# BIOCHEMISTRY AND BIOPHYSICS

THE DYNAMICS OF THE CHANGES IN THE SORPTION PROPERTIES OF TISSUES FOLLOWING THE ACTION OF  $\alpha\text{--RADIATION}$  ON THE ORGANISM

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In the development of changes occurring in the organism after irradiation, an important role is played by the disturbances in tissue permeability; this is regarded by many authors as one of the most characteristic and essential components in radiation illness [3, 7, 8, 9].

The works of D. N. Naconov and co-workers [2, 11, 12, 13] have convincingly shown that the permeability of cells depends on the sorption activity of the protoplasm and its more important component part—the proteins. Numerous investigations, performed in this direction, have indicated that under the influence of various external factors there occur definite physico-chemical changes in the cellular proteins, as a result of which the capacity of the protoplasm to bind vital stains increases. Intensification of the tissue sorption properties subsequent to injury is explained by denaturing changes in the protein structures of the cells.

It is known that disturbances in the protein metabolism regularly set in, and are the most important stage of radiation illness in the organism [5, 6, 10, 15].

Recently, isolated bodies of evidence have appeared in the literature on the genesis of denaturing changes in the protein molecules subsequent to the action of ionizing radiation on biological subjects. The occurrence of denaturation of the protein structures was discovered in an investigation of the binding of tagged methionine by irradiated proteins [13], by the use of immunological procedures [8], and by the study of the tinctorial properties of tissues [1, 3, 4]. However, the data obtained by the various investigators is, to an appreciable degree, contradictory.

In line with the above, we attempted, in this work, to elucidate how the sorption properties of experimental animals' tissues are modified under the influence of gamma-ray radiation, and how the observed changes are caused. For this purpose we used the method of supravital staining, a delicate and sensitive index of the physiological state of cells [11, 14].

### METHOD

The experiments were performed on white rats of both sexes, 165-250 grams in weight, maintained on a normal mixed diet. The work was carried out on 145 white rats, 25 of which served as the control. The animals were subjected to whole-body radiation with gamma-rays from radioactive cobalt, in doses of 800 r (first series of experiments), and 400 r (second series). The irradiation was accomplished on the GUT-Co<sup>60</sup>-400 apparatus, with a dose output of 35.5 r/min. At various intervals after the irradiation we studied the sorption properties of the tissues in the experimental animals relative to the basic vital stains—neutral red (granular stain) and chrysoidin (diffuse stain).

TABLE 1

Changes in the Sorption Properties of Tissues from White Rats at Varying Intervals following Irradiation with a Dose of 800 r (in %)

					H	Interval	following the	irradi	irradiation (in hours	(8)		
Stain	Tissue studied	Control	9		24		84		72		120	
		M±m	M±m	I	M±m	T	M±m	7	M±m	T	Μ±m	7
Neutral red	Liver Kidney Small intestine Spleen Lung Brain Adrenal Heart muscle	0001 10000 1	118 120,22 130,52,21 128,4,11 107,54,4 117,7,4 108,68 108,	0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0,	112,5±3,5 130,5±6,9 142,9±4,8 139,7±6,1 109,6±7,9 108,4±7,1 102,5±5,9	4284-1-0	117,0±7,8 128,7±6,0 163,3±4,7 158,0±5,9 126,9±4,5 103,1±6,8 80,1±7,7 95,4±5,3	7,6,7,4,0 7,6,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,0,	134,6±6,7 131,3±5,9 150,5±5,8 166,1±5,4 136,9±5,2 113,4±4,4 107,6±5,7	0,0,0,4,0,0,1 0,0,0,0,0,1	98, 3±5,2 90,1±6,2 107,6±5,7 119,2 = 5,2 108,5+5,5 77,3±7,4 78,2±9,1	00-0-0 
Chrysoidin	Liver Kidney Small intestine Spleen lung Brain Adrenal Heart muscle	100±4,0 100±3,8 100±3,8 100±3,5 100±3,1 100±3,4	108,6±8,5 113,5±8,6 123,4±5,1 121,0±4,9 111,0±6,4 106,9±7,5 93,1±5,7	0,	199,37 122,4 122,57 131,57 105,58 106,63 106,04 106	6,4,0,0,0,0 6,6,0,0,0,0,0,0,0,0,0,0,0,0,0,0	111,2±7,7 125,4±5,2 143,9±6,4 116,2±6,8 122,8±5,1 101,1±10,2 83,3±7,0 91,4±8,7		128,5±6,7 126,5±6,4 140,1±6,9 152,5±6,5 1129,5±6,5 106,4±5,5 106,4±5,5	00000000000000000000000000000000000000	105,3±6,9 90,3±7,1 98,3±8,1 96,0±5,7 92,1±6,4 72,4±6,9 78,4±6,9	0-004 <i>u</i> 0.0004 <i>u</i>

