THE EFFECT OF CYSTAMINE ON OXIDATIVE PHOSPHORYLATION IN THE SPLEEN OF IRRADIATED ALBINO RATS

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Recent investigations have shown that oxidative phosphorylation is disturbed in the tissues of the irradiated organism [2, 4, 10, 13, 14, 15]. The resistance of the organism to the action of ionizing radiation may be increased by the prophylactic administration of certain sulfur-containing compounds. We were therefore interested in the study of the effect of these compounds on the disturbances of oxidative phosphorylation caused by irradiation. In the present research we studied the effect of cystamine on oxidative phosphorylation in the spleen of irradiated albino rats.

EXPERIMENTAL METHOD

Experiments were conducted on male albino rats weighing from 90 to 150 kg. The animals received a normal diet. Radiation sickness was induced by whole-body roentgen-ray irradiation in a single dose of 600 r. Conditions of irradiation: current strength 15 mA, voltage 180 kV, dose rates 9.2 and 11.2 r/min, filters 0.5 mm Cu and 1 mm Al, skin focus distance 70 cm. The experimental rats were decapitated on the 1st, 2nd, 3rd, 6th, and 9th days after irradiation.

The rats used to investigate the effect of cystamine on the processes of oxidative phosphorylation received an intraperitoneal injection of a 1% neutralized solution of the hydrochloride of this compound, immediately before

TABLE 1. Changes in Oxidative Phosphorylation in Spleen Homogenates of Rats after Whole-Body Irradiation in a Dose of 600 r

Time after irradiation (in days)	No. of experimental animals	No. of analyses	Weight of combined phosphorus (μg)		Vol. of oxygen absorbed (μg)		Ratio P : O	
			arithmetical mean ±m	significance of changes (P)	arithmetical mean ±m	significance of changes (P)	arithmetical mean ±m	significance of changes (P)
Before irradiation	26	13	206 ± 11.7	–	73 ± 6	–	1.05 ± 0.05	–
1st	20	10	188 ± 10.6	Not significant	76 ± 7	Not significant	0.95 ± 0.09	Not significant
2nd	27	10	131 ± 12.8	< 0.001	94 ±8	< 0.02	0.52 ± 0.07	< 0.001
3rd	34	10	69 ± 9.9	< 0.001	64.± 5	Not significant	0.40 ± 0.05	< 0.001
6th	24	9	204 ± 21	Not significant	82 ± 6	" "	0.96 ± 0.17	Not significant
9th	18	9	180 ± 9.9	" "	98 ± 9.1	< 0.05	0.67 ± 0.04	< 0.001

irradiation, in a dose of 75 mg base per kilogram body weight. In this series of experiments the oxidative phosphorylation was studied on the 3rd, 6th, and 9th days after irradiation.

Splenic tissue was homogenized in a 0.25 M sucrose solution with the addition of versene (0.01 M) and NaF (0.1 M). The relative proportions of tissue and sucrose solution were 1 : 3. The oxygen absorption was measured

TABLE 2. Effect of Cystamine on Oxidative Phosphorylation in the Spleen of Irradiated Rats

Time after irradiation (in days)	No. of experimental animals	No. of analyses	Weight of combined phosphorus (μg)			Vol. of oxygen absorbed (in μl)			Ratio P:O		
			arithmetical mean ±m	percentage of value in irradiated animals of control group	significance of changes (P)	arithmetical mean ±m	percentage of value in irradiated animals of control group	significance of changes (P)	arithmetical mean ±m	percentage of value in irradiated animals of control group	significance of changes (P)
3	24	10	136 ± 12.1	199	<0.001	105 ± 5.4	164	<0.001	0.47 ± 0.04	117	Not significant
6	16	10	254 ± 16.6	124	Not significant	92 ± 6.2	111	Not significant	1.04 ± 0.09	108	" "
9	14	9	228 ± 9.8	126	<0.01	102 ± 8.4	104	" "	0.85 ± 0.08	127	<0.001

manometrically in a Warburg's apparatus. The composition of the incubation medium was as follows: potassium phosphate buffer 0.006 M, triethanolamine buffer 0.016 M, glucose 0.02 M, succinic acid 0.012 M, $MgCl_2$ 0.006 M, ATP 0.001 M, crystalline hexokinase 1 mg/ml of medium; the pH of the mixture was 7.6. The incubation time was 20 min at 36°.

