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INHIBITION OF THYMIDINE KINASE - DNA POLYMERASE ACTIVITY IN THE KIDNEY BY X-RADIATION BEFORE OR AFTER UNINEPHRECTOMY

by
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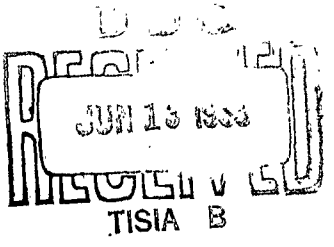

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EXPERIMENTAL PATHOLOGY BRANCH
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ADMINISTRATIVE INFORMATION

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ABSTRACT

Groups of rats were exposed to whole-body X-radiation (850 rad) at various time intervals either before or after unilateral (left) nephrectomy, and the remaining (right) kidney was removed 48 hours postnephrectomy. The specific activity of the kidney enzymes (thymidine kinase-DNA polymerase system) catalyzing DNA-synthesis was then assayed by measuring the incorporation of H^3-thymidine into DNA in vitro. The ratio of enzyme activity in right versus left kidney in each animal is termed the "induction ratio". The induction ratio was profoundly suppressed in rats irradiated at 10 minutes to 18 hours after uninephrectomy (values <1); when irradiated between 19 and 24 hours after unilateral nephrectomy there was a sharp and precipitous rise in the induction ratios (values up to 6.6).

When radiation exposure was carried out between 15 minutes and 22 hours before uninephrectomy, the induction ratios were depressed (< 1, and as low as 0.05). The evidence suggests that partial recovery from this latent radiation damage can occur if the time interval between irradiation and uninephrectomy is sufficiently prolonged; when this interval was 20 days, induction ratios attained values of 4.4.

The data indicate: (1) that the early steps in induction or formation of the thymidine kinase-DNA polymerase enzyme system are highly

radiosensitive; (2) that latent radiation-inducted damage to the enzyme-forming system occurs, i.e., when the radiation is applied before unilateral nephrectomy; (3) that this enzyme system in the kidney may be continuously renewed, with a turnover time of less than 48 hours.

SUMMARY

The Problem:

Although there is general agreement that ionizing radiation-induced damage in biological systems is manifested in changes in deoxyribonucleic acid (DNA) metabolism, there is no agreement on the actual biochemical lesion. It has been shown previously that *in vitro* X-ray exposure of an enzymatic system for DNA synthesis produces no inhibitory effect on DNA synthesis at doses ranging from 250 to 10,000 rad.

The Findings:

Rats were exposed to whole-body X radiation (850 rad) at various times before or after unilateral nephrectomy, and the other kidney was removed 48 hours later. The kidneys were homogenized in isotonic sucrose-tris buffer, and the homogenate was centrifuged at 105,000xg for 1 hour. The supernatants were assayed *in vitro* for the DNA polymerase enzymes which catalyze the incorporation of H^3-thymidine into DNA. The accumulation of these enzymes was profoundly suppressed in rats irradiated at 10 minutes to 18 hours after uninephrectomy; when irradiated between 19 and 24 hours after nephrectomy there was a sharp rise in enzyme activity. When irradiation was carried out between 15 minutes and 22 hours before uninephrectomy,

enzyme activity was also profoundly suppressed. The data indicate that the early steps in the induction or formation of the thymidine kinase-DNA polymerase enzyme system are highly radiosensitive.

INTRODUCTION

Although there is general agreement that alterations in deoxyribonucleic acid (DNA) are fundamentally involved in the action of ionizing radiations on living systems, the specific biochemical lesion(s) has not as yet been identified. Furthermore, the interpretation of experimental data on DNA is not unambiguous in studies on tissues (such as thymus and spleen) with high cell turnover, or in which rapid changes in cell population occur after radiation exposure (1). The discovery of the DNA-synthesizing enzyme system by Kornberg and coworkers (2) has opened up a new avenue for investigating the biochemical mode of action of ionizing radiation on DNA. Thus, it is now clear from several studies on regenerating rat liver, that radiation inhibits the formation of the thymidine kinase-DNA polymerase system (3,4,5), the enzymes involved in the biosynthesis of DNA. There are, however, questions as yet unresolved with respect to the time of irradiation relative to partial hepatectomy, and the degree of enzyme inhibition. Thus, according to Bollum, et al. (4), when X-radiation was given up to 13 hours after partial hepatectomy, the subsequent appearance of thymidine kinase-DNA polymerase in the liver was prevented; whereas, when the radiation was administered between 14 and 17.5 hours post-surgery, no inhibitory effect on the

