EXCRETION OF THYMIDINE, DESOXYURIDINE, AND β-AMINOISOBUTYRIC ACID BY RATS AFTER IRRADIATION

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In experiments on rats [1,4] and during radiation therapy of patients with malignant neoplasms [8], a marked increase has been observed in the urinary excretion of the specific DNA component thymidine, the excretion being proportional to the dose of ionizing radiation. A high level of thymidine in the urine may be employed as an indicator of radiation damage and a convenient early test for the diagnosis of radiation sickness [3]. However, the mechanism by which the increased excretion of thymidine in the urine is caused in the irradiated organism has not been clarified. Yu. A. Zharkov, T. A. Fedorova, and L. F. Mikhailova [4] suggested that the principal cause for increased thymidine excretion by rats in the first 24 h after irradiation is the breakdown of DNA in rapidly regenerating tissue. Moreover, the increased excretion of thymidine by irradiated rats, in the opinion of these authors, may be in part associated with inhibition of DNA synthesis and accumulation of unutilized DNA precursors in the tissues.

We have indicated [5] that an additional factor, leading to elevated thymidine excretion in irradiated rats, may be a disturbance in the degradation of desoxynucleosides to β-aminoisobutyric acid (BAIBA). The present series of experiments on the injection of irradiated rats with thymidine was designed to verify this theory.

The supplemental loading experiments with the substrate for the thymidine–BAIBA system after irradiation would indicate any insufficiency of the conversion if this existed. To assess the effectiveness of thymidine metabolism, the urine of the experimental rats was examined for thymidine, for desoxyuridine, its immediate precursor [17], and for BAIBA, one of the products of thymidine catabolism [12].

METHODS

Non-inbred male rats weighing 160-190 g were housed in metabolism cages and maintained on the conventional diet. The urine was collected every 12 h for 2 days before irradiation and at 6, 12, 24, and 48 h following irradiation, the volume was measured, the urine was filtered and then stored in a freezer at –16 to –20° until analysis. The animals were killed by decapitation. The experiments were carried out on 35 animals. The rats in the control group (5 animals) were each given 3 ml of physiological saline. Ten animals in the second group ("thymidine") received 6 μM thymidine intraperitoneally in 3 ml of physiological saline. Groups 3 and 4 (10 rats each) were given total radiation in the RUM-3 apparatus at a 650 r dose level (potential 190 kv, 15 ma current strength, filter 0.5 mm Cu and 1 mm Al, focal distance 40 cm, dose intensity 34 r/min). Immediately after irradiation, each animal in the third group ("irradiated") received 3 ml of physiological saline intraperitoneally, and those in the fourth group ("irradiated + thymidine") received 6 μM of thymidine in the same volume of physiological saline.

Thymidine and desoxyuridine were determined in the urine according to a method we described previously [5] with minor modifications. Estimation of BAIBA in urine was carried out by the method of Bawden [8] but with our fundamental alterations. The essential feature of this modification was the replacement of the cation exchanger with the anion exchanger Dowex-1 and the application of descending paper chromatography to the amino acid extract using the system n-butanol-formic acid-water (75: 15: 10)* as the developing solvent.

*The authors are grateful to O. Ya. Tereschchenko for the BAIBA preparation generously contributed for our work.

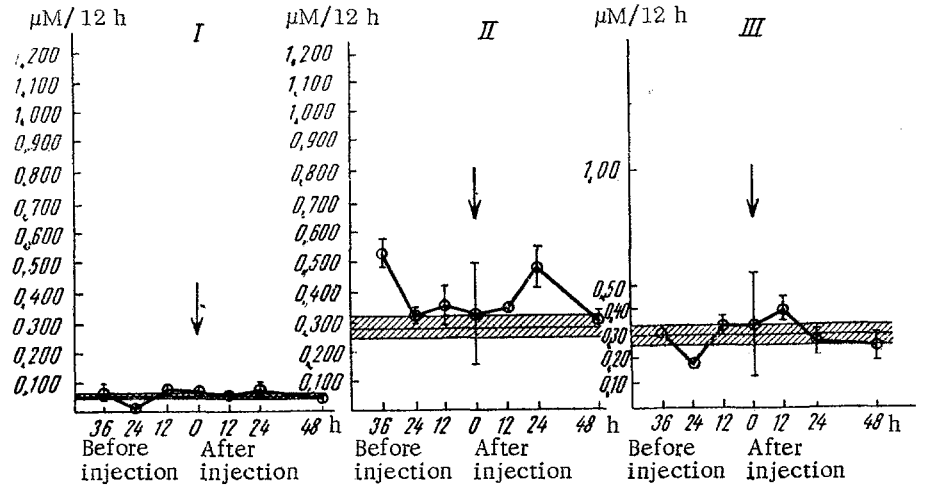

Fig. 1. Urinary excretion of thymidine (I), desoxyuridine (II) and BAIBA (III) by rats after intraperitoneal administration of physiological saline. The shaded portions show M ± m (normal); ⊙ indicates M ± m (data on the respective periods of observation); o indicates M ± m (deviation from normal being statistically significant). | shows time radiation and/or solutions were administered.

