BIOCHEMICAL ASSESSMENT OF THE LEVEL OF EARLY POSTRADIATION CELL DESTRUCTION IN RATS RECEIVING DIFFERENT DOSES

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The total level of cell destruction in normal and irradiated rats was determined by a biochemical radioisotope method. The number of cells destroyed in the body during the 8 h after irradiation in doses of 100, 300, and 900 R is 170, 260, and 630%, respectively, of the normal level.

There is no general agreement at the present time regarding the level of cell destruction in the tissues of an animal irradiated with different doses. Besides some facts indicating increased destruction of cells during the first few hours after irradiation [2, 7], there are others which suggest that increased destruction in the tissues of the irradiated animal plays no significant role and the mechanism of depopulation of the radiosensitive tissues is simply one of the suppression of cell repopulation [8, 9]. These contradictions can largely be explained by the deficiencies of the cytomorphological methods which are usually used to study this problem.

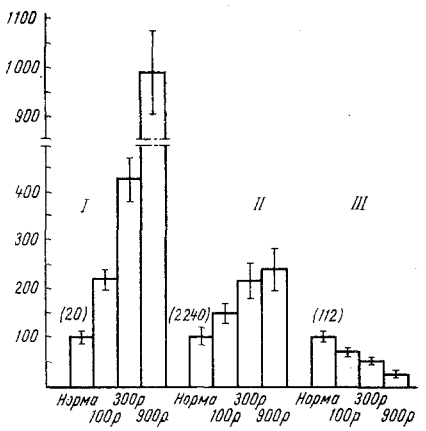

Fig. 1. Effect of irradiation of rats in various doses on content of thymidine (I) and its total (II) and specific (III) radioactivity in sample of urine (0-8 h) after injection of thymidine-C^{14}. Results given per animal as percentages of control. Numbers in parentheses give absolute values of indices: (I) in μg; (II) in $\mu Ci \times 10^{-4}$; (III) in $\mu Ci/\mu g \times 10^{-4}$.

The object of the investigation described below was to determine the number of cells destroyed in the body during the first few hours after exposure to various doses of ionizing radiation, using a biochemical radioisotope method for estimating the total cell breakdown [4]. It consists essentially of determining the endogenous level of a specific DNA metabolite by means of a radioisotope method and determining the fraction of the general background for which the breakdown of this biopolymer is responsible. From these parameters it is possible to determine the absolute quantity of DNA in the body broken down and this directly reflects the level of cell destruction.

EXPERIMENTAL METHOD

Experiments were carried out on 145 noninbred male rats weighing 180-200 g. The animals were irradiated on the ÉGO-2 apparatus (Co^{60} γ rays, 385-460 R/min) in doses of 100, 300, and 900 R. Immediately after irradiation the rats received an injection of orotic acid-2-C^{14} (specific activity 60 $\mu Ci/\mu g$) in a dose of 8-10 μCi per animal. Orotic acid-C^{14} is a common precursor of the pyrimidine nucleosides. It was injected so as to label both thymidine and deoxyuridine. In another series of experiments the rats were injected with thymidine-1-C^{14} (specific activity 54-34 μCi/mole) immediately after irradiation in a dose of 5 μCi per animal

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TABLE 1. Radioactivity of Thymidine and Deoxyuridine Excreted after Injection of Orotic Acid-C^{14} into Rats (results given in $\mu Ci/\mu g$ per 1 μCi orotic acid-C^{14} injected $\times 10^{-6}$*)

Dose of irradiation	Specific activity of deoxyuridine	Specific activity of thymidine	Specific activity of deoxyuridine / specific activity of thymidine
Control	4,07±0,31	1,66±0,12	2,5:1
100	3,82±0,41	1,25±0,25	3,0:1
300	3,99±0,48	0,92±0,09	4,3:1
900	3,46±0,27	0,40±0,03	8,6:1

*$P < 0.001$ for groups: control 300 R, control 900 R, 300-900 F.

