(Table 1). Our study confirmed that the incidence of abnormal signs increases in women with grave clinical manifestations of placental failure (Table 1).

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Morphological Assessment of Growth Capacity of the Central Nervous System Axons in a Peripheral Nerve

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Embryonal rudiments of rat neocortex and spinal cord survive for 60 days after subperineural transplantation into the distal part of crossed sciatic nerve in adult animals. Embryonal cell elements are differentiated from neuroepithelial cells and neuroblasts to mature neuro- and gliocytes. Transplanted spinal neuron axons migrate from the transplant and are myelinized with recipient peripheral nerve Schwann cells. A cavity lined with ependyma-like cells is often formed at the site of dying grafted cell elements.

Key Words: *transplantation; nerve tissue; peripheral nerve; regeneration*

Limited reparative regeneration of central nervous system (CNS) tissues prompts the search for new methods correcting and compensating for the lost nervous functions. Peripheral nerve lemmocytes can be employed as a conducting path for CNS axon growth. Schwann cells [7] or fragments of nerve stems of the peripheral nervous system [8,9,11-13] are transplanted into different compartments of damaged brain with this aim in view. We transplanted embryonal rudimental brain into crossed sciatic nerve of adult rats in order to investigate the relationships between CNS neurons and peripheral glia.

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MATERIALS AND METHODS

Fifty male and ten female Wistar rats weighing 200-250 g were used. The sciatic nerve was crossed under ether narcosis and the proximal and distal ends were ligated. Embryonal material was introduced under the perineurium of the distal part of a large nerve stem. Fourteen-day-old Wistar rat embryos were donors, from which brain sites with rudimental neocortex and the spinal cord were isolated. The animals were killed by ethyl ether overdose 7, 14, 30, and 60 days after transplantation. For histological studies, sciatic nerves were fixed in Bouin's fluid. Paraffin sections (5-7 μm) were stained with hematoxylin and eosin and toluidine blue by the method of Niessle. Nerve fibers were

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detected in the grafts by silver nitrate impregnation according to Bil'shovskii-Gros' method modified by E. I. Chumasov [4]. For electron microscopy, the material was fixed in 2.5% glutar aldehyde in 0.1 M phosphate buffer (pH 7.3) and then in 2% osmium tetroxide in 0.05 M cacodylate buffer for 1 h, dehydrated in ethanol and propylenoxide, and embedded in Epon-812. Ultrathin sections were cut in an LKB-III ultratome, contrasted with uranyl acetate and lead citrate, and examined in JEM-100B electron microscope at accelerating voltage 75 kV.

RESULTS

A fragment of the spine and anterior cerebral vesicle wall with rudimental neocortex consist of neuroepithelial cells and poorly differentiated neurofibroblasts. Developing spinal cord is differentiated more rapidly than the neocortex [1]. The number of neuroblasts in spinal rudiments used for transplantation was higher than in cortical rudiments. Seven days after sciatic nerve crossing its distal end is in a state of Woller degeneration. Axons in this portion of the nerve die during this period, and their myelin membranes degenerate. Myelin degradation products are seen in the cytoplasm of the endoneurim macrophage. Schwann cells in the distal end are viable, some of them are dividing. Fine-wall blood vessels are seen in the endoneurium. Nervous membranes and vessels at the site of embryonal tissue transplantation are hypertrophic and infiltrated with leukocytes and macrophages. Neocortical transplants during this period consist of neuroepithelial cells and neuroblasts. The latter elements possess large clear nuclei with well-defined nucleoli and a narrow cytoplasm rim. Neuroepithelium is seen at a remarkable distance as long cords; there are dividing cells. Neural elements of transplanted spinal cord are represented mainly by neuroblasts. We observed such anticipation in differentiation of cell elements in transplanted embryonal spine in comparison with the cortex in our previous studies, when we transplanted nerve rudiments into the proximal part of damaged sciatic nerve of rats [2,5]. After 14 days, the neurografts are clearly seen in the nerve together with myelin degradation products, inflammation infiltration, and well-developed vascular network. Neural elements of trans-