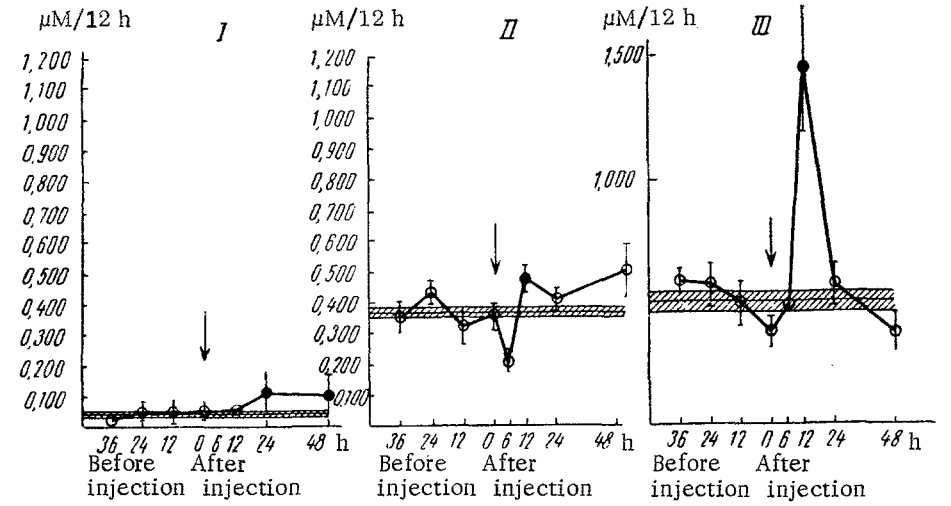

Fig. 2. Urinary excretion of thymidine (I), desoxyuridine (II), and BAIBA (III) by rats after injection of 6 μM thymidine. Points on the figure correspon ing to 6-h periods after irradiation or injection of solutions indicate M ± m (in μM/6 h). The remaining symbols are like those in Fig. 1.

RESULTS

It may be seen from Fig. 1 that administration of physiological saline did not change the desoxyuridine or BAIBA excretion by the rats.

After injection of thymidine into intact rats (Fig. 2), the thymidine excretion did not differ from the initial level during the first 12 h, the output of desoxyuridine increased 31% ($p < 0.02$) and the BAIBA excretion increased by 3 times ($p < 0.001$). In this period, the conversion of administered thymidine into BAIBA was calculated to be 16%. Twenty-four h after administration, the thymidine excretion was doubled ($p < 0.05$) and remained at this level to the end of observation. Thus, the administration of thymidine exerted no influence on thymidine output up to 24 h after injection but it caused a marked and rapidly appearing elevation of BAIBA excretion.

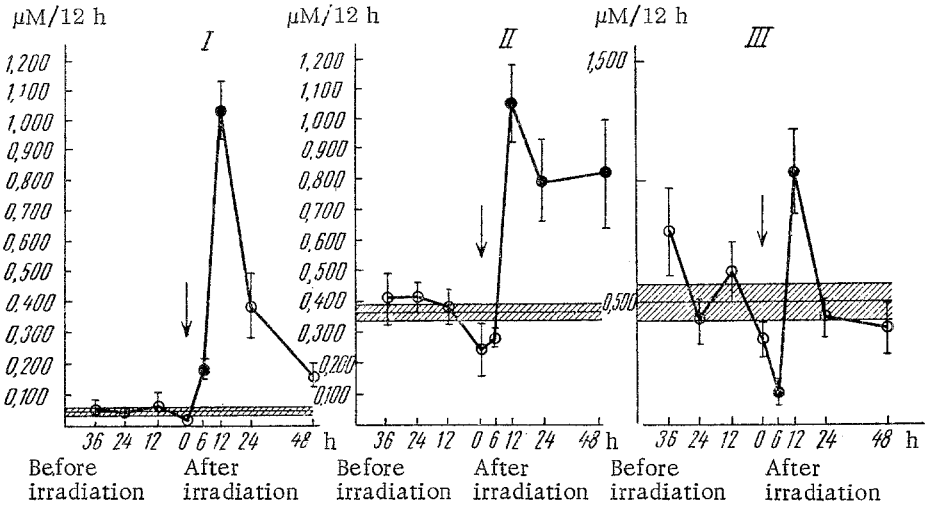

Fig. 3. Urinary excretion of thymidine (I), desoxyuridine (II), and BAIBA (III) by rats after irradiation at the 650 r level. Symbols are the same as in Figs. 1 and 2.

