REGENERATION OF NERVE CELLS AFTER THE ACTION OF CEREBROCYTOTROPIC SERA

N. A. Levkova, N. D. Skuba, and V. P. Lebedevskaya

UDC 612.8.014.2:612.6.03].014.46:365.81

Experiments on dogs have shown that after administration of cerebrocytotropic serum, regeneration of nerve cells is mainly of the intracellular type: hypertrophy of the bodies and processes of the neurons. Hypertrophy of neurons is accompanied by an increase in their content of DNA and RNA.

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The view has long been held, as some investigators [4] still maintain, that nerve cells are stable and cannot regenerate. However, the problem of regeneration of nerve cells in the central nervous system has not yet been finally solved [3, 6, 8].

The present investigation was based on the principle of specific action of cerebrocytotropic sera on brain tissue. It was assumed that large doses of these sera may produce destructive changes in the brain, as a result of which protein breakdown products will subsequently stimulate regeneration of nerve tissue elements still remaining intact.

EXPERIMENTAL METHOD

Experiments were carried out on 16 healthy young and adult dogs, receiving cerebrocytotropic sera by injection into the blood stream, in some cases (7 dogs) intravenously and in other (9 dogs) intra-arterially: the common carotid artery used for intra-arterial injection of the serum was exteriorized in a skin flap.

All the animals received cerebrocytotropic serum in a dose of 4 ml 5 times at intervals of 3 days. The reacting dose was injected in a volume of 5 ml 5 days after the last injection. After receiving the cerebrocytotropic sera the animals developed severe cytotoxic shock, from which 3 of the 9 dogs (intra-arterial injection) died.

The cerebrocytotropic sera were obtained by immunization of rabbits with dog brain tissue by the normal immunologic method. The sera thus obtained were tested by the complement fixation reaction; their serologic titers were 1:400, 1:600, and 1:800.

The animals were sacrificed 5, 10, 15, 20, 25, and 30 days after injection of the reacting dose. The brain was fixed in 12% neutral formalin solution. Pieces of different parts of the brain and spinal cord were embedded in paraffin wax. Sections were stained by Nisel's method and for nucleic acids (Feulgen and Brachet reactions and with gallocyanin).

EXPERIMENTAL RESULTS

Particular attention was paid to changes in the nerve cells. At short intervals after injection of the sera, degenerative and destructive changes were observed, principally in less resistant neurons. The more resistant cells remained intact, and they were subsequently responsible to some extent for the development of regenerative processes (20th-30th day after injection of the reacting dose).

Department of Pathological Anatomy, Zaporozh'e Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 68, No. 11, pp. 88-91, November, 1969. Original article submitted March 4, 1969.

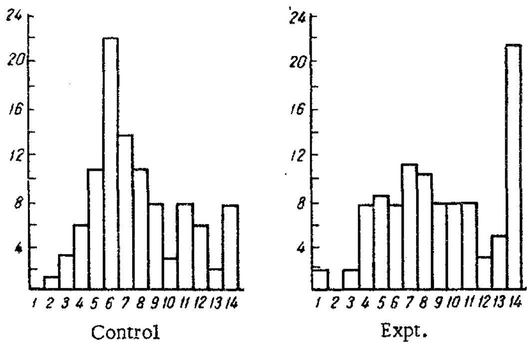

Fig. 1. Histograms of distribution of cortical neurons by size. Abscissa – classes of cells by size (in units of area): 1 (1-1.9); 2 (2-2.9); 3 (3-3.9); 4 (4-4.9); 5 (5-5.9); 6 (6-6.9); 7 (7-7.9); 8 (8-8.9); 9 (9-9.9); 10 (10-10.9); 11 (11-11.9); 12 (12-12.9); 13 (13-13.9); 14 (14 and over); ordinate – percentage of cells.

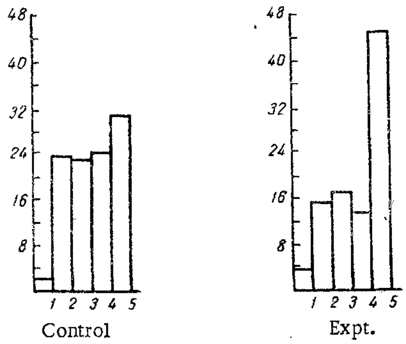

Fig. 2. Histograms of distribution of cortical neurons by their nucleic acid content. Abscissa – classes of cells by content of DNA + RNA (in conventional units): 1 (0.168-0.499); 2 (0.500-0.999); 3 (1.000-1.499); 4 (1.500-1.999); 5 (2.000 and above); ordinate – percentage of cells.

