CHANGE IN CEREBELLUM TISSUE METABOLISM WITH IRRADIATION BY X-RAYS

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Physiological investigations [2] show that local irradiation of the animal cerebellum causes the same disturbances in physiological functions as are observed on its surgical removal. When the cerebellum is subject to small doses of ray energy a gradual decline in the magnitudes of the conditioned reflexes occurs and towards the end of the 3rd day of radiation full suppression of the conditioned reflex activity takes place. At first its restoration is observed only 3-4 weeks after irradiation.

A protracted change in the functional state of this or that section of the brain allows one to conduct an investigation on metabolism in various periods of functional disturbance and to make clear the relationship between the biochemical changes and the functional state of the animal.

The object of the present work was to study the intensity of the processes of respiration, of aerobic and anaerobic glycolysis and also to determine the content of cerebellum tissue nucleic acids. A considerable number of these acids in the cerebral tissue allows one to assume that their volume is closely tied to the physiological activity of the cerebrum.

EXPERIMENTAL METHODS AND RESULTS

The investigation of the processes of respiration and glycolysis was conducted on homogenates of the cerebellum of hens, determination of nucleic acids content—ribonucleic (RNA) and desoxyribonucleic (DNA)—in the cerebellum of a rabbit. The stateof the animal after irradiation was evaluated according to the degree of suppression of conditioned reflex activity.* A sharp change in the conditioned reflex activity in the animal usually occurred on the 3rd day after irradiation with a 500 r dose, and on the 4th day after irradiation with 7000 r. In the experiments the cerebellums of two hens were used. After decapitation the cranium was quickly dissected in the cerebellum region with the aid of surgical forceps. The extracted and chilled cerebellum was freed as far as possible from blood vessels, washed with Ringer solution and the residues removed on filter paper. Then it was suspended and homogenized in a chilled phosphate-saline solution in the ratio of 1 : 4 for 10 seconds. The homogenate was pipetted into a small flask or vessel of the Warburg apparatus containing by volume an amount of phosphate-saline solution, with or without glucose, equal to that of the homogenate. 0.2% glucose was added to the experimental tests;the pH was 7.4-7.5. Incubation at 37°C lasted for 60 minutes. The fermentation process was stopped by tipping in an equal volume of 6 % solution of trichloracetic acid.

In the tests before incubation, the homogenate of the cerebellum was added to the earlier measured amount of phosphate-saline and trichloracetic acid solution. The rate of aerobic and anaerobic glycolysis in the cerebellum homogenate was estimated by the increase in amount of lactic acid and the diminution of glucose in the non-albuminous trichloracetic filtrate. Glucose was determined by the Hagedorn and Jensen method, lactic acid by a test with paraoxydiphenol, inorganic phosphorus by the color reaction with ammonium molybdate. The intensity of the respiratory processes was measured manometrically in an oxygen atmosphere.

* Physiological evaluation of the state of suppression of conditioned reflex activity of the animal was conducted by P. F. Minaev in the USSR AMS.

The experiments to determine RNA and DNA were arranged in four variants with the use in each variant of 4 to 6 rabbits: I. control; II -4th day after irradiation, dose 500 r; III -same day, dose 7000 r; IV -30th day after irradiation, dose 7000 r. The nucleic acids content in the tissues of the irradiated and non-irradiated cerebellums was determined by the method of Schmidt and Tannhauser (according to the amount of phosphorus in RNA and DNA).

The cerebellum of the rabbit was freed from blood vessels, suspended and ground in a mortar with chilled 0.1 N perchloric acid in the ratio of 1 :10, and with the greatest possible care transferred to a centrifuge tube of 50 ml capacity to which extracts of the cerebellum tissue lipoids were added. The albumin residue was dried to constant weight. The dry weight of the tissue was usually from 50 to 75 mg.

The results of the experiments conducted in aerobic conditions show that the glycolytic activity in the homogenate of the irradiated cerebellum (7000 r), estimated by the amount of lactic acid formed during the test period, was somewhat higher than the glycolytic activity in the homogenate of the non-radiated cerebellum (Fig. 1a).

