LONG-TERM TRANSPLANTATION OF DIFFERENTIATED SENSORY NEURONS

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The spinal ganglia of sexually mature cats were autografted into the mesocolon and investigated after intervals of between 1 day and 9 months. About 30% of differentiated sensory neurons survived for long periods after grafting. A distinguishing feature of the transplanted sensory neurons was the intensive regeneration of their processes. Total myelination of the regenerating nerves occurred after 3-5 months. Potential regenerative powers of differentiated sensory neurons and the possibility of their long-term transplantation were revealed. KEY WORDS: spinal ganglia; sensory neurons; mesentery; transplantation.

Attempts to carry out auto- and heterografting of spinal ganglia have been made since the beginning of this century [4-8]. It has been shown that in newborn animals some sensory neurons transplanted into the subcutaneous fatty areolar tissue and into the brain survive for 10 to 33 days [5-7]. In the last decade longer survival has been obtained of sensory ganglia transplanted into muscle tissues [1], the anterior chamber of the eye [3, 9, 10], and the mesocolon [2]. However, the question of the possible duration of survival of differentiated sensory neurons has not yet been finally settled. The morphology of transplanted neurons likewise has received little study.

The object of this investigation was the long-term transplantation of differentiated sensory neurons in adult animals and the detailed study of their morphological features and the regeneration and myelination of their processes.

EXPERIMENTAL METHOD

Chronic experiments were carried out on 35 sexually mature cats. The operations were performed under sterile conditions and the animals were anesthetized by intraperitoneal injection of 10% hexobarbital solution (1 ml/kg). Laminectomy was performed at the level L5-L6 and the spinal ganglion extirpated and the outer layers of its connective-tissue capsule removed. Laporotomy was then performed and the isolated ganglion placed between the two layers of the mesocolon where it was well vascularized. Material for investigation was taken after 1, 2, 3, 7, 10, 14, and 20 days, and 1, 1.5, 2, 2.5, 3, 4, 5, 6, and 9 months.

The grafts of ganglia together with the surrounding tissue of the mesocolon were fixed in 10% neutral formalin, 70% alcohol, and 2.5% glutaraldehyde solution made up in phosphate buffer, pH 7.4. The preparations were stained with hematoxylin-eosin, Sudan black by Becker's method, and toluidine blue by Nissl's method and impregnated with silver by the Bielschowsky – Gros method. The ÉVM-100 L instrument was used for electron microscopy.

EXPERIMENTAL RESULTS

Between one and three days after transplantation the structure of the ganglion was indistinguishable from normal. Staining by Nissl's method and impregnation with silver by the Bielschowsky–Gros method showed many neurons in the ganglion which were outwardly similar to intact neurons, situated between large myelinated nerve bundles and at the periphery of the ganglion (Fig. 1a). In some cells signs of retrograde changes were found, chromatolysis and pycnosis of the nucleus. On the tenth day a marked decrease in the number of

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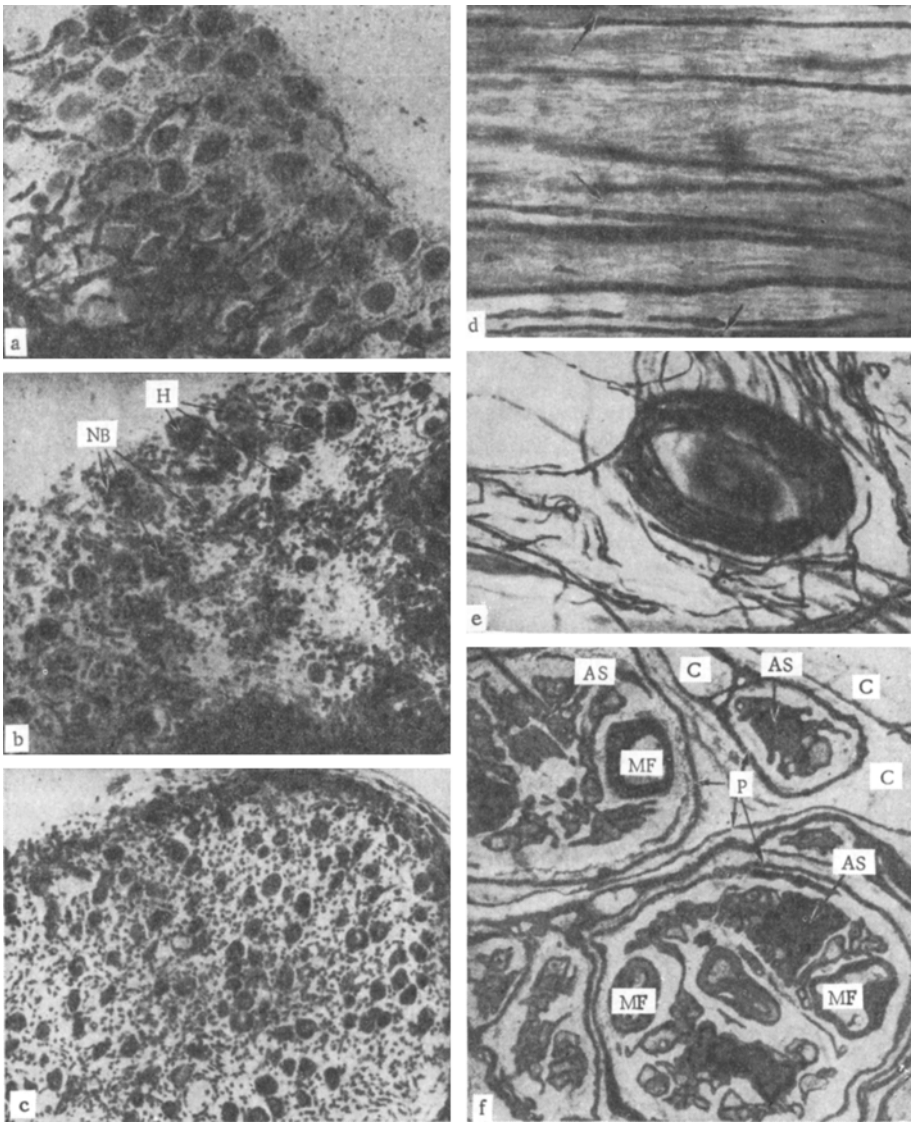

