implantation of embryonal brain tissue into regenerating peripheral nerve

E. S. Petrova, E. I. Chumasov, and V. A. Otellin

One of the most promising approaches to the solution of the problem of the restoration of injured nerve trunks is the construction of new relay and trophic centers for the recovery of the functions of injured conductive pathways of the central and peripheral nervous systems through the transplantation of nerve tissues [5]. In the last decade the method of transplantation of embryonal nerve tissues into sites protected from the action of the immune mechanisms, namely, the brain [1, 3, 4], and the anterior chamber of the eye [2], has been widely used. The implantation of embryonal nerve tissues into a peripheral nerve of adult animals has been carried out, and has demonstrated that implants of areas of the brain of 11 day old rat embryos containing the anlage of the cortex survive, increase in size, and differentiate both in the degenerating and the regenerating nerve [6-8]. However, the intertissue interrelationships, vascularization, interneuronal connections, and the myelinization of the axons are still inadequately studied.

The purpose of the present investigation is to elucidate the morphological features of the embryonal tissues of the CNS which have been implanted into the regenerating nerve of adult rats, and to study their interrelationships with the host tissue.

Materials and Methods. The study was carried out on Wistar rats weighing 200-250 g. An incision (1-2 cm) was made under ether anesthesia in the skin of the upper third of the thigh following epilation, the underlying muscles were dissected, the nerve was freed up from the loose connective tissue, and was injured by means of a dosed (for 30 sec) crush by a squeeze. A small incision in the coverings of one of the nerve trunks was made under an MBI-3 microscope 5 mm proximal to the injury site. The embryonal material was introduced by means of a glass cannula under the perineurium of the nerve trunk through this incision. Wistar rat embryos of 17 days development, in which a section of the brain containing anlage of the neocortex was isolated, served as the donors. The embryonal tissue was placed in sterile Petri dishes with nutrient medium (medium 199, Moscow, with the addition of streptomycin sulfate, 0.01 g per 10 ml of medium). Then it was broken up into small pieces, approximately 0.5 x 0.5 mm in size. The corresponding areas of the brain of 17 day old embryos were used as control. The animals were kept in the usual conditions until the 3rd week after transplantation.
Fig. 1. Initial (a) and implanted cerebral tissue two weeks after operation. a) Undifferentiated neuroblasts in the cortical angle of 17 day old rat embryos. Arrows point to mitotic figures in the germinative zone; b) general view of an implant in the sciatic nerve of an adult rat; c, d) neural and glial elements of the implant at various stages of differentiation. Nb represents neuroblasts; YN, young neurons; Np, neuropil; Gl, gliocytes; Mf, myelinated fibers of the host. Bouin; a, c, d) hematoxylin-eosin; b) toluidine blue by Nissl's method. a, c, d) Obj. 20, homal 4; b) obj. 3.7, oc. 10.

Vivarium conditions, and were sacrificed with ethyl ether 14, 30, and 60 days after implantation. The sciatic nerves were fixed in Bouin's fluid and in a 10% solution of neutral formalin for histological investigation. Paraffin sections 5 μm in thickness were stained with hematoxylin-eosin and toluidine blue by Nissl's method. Sections 15-30 μm in thickness, made up on the freezing microtome, were stained with Sudan black and were impregnated with silver nitrate by the Bielschowsky-Gros method.

Results of the Investigation and Discussion. The cerebral cortical anlage of the rat embryos of 17 days' development (initial material, Fig. 1, a) is represented by four zones: the germinative, the intermediate, the cortical lamina, and marginal membrane (veil). The germinative zone consists of undifferentiated cells, among which mitotically dividing cells are frequently encountered. The cells of the intermediate zone are arranged less densely, as compared with those of the germinative zone and the cortical lamina. The latter is represented by typical little-differentiated neuroblasts, 6 to 9 μm in size. They have large, round, light nuclei with one to two nucleoli and a thin rim of cytoplasm. In some of the neuroblasts the cytoplasm is located at the two poles, and are fusiform; in others it is localized at one pole. Such cells are pear-shaped. The layered distribution of neurons which is characteristic for the mature cortex is lacking. Besides the neuroblasts, small, sparsely distributed glioblasts with hyperchromic nuclei can be detected in the cortical anlage.