The resistance of the glycolysis enzyme system to the influence of rœntgen rays in doses of 500-7000 r is also borne out by the experiments conducted in anaerobic conditions (Fig. 1 b). The decrease in glucose in the oxygen atmosphere in the homogenate of the irradiated cerebellum was less and on average amounted to 25μ M, while in the controls decrease in glucose amounted on average to 37μ M. It should be noted that in the homogenate of the irradiated tissue of the cerebellum almost the total loss in glucose was accounted for by lactic acid formation (see Fig. 1 a). In the control tests as a rule the lactic acid was found to be significantly less than could be accounted for by the total amount of glucose utilized by the tissue. We shall refer to this discrepancy as "surplus loss" of glucose. The additon to the experimental tests of the glucose increased oxygen intake by the homogenates of the hens cerebellum in all the variants of the experiments (Fig. 2). This surplus respiration in the tests with the irradiated cerebellum tissue was somewhat less than in the control (Fig. 3). The increase in oxygen intake by the cerebellum homogenates in presence of glucose was apparently closely related to the oxidative transformations of the latter. However, the amount of surplus oxygen intake was insufficient for complete oxidation of the whole amount of the glucose loss in the experiments both in irradiated and non-irradiated tissue.

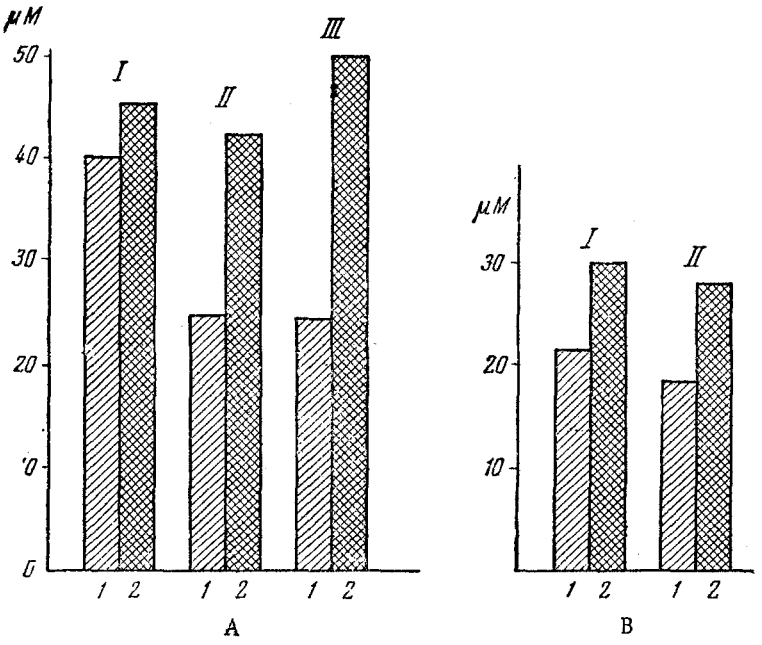

Fig. 1. Decrease in glucose [1] and increase in lactic acid [2] in the cerebellum tissue. a-in aerobic conditions (oxygen atmosphere): I -control, II -with irradiation of 500 r, III-7000 r; b-in anaerobic conditions I-control, II -with irradiation of 7000 r.

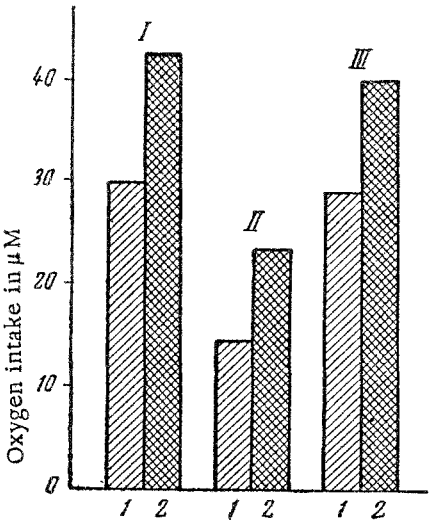

Fig. 2 Dynamic of oxygen intake by cerebellum tissue.
I-control; II -with irradiation of 500 r; III -with irradiation of 7000 r: 1-without glucose, 2-with glucose.