Fig. 1. Sensory neurons and regenerating fibers of spinal ganglia at various times after transplantation. a) Three days (impregnation with silver by Bielschowsky–Gros method, 130×); b) 10 days, H – hyperimpregnated neurons, NB) Nageotte's bracelets (impregnated with silver by Bielschowsky–Gros method, 130×); c) 4 months (stained by Nissl's method, 130×); d) 3 months, arrows indicate nodes of Ranvier (Sudan black, 515×); e) 5 months, myelinated fiber winds around body of nerve cell (Becker's method, 580×); f) ultrastructure of bundles of regenerating nerve fibers, P) perineural sheaths, MF) myelinated fibers, C) collagen, AS) axon-Schwann cell complexes of unmyelinated nerve fibers, 8500×.

neurons was observed as a result of death of some of them from hypoxia and deficient nutrition (Fig. 1b). At the sites of the dying neurons Nageotte's bracelets were formed – collections of small cells with poorly stained cytoplasm. In some of the residual neurons ischemic and retrograde changes were observed after staining by Nissl's method. Many neurons were hyperimpregnated with silver and reactive outgrowth of cytoplasm and excessive growth of initial glomeruli were found on them. Around the bodies of the nerve cells proliferations of satellite cells could be seen; they were 5 to 10 times more numerous then normally. Starting from the 20th day, however, most of the neurons which still remained were normal in appearance, and the number of pathologically changed neurons diminished gradually in the period after transplantation. Later, in the second and third months, the neuron population was stabilized and remained constant in the later periods of transplantation also. Counting the neurons showed that of the total number of nerve cells in the normal ganglion 30-32% sur-

vived in the graft. The neurons were thinly distributed and were located mainly near the fibrous capsule of the ganglion. Starting from two months, staining by Nissl's method revealed that many nerve cells had a cytoplasm and nucleus of normal structure, and a round or elongated body; they differed in size (Fig. 1c). The dimensions of the neurons varied depending on the time after transplantation. From 10 to 20 days after grafting they were most frequently 30-50 μ long, growing to 60-75 μ after three months. Statistical analysis of the morphometric data showed that after three months the volume of the nerve cells was significantly increased to 2048 ± 163 μ^3 compared with 1585 ± 114 μ^3 ($P < 0.01$), but by the fifth month the volume of the neurons had returned to its previous level, namely 1611 ± 112 μ^3 ($P > 0.05$). As a rule the nucleus of the neurons contained one nucleolus and in some neurons it lay in the center of the perikaryon, whereas in others it was eccentrically situated. The neurons preserved this morphological appearance in the late periods after transplantation until nine months.

