NEUTROTROPHIC INTERACTION BETWEEN SPINAL GANGLIA AND VARIOUS TISSUES

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Spinal ganglia L5-L6, together with the mesentery of the large intestine (innervated from these ganglia) and the epithelium of the tongue, were transplanted into the anterior chamber of the eye in cats. After 2 months survival of some of the sensory neurons and intensive regeneration of their nerve fibers were observed. Meanwhile the tissue of the mesentery together with its mechanoreceptors (Pacinian corpuscles) remained intact. The epithelium of the tongue was destroyed. The trophic influence of these particular sensory ganglia on the corresponding tissue ensured the structural integrity of that tissue and of its receptor apparatus. The need for metabolic conformity between the sensory ganglia and the tissue innervated by them is revealed.

KEY WORDS: *spinal gnaglia; anterior chamber of the eye; mesentery; trophic effect of sensory neurons; transplantation.*

Several different views are held on the formation of nerve endings. Supporters of the modulation theory consider that the tissue around the endings of a nerve fiber "imposes" the specific functional properties on the sensory neuron [8. 9]. Supporters of another theory (specific selectivity) postulate that the differentiating nervous center possesses initial specificity, as a result of which the growing nerve fibers form selective connections at the periphery with only those tissues that have definite biochemical properties [6, 12]. However, some investigations [1-4] have shown the need for some form of conformity between the tissue and the source of its innervation in order that the tissue can preserve its structural integrity and receptor elements.

The object of this investigation was to study *in vivo* the neutrophic influence of sensory neurons on tissues which normally obtain their afferent innervation from transplanted spinal ganglia L5-L6 (the mesentery of the large intestine and the Pacinian corpuscles contained in it) and also on tissues not characterized by this type of innervation (the epithelium of the tongue). The reason why the anterior chamber of the eye was chosen as the site for autotransplantation is that it contains a good nutrient medium and allows intravital observation of the development of the graft.

EXPERIMENTAL METHOD

Chronic experiments were performed on 18 sexually mature cats. The operations were performed under sterile conditions and the animals were anesthetized intraperitoneally with 10% hexobarbital solution (1 ml/kg). Laminectomy was carried out at the level L5-L6 and the spinal ganglion with its peripheral process 1.5 mm long was isolated. The connective-tissue capsule of the ganglion was removed. Under the MBF-2 microscope an incision was made in the cornea in the superolateral quadrant of the eye 3 mm from the limbus to open the anterior chamber. The spinal ganglion together with pieces of other tissues (mesentery of the large intestine and epithelium of the tongue) were introduced into it by means of a spatula. Pieces of these tissues measuring 2 × 3 mm were laid firmly against the peripheral process of the ganglion, partly enveloping it. Tissue of the mesentery of the large intestine only was transplanted into five control animals. The volume of fluid in the anterior chamber of the eye was restored by injection of physiological saline.

