# HISTOCHEMICAL STUDY OF THE LIPIDS IN THE NERVE CELLS OF RATS IN NORMAL AND HYPOXIC CONDITIONS

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In hypoxic conditions important changes take place in the phospholipid metabolism of the nerve tissue. The results of biochemical investigations have shown that, in hypoxia, there is a marked decrease in the intensity of the phospholipid metabolism in the central nervous system, as revealed by a decrease in the rate of incorporation of labeled precursors into phospholipids [1,3]. The considerable differences in the chemical structure and functions of the individual phospholipids, components of all cells without exception, including the cells of the nervous system, make it necessary to discover the cell structures in which phospholipid metabolism is most sensitive to oxygen lack. However, the methods of biochemical analysis, usually requiring pieces of tissue, can only give aggregated mean data. The neurons situated in different parts of the nervous system, and even neurons belonging to the same morphological formation, undoubtedly differ in their sensitivity to hypoxia. It is only by histochemical investigation that it is possible to find out in which nerve cells and subcellular formation of the neurons the effects of hypoxia are most clearly manifested.

Phospholipids are components of many different cell structures (the boundary layers of the cell and nucleus, the membranes and cristae of the mitochondria, the endoplasmic reticulum), mainly in the form of complexes with proteins and carbohydrates, and also as lipid cytoplasmic granules (lipochondria). The phospholipids evidently play an important role in the specific function of the nervous system, forming functionally active membranes — structures taking part in the phenomena of permeability and maintaining ionic gradients. It may be postulated that these intracellular structures differ in their reaction to hypoxia.

The object of the present investigation was to study the changes in the content of cytoplasmic lipid granules in the various structures of the nervous system in normal conditions and in acute hypoxia.

## EXPERIMENTAL METHOD

The phospholipids were demonstrated histochemically by the methods of Baker (with a pyridine extraction control) and Elftman [3,8]. It has recently been shown that these methods are not strictly specific in relation to phospholipids [5]; besides phosphatides, other substances of both lipid and nonlipid nature are also stained, and their differentiation is not yet possible. The brain and spinal cord and the spinal ganglia of the cervical division from control rats and rats subjected to acute oxygen deficiency were investigated. To create hypoxic conditions, the animals were placed for 2 h in a pressure chamber, the pressure within which was lowered for 20 min to 200-160 mm Hg. The rats were sacrificed either immediately after their stay in the pressure chamber or after an interval of 1, 3, and 7 days.

## EXPERIMENTAL RESULTS

By means of the methods used, phospholipids were revealed in the section by the appearance of a blue or grayish-blue color. Proof of the phospholipid nature of this stain was given by its absence after extraction with pyridine. The nerve fibers in the gray and white matter became bluish-gray in color. The nerve cells were stained most clearly in the spinal ganglia. In the control rats, uniformly distributed lipid granules measuring 1-2 $\mu$ in diameter, round or irregular in shape, and stained a blue color, were found in the cytoplasm of these neurons (see figure, A). Because these granules give a positive reaction with acid hemateins (Baker's method) and stain after controlled staining by Elftman's method with Sudan black B, but do not give these reactions after extraction of the fragments of brain tissue with hot pyridine, it may be concluded that they contain large quantities of phospholipids and are analogous to the lipochondria described by Baker and other authors [4,7,10]. Besides phospholipids, other

