The results of this quantitative electron-microscopic investigation thus demonstrate the influence of malnutrition on the basic structural elements of synapses, and that this influence is stronger in young than in adult animals. Meanwhile, malnutrition in young and adult mice produces significant changes in ultrastructure of synapses located on dendritic spines, and these changes may lead to depression of functional activity of the CNS.

LITERATURE CITED

1. N. I. Artyukhina, Structural and Functional Organization of Neurons and Interneuronal Connections [in Russian], Moscow (1979).
2. N. N. Bogolepov, Ultrastructure of Synapses Under Normal and Pathological Conditions [in Russian], Moscow (1975).
3. L. N. D'yachkova, Zh. Obshch. Biol., No. 5, 772 (1979).
4. G. M. Erastov, D. I. Medvedev, K. Yu. Reznikov, et al., Current Problems in the Etiology Pathogenesis, Clinical Picture, and Treatment of Tropical Diseases [in Russian], Moscow (1976), p. 170.
5. I. Z. Eremina, The Developing Brain [in Russian], Tbilisi (1984), p. 78.
6. E. V. Loseva and S. B. Stefanov, Byull. Éksp. Biol. Med., No. 5, 112 (1983).
7. D. I. Medvedev, I. I. Babichenko, I. Z. Eremina, and A. I. Kravtsova, Byull. Éksp. Biol. Med., No. 3, 108 (1983).
8. O. B. Savrova and D. I. Medvedev, Abstracts of Proceedings of the 6th All-Union Conference of Embryologists [in Russian], Moscow (1981), p. 159.
9. K. Akert, P. Strait, C. Sandri, et al., Schweiz. Arch. Neurol., 111, 227 (1972).
10. C. T. Cooke, T. M. Nolan, S. E. Dyson, and D. G. Jones, Brain Res., 76, 330 (1974).
11. B. G. Cragg, Brain, 95, 143 (1972).
12. P. Gambetti, L. Antilio-Gambetti, N. Lizzuto, et al., Exp. Neurol., 43, 464 (1974).
13. D. Jones and S. Dyson, Exp. Neurol., 51, 529 (1976).
14. D. Jones and S. Dyson, Brain Res., 208, 97 (1981).
15. M. Salas, S. Diaz, and A. Nieto, Brain Res., 73, 139 (1974).

ULTRASTRUCTURE OF THE CEREBRAL CORTEX AND HIPPOCAMPUS IN RATS IN THE EARLY PERIOD AFTER RESUSCITATION FROM TOTAL ISCHEMIA

Z. Ya. Esipova

UDC 616.831-005.4-008.66-092.9-07:[616.831.31+616.831.314]-091.8

KEY WORDS: brain; anoxia; clinical death; postresuscitation period.

The study of changes in the brain at the subcellular level is a leading approach to the elucidation of the mechanisms of development of brain damage in anoxia, knowledge of which is essential for the development of prevention and treatment of postresuscitation encephalopathy [6].

EXPERIMENTAL METHODS

The frontal cortex and area CA_1 of the hippocampus of 12 noninbred male rats weighing 180-200 g were studied. Systemic circulatory arrest was produced in seven animals for 10 min by retrosternal compression of the vascular bundle [4]; five rats remained intact. The brain of animals with complete visible restoration of their neurologic status on the 4th day after clinical death was investigated. Material was fixed in 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.6) and then treated with OsO_4, dehydrated in alcohols

Laboratory of Resuscitation Pathology of the Central Nervous System and Laboratory of Normal and Pathological Morphology of the Brain, Institute of General Reanimatology, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. A. Negovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 497-501, April, 1988. Original article submitted April 14, 1987.

