POLYRIBOSOME RESPONSE IN REGENERATING LIVER FOLLOWING IONIZING RADIATION*

Milton B. Yatvin and Thomas P. Lathrop

Radiobiology Research Laboratories,

University of Wisconsin Medical School, Madison, Wis.

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The inhibitory effect of x-irradiation on DNA synthesis in regenerating liver is well established (Kelly, 1954; Ord and Stocken, 1956; Beltz et al., 1957). However, there are conflicting reports regarding the effect of x-irradiation on RNA synthesis in regenerating liver. It has been suggested (Kelly, 1961) that the lack of uniform results may be due to the great metabolic and chemical heterogeneity of cellular RNA. For example, the interference by x-irradiation with the rate of nuclear RNA synthesis as measured by P^{32} uptake differed with the method used in extracting the RNA (Klouwen, 1960; Welling and Cohen, 1960). Lethal doses of whole body x-irradiation administered to partially hepatectomized rats reduced the incorporation of orotic acid-C^{14} into a rapidly labeled fraction of nuclear RNA (Uchiyama et al., 1965). In contrast, whole body irradiation did not decrease the incorporation of isotopically labeled precursors into cytoplasmic RNA of regenerating liver (Vermund et al., 1953; Thomson et al., 1954; Ord and Stocken, 1956; Fausto et al.,1964).

The correlation between radiation-produced inhibition of some enzyme biosynthesis in regenerating liver (Bollum et al., 1960) and the reduced synthesis of rapidly labeled nuclear RNA (Uchiyama et al., 1965) suggests that irradiation could be blocking the synthesis of new messenger RNA. In order to explore this

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possibility, experiments were designed to determine what effect γ-irradiation has on the polyribosomes which are believed to contain messenger RNA. Regenerating liver is particularly suited for such an investigation inasmuch as polyribosome sucrose density gradient profiles are characterized by a marked decrease in the monomers and smaller oligomers and a shift to heavier aggregates after partial hepatectomy (Cammarano et al., 1965; Webb et al., 1966). This report deals with the inhibitory effect of whole body irradiation on heavy polyribosome distribution in regenerating rat liver.

MATERIALS AND METHODS

Liver was obtained from 150 gm. male rats of the Badger strain (Badger Research Corp., Madison, Wis.). All rats were fasted 24 hours prior to killing but given water ad libitum. Partial hepatectomies were performed by the method of Higgens and Anderson (1931).

Whole body irradiation was delivered by a 2000 curie ^{137}Cs source emitting 662 KeV gamma rays. A copper field flattener was used to increase the uniformity of the dose throughout the radiation field. During irradiation the animals were kept unrestrained in a round plastic cage 18.7 cm in diameter and 9.9 cm high. The source to cage bottom distance was 35.9 inches and the dose rate 60 R/min.

Animals were killed by decapitation and the livers removed and quickly rinsed in cold buffer. The livers were then weighed, scissors-minced, and homogenized in a teflon Potter-Elvehjem type homogenizer (2 ml of buffer medium per gram of liver). After centrifugation the mitochondrial-free supernate containing ribosomes was adjusted to 1.7% with deoxycholate and either layered directly on linear sucrose gradients or else the polyribosomes (C ribosomes) were prepared according to the procedure of Wettstein et al.(1963). The polyribosome pellet was then carefully resuspended and centrifuged at 0-4°C in a linear sucrose gradient (10-40%) at 25,000 rev/min in a SW-25.1 rotor of a Spinco L-2 preparative centrifuge. Optical densities were read at 260 mμ in a Gilford recording spectrophotometer. All distribution patterns were from livers pooled from 2 or 3 animals.

