EFFECT OF IRRADIATION ON URINARY EXCRETION OF DEOXYCYTIDINE AND β-AMINOISOBUTYRIC ACID IN MONKEYS

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After whole-body γ-ray irradiation of monkeys (Macaca rhesus) in a dose of 620 R, the urinary excretion of deoxycytidine increased on the first to third, sixth to eighth, and 18th-24th days. The urinary excretion of β-aminoisobutyric acid was increased on the first to second and fifth to seventh days and returned to normal on the 24th-27th days.

An important problem in radiation biochemistry is the development of sensitive biochemical tests for use as indicators and dosimeters of radiation damage. A great step forward in this direction was the discovery of postradiational deoxynucleosiduria, the hyperexcretion of deoxynucleosides, which are DNA metabolites, in the urine. A study of the principles governing postradiational nucleosiduria in rats has shown that determination of the level of excretion of deoxycytidine and thymidine in the urine of this species of animals can be used as a diagnostic dosimetric test of radiation damage [1-3, 11, 12, 16-18]. The character of the postradiational deoxynucleosiduria in animals of other species has so far been investigated only superficially. Only two studies of the urinary excretion of DNA metabolites by irradiated monkeys has so far been published [9, 10].

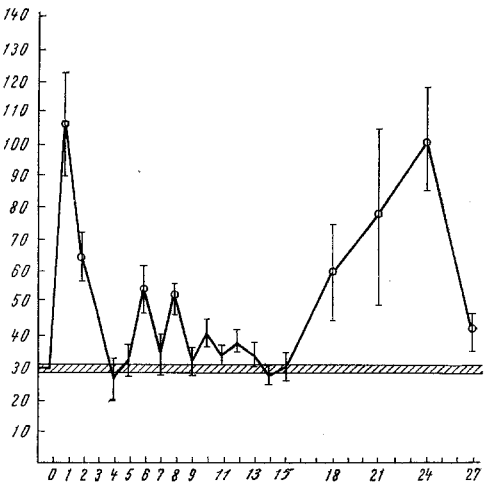

Fig. 1. Dynamics of urinary excretion of DC in monkeys after γ-ray irradiation in dose of 620 R. Empty circles denote M ± m after γ-ray irradiation; shaded strip shows normal range of M ± m. Abscissa, days after irradiation; ordinate, excretion of deoxycytidine (in μg/day).

The object of the present investigation was to study the level of excretion of deoxycytidine (DC) and of β-aminoisobutyric acid (BABA), a breakdown product of thymidine in monkeys, in the 24-h urine.

EXPERIMENTAL METHOD AND RESULTS

Tests were carried out on monkeys (Macaca rhesus; weight 2-2.5 kg) under normal conditions and after whole-body γ-ray irradiation in a dose of 620 R (LD 80-90/45) from the first to the 27th days. Fifteen monkeys were kept in metabolism cages singly before irradiation and in pairs after irradiation. The urine was collected two to three times before irradiation, and thereafter every day for 15 days, and also on the 18th, 21st, 24th, and 27th days after irradiation. The urine was filtered and the DC and BABA fractions were isolated from it by means of ion-exchange resins. DC was determined in the urine by the method of Tarakanova and Tereshchenko [7]. This method is based on the preparative isolation of the DC fraction by Dowex-1

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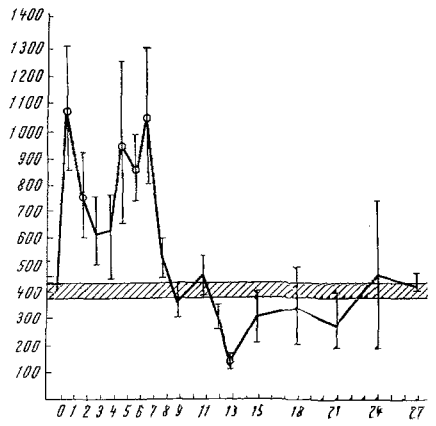

Fig. 2. Dynamics of urinary excretion of BABA by monkeys after whole-body γ-ray irradiation in dose of 620 R. Legend as in Fig. 1.

ion-exchange resin in OH^- form and Dowex-50 in the H^+ form, and by paper chromatography followed by spectrocolorimetric determination of DC as 2-deoxyribose in the reaction with 2-thiobarbituric acid. BABA was determined by the ninhydrin method after isolation of the compound from the urine with the aid of Dowex-50 resin in the H^+ form and paper chromatography in a system of n-butanol–formic acid–water (75 : 15 : 10).

The daily urinary excretion of DC by the irradiated monkeys is shown in Fig. 1. After irradiation of the monkeys, their urinary excretion of DC reached a maximum on the first day, when it was 3.6 times higher than the background level ($P < 0.001$); on the second day the urinary DC excretion had fallen, but was still twice its background level ($P < 0.001$). On the following days the DC content in the urine fluctuated: it increased on the sixth to eighth day ($P < 0.001$), and on the 18th–27th day, with a tendency to decrease toward the 27th day ($P < 0.05$); at all other times of investigation the urinary DC level fluctuated within normal limits.