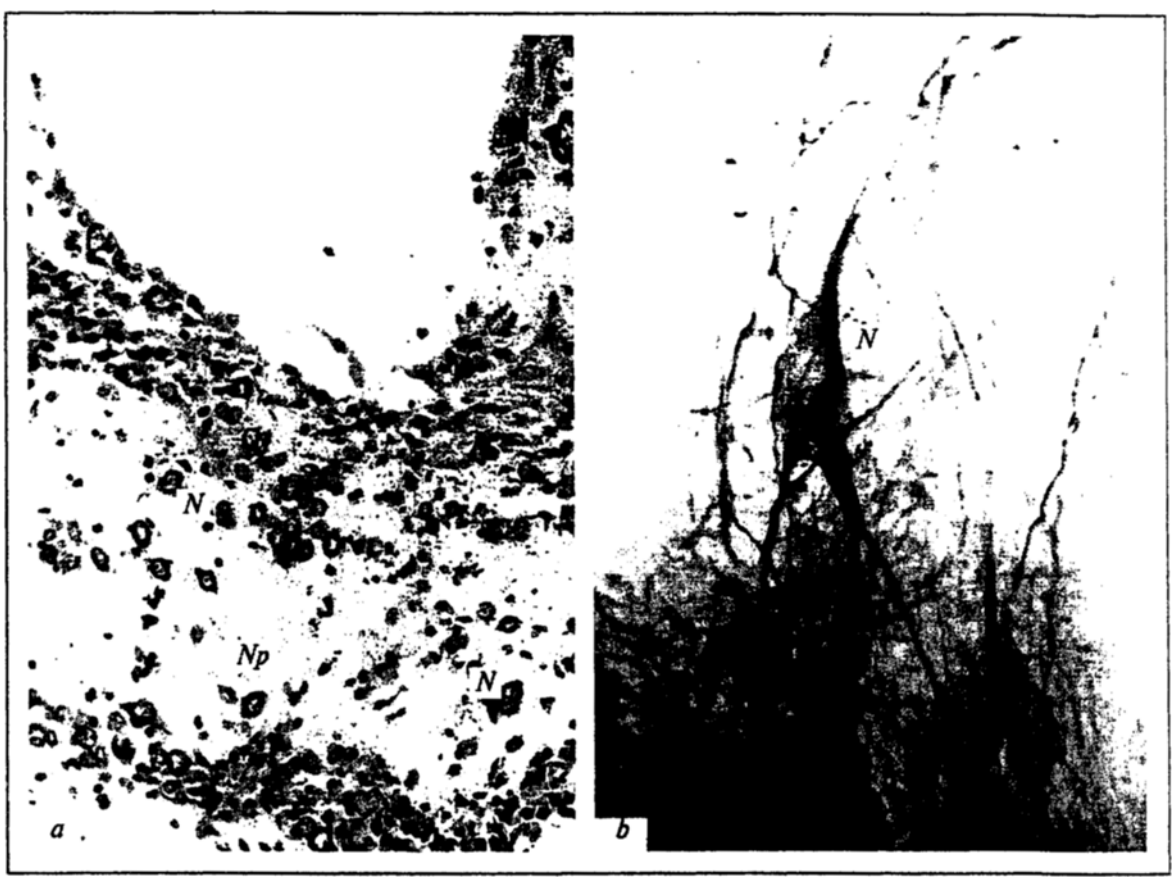

Fig. 1. Fragments of embryonal neocortex transplants 60 days after transplantation to the distal portion of crossed sciatic nerve of an adult rat. *a*) general, toluidine blue staining by the method of Niessle, ×100; *b*) pyramidal neuron, silver nitrate impregnation, ×250; *N*: neurons; *Np*: neuropile.

Fig. 2. Nerve fibers in 14-day-old rat embryonal spinal transplants 60 days after transplantation to the distal portion of crossed sciatic nerve. a) outgrowth of nerve fibers beyond the transplant. ×100; b) profiles of newly formed amyelinic and myelinized nerve fibers at a distance of 2 cm distal from the graft. ×20 000. Silver nitrate impregnation. *BM*: basal membrane; *A*: axons; *SC*: Schwann cell; *MF*: myelin fiber.

plants are represented by neuroblasts and young neurons. Small cavities lined with epithelium can be sometimes seen in the transplants at this period. At some places there are foci of transplanted tissue degeneration; some neurons die. After 30 and 60 days, the nerve stem is 3-4 times thicker at the site of transplantation. Examination of longitudinal histological sections often reveals large cavities formed in the transplant at these sites (Fig. 1, *a*). As a rule, the cavities in spinal transplants are smaller than in cortical transplants. Electron-microscopic examination showed that these cavities are lined with cells possessing cilia and microvilli, which points to their ependymoglial origin. Sites with normally developing tissues with intact neurons and neuroglia are neighboring the cavities. Mature neurons of different shapes and sizes, gliocytes, and blood vessels are discernible. Neurocortical transplants contain neurons with morphology similar to that of pyramidal neurons of adult rats (Fig. 1, *b*). These cells are triangular, have large clear nuclei with well seen nucleoli, and long apical and basal dendrites. Typical motor neurons are formed in spinal transplants. A dense network of nerve fibers varying in diameter is seen in both transplants on preparations impregnated with silver nitrate. Electron microscopy shows a well-developed neuropile with numerous axons, dendrites, and synaptic contacts in both types of transplants. Many axons are myelinized with oligodendrocytes after the central type. Axodendritic synapses can be found in the neuropile. The impregnation method revealed that some nerve fibers outgrow from spinal transplants (Fig. 2, *a*) 1.5-2 cm distal from it. Newly formed nerve fibers are dis-

cernible on semithin sections of the distal portion of the nervous stem endoneurium; after osmium tetroxide staining, myelinized membranes of these fibers looked like black rings 1-4 μm in diameter. The level of some myelin fibers maturation was rather high (Fig. 2, *b*). As a rule, there were amyelinic fibers of different diameters (0.1-1.5 μm) related to lemmocytes near myelin fibers.

Our results prove that axons of transplanted spinal neurons outgrow from the transplants, are myelinized by recipient nerve lemmocytes, and grow distally. We observed no axons at the periphery, no their relationship with lemmocytes [6,10]. Formation of glial lining at the interface with recipient tissues may prevent axon growth from transplanted cortical neurons [5]. Another cause is death of many large neurons and formation of cavities. Spinal axons are characterized by phylogenetic tropism to peripheral glia, whereas membranes of neocortical neuron axons may be devoid of receptors recognizing lemmocytes. Comparison of these findings with our previous data permits us to conclude that the proximal portion of damaged nerve is the most favorable site for the development of CNS neurons in a nerve stem [2, 3,5], since it contains numerous regenerating r cipient nerve fibers at the early stages.

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