Note: In the control group we used 25 animals, 12 in each of the experimental groups.

TABLE 2

		,			Inte	rval fo	Interval following the ir	radiati	irradiation (in hours)			
		Control	9		24		48		72		120	
Stain	Tissue studied	$M\pm m$	M±m	L	M±m	T	M±m	T	M±m	T	$M\pm m$	7
Neutral red	Liver Kidney Small intestine Spleen Lung Brain Adrenal Heart muscle	00101000000000000000000000000000000000	200 200 200 200 200 200 200 200 200 200	00000-00 \$\phi\$\phi\$\phi\$\phi\$	90,3±8,2 130,5±5,1 131,5±5,1 111,0±4,7 111,0±4,7 111,0±4,7 76,3±5,9 76,3±5,9	24.67.001.4.0 0.4.001.4.0	92,6±5,5 124,2±5,5 142,2±5,5 147,3±6,1 132,6±6,3 110,1±5,6 85,4±4,2 97,5±6,2	~w@4v~90 996/06/4	88 110,74 5,8 127,3 1 5,4 123,7 1 1 5,4 124,5 1 1 1 5,5 103,6 1 1 6,0 103,6 1 1 6,0 103,6 1 1 6,0 103,6 1 1 6,0	~.14.6.6.10.1 &rc.14.6.8.0.6	105.77 127.014 131.6144,0 135.3144,6 125,4144,6 118,4144,6 116,7444,6 116,7444,6	04004000 F40000000
Chrysoidin	Liver Kidney Small intestine Spleen Lung Brain Adrenal	100 100 100 100 100 100 100 100 100 100	86,1±10,8 98,4±4,9 116,3±5,4 115,4±8,6 102,4±6,9 102,4±4,7 94,2±9,2	-04-0000 46846646	87,4±6,9 120,3±3,4 127,7±5,0 127,4±4,7 107,3±5,1 79,3±7,2 84,6±4,7 100,7±7,2		97,3#4,1 135,0#4,1 138,1#5,6 138,3#45,0 90,8#4,0 92,1#10,1 86,2#4,0	0,0,4,4,0,00,— 70,5,4,0,00,00,00,00,00,00,00,00,00,00,00,00	93,4±7,8 120,1±4,2 119,4±4,2 110,0±4,4 100,0±10,3 98,4±5,1 96,4±5,1	0,0 w w w 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	110,6±6,6 123,2±5,3 126,8±4,3 129,3±5,4 125,6±4,1 116,1±11,9 111,7±4,3 116,5±7,4	0. 4. 0. 4 - 0 0 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0. 0.

Note: In the control group we used 25 animals, 12 in each of the experimental groups.

Within 6, 24, 48, 72 and 120 hours after the irradiation the animals were sacrificed by decapitation. The liver, kidneys, small intestine, spleen, lungs, brain, adrenals, and heart were quickly removed and place in Ringer's solution for 20-30 min. Then the material under investigation was transferred to a 0.01% solution of neutral red and chrysoidin for staining; the stains were prepared in Ringer's solution without sodium carbonate. After 30 min the organs were withdrawn from the stain soltuions, rinsed in Ringer's solution without sodium carbonate, and portions were dissected out from the middle which were transferred to a test tube for extraction of the stains. The test tube contained a determined, accurately measured, amount of 70° alcohol, acidified with sulfuric acid. Following extraction, which lasted 24 hours, the alcoholic extract was measured colorimetrically, using the concentration colorimeter KOL-1M, and the amount of stain taken up was calculated per 1 gram of weight of the organ. We set up the experiments with the non-irradiated rats under the same conditions.

