5'-NUCLEOTIDASE ACTIVITY OF TISSUE OF IRRADIATED RATS

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Activity of the 5'-nucleotidases of TMP, UMP, and DCMP in the small intestine and spleen of rats is increased 24 and 72 h after γ-irradiation in a dose of 850 R. The increase in activity of these enzymes in the liver was not statistically significant.

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Besides the inhibition of biosynthesis, another characteristic manifestation of radiation injury is stimulation of DNA catabolism, accompanied by the appearance of unusually large amounts of its metabolites – nucleosides and nucleotides – in the tissues and urine [2, 3, 10, 12]. The increase in nucleoside concentration indicates very high activity of dephosphorylation reactions which take place in the intact organism. For this reason the study of the group of enzymes responsible for dephosphorylation of nucleotides is of interest for its own sake. In earlier investigations [4-6] an increase in the 5'-nucleotidase activity of the tissues of irradiated animals was described in relation to dephosphorylation of only one nucleotide, namely, 5'-adenylic acid.

In the present investigation the specificity of the increase in 5'-nucleotidase activity after irradiation was studied. The object was to discover whether differences in the degree of increase of activity of 5'-nucleotidases dephosphorylating different nucleotides exist in the irradiated animal.

Since DNA synthesis requires as an essential condition strict correspondence between the levels of its precursors and may be inhibited as a result of increased degradation of one of the nucleotides, we investigated the specificity of the increase in level of 5'-nucleotidases dephosphorylating TMP* and its precursors – DUMP, DCMP, and UMP – after irradiation. The TMP level is known to determine the rate of DNA biosynthesis in the body, and the process of its formation plays the part of a control mechanism.

EXPERIMENTAL METHOD

Experiments were carried out on rats weighing 180-200 g after γ-irradiation (Co^{60}) in a dose of 850 R (dose rate 165 R/min). The 5'-nucleotidase activity of extracts of the liver, small intestine, and spleen was determined 3, 24, and 72 h after irradiation in the following system: 100 μmoles Tris-buffer, pH 7.8, 2.5 μmoles $MgSO_4$ solution; 2 μmoles nucleotide solution, and 0.2 ml tissue extract (5% for investigation of the liver and 10% for the small intestine and spleen). After incubation for 30 min at 37° and precipitation of the protein in the samples, the inorganic phosphate liberated during dephosphorylation of nucleotides was determined by the Fiske – Subbarow method. Activity of the enzyme was expressed in μmoles inorganic P/mg protein in the sample/30 min incubation.

EXPERIMENTAL RESULTS

A statistically significant increase in 5'-nucleotidase activity of extracts of the spleen and small intestine was found 24 and 72 h after irradiation (Fig. 1). The increase in activity of these enzymes in the liver extracts was not statistically significant. Differences in 5'-nucleotidase activity in relation to

* TMP – thymidine monophosphate, UMP – uridine monophosphate, DUMP – desoxyuridine monophosphate, DCMP – desoxycytidine monophosphate, GMP – guanosine monophosphate, DAMP – desoxyadenosine monophosphate.

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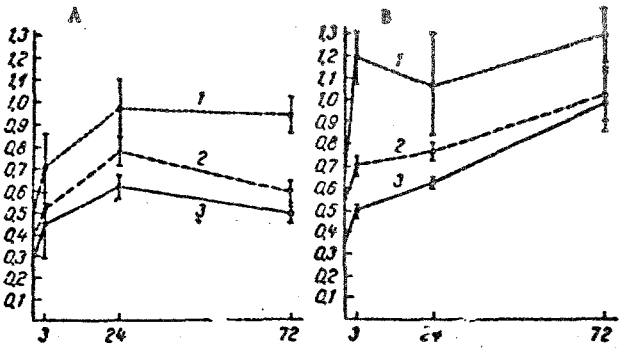

Fig. 1. 5'-Nucleotidase activity of small intestine (A) and spleen (B) of rats after γ-irradiation in dose of 850 R. Ordinate) inorganic phosphate (μmoles/mg protein/30 min incubation); abscissa) time after irradiation (in hours); 1) UMP; 2) TMP; 3) DCMP.

hydrolysis of different nucleotides and the absence of tissue specificity of their action were interesting discoveries. Uridyl nucleotides were dephosphoyrlated most actively in the extracts of all tissue tested (Fig. 1). Other authors [9] have also described that DUMP is dephosphorylated twice as fast as DCMP in rat liver. According to available data [7], the ctivity of DCMP 5'-nucleotidase amounts to 88% of the TMP enzyme activity, and this is consistent with our own observations.