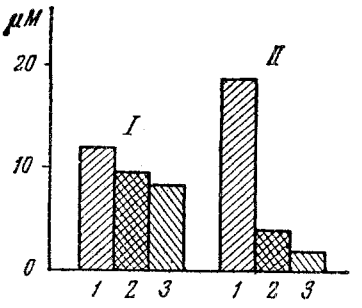

Fig. 3. Dynamic of surplus oxygen intake (I) and glucose loss (II) in cerebellum tissue.
1-control, 2-with irradiation of 500 r, 3-with irradiation of 7000 r.

As is clear from the experimental data, in irradiated cerebellum homogenates the possibility of oxidative transformation of the glucose was somewhat disturbed and the effective glycolytic course of decompositon was relatively poorly maintained.

The lessening in the oxidative mechanism effect with irradiation of the cerebellum can scarcely be explained simply as a disturbance of the capillary blood vessel network and by damage to the tissue, which took place with local irradiation since judging by the literature, [1] carbohydrate metabolism in brain tissue with temporary hypoxia returns to normal on the 2nd and 3rd days. In the conditions of our experiments 4 months after irradiation the rate of use of glucose by the homogenates of the irradiated cerebellum of hens did not reach the control values, though respiration was maintained.

As regards nucleic acids content, it is known from the literature that in the cerebellum it is higher than in the grey and white matter of the large cerebral hemispheres [3]. It was also noted that nucleic acids are subject to quantitative changes under the influence of roentgen rays on the cell.

We determined RNA and DNA in the tissue of the irradiated and non-irradiated rabbit cerebellums according to the amount of phosphorus in the compounds.

The results of the experiments are tabulated in micro-atoms (μa) of phosphorus per 1 gm crude weight. From Figure 4 it is clear that the amount of phosphorus in RNA in the irradiated tissue (500 and 7000 r) of the rabbit cerebellum remained practically unchanged. On the assay of total phosphorus content in the nucleic acids fraction a decline was observed in phosphorus content in irradiated cerebellum tissue. A larger decline in DNA phosphorus was discovered in the tissue of the rabbit cerebellum taken on the 30th day after irradiation with 7000 r. In this case some reduction in the phosphorus content of the phosphoproteins was also observed. But this degree of diminution of phosphorus (0.23 μ a) did not correspond to the diminution of total phosphorus of the nucleic acids fraction (5.2μ a). Apparently the change in the amount of phosphorus in the cerebellum tissue observed on the 30th day after irradiation with 7000 r, occurred basically on account of phosphorus in compounds unknown to us, which passed into the nucleic acids fraction on isolation by the Schmidt and Tannhauser method.

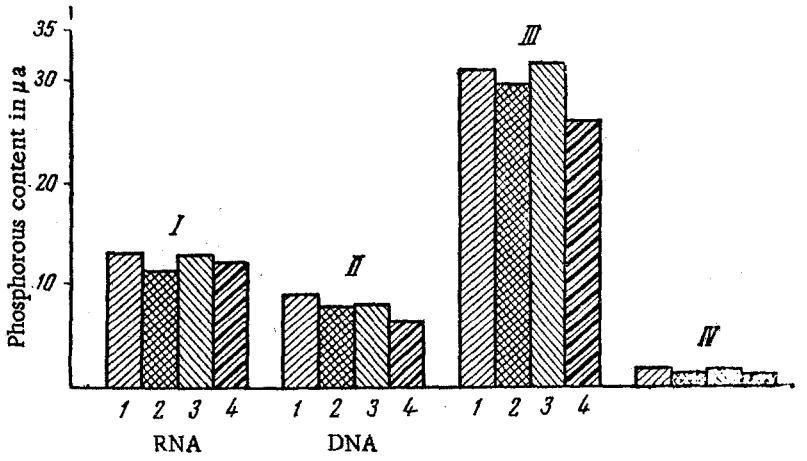

Fig. 4. Change in phosphorus content: I , in ribonucleic acid, II, in desoxyribonucleic acid, III, total phosphorus in nucleic acids fraction, IV, phosphorus content of phosphoproteins; 1-control, 2-with 500 r irradiation, 3-with 7000 r, 4-on 30th day with 7000 r.

LITERATURE CITED

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[3] Skvirskaya, E. B. and Silich, T. P., Doklady. Akad. Nauk SSSR, Vol. 93, No. 6, pp. 1073-1075 (1953).