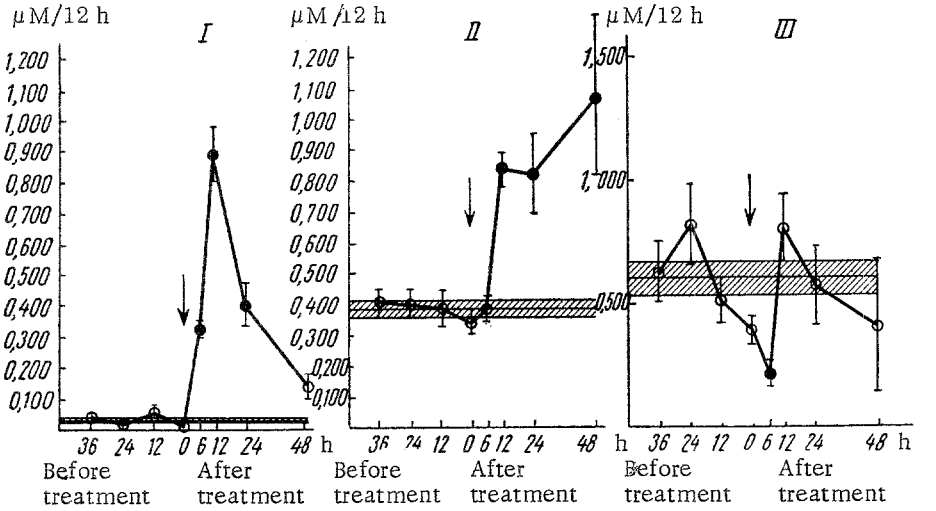

Fig. 4. Urinary excretion of thymidine (I), desoxyuridine (II), and BAIBA (III) by rats after irradiation at a dose of 650 r and injection with 6 μM of thymidine. Symbols are identical with those used in Figs. 1-3.

Irradiation applied to the rats at the minimum lethal dose level (Fig. 3) sharply increased the output of thymidine and desoxyuridine; the maximum quantity of these desoxynucleotides were found in that portion of urine collected in the interval of 6-12 h following irradiation. Subsequently, there was a rapid decline in urinary thymidine content. BAIBA excretion in the first 6 h after irradiation decreased by 50% ($p < 0.01$) and then abruptly increased at 12 h after treatment, attaining twice the initial level ($p < 0.01$). In later periods, it did not exceed normal. The desoxyuridine excretion fell somewhat in the 12-24-h period after irradiation compared with the first 12-h period, and then there was a renewed increase, the level at 48 h exceeding the normal by $2\frac{1}{2}$ times ($p < 0.02$). Consequently, irradiation resulted in a sharp decrease of urinary BAIBA in the first 6 h after treatment and this was followed by an increase in its excretion during the next 6 h; this was accompanied by a continuous steep increase in the urinary thymidine and desoxyuridine output.

Irradiation and administration of 6 μM of thymidine to the animals (Fig. 4) increased the thymidine excretion during the period of 0-6 h to a greater extent than did irradiation alone. In the next 6 h, the urinary thymidine excretion was lower than in the animals that received irradiation only. The urinary output of BAIBA in the 0 to 6-h

period after irradiation was decreased by 32% ($p < 0.01$), after 12 h it was increased but to a significantly lesser degree than in the group that was only irradiated (rising only to normal), and at all subsequent periods the excretion of this component did not differ from normal to any statistically significant degree.

The data presented herein indicate that, despite the excess of thymidine in the organism, conversion of this substance to BAIBA was disrupted just as it had been in animals that were simply irradiated and received no thymidine. But although in this latter group the disruption of thymidine to BAIBA conversion was temporary, disappearing after the sixth h following irradiation, the disruption was maintained for a longer time in the "irradiation + thymidine" group. This is indicated by the very limited peak of BAIBA excretion at 12 h after treatment.

It is interesting that, despite the administration of 6 μM of thymidine to the irradiated animals, the amounts of thymidine and BAIBA excretion in 2 days by the "irradiation + thymidine" group was practically the same as that observed in the "irradiation" group. Thus, the injected material is either absorbed by the organism or it is degraded by way of a reaction not leading to the formation of BAIBA. Both of these possibilities are likely. It was observed by Bens [9] that, during incubation in the presence of thymidine of bone marrow cells from guinea pigs irradiated at a 600 r dose level, there was more than a 2-fold increase in incorporation of thymidine into the reticular cells, these cells not having the requisite enzymes for pyrimidine synthesis. On the other hand, Cerecedo [12] as early as 1927 had shown the existence of an oxidative pathway for thymine degradation in the animal organism. Apparently, the oxidation of thymine in the final analysis results in formation of the α-amino acids [6,7] which are degraded by way of the tricarboxylic acid cycle.