If the 5'-nucleotidase activity for the nucleotides studied at different periods after irradiation was expressed as a percentage of that for TMP at the same times, these values were not significantly different from the corresponding figures under normal conditions. This indicated the nonspecificity of the increase in 5'-nucleotidase activity after irradiation and showed that one enzyme, whose activity is determined entirely by the structure of the substrate molecule, may be responsible for dephosphorylation of the nucleoside monophosphates.

It should be emphasized that the changes in 5'-nucleotidase activity after irradiation are secondary in character. It has been shown [11], for instance, that local irradiation of the spleen causes no increase in activity of 5'-nucleotidases or ATPase in the spleen, as after whole-body irradiation.

Ionizing radiation causes increased DNA breakdown in radiosensitive tissues, reaching a maximum 4-6 h after irradiation. As a result of increased DNA breakdown, products of degradation of the DNA molecules – nucleosides – accumulate in the tissues during this period and somewhat later (after 6-12 h) in the urine. We observed a statistically significant increase in 5'-nucleotidase activity of the tissues at later periods – 24 and 72 h after irradiation. The changes in earlier periods were less marked. However, it must be borne in mind that the 5'-nucleotidase activity of the tissue is extremely high under normal conditions also. According to some results [8], the enzyme content in homogenate of normal rat liver is between 18,000 and 25,000 units/mg DNA. For this reason, even without marked activation, these enzymes can bring about intensive hydrolysis of the quantity of nucleotides formed as a result of increased DNA breakdown, leading to the appearance of large quantities of nucleosides in the tissues and urine of the irradiated animals.

Only isolated hypotheses requiring experimental verification can be expressed regarding the mechanism of increase of 5'-nucleotidase activity of the tissues after irradiation. In the early periods after irradiation the increase in 5'-nucleotidase activity may be associated with an increase in permeability of the cell membranes and to liberation of some of the enzymes. Investigation of the 5'-nucleotidase activity in the separate microstructures of the cell after irradiation is interesting from this point of view. We know [8] that $^2/_3$ of the total 5'-nucleotidase activity of the cell is concentrated in the cytoplasmic granules, while $^1/_3$ is distributed evenly between the nucleus and cytoplasm. Differences have been found between the 5'-nucleotidase of the mitochondria, which dephosphorylates DCMP, DAMP, and GMP, and the microsomal enzyme, responsible for dephosphorylation of TMP and DUMP, [8].

In the later stages (24 and 72 h) after irradiation, breakdown of cell structures, with consequent changes in populations leading to an increase in the number of cells with an increased level of 5'-nucleo-

tidase activity, probably plays a leading role in the increase in activity of this enzyme. This hypothesis is supported by the fact that a similar relationship between changes in activity of other catabolic enzymes has been observed by several workers during the same times after irradiation. The increase in ATPase activity in the rat spleen was found to reach its maximum, for instance, 48-72 h after irradiation [5].

According to our findings, the increase in enzyme activity in the small intestine reached a maximum 24 h after irradiation, and it was less marked after 72 h. In the spleen a gradual increase in activity was observed, reaching a maximum after 72 h. The change in 5'-nucleotidase activity in the small intestine and spleen correlates with the destructive changes taking place in the cell structures of these tissues after irradiation [1]. The increase in 5'-nucleotidase activity in the spleen 24 and 72 h after irradiation coincides in time with destruction of the lymphocytes. This increase may be more marked in the small intestine during the first 24 h because destruction of lymphocytes is accompanied at this period by intensive disintegration of the highly radiosensitive epithelial cells. The small decrease in enzyme activity in the small intestine 72 h after irradiation compared with 24 h is probably connected with the onset of repair processes in this tissue. Repair processes in the spleen, as we know, do not begin until 5-7 days after irradiation.

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