INTERACTION BETWEEN TISSUES AND ACTIVE DYE AT DIFFERENT STAGES OF ACUTE RADIATION INJURY

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UDC 616-001.28-036.11-07:616-018-076.1

The sorptive power of most tissues is increased in rats irradiated in a dose of 800 R (experiments with the dye Procion bright red). The greatest increase in sorption was found in the small intestine and spleen.

Considerable experimental evidence of changes in the vital staining of tissues after exposure to radiation has been accumulated during recent years [4, 5, 7, 9, 12, 13, 18].

Recently, substances known as active dyes, with the ability to form stable covalent bonds with protein macromolecules of biological substrates, have begun to be used in cytochemical, histochemical, and biochemical research [1, 10, 11, 14-17].

The use of active dyes for vital staining of tissue structures have yielded more precise information concerning the responses of cytoplasmic substances to inadequate stimuli [6, 10, 14, 15].

The object of this investigation was to study the dynamics of changes in fixation of active dye by the tissues of experimental animals subjected to whole-body irradiation in a lethal dose.

EXPERIMENTAL METHOD

Experiments were carried out on 120 albino rats weighing 150-220 g, irradiated with x-rays in a dose of 800 R (180 kV, 15 mA, filters 0.5 mm Cu and 1 mm Al, skin-focus distance 40 cm). The rats were decapitated 6 h and 1, 3, 5, 7, and 10 days after irradiation, and the liver, kidneys, small intestine, spleen, brain, heart, and adrenals were quickly removed. After the organs had been kept for 30 min in Ringer's solution they were stained with the active dye Procion bright red, using the technique suggested for this purpose by Suzdal'skaya [14]. Pieces of tissue were placed in test tubes containing equal quantities of 0.06% solution of Procion bright red, made up in Ringer's solution. The test material was kept in the solutions of dye for 30 min, after which, in order to extract dye noncovalently bound by the tissues, the organs were transferred for 24 h into 80° ethyl alcohol, acidified with sulfuric acid (7%). The alcoholic extracts were examined colorimetrically in the FÉK-M3 photoelectric colorimeter and the quantity of dye weakly bound by the tissue was calculated. Meanwhile the content of Procion bright red in the original solution of dye and in the solutions in which the organs were stained was determined. The difference between the concentrations of the initial and working solutions gave the total quantity of dye absorbed by the tissue. By subtracting from this figure the content of dye which came out into the alcoholic extract, the quantity of dye covalently bound by the tissue was obtained. The quantity of Procion bright red bound weakly and strongly by the tissue was expressed per gram weight of organ. The tissues of unirradiated rats were stained under identical conditions.

EXPERIMENTAL RESULTS

An increase in the degree of binding of the weakly fixed fraction of active dye in the small intestine and spleen was observed 6 h after irradiation (Table 1). Sorption of the dye in the brain and adrenals was

Department of Roentgenology and Medical Radiology, Semipalatinsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR P. D. Gorizontov.) Translated from Byulleten' Éksperimental Biologii i Meditsiny, Vol. 69, No. 5, pp. 39-42, May, 1970. Original article submitted May 16, 1969.

TABLE 1. Fixation of Procion Bright Red by Tissues of Irradiated Albino Rats (in percent of degree of sorption in control animals)

Tissue investigated	Time of investigation after irradiation											
	6 h		1 day		3 days		5 days		7 days		10 days	
	M	*P*	*M*	*P*	*M*	*P*	*M*	*P*	*M*	*P*	*M*	*P*
Extractable fraction												
Liver	+8,3	>0,05	+26,4	<0,05	+37,3	<0,05	+16,4	>0,05	+13,6	>0,05	+27,2	<0,05
Kidneys	+11,2	>0,05	+27,6	<0,05	+50,0	<0,05	+12,3	>0,05	+15,4	>0,05	+27,8	<0,05
Small intestine	+37,5	<0,05	+55,3	<0,05	+84,9	<0,05	+37,5	<0,05	+26,3	<0,05	+29,1	<0,05
Spleen	+42,5	<0,05	+65,2	<0,05	+98,8	<0,05	+39,2	<0,05	+30,0	<0,05	+46,3	<0,05
Brain	−18,5	<0,05	+6,2	>0,05	+30,1	<0,05	+23,7	<0,05	+18,6	<0,05	+23,0	<0,05
Heart	+5,0	>0,05	+19,9	<0,05	+34,5	<0,05	+12,5	>0,05	+5,8	>0,05	+21,4	<0,05
Adrenals	−25,7	<0,05	−12,5	>0,05	+21,5	<0,05	+18,4	<0,05	+5,2	>0,05	−8,3	>0,05
Unextractable fraction												
Liver	+28,5	<0,05	+40,1	<0,05	+58,4	<0,05	+31,1	<0,05	+22,8	<0,05	+42,5	<0,05
Kidneys	+33,4	<0,05	+50,2	<0,05	+73,8	<0,05	+49,6	<0,05	+32,3	<0,05	+45,1	<0,05
Small intestine	+59,1	<0,05	+76,0	<0,05	+149,1	<0,05	+83,2	<0,05	+48,0	<0,05	+57,5	<0,05
Spleen	+70,2	<0,05	+87,3	<0,05	+173,4	<0,05	+75,7	<0,05	+55,9	<0,05	+69,8	<0,05
Brain	+6,5	>0,05	+14,8	>0,05	+58,7	<0,05	+35,2	<0,05	+42,4	<0,05	+34,3	<0,05
Heart	+12,7	>0,05	+30,6	<0,05	+53,2	<0,05	+24,9	<0,05	+11,6	>0,05	+29,5	<0,05
Adrenals	−17,8	<0,05	−4,7	<0,05	+42,5	<0,05	+29,5	<0,05	+14,1	>0,05	+7,4	>0,05