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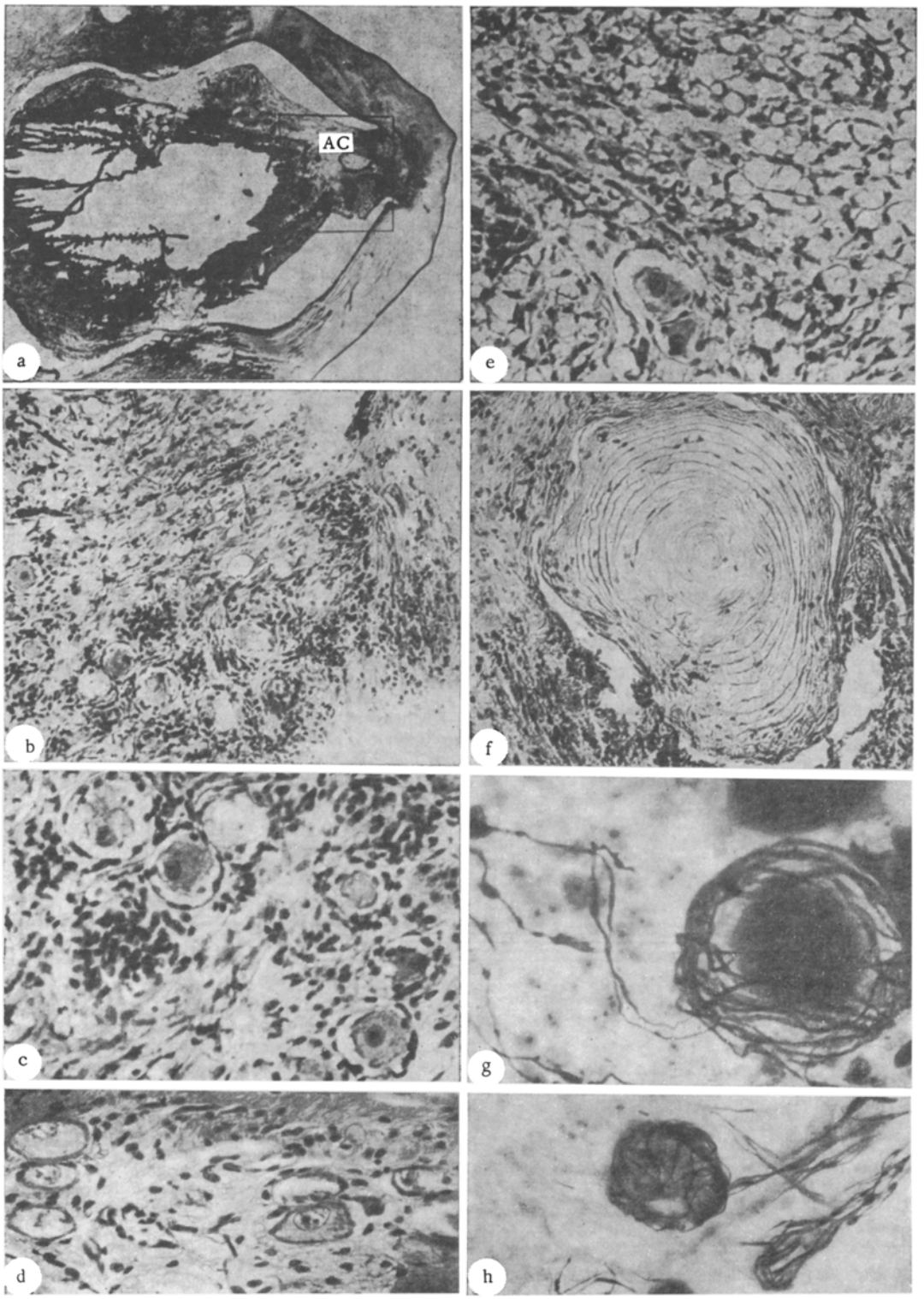

Fig. 1. Survival of grafts of spinal ganglia (L5-L6) and tissues of mesentery into anterior chamber of eye in cats: a) graft in anterior chamber of eye (AC) (hematoxylin–eosin, 3.5×); b) detail of Fig. 1a: neurons among tissues of graft, slight degree of infiltration by inflammatory cells (hematoxylin–eosin; 140×); c) ganglion cells with satellites (hematoxylin–eosin, 200×); d) the same (Nissl's method; 200×); e) neurons among tissues of mesentery (hematoxylin–eosin, 200×); f) Pacinian corpuscles with well-preserved lamellae of capsules (hematoxylin–eosin, 125×); g) excessive proliferation of initial bulb of neuron (impregnation with silver by Bielschowsky–Gros method; 280×); h) the same, varicose expansions along course of regenerating nerve fibrils (impregnation with silver by Bielschowsky–Gros method; 250×).

The state of the grafted tissues was observed intravitally with the MBF-2 microscope after preliminary anesthesia of the eye by instillation of 2% amethocaine solution. The eyes were enucleated for histological investigation after 3 weeks and 1, 1.5, and 2 months and fixed in a 10% solution of neutral formalin. The fixed eye was cut vertically along the naso-temporal line and the anterior portion was embedded in paraffin wax. Serial sections were cut from the block and each fourth section stained with hematoxylin–eosin, with picrofuchsin by Van Dieson's method, and with thionine by Nissl's method; sections also were impregnated with silver by the Bielschowsky–Gros method and en bloc by the Cajal–Favorskii method followed by preparation of serial sections.

EXPERIMENTAL RESULTS

During the 7-10 days after transplantation examination showed pericorneal injection of the eye and edema and opacity of the cornea in the region of the incision. The vessels of the iris in the inferomedial quadrant, where the grafted tissues were located, were dilated. The cornea became translucent 2 weeks after the operation. By the end of the first month organization of the graft was observed in all animals. It was surrounded by a connective-tissue capsule, penetrated by blood vessels, and somewhat pigmented.