0007-4888/88/0004-0600$12.50

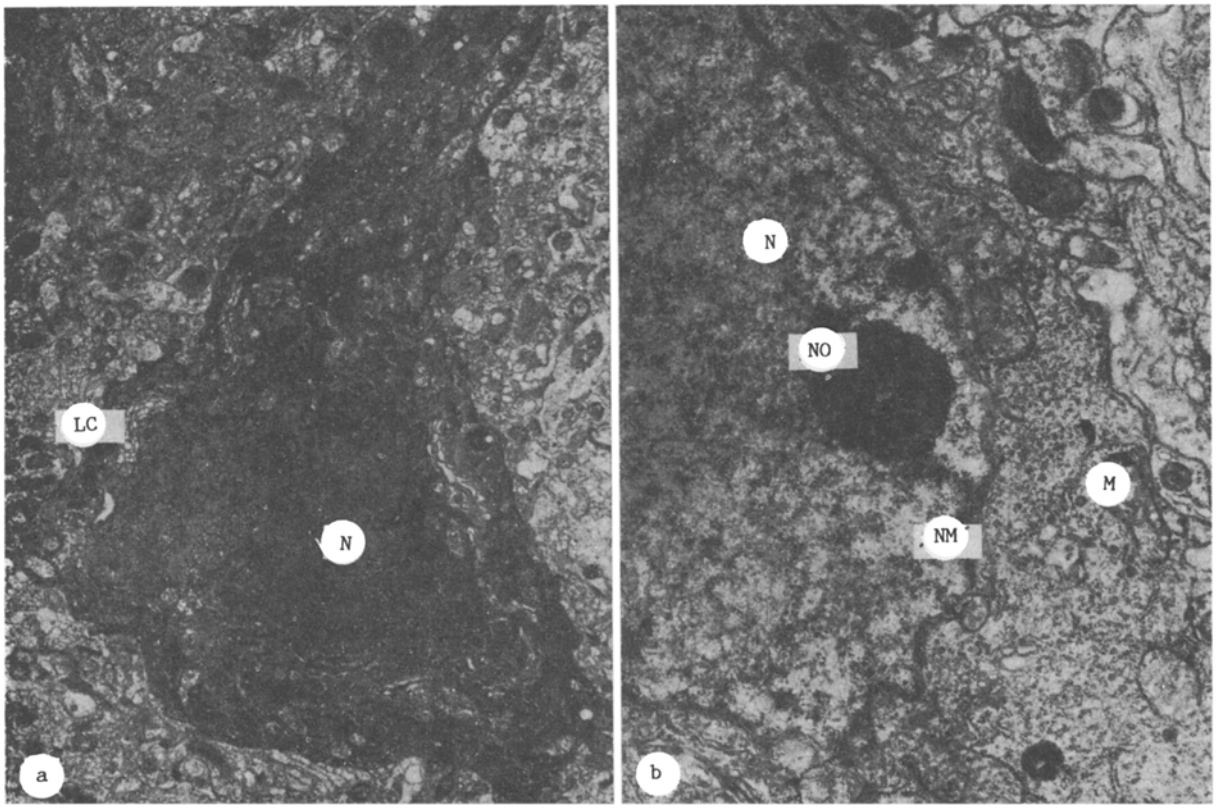

Fig. 1. Hyperchromic (a) and normochromic (b) neurons in layer III of the frontal cortex of a rat. N) Nucleus, NA) nuclear membrane; NO) nucleolus, LC) lamellar complex, M) mitochondria. Magnification: a) 1200, b) 4000. Here and in Figs. 2 and 3: 4 days after total ischemia for 10 min.

of increasing strength, and embedded in Araldite. Ultrathin sections were cut on the "Reichert" ultramicrotome, stained with uranyl acetate and lead citrate, and then examined in the Hitachi-9 electron microscope.

