EFFECT OF VARIOUS AGENTS ON STATE OF THE LYSOSOMES IN NERVE CELLS

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The neurons of intact animals usually contain only a few very small lysosomes.

During the massive development of lysosomes in cells of the nervous system taking place in animals under the influence of various agents, they can be found in the cytoplasm to the extent of 10-15 per section. The dynamics of lysosome development is seen most demonstratively after the action of pharmacological substances. The function of lysosomes in the central nervous system is to carry out intracellular digestion and autolysis of material penetrating into the cell or formed after injury to its ultrastructures.

Lysosomes developing in the tissues of the central nervous system under the influence of various factors are intimately connected with the mitochondria and the Golgi apparatus.

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Since lysosomes were first isolated from the cytoplasm of liver cells in 1955-56 [5, 8], many investigations have been published showing that lysosomes are particularly numerous and large in phagocytic cells (macrophages and leukocytes), the principal function of which is intracellular digestion. According to de Duve's theory [6], lysosomes are cytoplasmic structures measuring 0.25-0.8 μ, granular in structure, and containing hydrolytic enzymes with an optimum of activity in an acid medium; they possess a high concentration of acid phosphatase and other hydrolases. Harmful substances are ingested by the cell by endocytosis. A phagosome is formed, which undergoes digestion by means of enzymes contained in the lysosome. The lysosome fuses with the phagosome to form a digestive vacuole. The lysosome membrane isolates digestive enzymes capable of breaking down the large molecules of lipids, proteins, and nucleic acids, from the cell cytoplasm [7].

As Novikoff [9] points out, little work has been done to study the morphogenesis and function of the lysosomes.

During recent years the morphogenesis and functions of the lysosomes have been studied in various cells of the central nervous system in different animals under normal conditions and under the influence of various agents.

The results obtained are described in this paper.

EXPERIMENTAL METHOD

Electron micrographs of the brain of dogs with experimental neuroses produced by conditioned acoustic stimuli (pure tones of 15,000 Hz) and of rats irradiated with x-rays under standard conditions in a dose of 600-700 R, and also of animals receiving various pharmacological substances, were studied. The dogs were sacrificed 2 months and 1 year, and the rats 1, 3, 6, 24, 48, and 72 h and 1 month after irradiation. Intact animals were used as controls.

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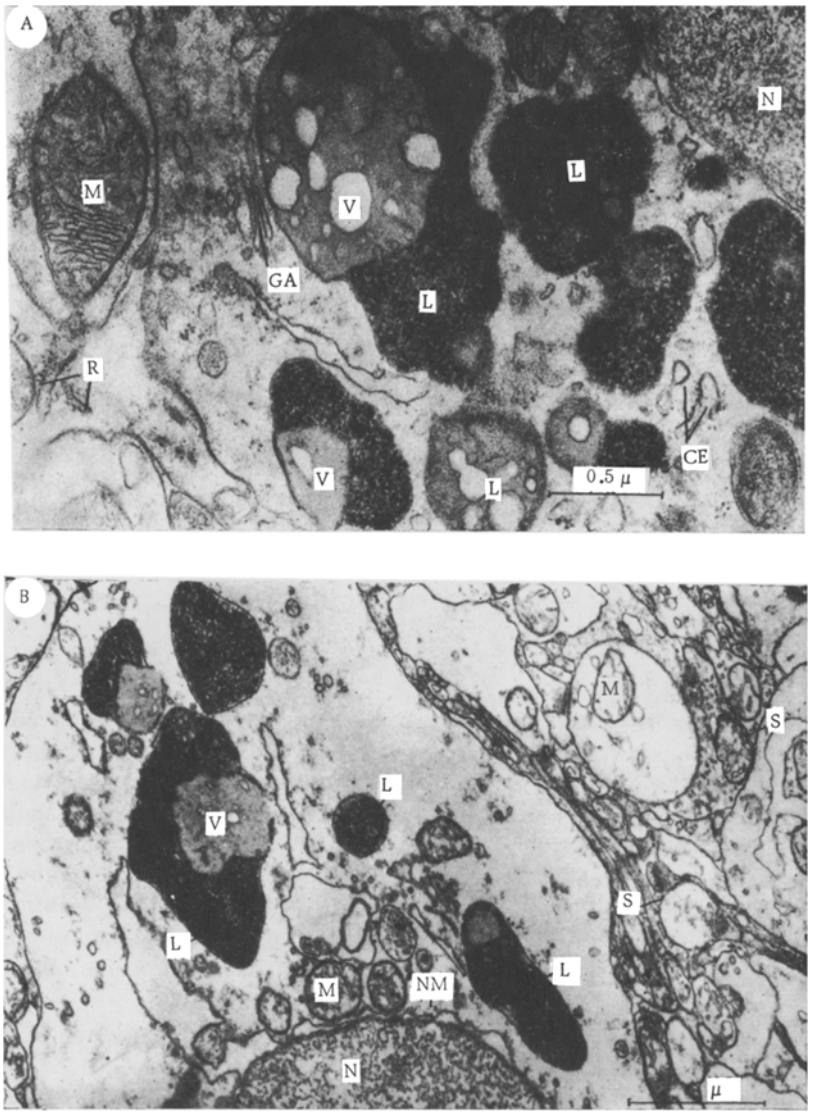