The regular course of the collagen fibers of the cornea in the zone of the incision was disturbed 20 days after transplantation, the endothelial layer was disorganized, and it was fused with the graft (Fig. 1a, b). The vessels of the iris were dilated and pigment cells were scattered haphazardly in the connective-tissue bands drawing the grafted tissues toward the conglomerate. The connective tissue replacing the graft developed mainly on the side of the iris.

At all periods of observation areas of ganglion cells (Fig. 1b, e, g, h) and of mesothelial and adipose cells of the mesentery (Fig. 1e) could be found in the grafts, and the mesentery contained its mechanoreceptors, or Pacinian corpuscules (Fig. 1f). Meanwhile the epithelium covering the tongue could be found in only one experiment and was in a necrobiotic state.

After transplantation one-third of the sensory neurons survived. Nerve cells died as a result of impairment of the blood supply in the central part of the ganglion. At the periphery of the ganglion, surviving neurons were arranged in groups or chains of two or three cells (Fig. 1b-e). They were round and elliptical in shape and the diameter of the neurons varied from 30 to 75 μ. In sections stained by Nissl's method the Nissl's substance at the periphery of the perikaryon was seen to be coarsely granular in structure, whereas in the center around the nucleus it was finely granular (Fig. 1d). Around the perikaryon of the neurons were many satellite cells (Fig. 1c). A characteristic feature of the transplanted neurons was the extremely intensive and excessive proliferation of initial bulbs in the form of a basket weave or cocoon (Fig. 1g, h). Not only the thick axial segment of the axon but also the very thin fibrils newly formed from the body of the neurons took part in the formation of this bulb. These branching nerve fibrils had fine varicose expansions along their course and they terminated after division in boutons, pinhead thickenings, and rings of different sizes (from 1 to 3 μ). The regenerating nerve fibers with endings of this type were observed both in the stroma of the ganglion and in the tissue of the mesentery. Similar intensive regeneration of the processes of the nerve cells also is observed in the ganglion in culture *in vitro*. However, only embryonic neurons, which subsequently undergo further differentiation, have been used for explantation *in vitro*. In the anterior chamber of the eye, however, nerve fibers of mature, differentiated neurons will regenerate.

In control experiments in which only the mesentery of the large intestine was transplanted into the anterior chamber of the eye no elements of that tissue were found; the graft was replaced by developing connective tissue. The results point to differences in the survival of different tissues in the presence of a particular source of innervation. The source of innervation for tissues of the mesentery of the large intestine is the lumbar spinal ganglia at the level L5-L6. After transplantation of the mesentery together with these ganglia, structural integrity of the mesentery, including such highly differentiated tissue mechanoreceptors as the Pacinian corpuscles, is observed. Meanwhile the epithelium of the tongue, which receives its innervation from the cranial ganglion nodosum, is rejected if transplanted together with the spinal ganglia. It can thus be postulated that specific metabolic conformity between the neuron and the tissue into which its endings penetrate is essential. Integrity of the mesentery with its receptor apparatus is determined by the trophic

influence of neurons of the corresponding ganglion. There is evidence in the literature of differences between the cranial sensory ganglia and the spinal ganglia as regards the character of their trophic influence on the epithelium of the skin and tongue with their receptor endings. After autografting of pieces of the epithelium of the tongue and skin into the enterior chamber of the eye in rats together with the ganglion nodosum, only the epithelium of the tongue preserved its integrity, and not only were its taste buds preserved, but new ones also were formed [12]. After the analogous transplantation, but only of the cervical and thoracic spinal ganglia, no taste buds were found in the tongue of the rat fetus [5]. The trophic action of sensory neurons on a peripheral tissue is considered to take place through the aid of the descending axoplasmic current in a direction opposite to the conduction of nervous impulses. Some workers have found that the trophic influence of the sensory ganglia is manifested not only by direct innervation, but it also spreads by the humoral route [10, 11]. Guth [7] defines the trophic action of neurons as prolonged interaction between nerve cells and other cells controlling modifications in target cells. Under experimental conditions, if the source of innervation is brought close to the tissue, specific mechanisms for these trophic influences determined by the metabolic characteristics of the sensory ganglia at different levels become apparent. To preserve the structural integrity and functional normality of a given tissue and its receptor apparatus, a particular source of afferent innervation must be present.

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