EXCRETION OF THYMIDINE AND DEOXYURIDINE BY IRRADIATED HAMSTERS AFTER ADMINISTRATION OF EXOGENOUS DNA

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Hyperexcretion of thymidine and deoxyuridine was found in the urine of hamsters during the first day after irradiation in doses of 600, 700, and 1000 R. Investigation of the dynamics of nucleoside excretion after irradiation (700 R) revealed an increase in their content in the urine on the 1st, 8th-10th, and 25th days. Administration of DNA to the hamsters 1 h after irradiation (1000 R) led to a decrease in the nucleoside level on the first day.

One of the manifestations of postradiation disturbances of DNA metabolism in mammals is an increase in the concentration of nucleosides in the urine [1, 4, 7, 9, 10], an early diagnostic sign of radiation damage. Metabolism of the pyrimidine nucleosides in man has been shown to differ significantly from their metabolism in rats, animals in which nucleosidurea has been investigated most fully, and in golden hamsters which resemble them closely [12]. It is therefore interesting to study the content of thymidine and deoxyuridine in the urine of hamsters after irradiation in various doses. Administration of DNA after irradiation leads to an increase in the survival rate of various species of animals [3, 6, 8]. Since the possible mechanisms of the therapeutic action of exogenous DNA are to some extent linked with overcoming disturbances of nucleic acid metabolism in the irradiated organism [5], it is logical to expect changes in the excretion of nucleosides by irradiated animals after administration of DNA.

In this investigation the excretion of thymidine and deoxyuridine in the urine of golden hamsters was studied during the first day after irradiation in various doses, the dynamics of the excretion of these nucleosides was investigated after irradiation in doses of 700 and 1000 R, and changes in these indices were examined after administration of DNA 1 h or 24 h after irradiation.

EXPERIMENTAL METHOD

Seventy golden hamsters weighing 80-100 g were used. The animals were kept under ordinary nursery conditions. The thymidine and deoxyuridine content in the 24-h specimen of hamster urine was determined by the writer's own method.

Preliminary purification of thymidine and deoxyuridine on Dowex-1 and Dowex-50 columns was carried out by the scheme described for determination of deoxycytidine [11]. Next, the thymidine and deoxyuridine fractions, which were not adsorbed on the ion-exchange resins, were separated by ascending (angle 35°) thin-layer chromatography in a system of isoamyl alcohol–acetone–water (3 : 2 : 1). Neutral alumina of grade II activity was used as the unbound layer. The plate size was 7 × 15 cm. The thymidine (R_f = 0.58) and deoxyuridine (R_f = 0.50) fractions were identified in UV light with the aid of a reference substance applied to the same plate, after which they were collected and their content determined by the reaction with 2-thiobarbituric acid for deoxyribose [2].

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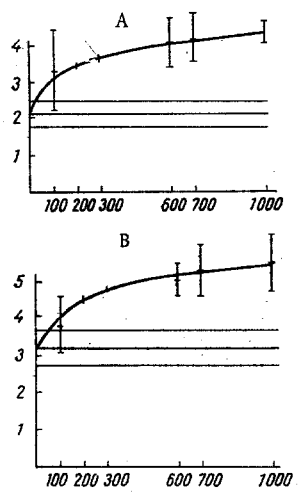

Fig. 1

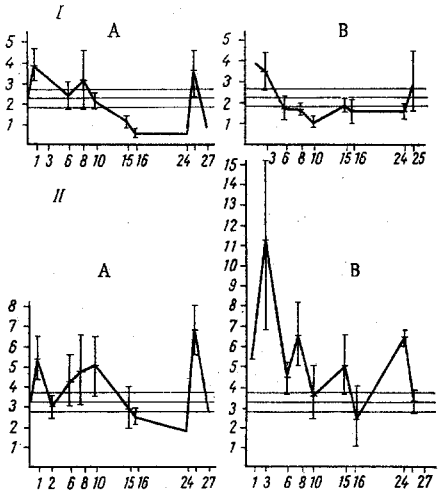

Fig. 2

Fig. 1. Excretion of thymidine (A) and deoxyuridine (B) in urine by hamsters during first day after irradiation as a function of dose. Control (0 R) consisted of 30 animals; 100 R received by 5 animals, 600 R by 5, 700 R by 16, and 1000 R by animals. Results subjected to regression analysis. Abscissa, dose (in R); ordinate, content of nucleoside in urine (in μg/24 h).

Fig. 2. Dynamics of excretion of thymidine (I) and deoxyuridine (II) by hamsters after irradiation in a dose of 700 R (A), and irradiation and administration of high-polymer DNA 24 h later (B). Here and in Fig. 3: abscissa, days after irradiation; ordinate, excretion of nucleoside (in μg/24 h).