RESULTS

Characteristic C ribosome distribution patterns of normal liver (NL) and regenerating liver (RL) (24 hours after surgery) are shown in Fig. 1. These

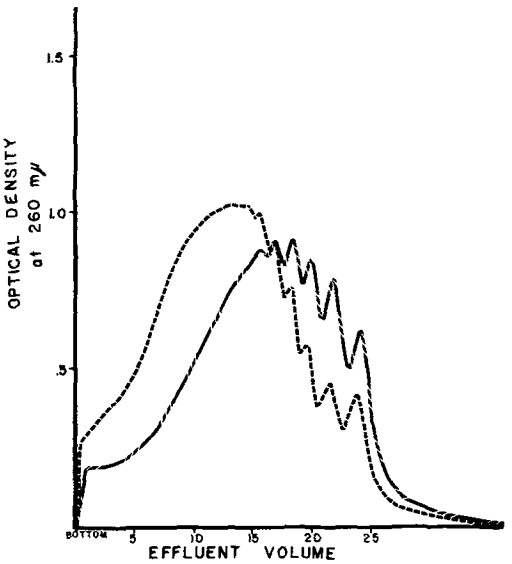

Fig. 1. The C ribosome pattern of normal rat liver (▬▬▬) and that from regenerating liver 24 hours after partial hepatectomy (▬ ▬ ▬). For density gradient analysis the C ribosomes were obtained after adjusting the post mitochondrial supernate to 1.7% with respect to DOC. The C ribosomes were layered on top of 27 ml of a linear (10-40%) sucrose gradient and centrifuged for 2 hours at 25,000 RPM in a SW-25.1 rotor at 0-4°C. Optical density was read at 260 mμ in a Gilford continuous flow spectrophotometer. Gradients were normalized by applying the same amount of ribosomal material, as measured by optical density, to each gradient tube.

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sedimentation patterns are in agreement with those depicted by Cammarano et al. (1965) for similarly prepared material. However, when rats with regenerating livers were exposed to whole body doses of 1500 or 6000 R of γ-radiation at either 6, 9 or 12 hours after partial hepatectomy and then killed 24 hours after surgery the characteristic RL pattern of polyribosome distribution was no longer observed, but rather one closely resembling that obtained from the unirradiated NL was now evident (Fig. 2A). Parallel results were obtained in the pattern of the total liver ribosomes from similarly irradiated rats that were killed 24 hours after partial hepatectomy (Fig. 3A). Thus the trend towards the accumulation of heavier ribosomal aggregates is inhibited by γ-irradiation.

