DEOXYRIBONUCLEASE ACTIVITY IN THE URINE OF MONKEYS AT VARIOUS STAGES OF ACUTE RADIATION SICKNESS

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Inhibition of deoxyribonuclease I (DNase I) in the urine of monkeys was found on the 1st-8th days after irradiation in a dose of 620 R ($LD_{80-90/45}$) and an increase in the activities of DNases I and II was observed on the 20th-30th days. Excretion of protein with the urine was indistinguishable from normal until the 8th day and above normal from the 10th to the 30th days. The increase in DNase activity during this period correlates with the beginning of regeneration of the hematopoietic tissue.

Acute irradiation of an animal leads to death of the cells and disappearance of the radiosensitive tissues, but after irradiation in sublethal doses these processes are replaced by a stage of intensive cell proliferation and regeneration. During the first days after irradiation, activation of enzymes causing fermentation of the DNA molecule, namely DNases I and II, is observed [6, 7, 10, 11, 13]. These results have been obtained on small laboratory animals. Few investigations have been carried out on higher mammals [2, 8, 9]. Species differences have not been studied, and activity of nucleases in the period of regeneration has received virtually no investigation. Analysis of the changes in activity of the DNases in the destructive phase as well as in the period of regeneration after irradiation is of undoubted interest.

The object of the investigation described below was to study activity of the DNases in the urine of monkeys with radiation sickness.

EXPERIMENTAL

Experiments were carried out on monkeys (Macaca mulatta) of both sexes weighing 2-4 kg and kept on an ordinary diet. The animals were irradiated in a dose of 620 R ($LD_{80-90/45}$, Co^{60} γ rays, dose rate 36.5 R/min). The 24-h specimen of the monkeys' urine was collected, 0.2 ml chloroform added as a preservative, and the fluid was filtered and frozen. Activity of the DNases was tested after 2-3 weeks, for which purpose the samples were thawed and filtered. This treatment of the material had no effect on the activity of these enzymes.

Activity of DNase I and DNase II was determined viscosimetrically [1], using the equation of Laskovsky and Zajdela in Bening's modification [1] for the calculations, and the results were expressed in conventional units based on the 24-h sample of urine. Protein was determined by Lowry's method [12] in aliquots of urine dialyzed against distilled water for 24 h.

EXPERIMENTAL RESULTS

It will be clear from Fig. 1 that the activity of DNase I in the urine of normal animals was 7220 ± 500 units per diuresis. Activity of DNase II was significantly lower, namely 1230 ± 340 units per diuresis. Irradiation of these animals caused sharp changes in the activity of these enzymes: activity of DNase I fell from the first until the 8th day after irradiation by 42-72% of the normal level, and increased by 96-244%

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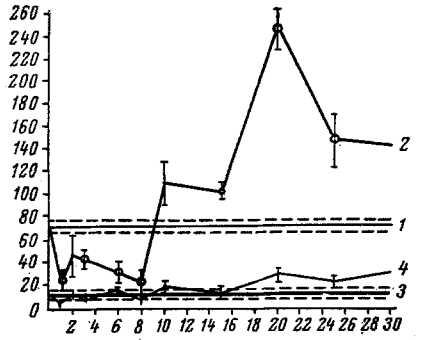

Fig. 1

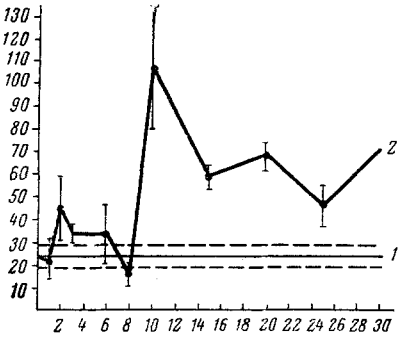

Fig. 2

Fig. 1. Change in activity of DNases in urine of monkeys at various stages of radiation sickness. Abscissa, time after irradiation (in days); ordinate, activity of DNases (in conventional units × 100). 1) Activity of DNase I in intact animals; 2) activity of DNase I after irradiation; 3) activity of DNase II in intact animals; 4) activity of DNase II after irradiation. Here, and in Fig. 2: broken lines denote scatter; points differing significantly from normal are circled.

Fig. 2. Excretion of protein with urine of monkeys during stages of radiation sickness. Abscissa, time after irradiation (in days); ordinate, protein content (in mg per diuresis). 1) Intact animals; 2) irradiated animals.

on the 20th-30th day ($P < 0.05$); activity of DNase II was indistinguishable from normal until the 15th day but 128% higher than normal between the 20th and 30th days of radiation sickness.

Changes in the protein content in the urine of the intact and irradiated monkeys are given in Fig. 2. The quantity of protein excreted with the urine of the irradiated monkeys from the 1st to the 8th day was 20-30 mg per diuresis, indistinguishable from normal. On the 10th day the protein concentration in the urine was raised to 106 mg, while between the 15th and the 30th days after irradiation it varied from 58.8 to 72 mg per diuresis ($P < 0.05$).

Comparison of the results given in Figs. 1 and 2 shows that the dynamics of the change in enzyme activity did not coincide with fluctuations in the protein concentration in the urine. The sharp inhibition of DNase I activity from the 1st to the 8th day took place against the background of a protein concentration indistinguishable from normal, while the period of maximal increase in activity of the enzymes (20th day) did not coincide with the time of the maximal increase in protein excretion with the urine (10th day).

The character of the changes in the activity of DNases I and II and in the protein concentration in the urine of the irradiated monkeys suggests that the mechanisms leading to an increase in the protein urea and an increase in the activity of these enzymes are different.

It is interesting to compare the changes in activity of the DNases in the urine of the monkeys with the changes in excretion of deoxycytidine (dC), one of the most important metabolites of DNA, described previously [3]. The workers cited report hyperexcretion of dC on the 1st, 6th, 8th, 18th, 21st, 24th, and 27th days after irradiation and they link the increased elimination of dC on the first day with intensive breakdown of DNA of the radiosensitive tissues, and in the period from the 18th to the 27th day with increased DNA metabolism following the beginning of regeneration of the hematopoietic tissue. No increased activity of the DNases was found in the monkeys' urine during the first day after irradiation. However, the DNase level under normal conditions was high enough to hydrolyze the DNA of the disintegrating tissues and to provide for the increased excretion of degradation products (dC) in the urine, as mentioned above. Inhibition of DNase I activity during the first day could be determined by liberation of the inhibitor of this enzyme from the disintegrating tissues, where it is present in large amounts. Probably the increase in DNase activity in the period from the 15th to the 30th day, coinciding with increased excretion of dC, owed its origin at this period to regenerative processes, for some investigators have noted a correlation between activity of the DNases and the level of mitotic activity [14], the intensity of DNA biosynthesis, and the rate of growth of the tissues [5].

Changes in the activity of the DNases in the urine of irradiated animals in the period of tissue breakdown thus differ in different species: in rats the activity of both DNase I and DNase II is increased [10, 11], in dogs the activity of only DNase II is increased [8], while in monkeys, as the present results show, DNase I is inhibited and no changes are found in the activity of DNase II. In the period of regeneration an increase in the activity of these enzymes was observed in both monkeys and dogs [2].*

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