Fig. 1. Electron micrograph of cortical neuron (A) and astrocyte (B) from auditory cortex of dog with experimental neurosis (experiment at 2 months). Various stages of lysosome formation are visible. Epon. Magnification, A 41,800 ×, B 24 640 ×. Legend, here and in Fig. 2: V) vacuoles; GA) Golgi apparatus; L) lysosomes; M) mitochondria; P) polysomes; PM) plasma membrane; S) synapse; R) ribosomes; ER) endoplasmic reticulum; CE) cisternase of endoplasmic reticulum; N) nucleus; NM) nuclear membrane.

Pieces of tissue were taken from the auditory cortex of the dogs and from various parts of the rats' brain, and fixed by Palade's method, then embedded in Vestopal and Epon. Ultrathin sections were cut on an LKB ultratome, negatively stained with lead citrate [10], and studied in the Hitachi NI electron microscope (voltage 75 kV). Acid phosphatase activity was detected by Gomori's method.

EXPERIMENTAL RESULTS

Profound disturbances of higher nervous activity were observed in the dogs with experimental neuroses (investigated jointly by M. M. Khananashvili and the writer). Trophic and degenerative changes developed in the cortical neurons and tracts of the auditory system, manifested by the formation of lipofuscin bodies, a decrease in the number of RNA macromolecules, vacuolation and the development of local foci

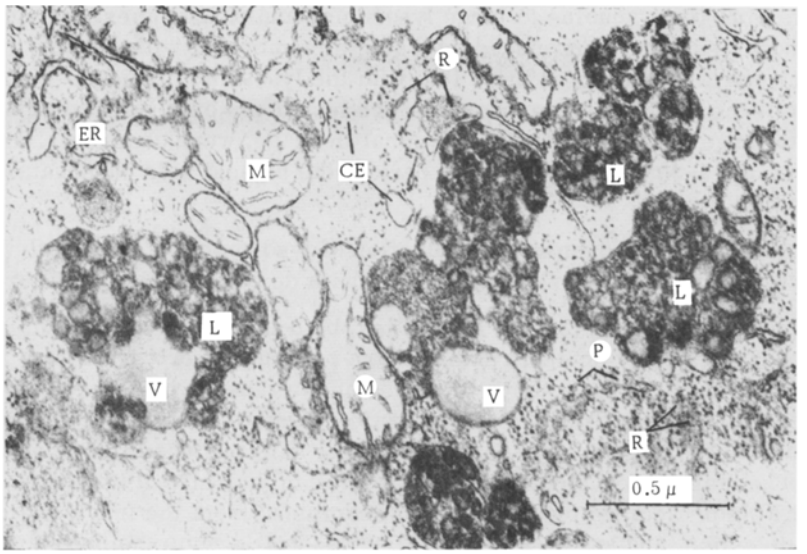

Fig. 2. Electron micrograph of neuron from motor cortex of rat 48 h after intravenous injection of imferon. Epon. 55,000 ×.

of degeneration in the cytoplasm and axons of the neurons. However, functional stress did not cause death of the nerve cells because of the simultaneous occurrence of regeneration of the cytoplasmic ultrastructures and the development of lysosomes, which have a protective function.

