SUCCINATE DEHYDROGENASE AND ALKALINE PHOSPHATASE ACTIVITY IN EXPERIMENTAL MYCOSES OF THE BRAIN

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In rats infected intracerebrally with a culture of Cladosporium trichoides, in the early stages of the disease a sharp decrease in succinate dehydrogenase activity and its redistribution are found in various parts of the brain. Alkaline phosphatase activity in the elements of the blood-brain barrier and area of infiltration is increased. In later stages a further decrease in respiratory activity takes place. Meanwhile, numerous macrophages with increased activity of these enzymes, and also giant cells specific for mycoses, with increased activity of these enzymes, appear.

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Few reports have been published on histochemical changes during development of mycoses of the brain, although the distribution of enzymes of the central nervous system under normal conditions and after exposure to various factors has been studied by many investigators [1-3, 5, 7, 8, 13].

EXPERIMENTAL METHOD

Experiments were carried out on 40 albino rats. The animals were injected intracerebrally with 0.1 ml of a suspension of a culture of the fungus Cladosporium trichoides, equivalent to 1 mg of dry culture. One of the distinguishing features of this fungus is its tendency to attack nervous tissue selectively [14]. The animals were sacrificed 1, 3, 6, and 9 days later. Activity of the succinate dehydrogenase system was determined by a modification of Nachlas's method with neotetrazolium in the incubation medium, and alkaline phosphatase activity was determined by the azo-coupling method. Quantitative analysis of activity of the enzymes of the succinate dehydrogenase system was carried out with the MF-4 photometer. The degree of alkaline phosphatase activity was assessed by visual comparison.

EXPERIMENTAL RESULTS

A sharp decrease in activity of the enzymes of the succinate dehydrogenase system was observed over the whole area of saggital sections of the brain and, in particular, in the region of infiltration, 24 h after injection of the culture of the fungus. Close to the zone of infiltration (one field of vision under a magnification of 10×10*), diformazan granules in the bodies of nerve cells were arranged extremely irregularly and occasionally were grouped in the region of the nuclear membrane. At a distance of 2-3 fields of vision away, in the motor lobe of the cortex, against a background of diminished activity of enzymes of the succinate dehydrogenase system in the interneuronal spaces, activity increased particularly clearly in circles around the nuclei and in the initial portions of the islets (Fig. 1).

Considerable changes in activity of the enzymes were observed in the cerebellum, where it fell below the normal level (in the granular layer by 46% and in the molecular layer by 38%). In the Purkinje cells, on the other hand, oxidative activity increased. The cell bodies were clearly outlined, and large formazan granules, angular in shape and of various sizes, became visible in the cytoplasm. At one pole of the cell the crystals merged, often continuing into the dendrites.

*Subsequently the field of vision is also given under magnification 10×10.

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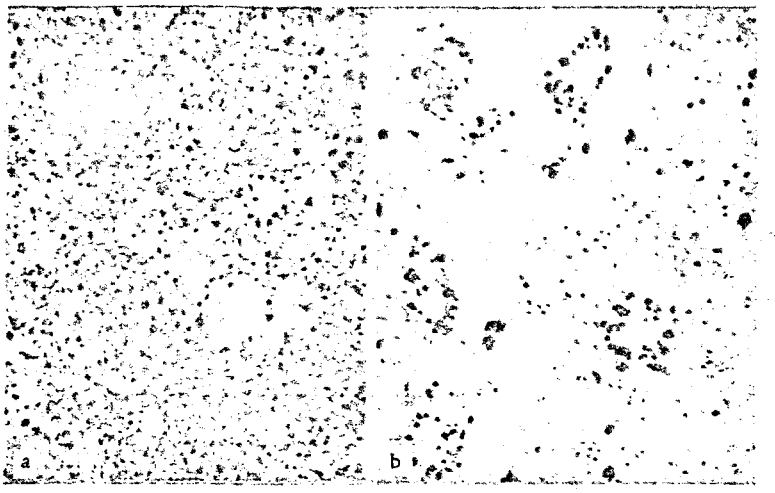

Fig. 1. Distribution of succinate dehydrogenase activity in cerebral cortex. a) Brain of intact rat; b) 24 h after infection. Diformazan granules in region of nuclear membrane. Sharp decrease in interneuronal activity. 60 × 10.

