DISTURBANCE OF THE DECOMPOSITION OF THYMINE IN RATS DURING RADIATION SICKNESS

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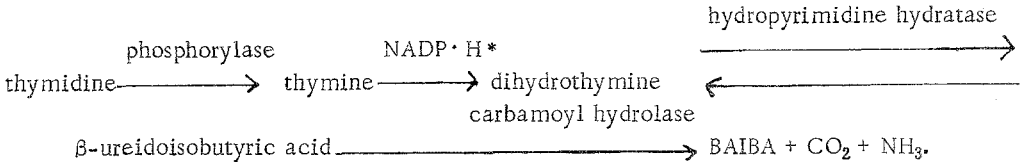
One of the early reactions of the intact organism to influence of ionizing radiation is a sharp increase in the level of DNA metabolites, deoxycytidine [4,8], thymidine [1,8], deoxyuridine [3], and β-aminoisobutyric acid (BAIBA) [13,15] in the urine. The thymidine and deoxycytidine content in the animals increases in proportion to the irradiation dose, and, in the opinion of a number of authors [2,14], may serve as a sensitive early test of radiation injury. Of special interest is the intensified excretion of thymidine with the urine. Massive loss of this biologically extremely important compound by the organism seems paradoxical, since thymidine is a specific component of DNA, and also participates in one of the control mechanisms of DNA biosynthesis [6]. This is evidence of profound changes in the DNA metabolism in the irradiated organism.

It is known that in the normal state, the degradation of thymidine is accomplished in several steps:



We have shown that irradiation sharply reduces the effectiveness of the conversion of thymine to BAIBA in the intact organism. We proposed that the disturbance of thymidine breakdown by irradiation is associated with a change in the effectiveness of the reduction of thymine to dihydrothymine, and, consequently, to a disturbance of the conversion of thymine to BAIBA.

In this work, we attempted to determine whether the degradation of thymine to BAIBA is actually disturbed after irradiation, and, if this is so, what is the degree of radiation disturbance of the reductive pathway of thymidine decomposition in the intact organism.

EXPERIMENTAL PROCEDURE

The content of thymidine, deoxyuridine, and BAIBA in the urine of intact and irradiated rats was determined after parenteral administration of thymine.

Nonpurebred male rats, weighing 190-260 g, were kept in metabolic cages on a normal diet. The urine was collected at 12-h intervals over a period of two days before irradiation and 6, 12, 24, and 48 h after irradiation; its volume was measured, and it was filtered and stored in a refrigerator at −16 to −20° until analysis. The animals were killed by decapitation. Experiments were conducted on 35 rats; five of them served as controls (they received intravenous injections of 3 ml of physiological saline). The 10 animals of the second group ("thymine") received injections of 6 micromoles of thymine in 3 ml of physiological saline. The rats of the third and fourth groups (10 animals in each) were irradiated totally on the RUM-3 apparatus (dose 650 R, voltage 190 kV, current strength 15 mA, filters 0.5 mm Cu and 1 mm Al, focal length 40 cm and dose rate 34 R/min). Immediately after irradiation, each

*NADP·H — reduced nicotinamide adenine dinucleotide phosphate.