appearance of the enzymes was observed. On the other hand, Beltz (6) found marked interference with the accumulation of thymidine kinase in the liver when 1500 r of X rayswere delivered 15-16 hours after partial hepatectomy; thymidylate synthetase activity was affected only slightly.

We have been interested in the biochemical basis of the inhibitory effect of whole-body X radiation, when applied before or after unilateral nephrectomy, on mitotic activity and hypertrophy of the remaining kidney (7,8). It was found that exposure of rats to a single dose of X rays (850 rad) 15 minutes to 22 hours prior to unilateral nephrectomy caused a profound inhibition of the system responsible for the induction of DNA polymerase and thymidine kinases in the kidney (9).

In the present study we have examined and compared the time course of the appearance of the thymidine kinase-DNA polymerase enzyme system when whole-body X radiation is applied at various early intervals either before or after unilateral nephrectomy in rats. This study has revealed a relatively sharp cut-off point in the inhibitory effect of the radiation when applied following uninephrectomy.

MATERIALS AND METHODS

X-Ray Exposure Following Uninephrectomy

The animals employed were female Sprague-Dawley rats, bred and raised in this Laboratory. Except when otherwise noted, the rats were 3-1/2 weeks old at the time of irradiation. Groups of rats were subjected to left unilateral nephrectomy under ethyl ether anesthesia,

following which they were exposed, at various time intervals, to a single whole-body X radiation dose of 850 rads (an LD_{100}). The radiation source was a 250-kvp Therapy Unit, and the dose rate was 30 rad per minute. The radiation characteristics were: 250 kv; 15 MA; filter 0.5 mm Cu plus 1 mm Al; HVL 1.28 mm Cu; TSD 100 cm; dose-rate 30 rad per minute, measured in air. The remaining kidney was extirpated 48 hours after nephrectomy. Immediately following extirpation, the left and the right kidney, respectively, of each animal was separately weighed and homogenized in cold tris-sucrose buffer (tris, 11 mM; sucrose, 0.23 M; pH 8.0) in the ratio of 9.0 ml buffer per g kidney tissue. Each homogenate was then centrifuged (5 C) at 105,000 xg for 1 hr, and without further treatment the supernate was used as the enzyme source. Each enzyme preparation was stored at -196 C until used for the assay. The frozen supernatants were then rapidly thawed and, after determination of protein concentration, each preparation was diluted to contain the same protein concentration (5.0 mg per ml). The supernatants from the left and right kidneys from the same rat were assayed simultaneously for enzyme.

The specific activity of the enzyme system (thymidine kinase-DNA polymerase) catalysing DNA synthesis was determined by measuring the rate of incorporation of tritiated thymidine into the acid-insoluble fraction (i.e., containing all the DNA) as described previously (9). The incubation mixture (0.50 ml) contained: (a) each of the 5'-deoxyribonucleotides of adenine, guanine and cytosine, 25 mμmoles; (b) mag-

nesium chloride, 3 μmoles; (c) adenosine triphosphate (tris form at pH 7.4), 3 μmoles; (d) tritiated thymidine (specific activity 3 c/mmole) 3.3 mμmoles; (e) tris phosphate buffer pH 7.4, 5 μmoles; (f) enzyme preparation, 1.25 mg protein; (g) DNA (calf thymus DNA (Worthington)) preheated 7 min at 100 C. Resulting specific radioactivity of the DNA fraction, expressed in cpm/mg protein, was determined by a modification of the liquid scintillation counting method of Main and Walwick (10). The data from each animal obtained under these experimental conditions are presented as a ratio of the specific enzyme activity determined in the supernatant of the right kidney divided by that from the left kidney. This ratio (cpm_r/cpm_l) is termed the "induction ratio". Although the absolute counts per minute per mg protein resulting from enzyme activity in the left kidney supernatants were relatively low, there was appreciable variation from animal to animal. Therefore, it was considered that the ratio of enzyme specific activities of the right to the left kidney from the same rat represented the most realistic value for the induction of new enzyme activity in the experimental animals.