EXPERIMENTAL RESULTS

Changes in the frontal cortex and in area CA_1 of the hippocampus were similar in character. After total ischemia the number of "dark" osmiophilic cells, corresponding to hyperchromic neurons at the light-optical level of investigation, was increased (Fig. 1a). Intracellular changes in the neurons were similar to those in normochromic neurons. Decondensation of chromatin, and ectopic and enlargement of the nucleolus, were observed in the nuclei of normochromic neurons. The degree of folding of the nuclear membrane was increased in both normochromic and hyperchromic cells. The cytoplasm of all cells was saturated with ribosomes: in normochromic cells they were grouped into polysomes, but free ribosomes predominated in the hyperchromic neurons. Cisterns of the rough endoplasmic reticulum were extensive in normochromic neurons, those of the smooth endoplasmic reticulum in hyperchromic cells. The lamellar complex in most cells was hypertrophied (Fig. 1a) and the cisterns were dilated. Many transport vesicles were identified close to the lamellar complex and lysosomes often were visible. In some cells cisterns of the lamellar complex were deformed, collapsed, and bent into semicircules; few vesicles were present. Mitochondria in the neurons and glia were distinguished by considerable polymorphism. Swollen mitochondria were found, sometimes with destruction of their cristae (Fig. 2a). These organelles were found more often in neurons of area CA_1 of the hippocampus than in the cortex. The appearance of small, newly formed mitochondria with a dense matrix, and also of hypertrophied filamentous organelles also was observed (Fig. 2b). The number of lipofuscin granules was increased in all the cells compared with the control animals. In addition, after clinical death lipofuscin with reactive surfaces appeared (Fig. 3), which was not found when the brain of the control animals was studied.

