IMPLANTATION OF EMBRYONIC ANLAGEN OF THE NEOCORTEX AND SPINAL CORD INTO AN INJURED ADULT RAT PERIPHERAL NERVE

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Regeneration of nerves and restoration of lost functions of the limbs are among the most important problems in medical biology. The use even of the most modern microsurgical techniques does not always lead to complete recovery of the integrity of nerve trunks and normalization of trophic influences in the tissues. Methods of neuroplasty have now been improved [3] and various microsurgical methods of uniting nerve trunks by suture have been introduced [2] and methods of oriented regeneration of injured nerves along artificially created pathways are being developed [7]. The creation of new relay and trophic centers in injured nerves with a view to stimulating their regeneration may turn out to be a promising approach. However, the solution of this problem requires elucidation of the possibility of survival and potential for development of different regions of the CNS under conditions of transplantation into peripheral nerves of adult animals. Analysis of the literature on transplantation of nerve tissues, summarized in monographs, collections, and articles provides evidence that most investigations have been devoted to transplantation into the brain and spinal cord, and there have been far fewer studies of other ectopic sites [1, 4-8, 13]. There have been only a few fragmentary studies of transplantation of CNS tissues into a peripheral nerve, and these have shown that embryonic anlagen of the cortex preserve their viability and their cells can differentiate to adult neurons and glia [9-12, 14]. Many aspects of CNS tissue transplantation into a peripheral nerve have not yet been studied. There are virtually no data on transplantation of spinal cord fragments into a peripheral nerve and no comparative morphological analysis of cortical and spinal cord tissues transplanted into a nerve has been undertaken.

The aim of this investigation was to study the possibility of survival and the time course of development of embryonic anlagen of the spinal cord and neocortex after their implantation into the injured peripheral nerve of adult rats.

EXPERIMENTAL METHOD

Experiments were carried out on 50 male Wistar rats weighing 200-250 g. Under ether anesthesia the spinal cord of the rats was injured by measured crushing with forceps; proximally to the site of injury embryonic material was injected by means of a glass cannula beneath the perineurium of a large nerve trunk. Embryos of Wistar rats (14 days of development), in which regions of the brain containing the anlage of the neocortex, and the spinal cord were excised, served as donors. Before implantation the embryonic tissue was placed in sterile Petri dishes with nutrient medium 199 and minced. The animals were killed with diethyl ether 7, 14, 30, 60, and 150 days after the operation. For histological study the sciatic nerves were fixed in Bouin's fluid. Paraffin sections 5-7 μm thick were stained with hematoxylin and eosin and with toluidine blue by Nissi's method. Material for electron microscopic investigation was fixed in glutaraldehyde in phosphate buffer (pH 7.3), postfixed with 2% osmium, dehydrated, embedded in Epon, and studied under the electron microscope.

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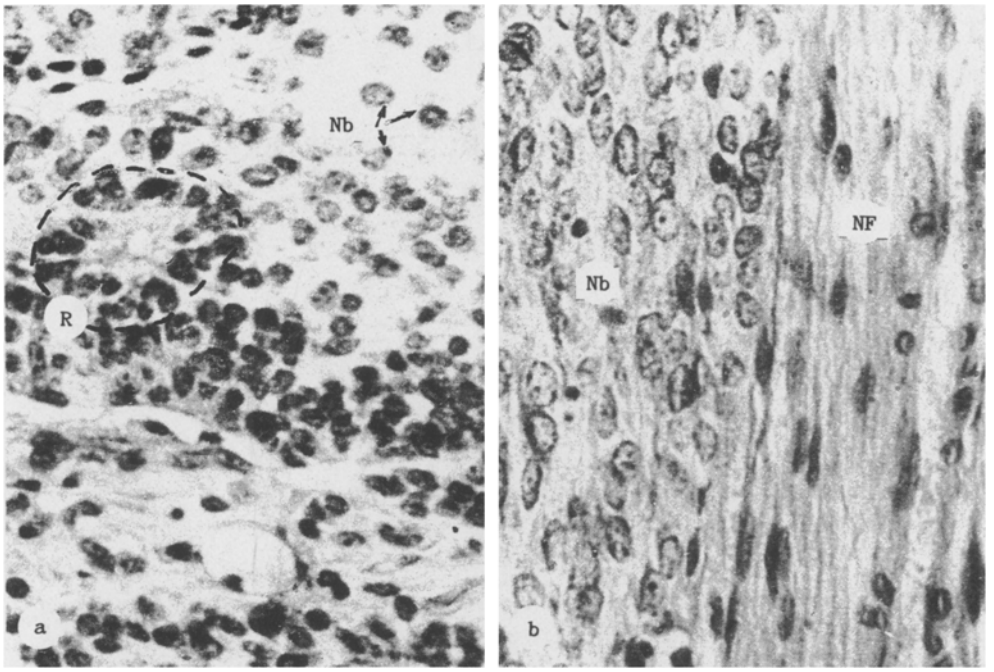

Fig. 1. Implants of cortex (a) and spinal cord (b) of 14-day rat embryos 7 days after transplantation into sciatic nerve. R) "rosette" of neuroepithelium, Nb) neuroblasts, NF) host's nerve fibers. Bouin's fixation, stained with hematoxylin and eosin. 300×.