X-ray Exposure before Uninephrectomy

In another series of experiments, rats were exposed to whole-body X radiation (850 rad) at various times prior to unilateral nephrectomy, and the enzyme activity of the left and right kidneys, respectively, was assayed according to the protocol described above.

RESULTS

As seen in the experimental data of Figure 1, there occurs a profound inhibition of incorporation of H^3-thymidine into DNA catalyzed by enzymes in the supernatants from the right kidneys of rats irradiated at 10 minutes to 18 hours after unilateral nephrectomy; that is, the induction ratio for the thymidine kinase-DNA polymerase enzymes was less than 1, and as low as 0.05. When the rats were X irradiated between 19 and 24 hours after unilateral nephrectomy, there was a sudden and precipitous rise in the values of the induction ratios, i.e., they were all above 1 and ranged up to 6.6. The specific activities for these enzymes in the kidneys of rats irradiated at 33 to 47 hours after uninephrectomy were even higher, i.e., induction ratios were up to 11.5. By comparison, the mean induction ratio for 10 nonirradiated 3-1/2 week old rats was 14.7, with a SD of 7.4. The corresponding mean value for 9 nonirradiated rats, in which both kidneys were extirpated simultaneously, was 1.01.

Effect of Irradiation Prior to Uninephrectomy

In this series of experiments, unilateral nephrectomy was carried out at 15 minutes, 30 minutes, 1 hour, 3 hours, 22 hours, 7 days, 20 days, or 34 days after the animals had been exposed to a single dose(850 rad) of X rays; the remaining kidney was removed 48 hours later. In the 3 latter cases the rats each received an intravenous injection of 8-10 x 10^7 bone marrow cells derived from normal Sprague-Dawley rats to afford survival against the otherwise lethal dose of radiation. It is