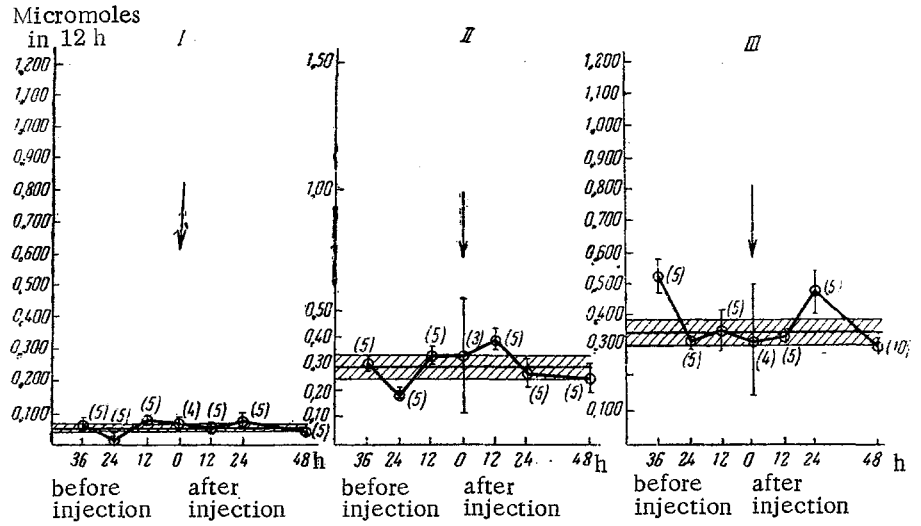


Fig. 1. Dynamics of the excretion of thymidine (I), BAIBA (II), and deoxyuridine (III) in rats after the injection of 3 ml of physiological saline. Here and in Figs. 2, 3, and 4, the excretion at the point "6 h after irradiation (or injection of solutions)" is given in micromoles in 6 h. Shaded portion, $M_{av} \pm m$ of the norm for each period of observation; the differences in comparison with the norm are statistically reliable; the number of determinations per point is indicated in parentheses; the arrow denotes the moment of irradiation and administration of the solution.

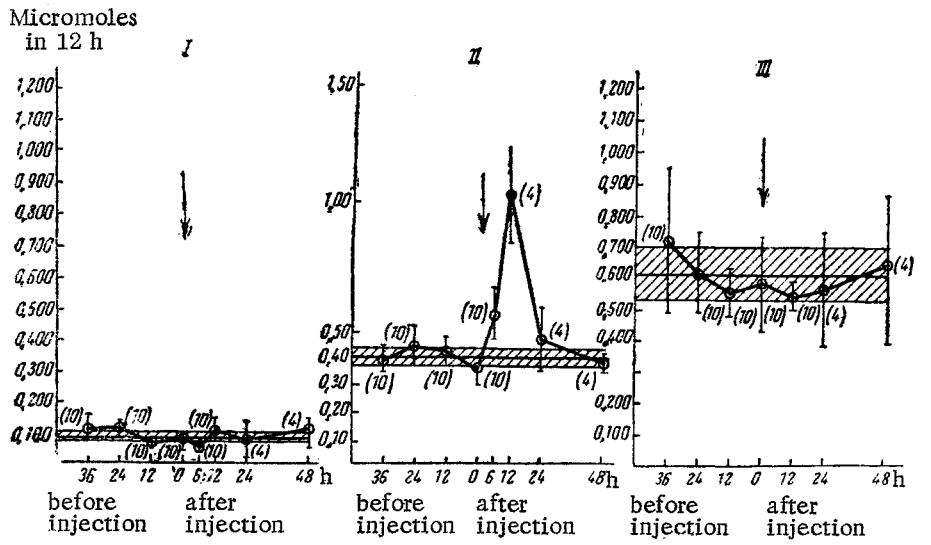


Fig. 2. Dynamics of the excretion of thymidine (I), BAIBA (II), and deoxyuridine (III) in rats after the injection of 6 micromoles of thymine.

animal of the third group ("irradiation") received an intravenous injection of 3 ml of physiological saline; the animals of the fourth group ("irradiation + thymine") received 6 micromoles of thymine in 3 ml of physiological saline. Thymidine and deoxyuridine were determined by the method that we described earlier [3] with slight modification, BAIBA by the Bawden method [7], which we modified (the cation exchange resin was replaced by the anion exchange resin Dowex-1, and the descending system n-butanol: formic acid: water, 75: 15: 10, was used for paper chromatography of the amino acid extract).*

The data obtained were treated statistically according to the Student method.

*The author would like to thank Senior Scientific Co-worker, O. Ya. Tereshchenko, for providing the BAIBA preparation.