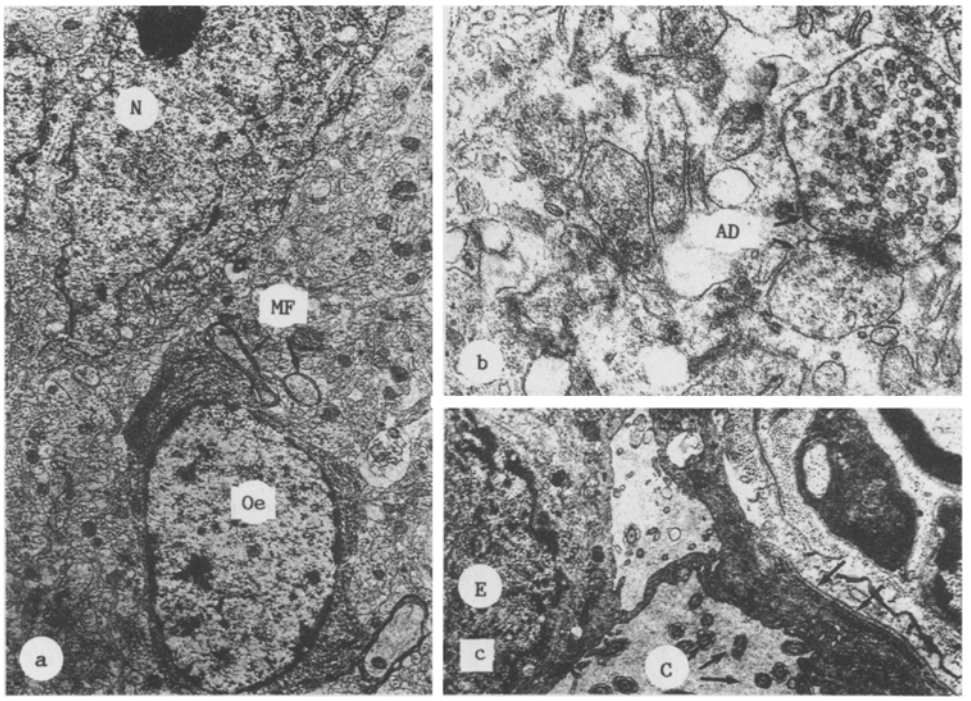

Fig. 2. Ultrastructure of spinal cord (a) and cortical (b, c) implants 30 days after operation. N) neuron, Ol) oligodendrocytes, MF) myelinated fibers in neuropil of implant, AD) axodendritic synapse; arrows indicate basement membrane on boundary between implant and host's tissues; E) ependymocyte, R) cilia of ependymocyte. Magnification: a) 2500×, b) 12,000×, c) 8000×

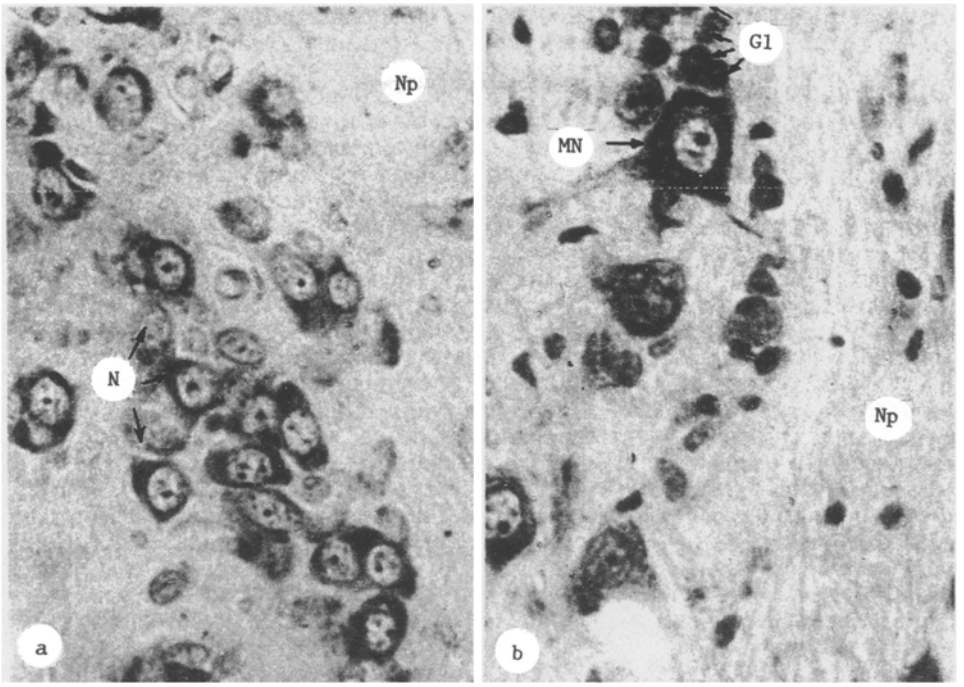

Fig. 3. Neurons of cortical (a) and spinal cord (b) implants 5 months after transplantation. N) cortical neurons, Np) neuropil, MN) motoneuron, Gl) gliocytes. Bouin's fixation, Nissl's toluidine blue. 300×.