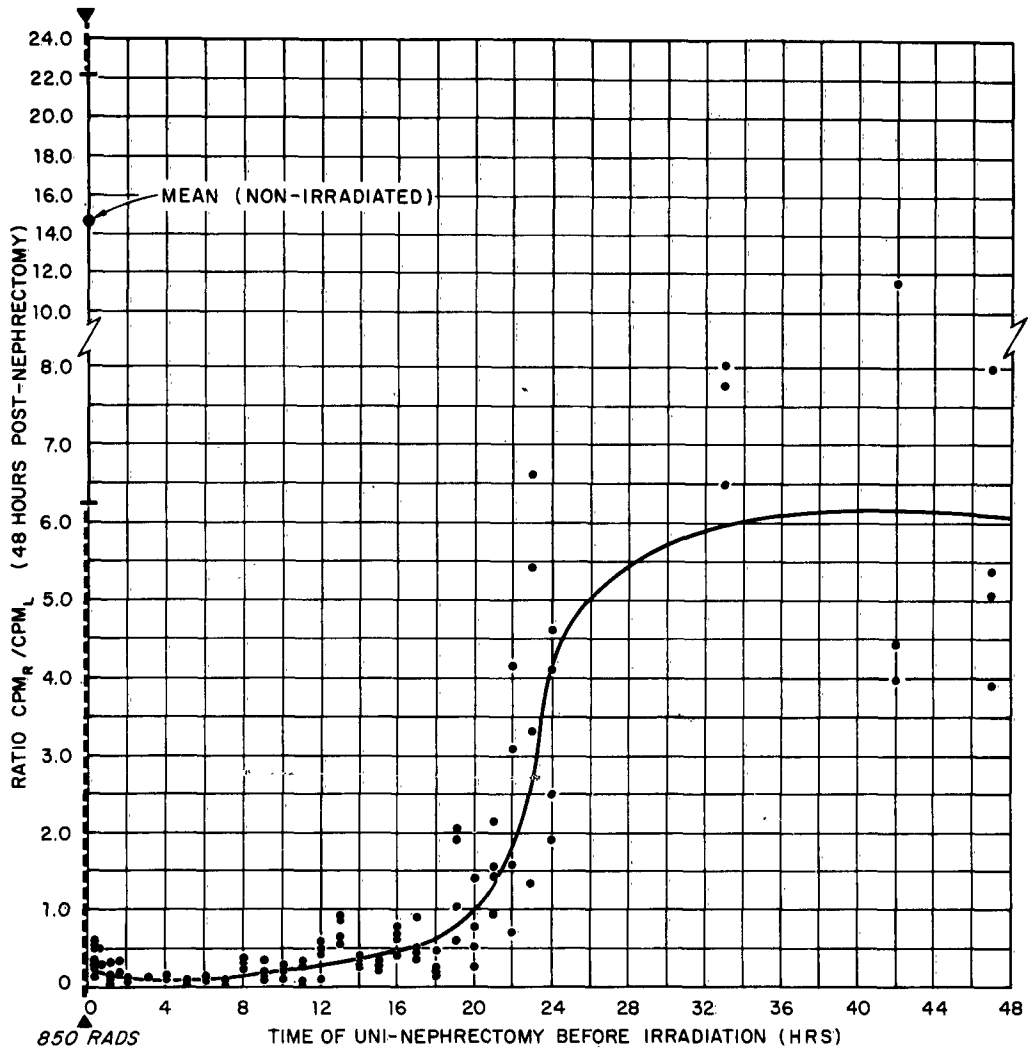

Fig. 1 Effect of whole-body X-radiation (850 rad), applied at various times after unilateral nephrectomy, on thymidine kinase-DNA polymerase enzyme activity in rat kidney supernatants. Each point represents one rat.

evident from the data (Fig. 2) that the specific activity of the DNA-synthesizing enzyme system did not increase when the radiation was applied up to 22 hours before uninephrectomy, i.e., the induction ratios were less than 1.0, and as low as 0.2. This suggests that the thymidine kinase-DNA polymerase enzyme-forming system was damaged by the radiation even before the stimulus to enzyme formation, i.e., uninephrectomy, was given. Similar latent radiation injury to DNA synthesis in rat liver in vivo has been reported by Albert and Bucher (11). The data indicate also that partial recovery from this latent damage can occur if the interval between irradiation and uninephrectomy is sufficiently prolonged. Thus, when the interval was 7 days, the induction ratio was greater than 1.0, namely 2.5, 1.4, and 1.8, respectively, for 3 rats; when the interval was 20 days the values were 4.4 and 4.1; and after 34 days the induction ratios were 2.1, 1.4, and 0.8. It should be mentioned, in this connection, that there appears to be an age dependence for induction of this enzyme system in the rat kidney: normal, nonirradiated rats 6-1/2 to 8-1/2 weeks old showed definitely lower values than those in 3-1/2 week old rats, i.e., a mean of 4.5, with a range of 2.5 to 6.8.

DISCUSSION

It is evident from the foregoing data that whole body X radiation (840 rad) applied during early periods (up to 18 hours), following uninephrectomy, prevents the appearance in the remaining kidney of the enzyme systems that catalyze the biosynthesis of DNA. When the radi-

