4. V. Z. Lankin, A. K. Tikhaze, A. L. Kovalevskaya, et al., Dokl. Akad. Nauk SSSR, 261, No. 6, 1467 (1981).
5. B. Chance, H. Sies, and A. Boveris, Physiol. Rev., 59, 527 (1979).
6. V. L. W. Go, R. G. Warnon, and J. B. Frith, Can. J. Biochem., 49, 753 (1971).
7. M. K. Holland and B. T. Storey, Biochem. J., 198, 273 (1981).
8. P. I. Jamage, T. Mori, and S. Matsushita, Agr. Biol. Chem., 35, 33 (1971).
9. R. Jones and T. Mann, Proc. R. Soc. London, B, 193, 317 (1976).
10. M. R. F. Mennella and R. Jones, Biochem. J., 191, 289 (1980).
11. R. G. Miller and R. A. Phillips, J. Cell. Physiol., 73, 191 (1969).
12. J. R. Prohaska, Biochim. Biophys. Acta, 611, 87 (1980).
13. J. R. Prohaska and H. E. Ganther, Biochem. Biophys. Res. Commun., 76, 437 (1977).
14. L. J. Romrell, A. R. Bellve, D. W. Fawcett, et al., Dev. Biol., 49, 119 (1976).

DYNAMICS OF NITRITE-INDUCED METHEMOGLOBIN FORMATION AFTER TOTAL GAMMA-RAY IRRADIATION OF RATS

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One trend in the design of atomic power stations (APS) based on fast neutrons is the development of APS in which dissociating nitrogen tetroxide is used as heat carrier and as working heat. The multifactor nature of the radiational and toxic action (ionizing radiation, nitrogen oxides and their transformation products, mainly nitrates and nitrites) determines the need for solution of a number of medical biological problems, one of which is the study of the combined action of ionizing radiation and of sodium nitrite on methemoglobin formation, associated with the action of nitrites as a metabolic product of nitrogen oxide metabolism.

EXPERIMENTAL METHOD

Experiments were carried out on male Wistar rats weighing 180-200 g. The animals were kept under animal house conditions on a standard diet. Single whole-body irradiation was given on the UGU-420 gamma-ray source in doses of 77.4 and 180.6 mCi/kg body weight with a dose rate of $1.64 \cdot 10^{-4}$ A/kg. Sodium nitrite (chemically pure) dissolved in physiological saline was injected intraperitoneally (7 mg/100 g body weight) 1, 3, 7, and 15 days after irradiation. The animals were killed under superficial ether anesthesia by bleeding from the heart. The methemoglobin concentration was determined 15, 45, 60, 90, and 180 min after injection of sodium nitrite [1].

The dynamics of methemoglobin formation and reduction was determined in hemolysates [2]. Oxidation of hemoglobin to methemoglobin was carried out with glucose and methylene blue [9-11].

The dynamics of changes in the methemoglobin concentration was monitored on a VSU-2-P spectrophotometer at 540-630 nm. The hemoglobin content in the hemolysates was determined by the cyanmethemoglobin method, the methemoglobin content by the method of Austin and Drabkin and by spectrophotometry [2].

EXPERIMENTAL RESULTS

Methemoglobin is present in small quantities (1-4%) in the blood of intact animals. The methemoglobin concentration in the blood of the irradiated rats increased with the time elapsing after iation in a dose of 180.6 mCi/kg, and was 4.45, 6.56, 12.2, and 8.3% on the

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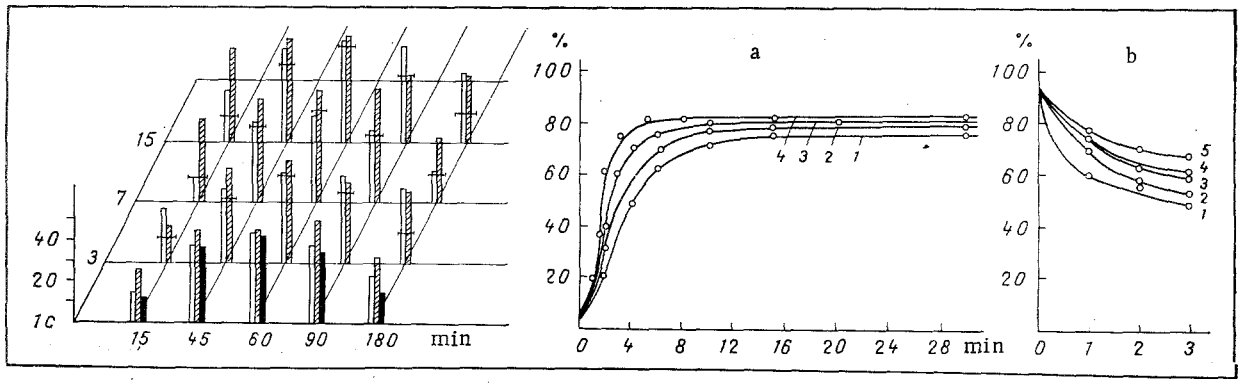

Fig. 1 Fig. 2

Fig. 1. Methemoglobin concentration (in %) in rats' blood on 1st, 3rd, 7th, and 15th days after irradiation and 15, 45, 60, 90, and 180 min after injection of nitrite. Black columns — control; white columns — irradiation in a dose of 77.4 mCi/kg; obliquely shaded — irradiation in a dose of 180.6 mCi/kg; horizontal line indicates methemoglobin level in control animals.

Fig. 2. Dynamics of nitrite oxidation (a) and reduction (b) of methemoglobin (in %) in hemolysates of blood of control rats and rats irradiated in a dose of 180.6 mCi/kg body weight. A: 1) Control, 2) on 3rd day, 3) on 15th day, 4) on 7th day after irradiation; B: 1) control; 2) on 1st day, 3) on 4th day, 4) on 15th day, 5) on 7th day after irradiation.