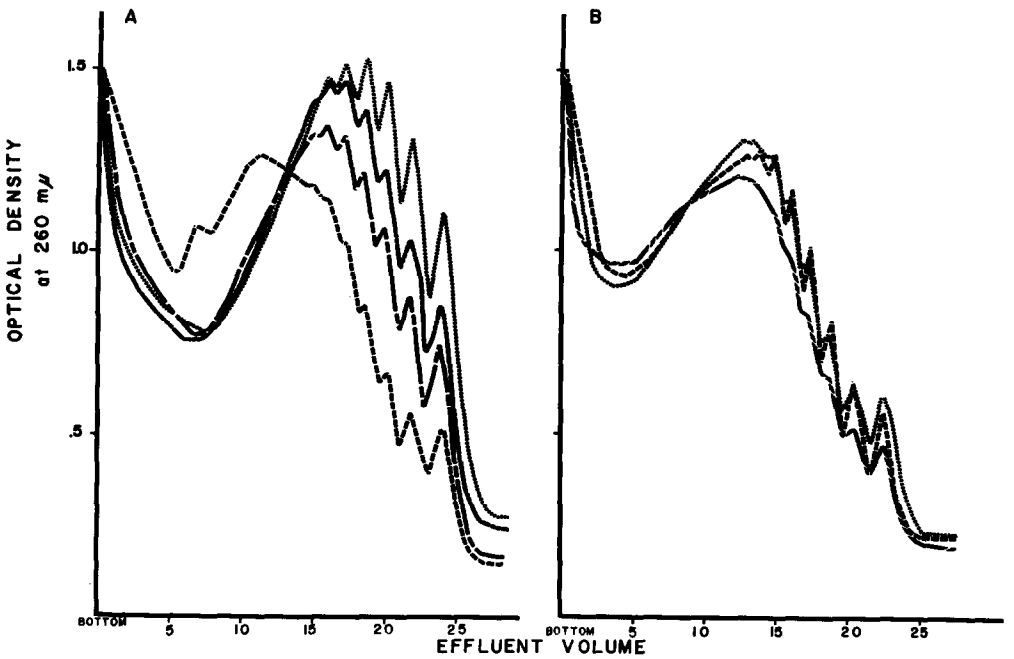

Fig. 2. A-The C ribosome pattern from regenerating liver from rats killed 24 hours after partial hepatectomy. Unirradiated (▬▬▬▬), irradiated with 6000 R, at either 6 hours (▬▬▬), 9 hours (▪▪▪▪▪▪▪▪▪▪▪) or 12 hours (▬▬▬▬▬) after surgery.

B-The C ribosome pattern from regenerating liver from rats killed 36 hours after partial hepatectomy. Unirradiated (▬▬▬▬), irradiated with 6000 r at either 9 hours (▪▪▪▪▪▪▪▪▪▪▪▪) or 12 hours (▬▬▬▬▬) after surgery. The conditions for density gradient analysis were the same as in Fig. 1 except that the linear gradient (26 ml of 10-40% sucrose) was cushioned by a 2 ml layer of 80% sucrose to reduce heavy aggregate loss. The optical density was read at 260 mμ in a Gilford recording spectrophotometer. Gradients were normalized by applying the same amount of material to each tube as measured by optical density at 260 mμ.

The γ-radiation did not alter the RL C ribosome pattern when it was delivered 24 hours after partial hepatectomy (a time when the characteristic RL pattern is well established) and the rats were killed either immediately or 6 hours after irradiation. It thus appears that irradiation inhibits the formation of the new heavier species of polyribosomes rather than causing a significant breakdown of those present at the time of exposure. In addition, C ribosome pellets obtained from RL have been subjected to as much as 20,000 R with no detectable decrease in the heavy ribosomal aggregates (Yatvin, unpublished).

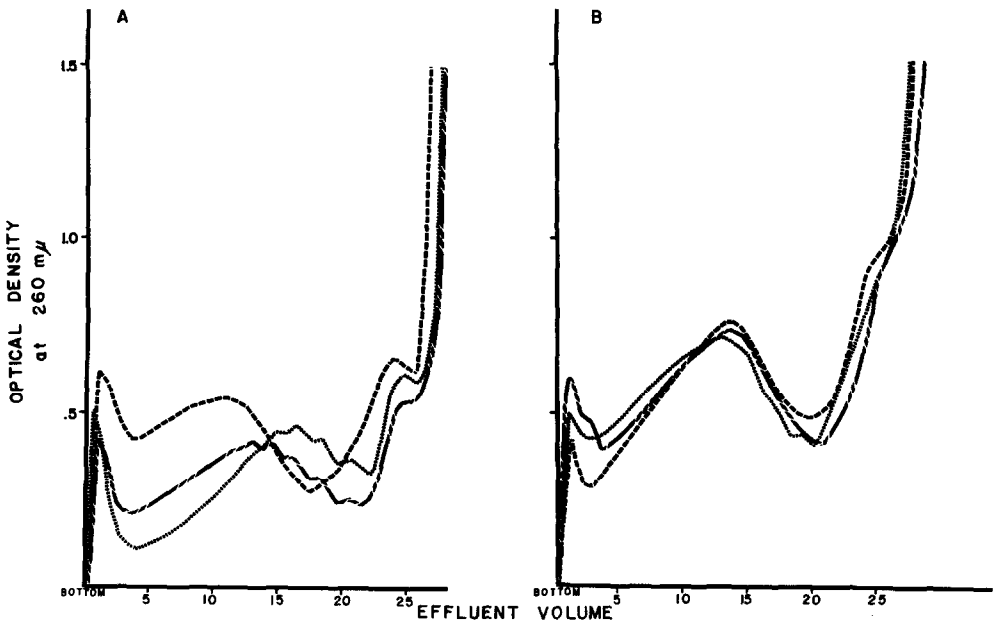

Fig. 3. A-The total liver ribosome pattern from regenerating liver from rats killed 24 hours after partial hepatectomy. Unirradiated (▬▬▬▬▬), irradiated with 6000 R at either 6 hours (▪▪▪▪▪▪▪▪▪▪▪▪▪) or 12 hours (▰▰▰▰▰) after surgery.