Measurements of the urinary excretion of BABA by the same monkeys are given in Fig. 2. On the first day after irradiation the urinary excretion of BABA was significantly increased (by about 2.7 times), while on the second, third, and fourth days the BABA level in the urine fell successively. On the fifth to seventh days a new wave of significant increase in the BABA level in the urine was observed. Starting from the ninth day, the BABA content in the urine showed a tendency to decrease to subnormal values (with a significant decrease to 30% of its initial level on the 13th day), and the normal level was not reached until the 24th–27th day.

The normal level of urinary excretion of DC in monkeys varies from 5.11 to 58.12 μg/day, with a mean value of 29.84 ± 1.48 μg/day. The 24-h urinary excretion of DC in rats is higher than in monkeys, varying on the average from 68.4 to 700 μg [6, 18]. This difference in the levels of DC excretion in monkeys and rats is probably due to species differences in the activity of deoxycytidylate deaminase, an enzyme catalyzing the conversion of DC into deoxyuridine at the nucleotide level, in the blood serum and tissues. The activity of this enzyme in rats is very low [14], whereas in animals of other species [8], in man [19] and, probably, in monkeys, it is considerably higher.

The BABA content in the urine of normal monkeys varies from 105 to 751 μg/day, with a mean value of 379 ± 29 μg/day, whereas in rats it varies from 6 to 98 μg/day, with a mean value of 50 μg/day [3], i.e., when calculated per kilogram body weight, the level of excretion of BABA by the animals of these two species is approximately the same.

The general rule of an increase in the excretion of DC during the 24 h after irradiation, established in rats, also holds good in monkeys. Unlike in rats, the increase in urinary excretion of DC in monkeys also continued in the later periods after irradiation. The sharp increase in the DC content in the urine of the monkeys observed on the first to third and sixth to eighth days after irradiation agrees well with the results of investigations of the effect of irradiation, in minimal absolutely lethal doses, on the urinary excretion of Dische-positive substances [10] and of DC [9] by monkeys.

What is the nature of the most characteristic changes in the excretion of DC and BABA observed in irradiated monkeys?

Soviet investigators [1-3, 6, 7, 11], whose results were subsequently reproduced and confirmed by Western workers [16, 17], have shown that the first peak of postradiation deoxynucleosiduria and excretion of BABA is characteristic not only of rats, but also of other species of mammals (mice, rabbits, dogs, monkeys) and man, and reflects increased breakdown of DNS [2] mainly as a result of the interphase death of cells of the hematopoietic organs [4], small intestine, and certain other organs. The size of this peak can give some idea of the extent of cellular destruction in these organs. The increased excretion of DC and BABA observed in monkeys on the first to third days after irradiation correlates closely with these findings and explains them.

Following DNA breakdown, the next phase of postradiation biochemical changes is the restoration of DNA metabolism. The following phenomena occur consecutively: a) an increase in activity of the enzymes

catalyzing biosynthesis and phosphorylation of nucleotides; b) gradual restoration of their polymerization, as shown initially by an increase in the incorporation of labeled precursors, and later by an increase in the DNA content (and, consequently, in the number of cells) in the hematopoietic organs. Hyperproduction of DNA precursors, especially deoxycytidyl and thymidyl nucleotides, is possible at this period.

In monkeys, foci of regeneration in the bone marrow are found after the ninth to 10th days after irradiation in doses of 570-650 R [5]. The biochemical reactions listed above precede the appearance of foci of regeneration and coincide in time with the second period of increased excretion of DC (sixth to eighth) and BABA (fifth to seventh days) in the irradiated monkeys. This suggests that the second wave of hyperexcretion of DNA metabolites is the result of the accumulation of pyrimidine deoxynucleotides, because of a discrepancy between their synthesis de novo and the rate of their incorporation into DNA molecules. This discrepancy is evidently corrected later, because the newly-formed cells (and DNA synthesis) in the hematopoietic tissue becomes detectable, and its rate increases sharply from the 10th to the 17th days [5], as a result of which an increase in the number of cells in the circulating blood is observed starting from the 15th day [15]. The increase in utilization of DNA precursors at this period (10th-15th days) is reflected in the decreased urinary excretion of DC in the monkeys to normal values, and of BABA to subnormal values.

Finally, the increase in DC excretion and restoration of the normal BABA excretion by the 24th-27th days are evidently due to hyperproduction of DNA precursors, for by the 30th day the number of many types of cells in the bone marrow becomes much greater than normal [5]. The differences in the levels of excretion of DC and BABA at this period are evidently attributable to the well-established fact of a difference in the rates of biosynthesis of cytidyl and thymidyl deoxynucleotides in the body: whereas the former is synthesized rapidly and, consequently, it may be formed in excess, the latter is synthesized much more slowly, and only in sufficient quantities to meet the demands for DNA synthesis [13].

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