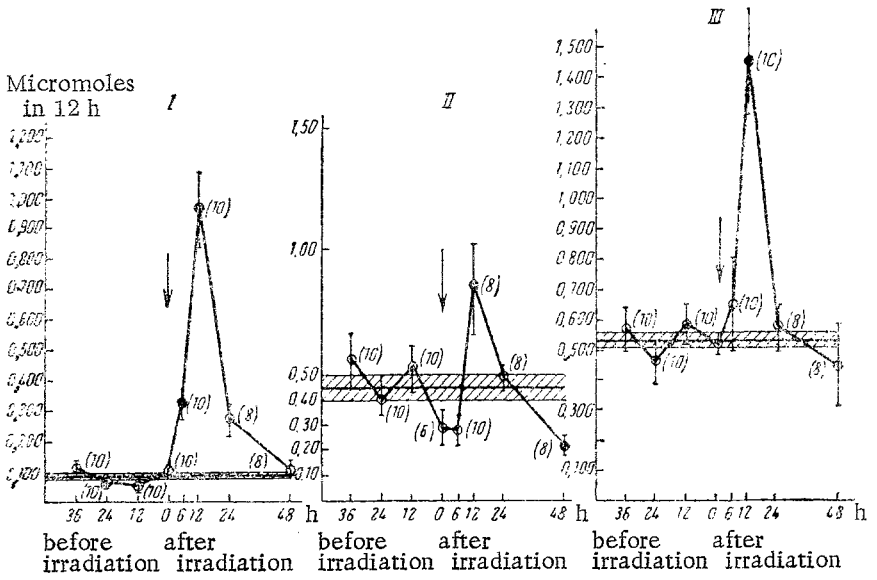


Fig. 3. Dynamics of the excretion of thymidine (I), BAIBA (II), and deoxyuridine (III) in rats after irradiation in a dose of 650 R.

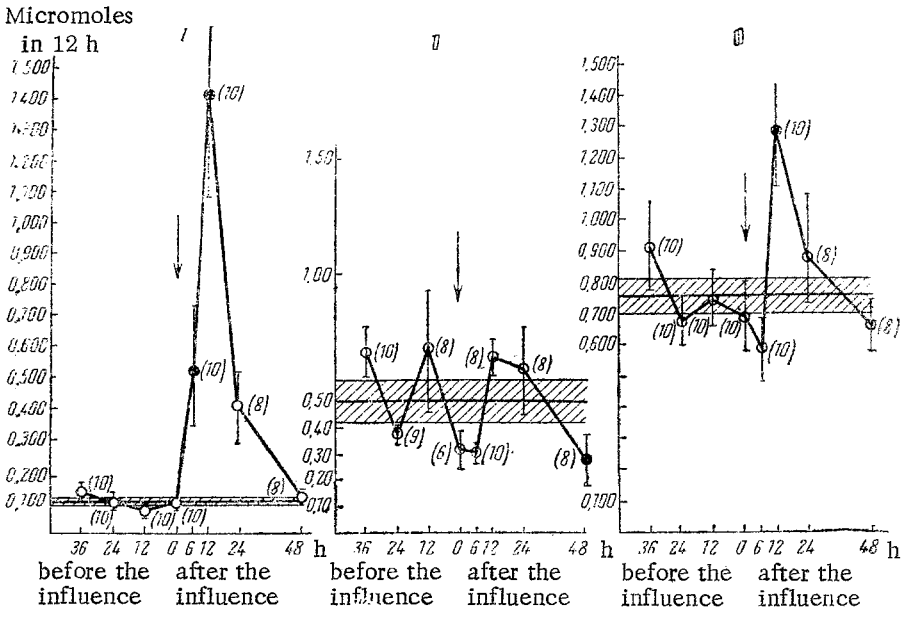


Fig. 4. Dynamics of the excretion of thymidine (I), BAIBA (II), and deoxyuridine (III) after irradiation and injection of 6 micromoles of thymine.

EXPERIMENTAL RESULTS

Figure 1 shows the dynamics of the excretion of thymidine, BAIBA, and deoxyuridine in rats in the normal state, and Fig. 2, that after the introduction of 6 micromoles of thymine; Fig. 3 shows the dynamics after irradiation in a dose of 650 R, while Fig. 4 shows that after irradiation and the administration of 6 micromoles of thymine.*

As can be seen from Fig. 1, the administration of physiological saline did not change the excretion of deoxynucleosives and BAIBA in rats.

*In the figure, the level of excretion of the investigated substances at the point "48 h after irradiation" was obtained by dividing the values of the excretion in the period of 24-48 h by two.

The injection of intact animals with thymine (Fig. 2) did not affect the deoxynucleosive content in the urine and caused a 2.5-fold increase in the excretion of BAIBA ($P < 0.01$) during the first 12 h after the injection. The effectiveness of the conversion of the introduced thymine to BAIBA of the urine was 11.6%. Thus, about 12% of the introduced thymine is decomposed in the normal state along a reductive pathway, with the formation of BAIBA.