Hypertrophy of intact neurons lying side by side with or a short distance away from dying nerve cells was observed to begin with. The nerve cell increased in size, its nucleus became large, as also did the nucleolus, and the processes became thicker and sometimes longer (Figs. 1, 2). The quantity of tigroid substance increased and the tigroid granules became larger. The RNA content in these cells increased. In sections stained by Brachet's method RNA appeared as bright red, large granules, more numerous than in the normal cell. Hypertrophied nerve cells were commoner in the basal ganglia and spinal cord. Some neurons were between twice and three times larger than their neighbors. The number of hypertrophied nerve cells increased in the later stages of the experiment.

To determine the extent of this phenomenon of hypertrophy of nerve cells, a cytospectrophotometric study was made of the total content of nucleic acids (DNA + RNA). This part of the investigation was carried out by a scanning method on the MUF-5 cytospectrophotometer. Sections were stained with gallocyanin. Photometric measurements were made at $\lambda = 578$ nm. Cortical neurons of the frontal lobes of control and experimental animals were investigated at random. Altogether 105 cells from each animal were studied.

The number of cells of increased size and with an increased nucleic acid content was found to be higher in the experimental animals.

The histogram (Fig. 1) shows that the number of enlarged cells (column 14 in the histogram) is 13.6% higher for the experimental animal than for the control (difference statistically significant). The other histogram (Fig. 2) shows that the number of cells with an increased content of nucleic acids (column 5 of the histogram) was 15.6% higher in the experimental animal than in the control (difference statistically significant).

The presence of hypertrophy of nerve cells is thus also confirmed by quantitative studies.

Hypertrophy of nerve cells can be regarded as one form of manifestation of what D. S. Sarkisov describes as intracellular regeneration.

Sarkisov [7] states that during hypertrophy specific protein structures accumulate in the cell. In his opinion, regeneration in some tissues takes place on the territory of intracellular structures. The content of those specific protein structures which participate in compensation of the function of dead cells is thereby increased. For this reason, the writers also regard this type of phenomenon as intracellular

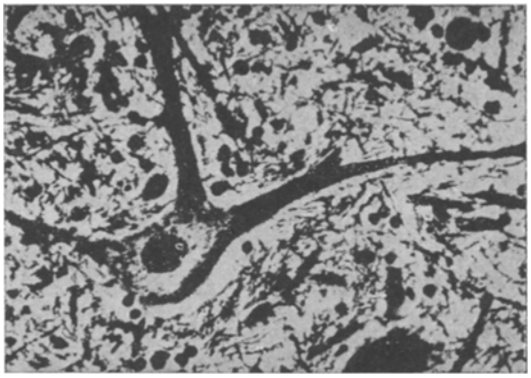

Fig. 3. Hypertrophy of processes of a nerve cell from the anterior horns of the spinal cord (20th day of experiment). Impregnation with silver by the Bielschowsky – Gros method. 500 ×.

regeneration. The nerve tissue in the present experiments is an example of this type of regeneration. In this context Zhinkin [5] states that the hypertrophied cell can perform the function of two or more cells.

Besides marked hypertrophy of the cell body, changes also took place in the processes (Fig. 3). Sometimes the cell did not increase in size, but it gave off one or several processes, sometimes ending in globular swellings. In some cases these processes touched the bodies of other cells. The formation of connections of this type by lengthening of the processes is another form of compensation occurring in nerve tissue.

In the cortex of young animals (2 dogs at the 30th day of the experiment), and also in some of the basal ganglia of the hypothalamic region, foci of different sizes, in some cases amounting to whole fields of proliferation of undifferentiated cells resembling medulloblasts were seen. Foci of this type were also present among the white matter of the cerebral hemispheres. Larger cambial cells (glial cells and neurons) were present here among the small cells. Among these undifferentiated cells, distinctively dividing cells could be seen.

When nerve tissue undergoes severe damage, the most primitive type of regeneration resembling the embryonic type thus reappears. Medulloblastic tissue proliferates, and as it differentiates it forms both neurons and glial cells. Evidently in this case the organism, confronted with a serious emergency, uses all its powers to bring about regeneration of the nervous system.

Binuclear nerve cells appeared in the cortex and subcortex of the brain in many animals, and this was evidently preceded by hyperchromatosis of the nucleus. Binuclear cells are larger than the ordinary mononuclear cells.

The widespread distribution of polyploid cells in nerve tissue has been demonstrated by cytophotospectrophotometric investigations [1, 2]. The possibility is likewise not ruled out that polyploid cells may also be formed following the action of cytotoxins on nerve tissue.

Regeneration of nerve cells following specific action on nerve tissue thus takes place principally by intracellular regeneration of neurons: hypertrophy of the body and processes with the appearance of new synaptic connections.

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