1st, 3rd, 7th, and 15th days, respectively. Irradiation in a dose of 77.4 mCi/kg caused no increase in the methemoglobin concentration in the blood compared with the control.

A diagram showing the relationship between nitrite-induced methemoglobin formation and dose and time elapsing after irradiation is shown in Fig. 1. It can be seen that the methemoglobin concentration when nitrite was injected after irradiation was much higher than values obtained when nitrite was injected into unirradiated animals. The maximal radiation effect was manifested in the first 15 min and toward 3 h after injection of nitrite. In the period of maximal development of methemoglobinemia (1 h) this difference disappeared, for the blood methemoglobin level can rise only to a limit (about 65%) beyond which the animals die. With an increase in the time after irradiation in a dose of 180.6 mCi/kg the methemoglobin concentration rose, to exceed the methemoglobin concentration in the blood of control animals with nitrite-induced poisoning by 240% on the 7th day and by 280% on the 15th day. According to the results, the expected blood level of methemoglobin after 15 min, based on summation of effects of their separate action (nitrites 10% and irradiation 12.2-8% on the 7th-15th day) ought to be 18 and 22%; in fact, it was 34 and 38%, i.e., the effect of combined action was more than additive.

One possible cause of this could be disturbance of the balance of the oxidation-reduction systems of the erythrocytes as a result of irradiation: an increase in the rate of generation of endogenous methemoglobin formers and a decrease in the rate of reduction of methemoglobin through inhibition of activity of the methemoglobin reductase system.

Data on methemoglobin formation in partially purified hemoglobin solutions during nitrite-induced oxidation in hemolysates of control rats and of rats irradiated in a dose of 180.6 mCi/kg, and on its reduction are given in Fig. 2. The rate of methemoglobin formation implies the time during which half of the hemoglobin is converted into methemoglobin. For the control animals this was 4 min. In irradiated animals the rate of oxidation of hemoglobin was higher and it increased with an increase in the time after irradiation. It reached peak values on the 7th day (108 sec), i.e., twice the rate of hemoglobin oxidation observed in the blood of intact animals. Later the rate of oxidation approached the normal value, and on the 15th day it was 144 sec.

The reduction process took place more slowly in irradiated animals. The rate of reduction decreased with an increase in the time elapsing after irradiation, to reach a minimum on the 7th day. A further increase in the time after irradiation led to an increase in the rate of reduction, which came close to the rate of reduction in hemolysates of blood from intact animals.

The coefficient of correlation between the rate of oxidation and the methemoglobin content in the hemolysates of irradiated animals, calculated from experimental values, was 0.91, and between the rates of oxidation and reduction it was also 0.91.

The results are evidence that processes of oxidation and reduction are interconnected and are determined by changes taking place in the erythrocytes during irradiation. This effect can be realized by several mechanisms. In particular, the strucutre of hemoglobin is changed during irradiation, and this is accompanied by disturbance of the physicochemical properties of the erythrocytes and of the amino-acid composition of the primary structure of the polypeptide chains of hemoglobin.

Under the influence of ionizing radiation excessive formation of hydrogen peroxide, an endogenous radiosensitizer and active methemoglobin former, takes place in the cells. High radiation radiosensitivity of protein sulfhydryl groups, which are endogenous radioprotectors (in particular, reduced glutathione, etc.), disturbs processes of detoxication of endogenous methemoglobin formers.

During irradiation, activity of glucose-6-phosphate dehydrogenase, which catalyzes the first oxidation reaction of glucose in the pentose phosphate cycle, an important step in the generation of reducing agents in the cells in the form of NADPH and NADH, decreases [8-10].

Increased sensitivity of irradiated animals to the action of sodium nitrite may thus be the result of a combination of radiation-toxic processes connected with disturbance of the internal structure of hemoglobin that maintains the integrity of biochemical processes and its resistance to oxidation-reduction factors, and also the result of additional formation of endogenous methemoglobin formers (hydrogen peroxide or other equivalent free-radical compounds), a decrease in the content of natural radioprotectors, and disturbance of intracellular generation of reducing agents necessary for the reduction of methemoglobin.

LITERATURE CITED

1. V. A. Boyarchik and A. G. Lutov, Gig. Tr., No. 3, 55 (1966).
2. M. S. Kushakovskii, Clinical Forms of Injury to Hemoglobin [in Russian], Moscow–Leningrad (1968).
3. S. E. Manoilov, Primary Mechanisms of the Biological Action of Penetrating Radiation [in Russian], Leningrad (1968).
4. E. N. Panasyuk and B. I. Zdravko, Fiziol. Zh. (Kiev), 28, No. 3, 327 (1982).
5. B. F. Sukhomlinov, A. V. Savich, M. I. Shal'nov, et al., Ukr. Biokhim. Zh., No. 6, 16 (1981).
6. M. I. Shal'nov and L. I. Olontseva, in: Problems in Radiobiology and the Biological Action of Cytostatics [in Russian], Vol. 5, Tomsk (1973), p. 37.
7. I. M. Shur'yan, in: Biophysics and Radiology [in Russian], No. 3, Kiev (1972), p. 26.
8. K. I. Betke, I. Greinacher, and F. Hecker, Arch. Exp. Pathol. Pharmakol., 229, 207 (1956).
9. E. Beutler and M. C. Baluda, Blood, 22, 323 (1963).
10. A. S. Kenneth, J. Appl. Physiol., 31, 154 (1971).
11. J. E. Smith and E. Beutler, J. Physiol. (London), 210 347 (1966).
12. E. R. Jaffe, in: Cellular and Molecular Biology of Erythrocytes, Baltimore (1974), p. 345.