Disintegration of myelinated fibers in the ganglion began at the fourth day and ended by the 18th-20th day after transplantation. Intact myelinated fibers were absent during this period, evidence of their total disintegration; disintegration of the myelin sheaths took place over the whole extent of the nerve fiber, starting from the divided segment and continuing as far as the first internodal segment. During this same period regeneration of the axons began and was manifested either as dichotomous division of the end of the divided axon into two thin branches or the formation of lateral shoots, given off from its main trunk. Often a short regenerating main axon divided into three branches not far from its cell body, and these in turn divided again within the region of the graft at different distances from each other. As a result of this frequently repeated division a mass of chaotically growing unmyelinated nerve fibers was formed. Regenerating axons formed many thin bundles which, crossing one another, formed a very dense plexus within the ganglion; some of them crossed into the mesocolon. By staining with Sudan black by Becker's method, after the 30th day these thin bundles could be seen to contain newly formed myelinated fibers (Fig. 1d). To begin with the myelination of the regenerating axons did not take place over their whole length but segmentally, and it began in bundles remote from the cell body. Along the axon altogether 5 or 6 myelinated segments, resembling thin tubes 0.8-1.2 μ in diameter, could be counted. Toward the end of the second month the number of myelinated fibers increased rapidly; myelin sheaths could be seen on long lengths of the nerve fiber as far as the cell body. The length of the internodal segments was closely connected with their diameter. For instance, the diameter of the nerve fibers in grafts 2-3 months old varied from 1 to 2.5 μ and the length of the internodal segments was 100-110 μ, or sometimes 120-156 μ. Starting from the fifth month, however, when the diameter of the fiber had increased from 3.5-4 μ the length of the internodal segments reached 238-400 μ. The newly formed myelinated fibers thus differed appreciably from intact fibers, whose diameter was 5-12 μ and whose internodal segments were 500-600 μ long. Total myelination in the graft usually occurred in the third to the fifth month, and it was so abundant that sometimes the initial glomerulus proliferating around the body of the neuron sometimes was myelinated also; the neuron appeared to be surrounded by a sort of coil formed by the myelinated fiber (Fig. 1e). The light-optical data were confirmed by submicroscopic observations which showed that initially many thin bundles of unmyelinated nerve fibers, distributed among the numerous bundles of collagen fibers, were formed in the graft. Each nerve bundle was surrounded by its own perineural sheath and consisted of bands of Schwann cells (lemmocytes), into the cytoplasm and processes of which thin axons (0.1-0.8 μ) were invaginated. The axon-Schwann ratio in the early period after transplantation was 10:1 or 30:1. After 18 days, the structures of the bundle changed during myelination, and in some of them axon-Schwann cell complexes were found in the ratio of 1:1. In grafts aged 3-4 months both myelinated and many unmyelinated axons were often present together in the same bundle: the unmyelinated axons were connected with the cytoplasmic processes of one Schwann cell, which could surround a myelinated fiber in a ring. Each axon–Schwann cell complex of the bundle was surrounded by a basement membrane, and the subperineural space was always filled with collagen fibers (Fig. 1f), running longitudinally. The perineural sheath also was surrounded by a continuous basement membrane, outside which collagen fibers were distributed. The inner subperineural collagen fibers, in the writers' opinion, were formed by Schwann cells, whereas the outer fibers were synthesized by perineural cells. The ultrastructure of the bundles of regenerating axons in the subsequent periods up to 9 months after transplantation remained the same, with only an increase in the total number of bundles and the number of myelinated fibers in them; the mass of collagen fibers around each discrete bundle also increased.

The results thus indicate that many differentiated sensory neurons of a ganglion transplanted from adult animals can survive for nine months and that their processes can regenerate intensively. Morphologically the grafted nerve cells were pseudounipolar in appearance and similar to normal sensory neurons. The reactive changes observed in the body of the neurons in the early period after transplantation, the increase in their volumes, and the excessive growth of the initial glomerulus did not prevent further long-term survival of the nerve cells after autografting. Regeneration of the axons was expressed as their excessive collateral division

and the formation of numerous discrete thin nerve bundles, whose fibers began to be myelinated at the 25th-30th days after transplantation. Isolation of the graft from the CNS was evidently the reason for the uncontrolled growth of the newly formed myelinated fibers in all directions and throughout long periods of observation (until nine months). The results indicate high functional activity of transplanted differentiated neurons and the potential powers of regeneration of differentiated nerve cells of adult animals. This long-term transplantation of sensory neurons can serve as a model for the study of the morphological and functional potential of differentiated neurons and for the creation of new nerve centers in the body.

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DIFFERENTIATION OF A TRANSFORMED CULTURE OF L-CELLS

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L-cells arising from subcutaneous connective tissue and adipose tissue of C3H mice were cultured in nutrient medium with the concentration of bovine serum increased to 60%. Differentiation of some of the cells into fat cells took place, with the formation of structures similar to normal adipose tissue. The occurrence of differentiation was judged from the formation of characteristic signet-ring cells, whose cytoplasm was filled with neutral fat, giving a positive reaction for lipids when stained with a mixture of Sudan III and Sudan IV. It is concluded that the long existence of the cells in vitro and the intracellular changes accompanying the transformation process, including those of mutation character, do not render the cells incapable of differentiation. KEY WORDS: L-cells; differentiation; adipose tissue; signet-ring cells; neutral lipids.

The concept of reversibility of malignant properties, affording new prospects for influencing the tumor process [3, 7, 9], is based on the epigenetic theory of malignant transformation of cells [5]. In some cases, however, malignant transformation is accompanied by changes of mutation character. For example, in normal cells in culture, kept in vitro for a long period of time, and during malignant transformation metabolic [6], antigenic [14], and genome [8] changes arise. In particular, for cultures of L-cells, which are extensively used in scientific research, considerable changes characteristically take place in the karotype, which distinguish these cells from their normal precursors [13]. Accordingly the problem of preservation of their ability to differentiate arises.

To study this problem experiments were carried out and their results are described below.

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