in order to determine the fraction of the total thymidine appearing in the body which was excreted. Thymidine and deoxyuridine were isolated from the urine collected over a period of 0-8 h and estimated quantitatively together with total and specific radioactivity. The thymidine and deoxyuridine were isolated from the urine by Mazurik's method [3]. Deoxyuridine was subjected to repeated chromatography in 1 M ammonia to purify it. To determine the radioactivity of the nucleosides aliquots of the fluids for analysis were evaporated to dryness, and the residues were dissolved in 0.8-1.0 ml distilled water and applied in amounts of 0.2-0.3 ml to aluminum targets measuring 14 mm^2. Radioactivity was measured on a "B" apparatus with end-window type counter.

EXPERIMENTAL RESULTS

Changes in the excretion of thymidine in the urine and its total and specific radioactivity in rats irradiated in various doses after injection of thymidine-C^{14} into the animals are shown in Fig. 1. The specific activity of the excreted thymidine fell with an increase in the dose of irradiation, but its concentration and total radioactivity rose. The decrease in the specific activity of thymidine-C^{14} is evidence of dilution of the injected isotope in the body with its endogenous analogue. The fraction of thymidine-C^{14} administered (5 μCi) which was excreted (and, consequently, the fraction of the total thymidine appearing in the body which was excreted) is normally 4.5%, but after irradiation in doses of 100, 300, and 900 R it was 6.6, 9.6, and 11%, respectively. It was assumed for this calculation that the injected thymidine-C^{14} is distributed uniformly throughout the body. On the basis of these values and the amounts of thymidine excreted under normal conditions and after irradiation given in Fig. 1, the quantity of thymidine appearing in the body was calculated. It was 0.43 mg for the unirradiated animals, and 0.66, 0.89, and 1.87 mg for the rats irradiated in doses of 100, 300, and 900 R, respectively.

The total thymidine in the body consists of two components: anabolic and catabolic. The ratio between these components of thymidine in the unirradiated and irradiated animals was determined from the ratio between the specific activities of deoxyuridine and thymidine excreted. Since deoxyuridine is virtually the sole precursor of thymidylate, the specific activity of deoxyuridine is identical with the specific activity of the thymidine formed de novo. It is clear from the results given in Table 1 that with an increase in the dose of irradiation the specific activity of thymidine fell, as a result of dilution of the label with unlabeled catabolic thymidine. The quantity of thymidine appearing in the body as a result of DNA breakdown in 8 h* is 0.26 mg under normal conditions, and it was 0.44, 0.68, and 1.65 mg in the same period after irradiation in doses of 100, 300, and 900 R, respectively, while the quantity of DNA† broken down during the same time interval was 1.07 mg under normal conditions and 1.8, 2.8, and 6.7 mg after irradiation in the above-mentioned doses respectively.

By virtue of the metabolic stability of DNA in the cell, the breakdown of this biopolymer takes place only on death of the cells (whether physiological revewal or a pathological destructive process). The DNA content per cell of the adult rat does not vary significantly in different types of tissues, and its mean value is $6.7 \text{ mg} \times 10^{-9}$ [6]. Hence, the number of cells destroyed in the normal rat in the course of 8 h is about 160 million. During the same period of time after irradiation the number of cells destroyed in the body increased to 270 million, 420 million, and 1,000 million or 170, 260, and 630% of the normal level after irradiation in doses of 100, 300, and 900 R, respectively.

These results, demonstrating the intensified destruction of the cells during the first few hours after irradiation, are in good agreement with the view that interphase cell death plays an important role in the general postradiation depopulation of radiosensitive tissues in the early period after exposure to ionizing radiation [1, 2, 5, 7].

*The accumulation of deoxy-compounds in lymphoid organs, blood serum, and urine takes place virtually simultaneously [10].

†This value was found by multiplying the quantity of catabolic thymidine by the reciprocal of the fraction of thymidine by weight (0.244) in DNA.

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