After irradiation of the rats in the minimum absolute lethal dose (see Fig. 3), the excretion of thymidine and deoxyuridine was sharply increased; the maximum amount of these substances was detected in the urine during the period 6-12 h after irradiation. Subsequently, their content dropped rapidly, and by 48 h did not differ from the normal. The excretion of BAIBA during the first 6 h after irradiation was unchanged; by 12 h, it exceeded the initial level by 88% ($P < 0.05$), followed by a decrease to normal (24 h) and below the original level. By 48 h after irradiation, the excretion of BAIBA was only 48% ($P < 0.001$) in comparison with the norm. Consequently, during the first 12 h after irradiation, the excretion of the three compounds studied with the urine is substantially increased.

The administration of 6 micromoles of thymine to the irradiated animals did not change the nature and degree of increase in the excretion of thymidine and deoxyuridine with the urine. However, irradiation disturbed the excretion of BAIBA after the injection of thymine, so that in not one of the periods, with the exception of 48 h, did the content of this amino acid in the urine differ from the norm. By 48 h after the influence, the excretion of BAIBA was lowered to 46% of the initial level ($0.05 > P > 0.02$). During the first 12 h after irradiation, only 2.5% of the thymine (of the introduced amount) was converted to BAIBA in the rat organism.

Thus, supplementary thymine loading of the thymine-BAIBA system of the irradiated organism permitted the detection of insufficiency of this system. The indicated insufficiency is characterized by the absence of any increase in the excretion of BAIBA during the first 12 h after irradiation and by a significant lowering of the effectiveness of the conversion of the introduced thymine to BAIBA after irradiation, but not by stimulation of further conversions of BAIBA, since this amino acid is one of the final products of the decomposition of thymine [9,10].

Consequently, the degree of radiation disturbance of the decomposition of thymidine in the rat organism is characterized by a sharp decrease in the effectiveness of the reductive pathway of the degradation of thymine during the first h after the radiation influence.

Bates, Smith, and Smith [6] administered 13-80 μg of labeled thymine to rats several h (up to 24 h) after irradiation and arrived at the conclusion that radiation does not disturb the process of reductive cleavage of the thymine ring.

Our data on the excretion of BAIBA with the urine, if represented in the form of excretion in micromoles in 24 h before and after irradiation, also show that irradiation not only does not lower the effectiveness of the reductive pathway of thymine decomposition, but also gives rise to a certain intensification of the formation of BAIBA (parallel with a sharp increase in the excretion of thymidine) form 0.9 micromoles in 24 h in the normal state to 1.34 micromoles in 24 h after irradiation. However, fractionated collection of the urine after short periods of time, used in our experiments, as well as the use of loading of the intact organism with thymine in the normal state and after irradiation, revealed a disturbance of the reductive conversions of thymine during the first h after irradiation. This disturbance, as can be seen from Fig. 3, is an early reaction, is temporary, and is rapidly eliminated. Consequently, the conclusion drawn by Bates et al. [6] is correct only for several h after the radiation influence. Thus, the data obtained by the indicated authors are refined by the results of our work.

In our earlier experiments with thymidine loading of irradiated rats, it was found that the formation of BAIBA from thymidine is substantially reduced during irradiation. We propose that, in addition to a disturbance of the conversion of thymine to dihydrothymine, this is due to a partial inhibition of the thymidine phosphorylase activity on account of the accumulation of thymine in the tissues. Actually, in experiments in vitro [11,12] it was shown that an increase in the thymine concentration in the incubation medium leads to an inhibition of the thymidine phosphorylase activity and lowers the rate of phosphorylytic cleavage of thymidine. However, the disturbance of the decomposition of thymidine to BAIBA after irradiation evidently is not associated with any inhibition of the phosphorylase activity, since after the administration of thymine to irradiated rats, the excretion of thymidine statistically did not exceed the excretion of this deoxynucleoside in the irradiated rats that did not receive thymine injections.

Thus, it may be assumed that the disturbance of the decomposition of thymidine to BAIBA in rats during radiation sickness is not associated with any change in the phosphorylase activity, but is determined by the substantial decrease in the effectiveness of the reductive pathway of the degradation of thymine during the first h after the

radiation influence. As for the "fate" of thymine introduced into the irradiated organism, this will require a special study. It may be assumed that most of the thymine is decomposed by an oxidative pathway, the effectiveness of which may increase after irradiation [7].

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