The intensity of oxidation and phosphorylation was estimated from the utilization of oxygen and inorganic phosphorus. The phosphorus was determined by the Fisk–Subbarow method. The results were analyzed statistically; the changes were considered significant if $P \leq 0.05$ (P is the probability of a chance occurrence).

EXPERIMENTAL RESULTS

On the 2nd day after irradiation of the rats the level of esterification of inorganic phosphorus fell by an average of 37%, while the oxygen absorption increased by 29%. Since the changes in oxygen absorption and esterification of phosphates were in different directions, the value of the P : O ratio was reduced on the average by half (Table 1).

A still more marked depression of oxidative phosphorylation was obtained in rats on the 3rd day after irradiation. At this time the esterified inorganic phosphate was reduced to one-third, and the value of the P : O ratio had fallen by more than 60% by comparison with the corresponding values obtained in nonirradiated animals. One of the causes of the marked depression of phosphorylation must have been the destruction of the lymphoid tissue, leading to changes in the cellular composition of the spleen. On the 6th day after irradiation, and the average value of the P : O ratio was normal in some cases, although in others it varied between wide limits (0.34-1.5). At the crisis of radiation sickness (the 9th day after irradiation) the level of combination of phosphate again fell and the P : O ratio was lowered.

Next we studied the protective action of cystamine on oxidative phosphorylation in irradiated rats by administering it in prophylactically effective doses.

The results given in Table 2 show that in the animals protected by cystamine, on the 3rd day after irradiation the weight of inorganic phosphate combined by the splenic tissue was almost twice as great as in the irradiated animals of the control group. However, because this increase was accompanied by a simultaneous increase in the oxygen consumption (by 64%), the P : O ratio was almost unchanged.

The effect of cystamine on oxidative phosphorylation in subsequent periods of the investigation was similar. On the 6th day after irradiation the level of esterification of inorganic phosphorus and of absorption of oxygen in the spleen of the rats protected by cystamine rose by 24% and 11%, respectively. The intensity of tissue respiration and the fixation of inorganic phosphorus in this case actually exceeded the level obtained in the nonirradiated animals, although the correlation between these processes remained the same as in healthy rats. Finally, on the 9th day, the protective action of cystamine was demonstrated not only by an increase in the level of esterification of inorganic phosphate, but also by an increase in the degree of correlation between phosphorylation and tissue respiration.

Hence the level of phosphorylation and its correlation with tissue respiration are lowered in the spleen of animals irradiated with ionizing radiation. Protection by cystamine diminishes but does not completely abolish these disturbances.

The mechanism of the disturbance of oxidative phosphorylation in irradiated animals has been insufficiently studied. There are indications [9] of depression of phosphorylation in the mitochondria of the liver and spleen during irradiation, attributed to increased secretion of thyroxin and corticosteroids. It is interesting to note that the dissociation between respiration and phosphorylation caused by the action of various factors (hypothermia, thyroid hormone) is not always an irreversible disturbance [5-8], and the lowering of the P : O ratio in some cases may actually be regarded as a compensatory mechanism responsible for ensuring thermo-regulation or the rapid accumulation of oxidation products providing the raw material for biological syntheses. However, the stability of the lowered P : O ratio and of the lowered concentration of high-energy phosphates in irradiated tissues suggests that the disturbances of oxidative phosphorylation are serious. We know that oxidative phosphorylation is closely connected with the synthesis of nucleic acids.

In A. M. Kuzin's opinion [3], during exposure to ionizing radiation the microstructures built up of high-polymer nucleic acids suffer most. It is obvious that even very slight injuries to the structures of the nucleus and mitochondria may lead to disturbance of the biochemical processes responsible for oxidative phosphorylation and DNA synthesis.

The protective action of cystamine on nucleic acid metabolism in different organs of irradiated animals has been described elsewhere [1, 11, 12]. The experimental facts obtained in the present investigation demonstrate that the prophylactic administration of cystamine also lessens the changes in oxidative phosphorylation in the spleen of irradiated rats.

SUMMARY

In this work, performed on albino rats, the author demonstrated that x-irradiation in a dose of 600 r leads to the change of the oxidative phosphorylation level in the spleen. In these conditions there occurs a drop of the phosphorylation level and its dissociation from the tissue respiration. Administration of cystamine (in prophylactic effective doses) to the animals prior to the irradiation with ionizing radiation decreases the changes of the oxidative phosphorylation in the spleen, without eliminating them completely.

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