Note. In control group 30 rats were investigated, and 15 in each experimental group. +, − denote increase and decrease, respectively, in sorption of dye; M denotes arithmetic mean change in fixation of dye in experiment as a percent of control.

reduced at this time. The binding of the extractable fraction of Procion bright red by the tissues of the liver, kidneys, small intestine, spleen, and heart muscles was increased 24 h after irradiation. The sorptive properties of the brain and adrenal tissues at this period were not significantly different from the controls. A marked increase in the sorptive power of all investigated tissues relative to the readily extractable fraction of active dye was observed 3 days after irradiation. These changes were especially marked in the small intestine and spleen. The sorption of the weakly fixed fraction of Procion bright red by the tissues of the experimental animals was reduced 5 and 7 days after irradiation. In some organs (liver, kidneys, heart) it was not significantly different from the control, but it exceeded normal in the small intestine, spleen, brain, and adrenals (5 days after irradiation). By the 10th day the level of fixation of dye had again increased in all studied organs except the adrenals, in which it corresponded to the control level.

Investigation of the character of covalent fixation of dye revealed the following results.

The content of firmly fixed fraction of Procion bright red was increased 6 h after irradiation in the liver, kidneys, small intestine, and spleen. Accumulation of the unextractable fraction of dye in these organs was combined with lowering of the level of sorption in the adrenals and by absence of significant changes in sorption in the brain and heart. An increase in covalent binding of active dye was found 24 h after irradiation in the liver, kidneys, small intestine, spleen, and heart muscle. The binding of Procion bright red was not significantly changed in the brain and adrenals. The most marked increase in the quantity of firmly fixed dye in all the investigated organs was discovered three days after irradiation, the level of fixation of the unextractable fraction of Procion bright red in the small intestine and spleen being much higher than in the other organs. On the 5th and 7th days after irradiation, the changes in sorption of dye were slightly reduced. Accumulation of dye in the tissues of the heart and adrenals was reduced (after 7 days). Ten days after irradiation an increase was observed in the covalent binding of dye in most of the investigated organs, except the adrenals where the indices of sorption corresponded to those in the control.

Whole-body irradiation of albino rats with x rays in a dose of 800 R thus causes the disturbance of sorption of active dye by the tissues throughout the studied period of observation. Covalent fixation of dye is predominant, and is especially marked in the radiosensitive tissues – the small intestine and spleen.

Considering that active dyes form stable covalent bonds with amino- and imino- [2, 3, 14, 15, 19] and, possibly, with sulfhydryl groups [8], it can be assumed that the development of lethal radiation injury is accompanied by the onset of severe structural changes in the cytoplasm, due to the liberation of additional reactive groups and radicals, and to disturbance of the conformation and order of the protein complexes. These changes can provide the basis for pathological reactions in individual organs and in the body as a whole, and they largely determine the development of radiobiological effects.

Comparison of these results with those of the investigations cited above, in which ordinary vital dyes were used to study the sorptive properties of the organs of animals irradiated in comparable doses, shows that the use of an active dye can reveal earlier, more severe, and more prolonged disturbances of the structural state of the tissues.

The results of this investigation, like those of earlier work to study the fixation of Procion bright red by the tissues of animals irradiated in a sublethal dose [6], demonstrate the desirability of using active dyes for the detection and analysis of fine physicochemical changes in the cytoplasm after exposure to irradiation.

The author is grateful to Professor V. P. Paribok, Doctor of Biological Sciences I. P. Suzdal'skaya, and Candidate of Biological Sciences V. P. Troshina for their help and advice with this investigation.

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