mg of purified DNA was used instead of nuclei). The irradiated animals received 1500 R 6 h before sacrifice.

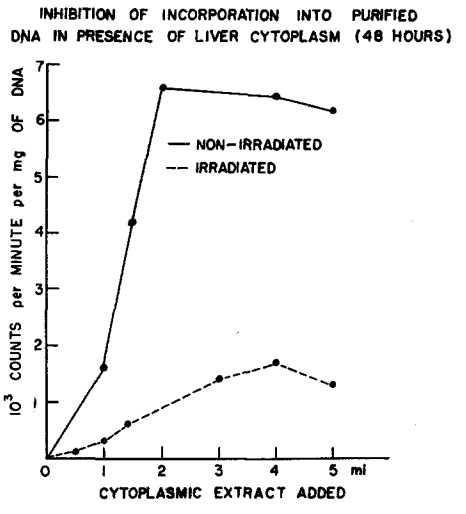

Fig. 4. A 48-h regenerating liver cytoplasmic extract of irradiated (4.72 mg of nitrogen/ml) or non-irradiated (4.65 mg nitrogen/ml) animals was prepared and incubated as in Fig. 3. The irradiated animals received 1500 R 24 h before sacrifice.

DISCUSSION

The inhibition of DNA synthesis in regenerating liver[9–11] or other actively growing tissues[12–21] by X-radiation has been demonstrated in numerous laboratories with a variety of precursors. It appears that X-radiation affects the incorporation of precursors into DNA more than it affects the incorporation of precursors in other major cellular constituents, such as, RNA[6,22] or protein[23]. Our studies were, therefore, mainly concerned with the elucidation of the mechanism of interference of X-radiation with DNA biosynthesis. This effect on both cellular constituents is not an isolated observation. Indeed, various investigators have made similar observations by studying the effect of u.v. or X-radiation on preparations ranging from amebae[24] to isolated mammalian[25] nuclei. Incorporation into DNA is more inhibited into reconstituted homogenates in which the cytoplasmic fraction was prepared from irradiated animals, than in those in which the nuclear fraction came from irradiated animals. The effect on the cytoplasm suggests that X-radiation markedly reduces

the activity of one or more enzymes involved in the utilization of nucleotides or nucleosides for DNA biosynthesis. It is however not known whether X-radiation directly affects phosphorylating enzymes or polymerase, either by interfering with their biosynthesis, or by inactivating the enzyme. Indeed POLARD AND WITHMORE[26] explain the inhibition of antigen formation after irradiation of *E. coli*, by interference with their biosynthesis. The inhibition of carboxypeptidase[27] activity is ascribed to the denaturation of the protein by X-radiation. The oxidation of the SH group has been suggested to explain the inhibition of numerous SH enzymes by X-radiation[28]. It is thus possible that in our experiments X-radiation interferes with the active centers of the cytoplasmic enzymes involved in DNA synthesis.

Furthermore, interference of X-radiation with normal function of numerous other biochemical systems has been demonstrated previously and such alterations as uncoupling of oxidative phosphorylation[29], increased activity of deoxyribonuclease[30–32], ribonuclease[33], adenosinetriphosphatase[34,35], or of enzymes involved in nucleotide or nucleoside breakdown[36] might well explain the present findings. Thus, comprehensive interpretation of the present data will have to wait for further investigation on the effect of X-radiation on these various biochemical systems in regenerating rat liver. Such studies are in progress in this laboratory and will be published in detail later.

The nature of the effect of X-radiation on the nuclei remains unexplained. LEHMAN[37] and BOLLUM[38] have studied the effect of heat denaturation on its capacity to act as a primer and report an increase of net DNA synthesis or incorporation of precursors into the DNA under such conditions. It is well known that X-radiation causes DNA denaturation[39,40]. If denaturation occurred after irradiation *in vivo* to a degree similar to that observed in heated DNA, one would expect an increased incorporation of [^{3}H]TDR under these conditions. In fact, *in vivo* irradiation inhibits the incorporation of [^{3}H]TDR into irradiated nuclei incubated with normal cytoplasm. If, as suggested by LEHMAN[37], macromolecular alteration of DNA is needed for it to function as primer, the decreased incorporation after irradiation may be caused by interference with the modification of the macromolecule which accompanies DNA synthesis. CREASY AND STOCKEN[41], who studied the effect of X-radiation on thymus nuclei, suggest that X-radiation mainly interferes with the oxidative phosphorylating properties of the nucleus. Such an interpretation cannot apply to our findings; indeed, according to the same authors liver nuclei are not capable of oxidative phosphorylation.

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