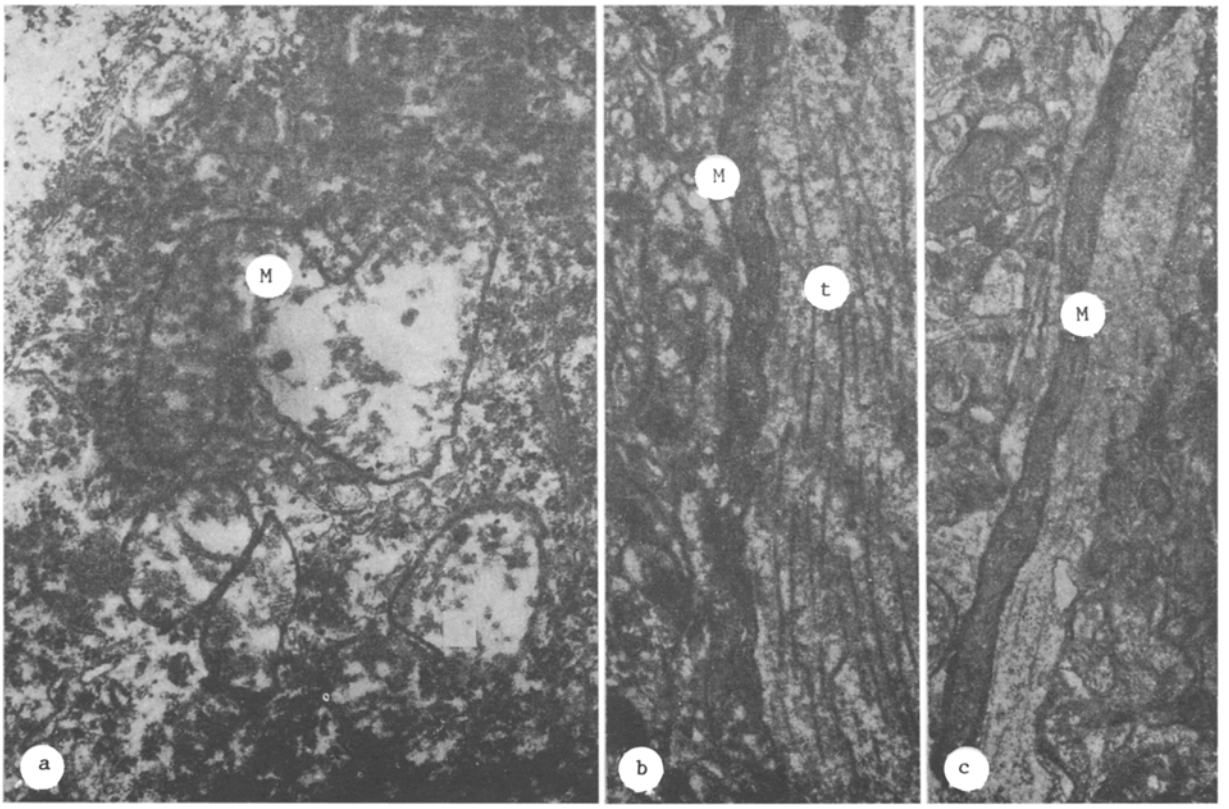

Fig. 2. Fragment of neuron in area CA_1 of hippocampus (a) and neuropil of layer III of frontal cortex of a rat (b). M) Mitochondria, T) tubules. Magnification: a) 10,000×, 6) 8600×.

Destructive processes were found in the cells, but they were neither widespread nor severe. Besides swelling of the mitochondria and destruction of their cristae, the outlines of the membranes were indistinct, synaptic vesicles fused with each other, and presynaptic condensation was modified. Swelling of the processes of astrocytes was observed, and was more marked in the hippocampus. In addition, signs of disturbance of the microcirculation in the form of sludging and microthrombosis were found in area CA_1 of the hippocampus.

The investigation thus showed that after systemic circulatory arrest in animals changes in the brain consist of a combination of compensatory-reparative and destructive processes, in agreement with data obtained on other experimental models [1, 8, 12, 15]. In this investigation compensatory and reparative processes were predominant. Structural changes found in the nucleus and cytoplasm indicate activation of repreative processes, beginning with restoration of nuclear synthesis of nucleic acids and their transport into the cytoplasm [12], followed by intensification of protein synthesis; activation of the nucleus, expansion of the cisterns of the endoplasmic reticulum, saturation of the cytoplasm with ribosomes, hypertrophy of the lamellar complex, and the appearance of large number of transport vesicles also were recorded.

Destructive changes were found to be more marked in area CA_1 of the hippocampus, in agreement with the view that certain parts of the hippocampus are more vulnerable in the postresuscitation period [8, 15]. One possible cause of the postanoxic damage to pyramidal neurons is the inadequate blood supply to the hippocampus after ischemia [8]. This was also confirmed by our results demonstrating evidence of disturbance of the microcirculation in area CA_1 of the hippocampus.

We found that after clinical death there was an increase in the number of "dark," osmiophilic cells in the brain, in agreement with data obtained by other workers, who observed an increase in the number of "dark" neurons in response to anoxia [1, 8, 12]. From the functional point of view, these cells are inactive as regards RNA synthesis and its transport into the cytoplasm [9], and the appearance of "dark" neurons is regarded as a universal, nonspecific response of nerve cells to physiological and pathological influences [3]. The

