TIME COURSE OF CESSATION OF BIOSYNTHESIS BY BRAIN CELLS AFTER DEATH

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Brain death is a fundamental problem in resuscitation and transplant surgery. Recent clinical, biochemical, and morphological investigations have led to progress in the solution of this problem, but some questions still remain unanswered, connected with the times of resuscitation measures, and with the actual definition of the terms "biological" and "clinical" death [3, 8, 9]. The degree of disturbance of metabolic processes during the development of the terminal state ultimately determines both the depth and the irreversibility of changes taking place in the body. Biochemical processes taking place in brain cells must also be taken into account: the effect of free radicals and of biologically active products of uncoordinated oxidation, and tissue products of unbalanced metabolism on brain tissues, the effects of exposure to toxins, disturbance of the blood supply to the tissues, neuroendocrine mechanisms, postresuscitation disturbances of the immunologic defense system, and many others [1, 10]. All these pathogenic factors create feedback chains which interact with adaptive processes in the CNS, and for that reason, it is essential to examine them from the standpoint of the theory of intracellular regeneration [2, 3, 4, 6].

To study the problem of RNA synthesis in human brain cells after death we decided to use the method of electron microscopic autoradiography in order to examine the ability of the cells of the CNS to incorporate the RNA precursor ^{3}H-uridine at different times after cessation of the circulation in the brain.

EXPERIMENTAL METHOD

The experimental material consisted of pieces of brain tissue obtained at autopsy on seven cadavers, the immediate cause of death being acute cardiopulmonary failure. The time from death until removal of the brain specimens (from the frontoparietal region) was between 4 and 8 h. The brain fragments were cut into pieces measuring 0.2-0.5 mm^3, and were incubated at 37°C for 80 min in medium 199 containing ^{3}H-uridine 100 μCi/ml (specific activity 26 Ci/ml). Subsequent treatment of the material was described in a textbook of electron microscopic autoradiography [5]. Material for this process was selected on the basis of semithin sections (autoradiographs), stained with cresyl violet. Under light optical microscopic control, areas of the semithin sections were chosen for future investigation. In the description of the structural changes in brain cells we used the following plan: capillary – neuron – glia.

EXPERIMENTAL RESULTS

In semithin sections from material obtained at autopsy we observed structural changes characteristic of cerebral ischemia: focal necroses, marked widening of the space between the cellular structures of the brain. The state of the capillaries depended on the time factor (Fig. 1).

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Fig. 1. Marked edema and swelling of brain tissue, widening of space between cellular structures. Capillary with preserved lumen in cerebral hypoxia. Semithin section. Stained with cresyl violet.

