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EFFECT OF X RAYS ON DNA CONTENT AND SIZE OF CELL NUCLEI IN REGENERATING RAT LIVER

B. Fabianova, E. Mišurova, and K. Kropačova

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Besides other changes, ionizing radiation also causes inhibition of DNA synthesis and mitosis in the liver of intact animals, to a degree which depends on the dose and the age of the animals [12, 13]. These changes are expressed more clearly in the regenerating liver [6, 8, 10]. Data of most investigations are concerned mainly with changes found in irradiated animals during 2 or 3 days or regeneration.

This paper describes the results of a study of the DNA content in the nuclei and measurement of the size of the nuclei in regenerating liver cells of rats irradiated for a period of 21 days after partial hepatectomy (PH), i.e., throughout the period of regeneration.

EXPERIMENTAL METHOD

Two series of experiments were carried out on 130 adult male Wistar albino rats weighing 200-220 g. In series I the rats were irradiated in a dose of 154.8 mCi/kg body weight, i.e., 600 R (on the TUP-T-250 x-ray therapy apparatus, 200 kV, 16 mA, dose rate 10.32 mCi/kg body weight/min, i.e., 45 R/min) and PH was performed on them 10-30 min later by the usual method [7]. In series II the same determinations were made on unirradiated animals subjected to PH (control).

The experimental and control rats were killed between 18 h and 21 days after the operation, always in the morning (between 6:30 and 7:30 a.m.). Films were made from liver cell suspensions [3] and stained under standard conditions by Feulgen's method. The DNA content in the nuclei was determined cytophotometrically (on the Chirana 11 cytophotometer), by a two-wave method and calculated in relative units (rel. u.). In each group, consisting of five animals, 250 measurements were made of extinctions and of the dimensions of the same nuclei. The nuclei were measured in two mutually perpendicular directions by means of an

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Department of Normal and Topographical Anatomy, Medical Faculty, and Department of General Biology, Faculty of Natural Sciences, P. J. Šafarik University, Košice, Czechoslovakia.
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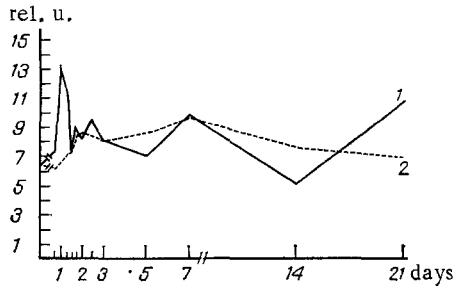

Fig. 1

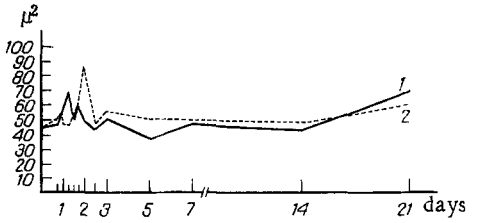

Fig. 2

Fig. 1. Mean DNA content (in rel. u.) in cell nuclei of regenerating liver of control (1) rats and rats irradiated in a dose of 154 mCi/kg (2).

Fig. 2. Mean area of cell nuclei (in μ^2) in regenerating liver of control rats (1) and of rats irradiated in a dose of 154.8 mCi/kg (2).

adapted screw-operated ocular micrometer (Meopta), which was used instead of the ocular part of the cytophotometer. The area of the nuclei was calculated by the equation for an ellipsoid.

On the basis of the results of determination of the DNA content in the nucleus and its area, the nuclei were distributed among 25 classes and this distribution was represented graphically by histograms; the mean values of the above-mentioned indices also were calculated for each group, respectively.

EXPERIMENTAL RESULTS

The mean DNA content in the nucleus of the intact liver of unirradiated rats was 6.5 rel. u. (Fig. 1) and the corresponding area of the nucleus was 45 μ^2 (Fig. 2). The values obtained for the size of the nuclei agrees with data in the literature [2].

After removal of approximately two-thirds of the liver by PH in the unirradiated rats an increase was found in the number of nuclei with high content of DNA as the result of premitotic DNA synthesis, which began 14-18 h after the operation [4, 14]. The histograms in Fig. 3 show that the progressive rise in the DNA level in the nucleus reached a maximum 24 h later, and this was accompanied by an increase in size of the nuclei (Fig. 4). In the overwhelming majority of nuclei the DNA content was doubled; the mean DNA content in the nucleus at this time was 13.4 rel. u., i.e., twice as high as in the intact liver. The same conclusion was reached by Lancker and Sempoux [9] who used biochemical methods of investigation. The histogram of distribution of the nuclei by DNA content after 24 h shows that most nuclei took part in the first wave of DNA synthesis (about 83-90%). These data confirm the results of an investigation by Grigor'ev et al. [1]. According to data in the literature [5, 6], after the first wave of DNA synthesis the degree of synchronization of entry of the hepatocytes into individual phases of the cell cycle is reduced, and far fewer cells are involved in the 2nd and 3rd waves of DNA synthesis than in the first. The distribution of nuclei by DNA content on the histogram and the mean values of this parameter confirm that these waves of synthesis are accompanied by an increase in the DNA content only in a certain number of nuclei. On the 14th day after PH most nuclei had reverted to their original size, evidence that the principal stage of regeneration in the liver had ended. The increase in the DNA content in the nuclei on the 21st day, in the writers' opinion, may be due to polyploidization.