Massive formation of lysosomes in the experimental dogs was discovered both in neurons and in glial cells, parallel with destruction of the cytoplasmic ultrastructures. Lysosomes developed in the cytoplasm as dense formations with a granular structure. They attained a diameter of 150-200 Å. The lysosomes were surrounded by a membrane 70-95 Å thick; small lysosomes frequently merged with phagosomes and vacuoles, and also joined together to form larger complexes and conglomerations, attaining a diameter of 0.4-0.8 μ or more; these possessed high osmiophilia. Sometimes the number of lysosomes per section reached 10 or more, and they occupied a large part of the neuron body. In their morphology, the lysosomes of neurons and glial cells were similar (Fig. 1), although in the former they were larger and more numerous.

After whole-body x-ray irradiation of rats [2], the formation of lysosomes in the cells and tracts of the brain was also observed during the development of ultrastructural changes. From 2 to 3 days and during the month after irradiation, medium-sized lysosomes (0.4-7.0 μ in diameter) were found in the cortical neurons. With the cessation of degenerative changes in the cells of the central nervous system, only solitary lysosomes were detected. They differed from those described in dogs by the denser distribution of granules, their high osmiophilia, and the almost total absence of vacuoles. They varied in shape, and sometimes the lysosomes contained fragments of mitochondria, of membranes of the endoplasmic reticulum, and other ultrastructures undergoing destruction. During the first two weeks of the experiment, as the writer described previously [3], an intimate connection was seen between the lysosomes and Golgi apparatus. The Golgi apparatus was horseshoe-shaped or ellipsoidal, with the formation of a "GERL" complex described by Novikoff [8] during the development of a pathological process in cells of the nervous system (the name of this complex is derived from the initial letters of the three components concerned in its formation: Golgi apparatus, endoplasmic reticulum, and lysosomes).

The formation of lysosomes and their functions were most clearly demonstrated after administration of pharmacological agents. For instance, 6 h after injection of imferon,* pinocytotic vacuoles, 350-450 Å in diameter and heterogeneous in density, were found in the cytoplasm of the cortical neurons, concentrated in the hyaloplasm and cisternae of the endoplasmic reticulum. The membranes of the endoplasmic reticulum lost their ribosomes and surrounded these structures; sometimes these membranes still kept their ribosomes here and there. At 24 h and, in particular, 48 h of the experiment, the photomicrographs clearly revealed that lysosomes were formed by pinocytotic vacuoles which had ingested imferon, and by the endoplasmic reticulum (Fig. 2). A large part of the neuron body was occupied by lysosomes in various

*One-half milliliter of imferon contains 25 mg ferrous hydroxide with the low-molecular-weight fraction of dextran.

stages of development: from tiny vacuoles to large lysosomes and phagosomes, forming complexes and conglomerations. The lysosomes were surrounded by a single membrane, with a dense granular matrix. The concentration of mitochondria and their intimate link with the lysosomes indicate intensification of energy production in this part of the cell. Small lysosomes were less commonly found after 48 h. Their condensation and unification into large conglomerations, polygonal in shape, were observed. Sometimes their number in a single section was 15-20 or more. At this period, the highest acid phosphatase activity was detected in the lysosomes.

The data concerning the morphogenesis and function of lysosomes in cells of the central nervous system obtained in the writer's laboratory are in agreement with de Duve's theoretical arguments [6].

The writer has previously shown [3] that lysosomes, both in neurons and in glial cells of the nervous system, are normally comparatively small in size and are present in very small numbers. Their structure is basically similar to that of lysosomes in other tissues and organs. Lysosomes develop in the central nervous system of different animals from the endoplasmic reticulum and other ultrastructures after their injury or after penetration of foreign substances into the cell in cases when agents of many different types act on the organism. The massive development of lysosomes in the cells of the central nervous system must be regarded as a general biological protective reaction, combined from the functional point of view with endocytosis, intracellular digestion, and autolysis of material penetrating into the cell or formed as a result of damage to its ultrastructures. The morphology, size, and number of the lysosomes vary depending on the agent concerned and the degree of destruction of the cell ultrastructures. If destruction is extensive, many large lysosomes intimately connected with the Golgi apparatus and mitochondria develop in the neurons and glia.

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