NEUROHISTOLOGICAL INVESTIGATION OF HOMOGRAFTED LIMBS IN TOLERANT DOGS

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The peripheral nervous system was investigated in homografted limbs of two dogs in which artificial tolerance was produced during the first days after birth by replacement transfusion. Regeneration of the nerve trunks followed the general rules of regeneration in the peripheral nervous system. Typical nerve endings—motor end-plates and neuromuscular spindles—were formed in the homografted limb.

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Data on the state of the peripheral nervous system in the homografted dog's limb could be found only in one paper by Khristich [14]. This limb was transplanted to the mother from its puppy, one year old. On the 22nd day after transplantation, profound trophic disturbances began to appear, leading rapidly to death of the limb. In three other dogs on which Khristich operated, the homoplastic limbs survived for an even shorter time: rejection always took place because of biological incompatibility of the donor's and recipient's tissues.

The object of the present investigation was to study the peripheral innervation of homografted hind limbs of two dogs (Bratik and Jupiter) on which operations were carried out at the Central Institute of Traumatology and Orthopedics by A. G. Lapchinskii and collaborators.

Artificial tolerance to the homograft was created in these dogs by subtotal replacement transfusion during the first days after birth of the puppy using blood taken from the future donor of the limb—an adult unrelated dog. As a result, permanent survival of the limbs was obtained [2-5]. The dog Bratik, undergoing operation on January 7, 1964, still lives with its grafted limb (more than 4 years later). The dog Jupiter, undergoing operation on March 22, 1965, survived for 335 days and died from thromboembolism of the pulmonary artery.

EXPERIMENTAL METHOD

The test object consisted of pieces of skin and muscles of the homografted limb taken at biopsy from different segments of the limb from dog Bratik on the 101st, 313th, and 904th days, and from the dog Jupiter on the 191st day after transplantation. Tissues obtained at necropsy from the dog Jupiter on the 335th day after the operation also were studied. The material was fixed in 15-20% neutral formalin. Frozen sections were impregnated with silver nitrate by Campos's method and also stained with hematoxylin-eosin, by Van Gieson's and Spielmeyer's methods, and with Sudan III and Sudan black.

EXPERIMENTAL RESULTS

At the first biopsy performed on Bratik on the 101st day after homografting, hardly any epidermis was found in the skin fragments at the mid-thigh level and in the upper third of the leg of the transplanted limb, for by the 59th day lysis of the superficial layers of the skin had taken place as a result of an allergic crisis (host versus graft reaction). Structureless necrotic masses were found on the skin surface, beneath which was a leukocyte barrier, separating them from viable tissues. Part of the superficial zone was occupied by granulation tissue. In the deep parts of the dermis the structure of the reticular layer remained intact. Extensive foci of infiltration, consisting of lymphocytes, plasma cells, and neutrophils, were observed around

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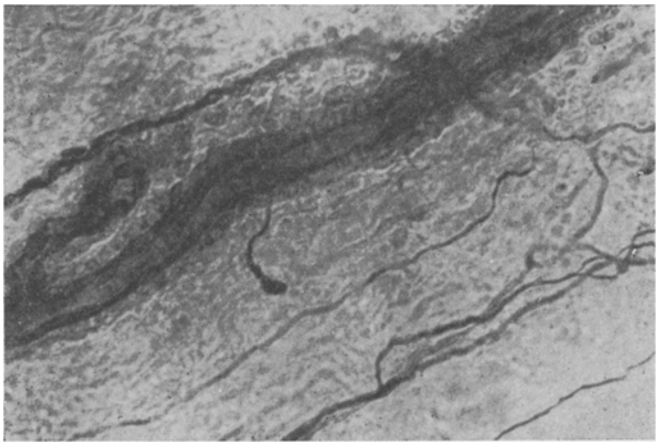

Fig. 1. Regenerating axons with bulbs of growth in dermis of leg. Dog Bratik. Biopsy on 101st day after homografting. 400 ×.

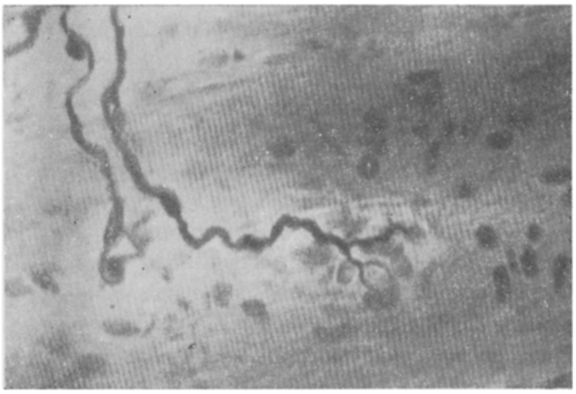

Fig. 2. Motor end-plate in leg muscles of grafted limb. Dog Bratik. Biopsy on 313th day after homografting. 400 ×.

the blood vessels and nerve trunks. Small bundles of striated muscle fibers, present in the sections, appeared atrophied and varied in diameter. Nerve trunks, consisting of 10-20 thin, medullated and nonmedullated fibers, and also solitary regenerating axons with typical bulbs of growth at their ends (Fig. 1), were detected by neurohistological methods in sections from material taken from the lower third of the thigh and the upper third of the leg, in the deep layers of the dermis. Meanwhile, in a few of the nerve fibers, various stages of Wallerian degeneration were observed (fragmentation of axons with granule formation, destruction of myoneural synapses).