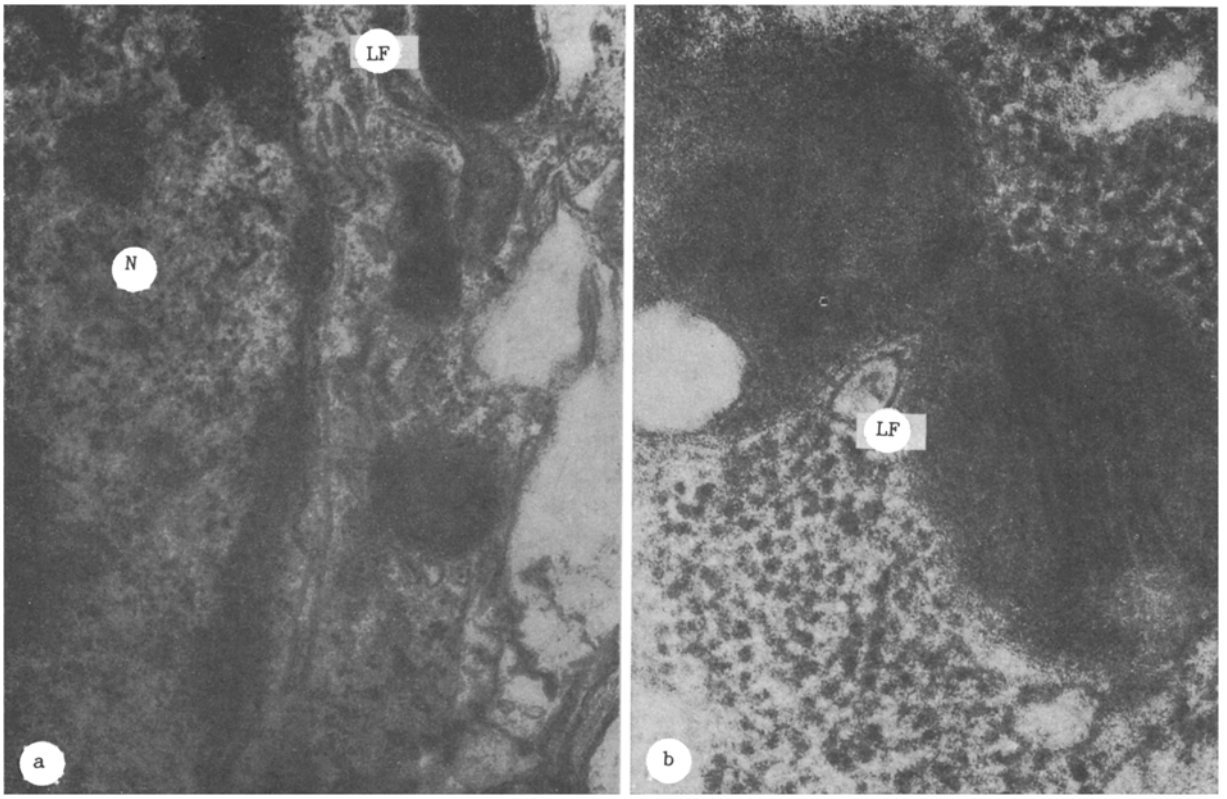

Fig. 3. Fragments of an oligodendrogliocyte (a) and a hyperchromic neuron (b) in layer III of the frontal cortex of the rat brain. N) Nucleus, LF) lipofuscin. Magnification: a) 12,000×, b) 36,000×.

response of brain cells to anoxia thus becomes heterogeneous: some cells respond to ischemia by increased synthesis of nucleic acids and protein, whereas other cells change into inactive neurons. Changes in functional activity of the cells may lead not only to ultrastructural disturbances, but also to a change in the animals' higher nervous activity [7].

We observed hypertrophy of some mitochondria and the appearance of long filamentous organelles. The appearance of mitochondria of these forms has been described in cells of various tissues in response to extremal influences, including anoxia, and it is regarded as an adaptive reaction of the organelles to altered conditions of existence [11]. High activity of enzymes of energy metabolism is known to be observed in such mitochondria [10]. In our view the appearance of lipofuscin granules in the brain is very important. Many workers regard the appearance of this pigment in cells as an adaptive response to anoxia [2, 5, 10, 13, 14]. It has been shown that lipofuscin is an intracellular oxygen depot and that it contains a terminal oxidation system, which functions in a pulsed manner during anoxia [2]. Cytochrome oxidase activity also has been detected in lipofuscin [10]. Thus lipofuscin granules, together with hypertrophied forms of mitochondria, can take over the function of supplying the brain cells with energy in the postanoxic period, at a time when the damaged organelles are undergoing repair.

These results indicate that compensatory-reparative, destructive, and adaptive processes are observed in the brain in the early postresuscitation period after systemic circulatory arrest, and knowledge of this fact is essential for the search for ways of preventing destructive processes and of stimulating reparative and adaptive processes, with the aim of preventing postanoxic encephalopathies.