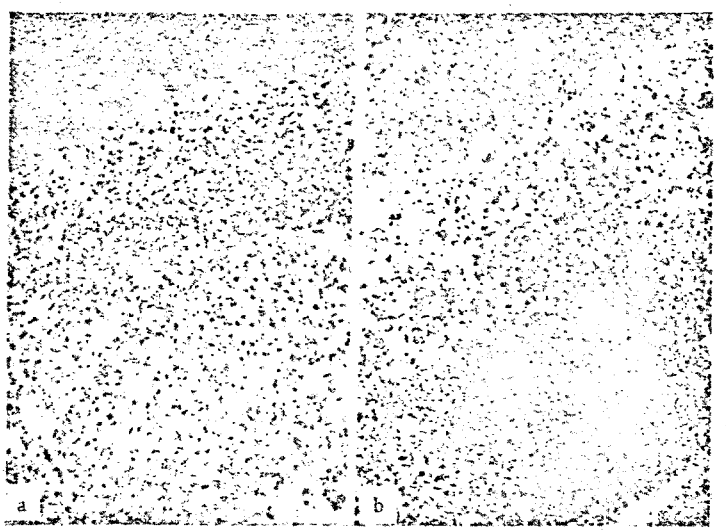

Fig. 2. Distribution of succinate dehydrogenase activity in cerebellar cortex. a) Cerebellum of intact rat. Largest number of diformazan granules in granular layer; b) 3 days after infection. Maximal number of granules in molecular layer. 10 × 10.

After 24 h of the experiment, the alkaline phosphatase activity was increased in nearly all parts of the brain: in the vascular endothelium, choroid plexus, and ependyma, probably indicating an increased load on the blood-brain barrier. In the choroid plexuses of the ventricles, showing infiltrative changes, alkaline phosphatase activity was highest. The swollen cells of the choroid epithelium contained large amounts of enzyme in its basal portions.

The zone of infiltration formed (in the region of the corpora quadrigemina) at this period of the experiment consisted mainly of neutrophils with a few lymphocytes. Activity of the enzymes in the zone of infiltration was moderate and was concentrated mainly in the cells surrounding the fungus. In areas bordering the zone of infiltration (cortex of the occipital lobe, corpora quadrigemina), alkaline phosphatase activity was localized in the walls of the vessels and in hematogenous cells, and sometimes in glial cells.

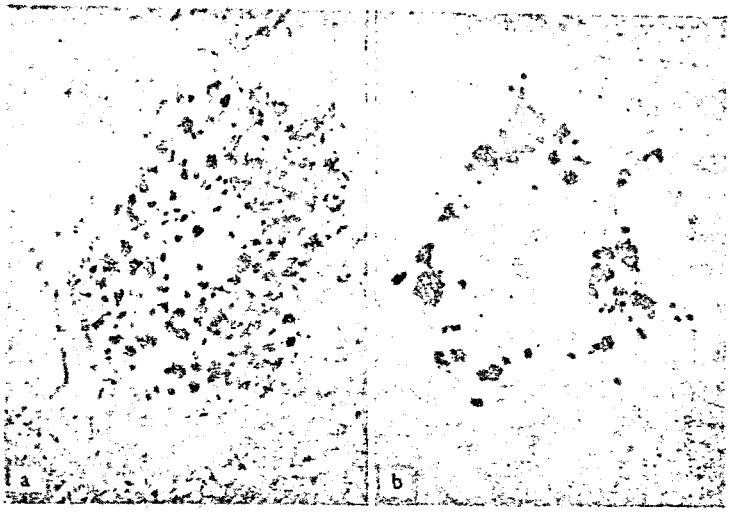

Fig. 3. Distribution of succinate dehydrogenase activity in neurons of pontine reticular formation. a) Pontile reticular formation of intact rat; b) 3 days after infection. Gross swelling and redistribution of diformazan granules throughout cell body. 60 × 10.

After three days, activity of the succinate dehydrogenase system around the zone of infiltration in different parts of the brain was greatly reduced: in the superior colliculi by 54%, in the anterior nuclei of the thalamus by 92%, and in the ganglion cells of the hippocampus by 85%. At a distance of 2-3 fields of vision from the focus, in the motor cortex, activity of enzymes of the succinate dehydrogenase system remained the same as after 24 h. In the granular layer of the cerebellum, activity of the enzyme continued to fall down to 51%. The coarse diformazan granules merged to form large granules and crystals were thinly scattered. Activity of the molecular layer was almost indistinguishable from that after 24 h (36% below the normal level). Sometimes the intensity of staining of the molecular layer was greater than that in the granular layer (Fig. 2).

