EFFECT OF HALASCORBIN ON OXIDATIVE PHOSPHORYLATION IN BRAIN MITOCHONDRIA OF IRRADIATED ANIMALS

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The intensity of respiration and phosphorylation in the brain mitochondria is reduced in rats irradiated with 600 R. Administration of halascorbin to the animals increases the oxygen absorption and esterification of inorganic phosphate and raises the coefficient of phosphorylation.

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Recent investigations [1, 2, 6] have demonstrated the beneficial effect of vitamins C and P on the course and outcome of radiation sickness in experimental animals. This effect is evidently associated with the participation of these vitamins in oxidation-reduction reactions in the body. According to some workers [10, 13] the ascorbic acid–monodehydroascorbic acid system participates in electron transport in the respiratory chain. Following exposure to ionizing radiation, the oxygen consumption by the tissues falls significantly and the process of oxidative phosphorylation is disturbed [3, 5, 12].

The object of the present investigation was to study the effect of halascorbin, a combination of vitamins C and P, on respiration and oxidative phosphorylation in the brain mitochondria of irradiated animals.

EXPERIMENTAL METHOD

Experiments were carried out on albino rats weighing 180-200 g. The animals were irradiated with x rays using the RUM-11 apparatus. The total dose of irradiation was 600 R.

The rats were divided into three groups: group 1–normal animals acting as control; group 2–irradiated animals; group 3–irradiated animals receiving halascorbin.

Halascorbin was given daily, starting from the first day of irradiation, by subcutaneous injection in a dose of 5 mg/100 g body weight. On the day of the experiment the product was injected 2-3 h before the experiment.

The rats were sacrificed on the 7th, 14th, and 21st day after irradiation. Mitochondria were isolated by the usual method. The isolated mitochondria were incubated in the small vessels of a Warburg's apparatus where the oxygen absorption was determined. The intensity of phosphorylation was estimated from the decrease in inorganic phosphate determined by the Fiske–Subbarow method. In all the experiments the P/O ratio was calculated. Values obtained in the experiments were calculated per mg protein. Protein was determined by the biuret method. The results were analyzed by statistical methods.

EXPERIMENTAL RESULTS

The results given in Table 1 show that oxygen absorption by the brain mitochondria of the irradiated animals fell appreciably. This decrease was seen most clearly on the 7th day and amounted to 31% of the oxygen absorption by brain mitochondria of normal animals. The difference is statistically significant. Binding of inorganic phosphorus was reduced by a still greater degree (58%), so that the phosphorylation coefficient fell from 1.64 to 1.07. Consequently, dissociation of oxydation and phosphorylation was observed in the brain mitochondria after irradiation of the rats (600 R). This period of maximal decrease in oxidative

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TABLE 1. Oxidative Phosphorylation in Brain Mitochondria of Normal Rats and Rats Irradiated in a Dose of 600 R (ΔP and ΔO in μatoms/mg protein)

Normal (8)	ΔP				ΔO				P/O			
	Irradiation (6)			Normal (8)	Irradiation (6)			Normal (8)	Irradiation (6)			
	7-th day	14-th day	21-st day		7-th day	14th day	21st day		7-th day	14-th day	21-st day	
1,4±0,08	0,59±0,05	0,68±0,03	0,82±0,04	0,81±0,01	0,56±0,01	0,59±0,61	0,72±0,01	1,64±0,07	1,07±0,07	1,14±0,04	1,17±0,03	

Note. Number of experiments given in parentheses.

TABLE 2. Effect of Halascorbin on Oxidative Phosphorylation in Brain Mitochondria of Irradiated Animals (ΔP and ΔO in μatoms/mg protein)

ΔP				ΔO				P/O			
7-th	14-th	21-st		7-th	14-th	21-st		7-th	14-th	21-st	
day				day				day			
0,91±0,08	1,03±0,06	1,12±0,01	0,71±0,02	0,74±0,02	0,79±0,02	1,32±0,05	1,37±0,04	1,46±0,06			

Note. Five experiments were carried out in each series.

phosphorylation in the brain of the irradiated animals corresponded in time to the climax of radiation sickness. By the 14th-15th day after irradiation, i.e., by the beginning of the recovery period, no significant changes in the direction of an improvement in respiration and phosphorylation could yet be observed. The phosphorylation coefficient remained low (1.14). By the 21st day the oxygen absorption by the brain mitochondria of the irradiated animals had increased compared with the 7th day after irradiation; the esterification of inorganic phosphate showed a corresponding increase. A tendency toward improvement of the linking of oxidation and phosphorylation was observed. The phosphorylation coefficient on the 7th day after irradiation was 1.07, and on the 21st day 1.17.

Observations on the animals of group 3 revealed the beneficial effect of halascorbin on respiration and oxidative phosphorylation. Oxygen absorption by the mitochondria of the brain of these animals was increased by 27% on the 7th day compared with the oxygen absorption by the mitochondria of irradiated animals not receiving halascorbin. Binding of inorganic phosphate particularly was increased at this period, to the extent of 40%. This difference is statistically significant, as is clear from Table 2.

This pattern remained on the following days, i.e., esterification of inorganic phosphate increased somewhat more than the intensity of oxygen absorption. The coefficient of phosphorylation P/O was therefore higher in the animals receiving halascorbin at all stages of the investigation than in irradiated animals not receiving this product.

The experimental results thus indicate a considerable depression of respiration and dissociation of oxidative phosphorylation in the brain mitochondria after irradiation.

The work of Shamrai [7, 9] and Platonov [4] has shown that vitamin P (polyphenols), which participates in the enzymic oxidation of ascorbic acids into dehydroascorbic acid, which is readily transported through biological membranes, is essential for the assimilation of vitamin C by the body. Investigations by Martin and co-workers [11] and by Shamrai and co-workers [8] have shown that dehydroascorbic acid penetrates readily through the blood-brain barrier and accumulates rapidly in the brain tissues, undergoing reduction in the cells into ascorbic acid.

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