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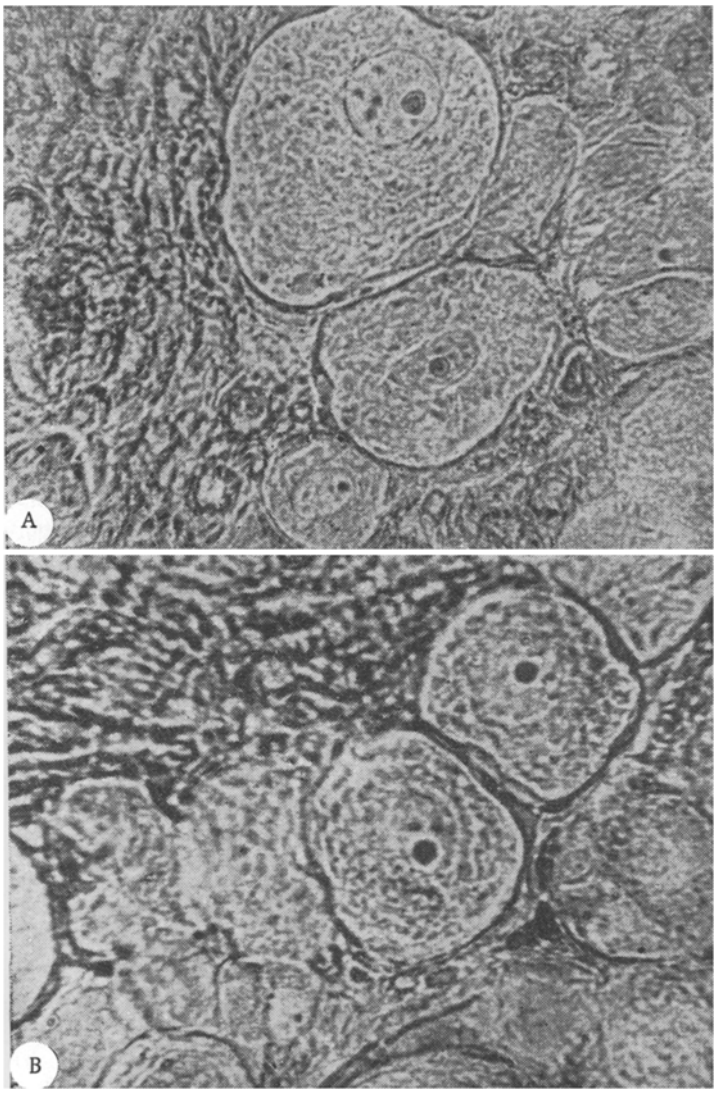


Lipid granules in the neurons of a spinal ganglion of a rat in normal conditions (A) and 24 h after acute hypoxia (B). Baker's method. Objective 40, ocular 10.

complex lipids are concentrated in the granules, but with modern histochemical methods it is impossible to observe structural and chemical differences between the lipid cytoplasmic inclusions.

In the ventral horns of the spinal cord, the medulla, and the nuclei of the cerebellum, only a few neurons contained lipid granules, and in much smaller amounts than the spinal ganglion. The remaining cells in these portions of the nervous system, and also the neurons of the cerebral cortex and the Purkinje cells of the cerebellum remained unstained, their cytoplasm was homogeneous and pale yellow in color, and did not contain granules. Individual neurons in the cerebral cortex and cerebellum were brown in color, but control extraction with pyridine did not confirm that these cells contained phospholipids, so that Brodal and Harrison were probably correct when they considered that the reason for the appearance of the brown stain was mechanical injury to these superficially lying neurons [6].

Acute hypoxia for 2 h was followed by the discovery of lipid granules in the neurons of the spinal ganglia in a still larger number of cells, the number of granules detected in each cell was increased, they stained more intensively, and their outlines became clearer (see figure, B). These changes were found immediately after the period of acute hypoxia, but were clearest 24 h later. After 3 and 7 days, the number and appearance of the granules in the neurons of the spinal ganglia were indistinguishable from the controls. In the neurons of the investigated portions of the central nervous system, hypoxia revealed no visible changes in the phosphatide-containing structures examined.

To interpret the results, the method of demasking the lipids by means of phenol was used. This causes liberation of the lipids from the lipoprotein complexes [9]. After fixation for 6 h in a formalin-calcium solution, the pieces of nerve tissue of the normal rats were immersed in 1% phenol solution for 24 h at 37°, and then subjected to the usual stages of the staining methods used: chroming, staining, differentiation.

Under the action of phenol, the lipid granules in the cytoplasm of the neurons of the spinal ganglia showed visible changes; they became coarser, they increased in number and in the intensity of their staining properties; i.e., changes of the same character appeared as were observed in the spinal ganglia 24 h after exposure to acute hypoxia. The similarity between these changes suggests that, as a result of acute hypoxia, as after the action of phenol, rupture of the lipoprotein complexes takes place with liberation of their lipid component, which becomes more accessible for detection by specific histochemical methods. This dissociation of the lipoprotein complexes caused by hypoxia is evidently a reversible process, because in the later stages of the post-hypoxic period (3 and 7 days) the content and distribution of the lipid granules in the neurons of the spinal ganglia were indistinguishable from those in the controls.

The question arises why the changes in the lipid granules were observed only in the neurons of the spinal ganglia, whereas other neurons also experienced hypoxia. These changes are probably not the result of hypoxia of the neurons of the spinal ganglia, but reflect the reception by these cells of impulses arriving from the organs and tissues exposed to the state of oxygen lack, causing metabolic disturbances.

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