LITERATURE CITED

1. N. N. Bogolepov, Brain Ultrastructure in Anoxia [in Russian], Moscow (1979).
2. V. N. Karnaukhov, Functions of Carotenoids in Animals Cells [in Russian], Moscow (1973).
3. N. S. Kolomeets, "The state of ribonucleoproteins and chromatin in neurons of the rat cerebral cortex during exposure to various influences in the antenatal and postnatal periods," Author's Abstract of Dissertation for the Degree of Candidate of Biological Sciences, Moscow (1985).

4. V. G. Korpachev, S. P. Lysenkov, and L. Z. Tel', Patol. Fiziol. Éksp. Ter., No. 3, 78 (1982).
5. I. G. Lyudkovskaya, "Morphology and pathogenesis of changes in the brain and spinal cord during respiratory resuscitation after stroke," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Moscow (1984).
6. V. A. Negovskii, Essays on Reanimatology [in Russian], (1986).
7. N. I. Nezlina, Results and Prospects for the Development of Modern Reanimatology [in Russian], Moscow (1986), p. 80.
8. N. K. Permyakov, A. V. Khuchua, and V. A. Tumanskii, Postresuscitation Encephalophy [in Russian], Moscow (1986).
9. Z. Ya. Rublëva, Yu. I. Savulëv, and A. S. Pylaev, Zh. Nevropatol. Psikhiat., No. 7, 966 (1977).
10. Z. Ya. Rubleva, Functions of Neuroglia [in Russian], Tbilisi (1984), p. 56.
11. Z. Ya. Rubleva, Tsitologiya, No. 6, 652 (1985).
12. V. V. Semchenko, "Principles of structural changes in the cerebral cortex in the recovery period after short-term total ischemia," Author's Abstract of Dissertation for the Degree of Doctor of Medical Sciences, Novosibirsk (1984).
13. Zh. V. Solov'ev and D. D. Orlovskaya, Zh. Nevropatol. Psikhiat., No. 7, 1040 (1977).
14. D. J. Malinska, W. Boellard, and W. Schlote, Acta Neuropath., 3, 222 (1984).
15. C. K. Petito and W. A. Pulsinelli, J. Cereb. Blood Flow Metab., 4, 194 (1984).

VASCULAR CHANGES IN MATURING GRANULATION TISSUE

Academician D. S. Sarkisov,* E. G. Kolokol'chikova, R. I. Kaem, and A. A. Pal'tsyn

UDC 616.003.92-005-091-07

KEY WORDS: granulation tissue; capillary; electron-microscopic autoradiography.

The morphology of granulation tissue has been studied in numerous investigations of wound healing during war and peace [1-3, 6-8]. In these investigations, conducted at the light-optical and electron-microscopic levels, the time course of structural changes in the cells and ground substance of granulation tissue during its maturation has been studied in fair detail. As regards the vascular network of granulation tissue, all that is known about it is that it undergoes considerable reduction during scar formation. However, the actual process of this reduction, i.e., how gradually the majority of these vessels of granulation tissue disappears, has not yet been studied.

The aim of the present investigation was to undertake an electron-autoradiographic study of granulation tissue at different stages of scar formation, paying particular attention to transformation of its capillary network during this process.

EXPERIMENTAL METHODS

Granulation tissue was studied in rats on the 20th and 40th days after wounding. A piece of skin with the underlying connective tissue, measuring 3 × 4 cm^2, was excised from the dorsal region of the animals under ether anesthesia. Material for histologic examination was embedded in paraffin wax and sections were stained with hematoxylin and eosin, with picrofuchsine by Van Gieson's method, and with toluidine blue.

For the electron-autoradiographic study pieces of skin measuring 1 mm^3 were excised and incubated at 37-38°C in medium 199 containing 20 μCi/ml of ^{3}H-thymidine (specific activity 21.6 Ci/mmole) for 1.5 h. At the end of incubation the material was washed to remove unin-

*Academy of Medical Sciences of the USSR.

Department of Pathological Anatomy, A. V. Vishnevskii Institute of Surgery, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 105, No. 4, pp. 501-503, April, 1988. Original article submitted August 14, 1987.

 0007-4888/88/0004-0604$12.50