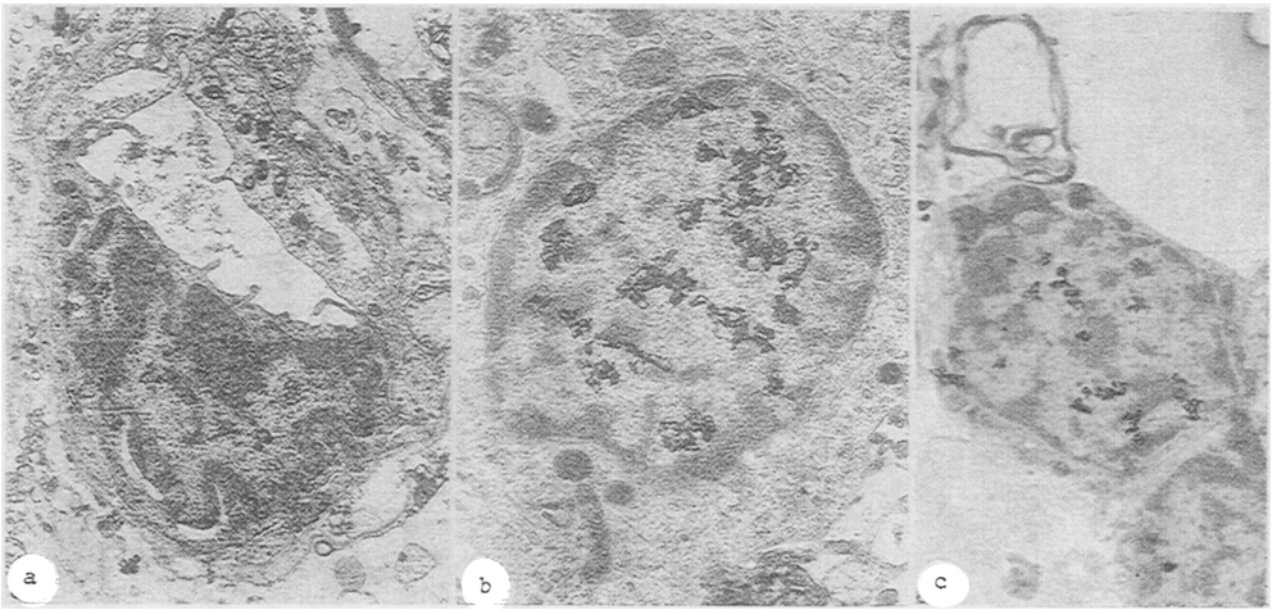

Fig. 2. Changes in ultrastructure of cells, RNA synthesis in human brain 4 h after death. 12,000×: a) Endothelial cell incorporating ^{3}H-uridine, evidence of active RNA synthesis in nucleus, with relatively intact ultrastructure; b) many grains of silver above nucleus of ischemic neuron; c) oligodendrocyte. Incorporation of ^{3}H-uridine shown by presence of grains of silver above nucleus.

The endothelial cells 4 h after death were swollen, the distribution of chromatin in their nuclei was disturbed, the number of pinocytotic vesicles was increased, and the outline of the basement membrane was uneven. The number of labeled and unlabeled endothelial cells was about equal, irrespective of the caliber of the vessels. No significant difference could be found in the ultrastructural changes and RNA synthesis between the endothelial cells (Fig. 2).

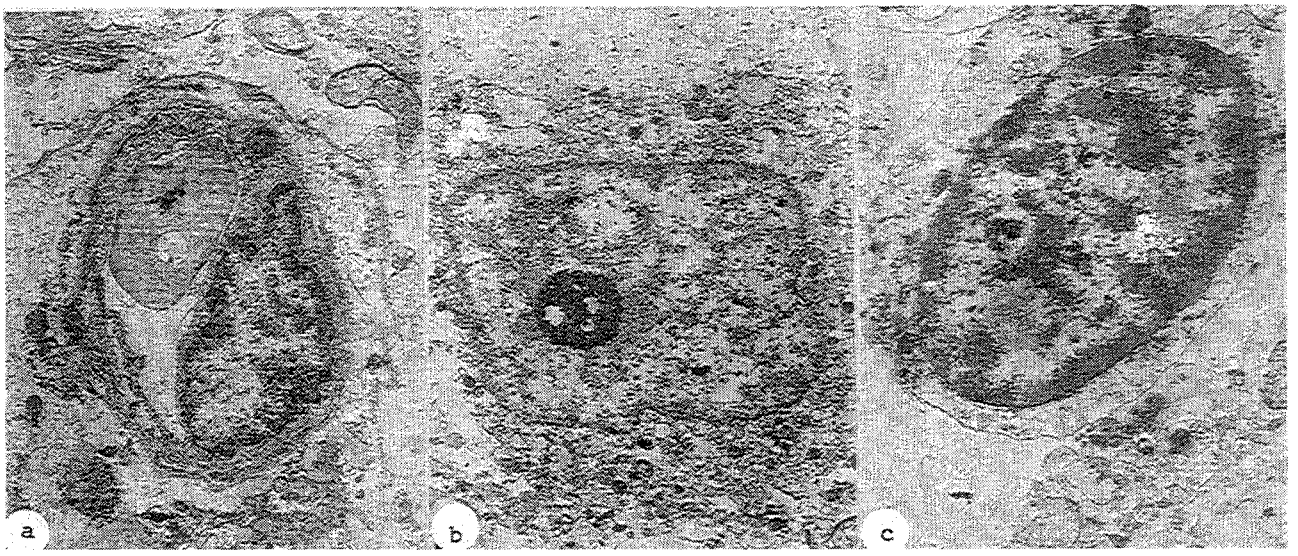

Fig. 3. Changes in ultrastructure and RNA synthesis in human brain cells 8 h after death. 12,000×: a) Endothelial cell with marked destructive changes in nucleus and cytoplasm; b) floccular deposits of chromatin in karyoplasm, many fragments of destroyed organelles present in cytoplasm of neuron; c) incorporation of ^{3}H-uridine in oligodendrocytes slight, only a few grains of silver can be seen above the nucleus, destruction of organelles.