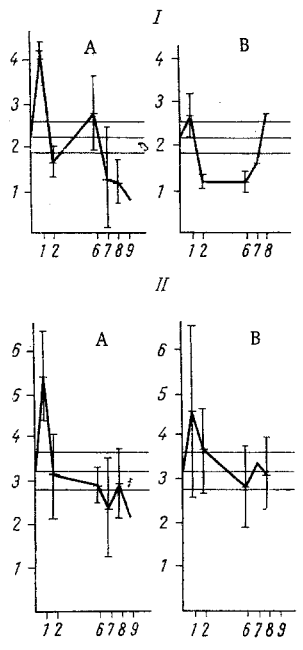

Fig. 3. Dynamics of excretion of thymidine (I) and deoxyuridine (II) by hamsters after irradiation in a dose of 100 R (A) and irradiation followed 1 h later by injection of high-polymer DNA (B).

The animals were irradiated on the EGO-2 apparatus (Co^{60}, 312 R/min) in doses of 100, 600, 700, and 1000 R. High-polymer ($1.5 \cdot 10^7$ dalton) native DNA from calf thymus was injected subcutaneously in a dose of 2.48 mg per animal 1 or 24 h after irradiation. Statistical analysis of the results was carried out by Student's method.

EXPERIMENTAL RESULTS

Tests on 30 unirradiated hamsters showed that the daily excretion of thymidine is 2.23 ± 0.37 and of deoxyuridine 3.23 ± 0.45 μg. These data for the excretion of deoxyuridine by hamsters agree well with those given in the literature [7].

The excretion of thymidine and deoxyuridine during the first day after irradiation is shown as a function of the dose in Fig. 1. With an increase in the dose of irradiation the concentration of nucleosides in the urine clearly increased. Their excretion after irradiation in a dose of 600 R or more differed significantly from normal. In hamsters, as in other species of animals, the causes of the increased excretion of thymidine and deoxyuridine after irradiation are breakdown of DNA in dying radiosensitive cells and mismatching between the intensity of synthesis of DNA and its precursors. The number of times by which the excretion of thymidine and deoxyuridine by hamsters increased after irradiation was small, less than in rats, and it was approximately the same for the two nucleosides. The smaller degree of increase in thymidine and deoxyuridine excretion after

irradiation in hamsters compared with rats can probably be explained by the fact that the interphase cell death during the first day after irradiation was less marked or by differences in nucleoside metabolism in hamsters and rats (the further degradation of the nucleosides is possibly more intensive in hamsters), or indeed by both these factors.

The dynamics of excretion of the nucleosides during the 27 days after irradiation (700 R) and after irradiation followed 24 h later by injection of DNA is shown in Fig. 2. In the control animals the excretion of thymidine and deoxyuridine in the urine was increased on the first day ($P < 0.05$) and the excretion of deoxyuridine was increased on the 25th day ($P < 0.05$). The increase in the excretion of deoxyuridine on the 8th-10th day was not significant, possibly because by this time the block to mitosis had been removed and the defective cells were dying immediately after division. On the 15th-24th day after irradiation a decrease in the excretion of both nucleosides in the urine of the control animals was observed. The increase in their content on the 25th day in the urine evidently arose on account of the possible regeneration of hematopoiesis at that time. Administration of high-polymer DNA to the hamsters 24 h after irradiation in a dose of 700 R restored the thymidine excretion to normal on the 15th-25th day.

The excretion of the nucleosides by the hamsters during the 9 days after irradiation (1000 R) and irradiation followed 1 h later by injection of DNA is shown in Fig. 3. An increase in the urinary excretion of thymidine and deoxyuridine was observed by the control animals on the first day, but from the 7th to the 9th days there was a progressive decrease in the quantity of nucleosides excreted (on the 10th day all the animals had died).

After administration of DNA the excretion of thymidine was reduced and the excretion of deoxyuridine showed a tendency to decrease during the first day after irradiation. In the control animals the excretion of thymidine and deoxyuridine at this time was 4.24 ± 0.21 and 5.47 ± 1.0 μg, respectively, 82 and 70%, respectively above normal; in the hamsters receiving DNA the excretion was 2.7 ± 0.53 and 4.64 ± 2.0 μg, respectively, 13.6 and 43% above normal (difference not significant). The difference in the excretion of thymidine on the 1st day after irradiation by the control hamsters and the hamsters receiving DNA was significant. These results are evidence that if DNA is administered 1 h after irradiation, interphase death of the cells is reduced in the recipient.

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