B-The total liver ribosome pattern from regenerating liver from rats killed 36 hours after partial hepatectomy. Unirradiated (▬▬▬▬▬), irradiated with 6000 R at either 9 hours (▪▪▪▪▪▪▪▪▪▪) or 12 hours (▰▰▰▰▰) after surgery. An aliquot of the total liver ribosomes (corresponding to 110 mg of tissue) after adjustment to 1.7% with respect to DOC was layered on top of a 27 ml linear sucrose gradient (10-40%) and spun in a SW-25.1 rotor for 175 minutes. The optical density was read at 260 mμ in a Gilford recording spectrophotometer.

The inhibition by γ-radiation of the appearance of heavy ribosomal aggregate distribution patterns of both C ribosomes and total RL ribosomes is apparently transient. γ-irradiation at 6, 9 and 12 hours after partial hepatectomy did not prevent the appearance of either the characteristic RL C ribosome pattern (Fig. 2B) or the total RL distribution pattern when the animals were killed 36 hours after surgery (Fig. 3B).

DISCUSSION

The first part of this study demonstrates that the characteristic increase in heavy polyribosomes observed in RL (Cammarano et al., 1965; Webb et al., 1966) is

inhibited by whole body γ-radiation doses of either 1500 or 6000 R. This γ-ray induced inhibition in the appearance of heavy polyribosome species corresponds well with the radiation produced inhibition of the expected increase in thymidine kinase and DNA polymerase in RL 6 - 24 hours after partial hepatectomy (Bollum et al., 1960). This correlation between inhibition of heavy polyribosome formation and, by inference, inhibition of messenger RNA production and the failure in enzyme synthesis is not an unexpected result. Similar results have been observed in RL with actinomycin D, a known inhibitor of messenger RNA synthesis. When this drug was administered within 12 hours after partial hepatectomy there was an almost complete inhibition of the usual rise in DNA polymerase (Giudice and Novelli, 1963). A similar correlation in RL between x-radiation interference of synthesis of a rapidly labeled nuclear RNA fraction and enzyme biosynthesis has been reported (Uchiyama et al., 1965). Furthermore, in bacterial systems it has been suggested that transcription of the genetic message from DNA to RNA is sensitive to ionizing radiation (Clayton and Adler, 1962; Pollard, 1964).

In the second phase of this study in which a longer interval between irradiation and killing was allowed, the characteristic heavy profile was again observed, suggesting that at least some repair of the transcription process had occurred. This recovery is not entirely unexpected since rapidly labeled RNA is not necessarily the primary target of ionizing radiation, and both Beltz et al. (1957) and Albert and Bucher (1960) have observed partial recovery in DNA synthesis in RL of irradiated rats. Whereas, Dickson and Paul (1961) found an initial depression of DNA synthesis in Strain L mouse cells, when the cells were allowed to grow for several days following irradiation the synthesis of DNA returned to the normal level. Thus the initial failure of heavier polysome species to appear in 24 hour irradiated RL could be related to the well documented inhibition of DNA synthesis. This inhibition could result in the inability of the DNA to direct the synthesis of new messenger RNA. However, as the DNA "recovers" the new species of messenger RNA associated with RL appear to be synthesized as evidenced by the characteristic RL polyribosome patterns obtained 36 hours after surgery.

An alternate explanation may lie in the fact that there are two major cell populations in RL which are present in nearly equal numbers and have different times of both DNA synthesis and periods of radiosensitivity (Kelly, 1961). It is therefore possible that the bulk of the heavy polyribosomes are obtained from less radiosensitive cells at 36 hours after partial hepatectomy.

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