Examination of the neurons (Fig. 2b) showed increased electron density of the nucleus, and the appearance of many mitochondria in the cytoplasm with swelling and fragmentation of the cristae, and accumulation of ribosomes and polysomes into conglomerates of different sizes. Besides mitochondria partially denuded of their cristae, numerous tiny vacuoles also could be seen, with dilated cisterns of the endoplasmic reticulum and redistribution of polysomes and ribosomes, i.e., while all signs of an ischemic neuron were present the nucleus remained functionally active and capable of synthesizing RNA, as shown by the presence of the label.

RNA synthesis in the neuron must be examined in close association with metabolism of the glial cells (oligodendrocytes) and their ultrastructural changes. Structural changes took place in the cytoplasm of the oligodendrocytes: the number of vacuoles was greater than normal, swelling of the mitochondria and deformation of the cisterns of the smooth reticulum were observed, and the number of lysosomes and glycogen granules was increased. The nucleus of the oligodendrocytes contained unevenly distributed chromatin granules The continuation of RNA synthesis could be judged from incorporation of ^{3}H-uridine (Fig. 2c). At the times observed RNA synthesis in the oligodendrocytes continued to proceed at a high level.

In material obtained 8 h after death a decrease in intensity of RNA synthesis in the endothelial cells was observed. Ultrastructural analysis revealed the formation of large vacuoles and pinocytotic vesicles; the distribution of chromatin in the nucleus was disturbed; many mitochondria were swollen and had lost their cristae, and their matrix was translucent. The basement membrane was unevenly thickened, and in some places its fibers were separated. Despite ultrastructural changes in the endotheliocytes the capillaries continued to preserve their lumen, in which blood cells could be found (Fig. 3a).

The cytoplasm and nucleus of the neuron underwent considerable ultrastructural changes (Fig. 3b). Redistribution of chromatin took place in the nucleus, in which it was condensed in the form of floccules in the karyoplasm. The cytoplasmic matrix was highly translucent. In most mitochondria the cristae were reduced or had undergone complete lysis, with the formation of large cavities; elements of the rough and smooth endoplasmic reticulum were virtually absent. The cytoplasm contained many fragments of membranes of the destroyed organelles and also a concentration of lipofuscin granules. An increase in the degree of destructive changes was observed in the glial cells compared with the material examined at an earlier stage (Fig. 3c). The increase in volume of the cytoplasm reflects destructive changes in the cell: large quantities of cellular debris with fragments of membranes, cytogranules, and vacuoles. Hyperchromatosis and deposition of masses of chromatin in the karyoplasm were observed in the nucleus of the oligodendrocytes. The perinuclear space was appreciably widened. Solitary glial cells which had incorporated ^{3}H-uridine into their nucleus were seen in the material studied, evidence of continuing weak RNA synthesis.

Thus individual brain cells, both ganglion and glial cells, preserve their ability to synthesize RNA for more than 5 h after cessation of the general circulation, and it is possible to evaluate the brain damage by the method of electron-microscopic autoradiography The facts described above demonstrate that further research into the problem of biological death and brain death during resuscitation from the clinical and morphological aspect can be undertaken.

REFERENCES

1. A. M. Gurevich, Patol. Fiziol., No. 2 (1988).
2. O. A. Zakharova and A. A. Pal'tsyn, Byull. Éksp. Biol. Med., No. 6 (1991).
3. V. A. Negovskii, Outlines of Resuscitation [in Russian], Moscow (1986)
4. D. S. Sarkisov, A. A. Pal'tsyn, and B. V. Vtyurin, Adaptive Reorganization of Biorhythms [in Russian], Moscow (1975).
5. D. S. Sarkisov, A. A. Pal'tsyn, and B. V Vtyurin, Electron-Microscopic Autoradiography of the Cell [in Russian], Moscow (1980)
6. V. P. Tumanov and M. D. Malamud, Changes in the Central Nervous System in Response to Thermal, Radiation, and Combined Trauma [in Russian], Kishinev (1977).
7. A. E. Walker, Brain Death [Russian translation], Moscow (1988).
8. S. Araujo, J. F. K. Saraiva, M. J. Gomez, and R. G. G. Terzi, Intens. Care Med, **16**, No. 1, 55 (1990).
9. P. Black, Am. Heart J., **99**, No. 3, 279 (1980).
10. T. L. Petit and J. C. Le Boutillier, Brain Res., **517**, No. 1-2, 269 (1990).