The large neurons of the mesencephalon, pons, and medulla and the Purkinje cells of the cerebellum showed the formation of large formazan granules and redistribution of formazan throughout the cytoplasm into compact groups (Fig. 3). Three days after infection, the zone of infiltration consisted mainly of polyblasts, and macrophages, grouped around the cells of the fungus and possessing high alkaline phosphatase activity (in agreement with data [4] indicating an increase in activity of this enzyme in the series lymphocyte–polyblast–macrophage). The preparations showed clearly that in its course toward the walls of the zone of infiltration, the fungus was stretched along the young collagen fibrils forming the capsule. Alkaline phosphatase activity was most distinctly increased here. At the border with the focus in the superior colliculi, the posteromedial thalamus, and the occipital cortex, macrophages were clearly seen which had migrated into the tissue and possessed high alkaline phosphatase activity. Sometimes a positive reaction was associated with hypertrophied glial cells.

On the 6th day of the experiment no significant changes in activity and localization of enzymes of the succinate dehydrogenase system and alkaline phosphatase were observed compared with the situation on the 3rd day.

Between 9 and 15 days after infection the fungus had completed its penetration of the capsule surrounding the focus and had emerged into the nerve tissue. Two-thirds of the brain was affected by infiltrative changes. In all parts diminished respiratory activity of the nerve tissue was observed as before. However, the results of photometry showed that the total activity 9 days after infection was slightly increased on account of migration of various phagocytic cells into the brain tissue and a well marked glial reaction. In preparations stained by Cajal's gold-mercuric chloride method, a sharply hypertrophied glia was seen at this time, in agreement with published reports [5, 11, 12] of an increase in activity of oxidative enzymes in glial cells after brain injury. In some Purkinje cells and in large neurons of the pons, numerous diformazan granules could be clearly seen in the cytoplasm. In other neurons in these regions no enzyme activity could be detected.

Around the filaments of fungus invading the tissue specific reactive processes developed, characterized by the formation of giant cells which, blocking the mycellium of the fungus exactly, surrounded it with a thin layer of cytoplasm with a mass of nuclei. Nine days after infection these cells were in the initial stages of formation. They possessed moderate respiratory activity. The giant cells which were formed in the brain possessed almost no succinate dehydrogenase activity, in agreement with data [4, 10] concerning their development in other tissues.

On the 9th day extensive areas of perivascular infiltration were formed, merging in some places and penetrating the nervous tissues in continuity. Among the cells of the infiltrated area were numerous macrophages, giving an intense reaction for alkaline phosphatase. These cells formed groups near the capsule of the focus, and at places where the fungus emerged into the brain tissue they were grouped around the infective agent. Next to the focus of injury (corpora quadrigemina, lateral ventricles) the earliest stages of giant cell formation were seen—adhesion of macrophages to the walls of the fungus. They showed very high activity. In the fully formed giant cell, round in shape with a mass of nuclei at the periphery (resembling cells of Langhans type), alkaline phosphatase activity was either absent or greatly reduced.

Hence, the development of experimental mycosis in the central nervous system of rats is characterized by lowering of the intensity of energy metabolism linked with the Krebs cycle, as expressed by a sharp decrease in succinate dehydrogenase activity in all the brain structures. In brain tissue not directly associated with infiltration, in the early stages of development of the disease various reactive changes were observed, characterized by a specific redistribution of respiratory activity [1-3]. In later periods reactive processes gave way to injury to the nerve cells, as a result of which activity of enzymes of the succinate dehydrogenase system fell sharply in the neurons of these regions. At a time when several brain regions, especially the components of the zone of infiltration, were in anaerobic conditions, the level of energy metabolism for performance of phacocytic functions was probably maintained sufficiently high by an increase in the intensity of glycolysis, indirectly facilitated by the increase in alkaline phosphatase activity in these regions, providing large quantities of inorganic phosphate required for ATP synthesis. A link between the activity of this enzyme and the process of transfer of sugars through membrane structures has been demonstrated experimentally [6, 9]. In the light of this we can understand the important role of dephosphorylation processes in the function of the blood-brain barrier [9, 13], which is confirmed by the increased activity of this enzyme in the capillary endothelium, ependyma, and choroid plexuses in the course of the disease.

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