EXPERIMENTAL RESULTS

The wall of the forebrain vesicle, containing the anlage of the neocortex, and the spinal cord of 14-day rat embryos used for implantation consist of germinative or ependymal and mantle layers and a marginal velum. They contain neuroepithelial cells and undifferentiated neuroblasts. The implants 7 days after transplantation were found beneath the perineurium or in the center of the nerve trunk, in the form of elongated small-cell formations. Cortical implants consisted mainly of neuroepithelial cells and neuroblasts. The former formed characteristic structures in the form of "rosettes" and in "bands" (Fig. 1a). Their cells were prismatic or cubical in shape, with hyperchromic nuclei and cilia. Mitotically dividing neuroepithelial cells could often be seen. The neuroblasts were arranged loosely or in the form of rows of cells around "rosettes" of neuroepithelium. They had large, pale nuclei with 2 or 3 nucleoli, and their cytoplasm was palely stained. The glioblasts were distinguished by their smaller size and oval hyperchromic nuclei, which were still difficult to distinguish from nuclei of the endothelium of capillaries invading the implant. Around and inside the implant were mononuclear cells. Spinal cord implants differed in their morphology from cortical implants (Fig. 1b). They consisted mainly of larger neuroblasts, with chromatin-deprived round or oval nucleus, with one or two nucleoli, and finely dispersed granules were visible in their cytoplasm stained by Nissl's method. It must be emphasized that, unlike the cortex, in spinal cord implants at this time there were no neuroepithelial cells, no "rosettes" were formed, and mitotic figures were extremely rare.

After 14 days further maturation of neural and glial cells was observed in the cortical and spinal cord implants. Young neurons appeared among the neuroblasts. Small clumps of chromatophilic material could be seen in their cytoplasm, and short dendrites were visible in some of them. Both in the cortex and in the spinal cord binuclear nerve cells were seen, which had been absent at the earlier period. The number of glial cells was increased: astrocytes and oligodendrocytes. A network of capillaries formed inside the implants. Besides erythrocytes, mononuclear cells also were seen in the capillary lumen.

After 30 and 60 days the implant contained differentiated nerve and glial cells. Between them there were wide spaces in the developing neuropil. Electron-microscopic investigation showed that it consisted of a very large number of very thin and glial processes. Many axons were myelinated. Their diameter was 1-2 μm. They were most frequently located close to processes and bodies of oligodendrocytes (Fig. 2a). In both cortical and spinal cord implants at this time synapses were frequently seen, mainly axodendritic (Fig. 2b). A few rosettes of ribosomes, large mitochondria, and numerous microfilaments were present in the postsynaptic boutons. A lining of ependymocytes was present on the boundary with the host's tissues (Fig. 2c).

After 5 months the implants preserved their viability and small, medium-size and large nerve cells of various shapes could be identified in them. Cells with morphological features similar to those of pyramidal and multipolar neurons predominated in the cortical implants. They were characterized by a large pale nucleus with one or two distinct nucleoli, by the small volume of their finely granular chromatophilic cytoplasm, and the presence of short apical and basal dendrites (Fig. 3a). Often, the neurons lay close together, with a dense neuropil around them. Large cells with characteristic signs of motoneurons could be seen at this time in the spinal cord implants (Fig. 3b). From their perikarya they gave off thick dendrites, which could be traced for a considerable distance. Chromatophilic material was clearly distinguishable in the cytoplasm and clumps of it were located not only in the perikaryon, but also actually in the dendrites. Besides motoneurons, small and medium-sized bi- and multipolar cells could be seen in both the spinal cord and cortical implants. Satellite cells around the spinal cord neurons were more numerous than in the cortical implants. Among the glial cells were astrocytes, oligodendrocytes, and microglia. The large number of glial cells, especially oligodendrocytes, in the spinal cord implants can be explained by the presence of numerous myelinated fibers. Unlike the cortical implants, on the boundary between the spinal cord implants and host's tissues a glial layer was absent in many cases. In these regions bundles of myelinated fibers were observed, connecting the implants with the host's nerve fibers. All the implants were well vascularized.

The results of this investigation showed that implanted embryonic anlagen of both cortex and spinal cord can maintain their viability for a long time (5 months of observation) in an injured peripheral nerve and their cells continued to differentiate. Comparative morphological analysis showed that in the early period after transplantation, neural elements of the spinal cord differentiate earlier than cortical cells, due to the heterochronicity of development of these brain structures in ontogeny. The implants 30-60 days after the operation contained mature neurons with the characteristic histotypical features of each part of the brain. Evidence of the degree of maturity of the neurons and their functional activity was given by the presence of myelinated axons and synapses. On the basis of this analysis we consider that spinal cord implants can be used in further research aimed at improving the regeneration of injured peripheral nerves, for the majority of nerve fibers composing them are motoneurons, and the myelin-forming cells of the peripheral axons are Schwann cells.

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