Irradiation in a dose of 154.8 mCi/kg caused an increase in the DNA content in some nuclei in the intact liver (after 18 h), as a result of which the DNA content as a whole was increased to 7.5 rel. u. compared with 6.5 rel. u. in the intact, unirradiated rats. This probably took place on account of the influence of nuclei in binuclear hepatocytes [11].

The content of nuclear DNA in the regenerating liver of the irradiated rats was basically unchanged until 30 h after PH. These results unambiguously confirm data obtained by other methods [5, 10], according to which irradiation inhibits predominantly the first wave of synthesis. A marked increase in the DNA content in the nucleus was observed in subsequent intervals with a maximum at about 42 h. From this time until the 14th day the trend of changes in the irradiated and unirradiated animals was similar.

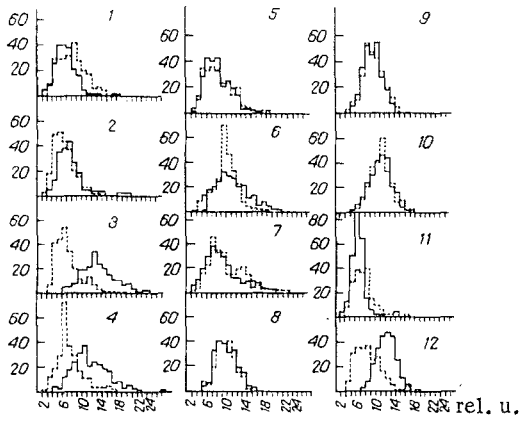

Fig. 3

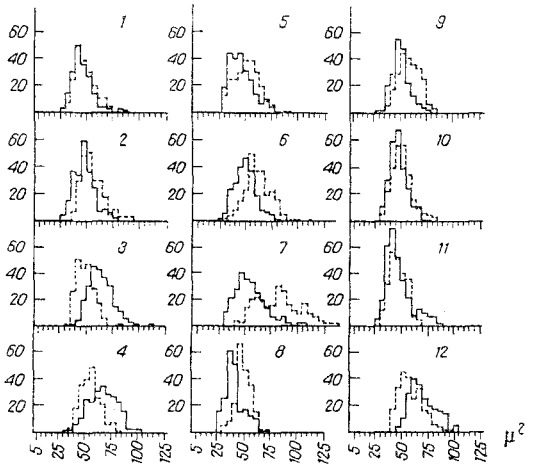

Fig. 4

Fig. 3. Distribution of cell nuclei by DNA content in regenerating liver of control (continuous line) rats and rats irradiated in a dose of 154.8 mCi/kg (broken line). Abscissa, distribution of nuclei among classes by DNA content (in rel. u.); ordinate, number of nuclei. 1) Intact liver; 2-12) regenerating liver 18, 24, 30, 36, 42, 48, and 60 h and 3, 7, 14, and 21 days, respectively, after PH.

Fig. 4. Distribution of cell nuclei by area in regenerating liver of control rats (continuous line) and rats irradiated in a dose 154.8 mCi/kg (broken line). Abscissa, distribution of nuclei among classes by area (in μ^2); ordinate, number of nuclei. Remainder of legend as in Fig. 3.

Changes in the size of the nuclei in the regenerating liver of irradiated animals preceded changes in the DNA content. An increase in size of some nuclei was observed as early as after 18 h, and a maximum was reached after 48 h; at that time the nuclei in the liver of the irradiated rats were almost twice as large as those in the regenerating and intact liver of control rats. Comparison of histograms of distribution by DNA content and by area of nuclei shows that in most nuclei an increase in size was not accompanied by any corresponding increase in DNA content. In the period from the 3rd to the 14th day there was no significant change in the size of the nuclei; at this stage of regeneration the nuclei of the irradiated animals were on average 5-15 μ^2 larger than the corresponding controls.

Consequently, the results showed that irradiation of rats (154.8 mCi/kg) for a few minutes before PH prevents an increase in the DNA content in the nuclei of the regenerating liver for the first 30 h. The increase in the DNA content in the nucleus is delayed by 12-18 h compared with the corresponding indices in the regenerating liver of the unirradiated animals and it takes place in fewer cells. The increase in size of the nuclei, predominantly 48 h after PH, was not accompanied by a corresponding increase in the DNA content.

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