The amount of bonded stain in the tissues of the irradiated animals was expressed in percents of the figures for the controls, the latter being taken as 100%.

All the data obtained in this investigation were subjected to variation-statistics treatment. Shifts in the sorption activity of the tissues were considered to be valid when the criteria of statistical significance for the different figures was equal to or greater than 3.

### RESULTS

The data on the binding of neutral red and chrysoidin by the organs and tissues of the white rats at varying periods after the irradiation are presented in Tables 1 and 2.

In the rats irradiated with a dose of 800 r (Table 1) there was a statistically significant elevation in the absorption of the stains in the liver, kidneys, small intestine, spleen and lungs.

The nature of changes noted in the different intervals of the investigation was varied. Within 6 hours after the irradiation a statistically significant intensification of the sorption properties took place only in the small intestine and spleen.

After 120 hours the changes in the sorption level for the majority of the organs investigated were insignificant.

A statistically reliable elevation in the staining capacity of the tissues was basically observed 24-72 hrs after the irradiation. In this period the elevation in sorption activity was observed in the small intestine, spleen, kidneys (after 24, 48, 72 hr), lungs (after 48 and 72 hr), and liver (72 hr after irradiation). In comparing the sorption properties of the tissues the most intensive accumulation of stains was noted in the small intestine and spleen. Elevation in the binding of the stains attained its maximum on the 3rd day after the irradiation.

We did not observe an increase in the absorption of the stains by the tissues of the adrenals. Within 120 hr after radiation exposure we noted a statistically significant lowering of the sorption level for this tissue.

No disturbances in the tinctorial properties of the brain and heart muscle were found in any of the periods of observation.

Under conditions of irradiation with a sublethal dose of gamma-rays (second series of experiments) the picture of the changes in the tissue sorption properties was somewhat different. In this cas the most manifest and regular changes were noted 48 and 120 hrs after irradiation. Statistical analysis showed that the elevation in the sorption level was significant for the small intestine and spleen (24, 48, 72 and 120 hr after irradiation), the lungs (after 48, 72, and 120 hr) and the kidneys (after 48 and 120 hr). In the remaining organs investigated (liver, brain, adrenals, heart) the difference between the experimental animals and the control was statistically insignificant by the variation method. As can be seen from Table 2, the accumulation of stains in the tissues of the animals taking part in this series of experiments was smaller than with the irradiation at a higher dose. Twenty-four hours after the irradiation a significant lowering was noted in the staining capacity of the adrenals.

Analysis of the results obtained shows that following the action of gamma-radiation on the organism pathological changes arise in a number of vital internal organs, which are manifest by an elevation in the sorption activity of the tissues in relation to vital stains. Changes in the sorption capacity of the cellular elements takes place even in the early stages of acute radiation illness and increases in the later stages of the disease. The alterations noted bear a phasic character, and depend on the seriousness of the radiation illness.

In accordance with the data in the literature, the observed changes in the tinctorial properties of the tissues can be explained by denaturing changes in the cellular proteins, which arise in the organism after exposure to radiation.

Thus, the observed changes in the sorption properties are related to the varying depth of the tissue injury, and reflect the dynamics and character of the pathological disturbances in the organism resulting from the progression of acute radiation sickness.

#### SUMMARY

Experiments were conducted on albino rats irradiated with various doses of gamma rays (800 and 400 r). The method of supravital staining was used to study the sorptive properties of tissues at different periods after irradiation. In 6-120 hours after irradiation, the tissues of the small intestine, spleen, kidneys, lungs and liver showed increased sorption of the stain. Denaturing changes of the cellular proteins were present in the irradiated organism. The character and the extent of the changes detected depended on the dose and the interval after the irradiation.

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