The excretion curve for desoxyuridine in the "irradiated + thymidine" group differs from that for the "irradiated" group in a somewhat smaller rise at 12 h after treatment, and a continuous rise in the subsequent periods throughout the experiment.

The increased desoxyuridine output after irradiation or after irradiation with thymidine administration may be explained as a reflection of a change in the supply of free thymidine in the organism. It could be caused by an increase in transglucosidation between thymidine and uridine [14] or, what seems more probable, a disturbance in the methylation of desoxyuridine, a process regulated by the concentration of reduced nicotinamideadeninedinucleotide phosphate (NADP-H) [22].

Regarding the cause for increased thymidine excretion, the following should be noted. As is known, one of the numerous biochemical reactions in the irradiated organism is the inhibition of DNA synthesis, as determined by a diminished incorporation of labeled precursors into tissue DNA [16-18,20]. This inhibition attains a maximum 2 h after irradiation and may be responsible for the elevated output of thymidine. Beginning at 8 h after irradiation, at doses close to that employed by us, the DNA synthesis in rapidly regenerating tissues (spleen, mucous membrane of the small intestine) gradually becomes reestablished. Thus, the increased thymidine excretion in rats during the early h after irradiation is probably in part associated with thymidine accumulation, because of inhibited DNA synthesis and, as the results of the present work show, to a great extent it appears to be a consequence of interference with the degradation of this desoxynucleoside to BAIBA. The subsequent elevation in thymidine, desoxyuridine and BAIBA in the 6-12-h period after irradiation appears to be due to DNA breakdown. Actually, the first signs of degradation of polymerized DNA in vivo are observed in the rapidly regenerating tissues as early as 2 h after irradiation [13], and the maximum accumulation of nonpolymerized DNA fragments in these tissues is reached at 6 h after radiation damage [2,11,21]. A calculation based on our data shows that only 18.1% of the total thymidine excretion, occurring in 12 h, takes place in the first 6 h. Consequently, about 80% of the thymidine lost to the organism in the first 12 h after irradiation is derived from DNA degradation in the most radiation sensitive tissues. The comparison of intact rats with animals of the "irradiated + thymidine" group, in regard to capacity for converting injected thymidine into BAIBA in 12 h, indicates that the efficiency of BAIBA formation is 16% in the normal and falls to 3% after irradiation, i.e., by a factor of more than 5.

Thus, the data obtained in our experiments on the whole organism, in contrast to data in the literature, indicate that the increased DNA degradation in radiation-sensitive tissues and the disruption of thymidine to BAIBA conversion are apparently the principal causes for the elevated excretion of this desoxynucleoside by rats after irradiation.

LITERATURE CITED

1. P. D. Gorizontov, T. A. Fedorova, Yu. A. Zharkov, et al., Radiobiologiya, 4, 514 (1963).
2. N. V. Ermolaeva, Radiobiologiya, 5, 670 (1961).

3. Yu. A. Zharkov, Labor. delo, 5, 296 (1964).
4. Yu. A. Zharkov, T. A. Fedorova, and L. F. Mikhailova, Radiobiologiya, 5, 563 (1965).
5. V. K. Mazurik, Byull. éksp. biol., 2, 59 (1965).
6. Yu. P. Shvachkin and M. A. Prokof'ev, Vestn. Moskovsk. un-ta. Khimiya, 2, 3 (1964).
7. T. H. Bates, C. J. Smith, and H. Smith, Nature, 203, 843 (1964).
8. D. Bawden, J. clin. Path., 16, 284 (1963).
9. L. Benes, Folia biol. (Praha), 10, 124 (1964).
10. H. K. Berry et al., Science, 142, 396 (1963).
11. C. W. Bishop and J. N. Davidson, Brit. J. Radiol., 30, 367 (1957).
12. L. R. Cerecedo, J. biol. Chem., 75, 661 (1927).
13. L. J. Cole and M. E. Ellis, Radiat. Res., 7, 508 (1957).
14. C. H. DeVerdier and V. R. Potter, J. nat. Cancer Inst., 24, 13 (1960).
15. K. Fink et al., J. biol. Chem., 187, 441 (1952).
16. O. F. Nygaard, Fed. Proc., 18, 1, 295 (1959).
17. O. F. Nygaard and R. L. Potter, Radiat. Res., 10, 462 (1959).
18. R. L. Potter, Fed. Proc., 18, 304 (1959).
19. P. Reichard, Acta chem. scand., 9, 1275 (1965).
20. F. G. Sherman and H. Quastler, Radiat. Res., 9, 182 (1958).
21. J. Soska and L. Soskova, Folia biol. (Praha), 5, 425 (1959).
22. A. J. Wahba and M. Friedkin, J. biol. Chem., 236, 11 (1961).

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