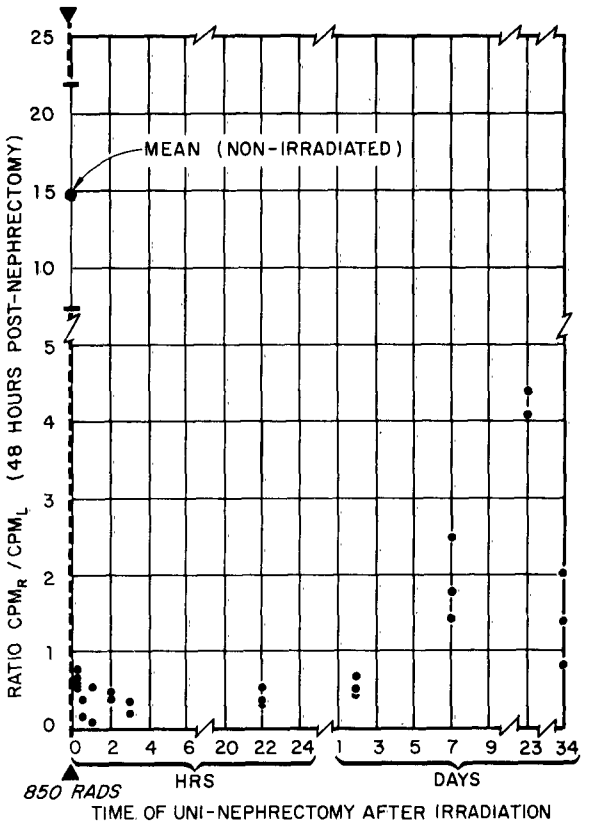

Fig. 2 Effect of whole-body X-radiation (850 rad), given prior to unilateral nephrectomy, on thymidine kinase-DNA polymerase enzyme activity system in rat kidney supernatants. Each point represents one rat.

early steps in enzyme induction have already been initiated at the time of irradiation. The basis of this latent damage is not as yet known. However, the question may be raised as to whether this damage might not well involve some event prior to the formation of the enzyme-forming system; possibly we are dealing here with a radiation-induced biochemical lesion to the macromolecular template-RNA, or DNA-dependent RNA (13), which governs and confers the specific information to the sites of enzyme synthesis (cf. 14). Current studies in this Laboratory are being directed towards a resolution of this hypothesis.

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6. Biosynthesis.
I. Main, R. K.
II. Cole, L. J.
III. Walwick, E. R.
IV. Title.
V. MR005.08-1200

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Naval Radiological Defense Laboratory

USNRDL-TR-640

INHIBITION OF THYMIDINE KINASE-DNA POLYMERASE ACTIVITY IN THE KIDNEY BY X-RADIATION BEFORE OR AFTER UNINEPHRECTOMY by R. K. Main, L. J. Cole and E. R. Walwick 23 April 1963 21 p. illus. 14 refs. UNCLASSIFIED

Groups of rats were exposed to whole-body X-radiation (850 rad) at various time intervals either before or after unilateral (left) nephrectomy, and the remaining (right) kidney was removed 48 hours postnephrectomy. The specific activity of the kidney enzymes (thymidine kinase-DNA polymerase system) catalyzing DNA-synthesis was then assayed by measuring the incorporation of H^3-thymidine into DNA in vitro. The ratio of enzyme activity in right versus left kidney in each animal is termed the "induction ratio". The induction ratio was profoundly suppressed in rats irradiated at 10 minutes to 18 hours after uninephrectomy (values <1); when irradiated between 19 and 24 hours after unilateral nephrectomy there was a sharp and precipitous rise in the induction ratios (values up to 6.6).

When radiation exposure was carried out between 15 minutes and 22 hours before uninephrectomy, the induction ratios were depressed (<1, and as low as 0.05). The evidence suggests that partial recovery from this latent radiation damage can occur if the time interval between irradiation and uninephrectomy is sufficiently prolonged; when this interval was 20 days, induction ratios attained values of 4.4.

The data indicate: (1) that the early steps in induction or formation of the thymidine kinase-DNA polymerase enzyme system are highly radiosensitive; (2) that latent radiation-inducted damage to the enzyme-forming system occurs, i.e., when the radiation is applied before unilateral nephrectomy; (3) that this enzyme system in the kidney may be continuously renewed, with a turnover time of less than 48 hours.

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2. X-radiation.
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