In material of the second biopsy specimen taken from Batik on the 313th day, cutaneous epithelium, regenerating after lysis and consisting of 15-30 layers of cells, was detected. The stratum germinativum, stratum granulosum, and the thin stratum corneum were clearly distinguishable. At the boundary between the epidermis and connective tissue, a smooth basement membrane without papillary processes was observed. The material investigated contained neither hairs nor cutaneous glands. Most muscle fibers in fragments taken from the thigh and leg of the homograft were normal in structure. However, individual muscle fibers were atrophied, their myofibrillary structure was ill-defined, and they contained an increased number of nuclei. On neurohistological investigation, nerve trunks consisting of 10-30 medullated fibers, and individual regenerating axons with bulbs of growth were found in the deep layers of the dermis in the lower third of the thigh and upper third of the leg of the transplanted limb by neurohistological methods. Larger trunks, consisting of 100-200 nerve fibers, mostly medullated, were present in the intermuscular connective tissue. Some nerves were surrounded by massive collections of lymphocytes and plasma cells forming cuffs surrounding the epineurium. Foci of infiltration also persisted along the course of the blood vessels. Trunks of smaller caliber, extending through the perimysium and endomysium, and also along the adventitia of the blood vessels, were given off by the large nerve trunks. Single nerve fibers, approaching the striped muscle fibers at different angles, formed endings of varying complexity on them: simple endings consisting of very thin filaments with loops or boutons at their end, primitive motor end-plates, definitive end-plates with characteristic arborization of the terminal fiber, and a large number of glial nuclei, forming their base (Fig. 2).

In material taken from the homografted limb of the dog Bratik at the third biopsy on the 904th day after the operation, large and small nerve trunks were found at the same levels, lying in the wide bands of inter-

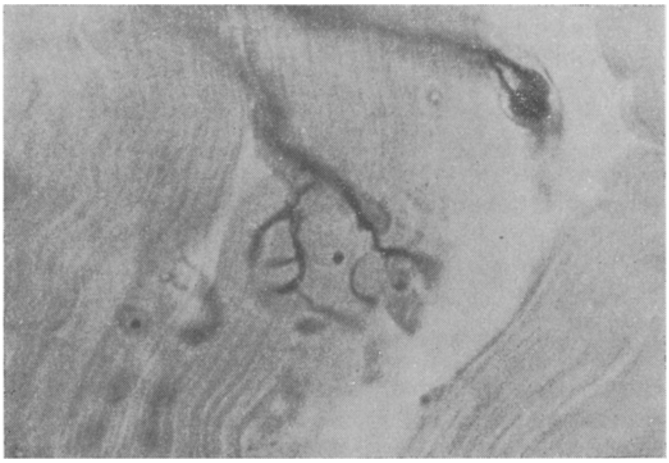

Fig. 3. Motor end-plate in leg muscles of the grafted limb. Dog Jupiter. Biopsy on 335th day after homografting, 900 ×.

muscular connective tissue. Foci of infiltration persisted around some of the nerve trunks, consisting of lymphocytes and plasma cells. As well as nerve fibers of normal structure, the trunks also contained fibers in a state of degeneration (swelling, formation of varicosities, fragmentation). In parts of the muscles falling into the sections, motor end-plates of normal structure were seen. However, atrophied motor end-plates with a shrunken base, pycnotic nuclei of the special cells, and mummified terminal fibers also were observed. In the skin, without glands or hair as before, small nerve trunks were found, breaking up into single fibers reaching almost to the basement membrane.

In the dog Jupiter on the 191st day after the operation, the epidermis appeared normal in fragments taken at biopsy from the lower third of the thigh and upper third of the leg of the grafted limb. The stratum germinativum, stratum granulosum, and stratum corneum were clearly distinguished. Derivatives of the skin also remained intact and appeared normal. The papillary and reticular layers were clearly discernible in the dermis. Perivascular foci of infiltration also were observed here, consisting mainly of lymphocytes and plasma cells. Most muscle fibers falling into the sections had a well marked cross striation. Some difference in the thickness of the muscle fibers was noted. At these same levels neurohistological methods revealed nerve trunks and many single nerve fibers located in the reticular and papillary layers of the dermis. Many axons ended in typical bulbs of growth. Nerve trunks containing as many as 30-50 fibers were found in the intermuscular septa of the thigh and leg muscles. Single nerve fibers formed both primitive endings of bouton or loop type on the muscle fibers or structures resembling motor-end plates of the normal pattern.

The study of necropsy material obtained from Jupiter on the 335th day after operation showed that many muscle fibers retained their normal structure, while other parts of the muscle of the homografted limb were atrophied. In these zones the muscle fibers were thin and contained chains of nuclei. Between them were wide spaces filled with adipose tissue. Nerve fibers approaching the unchanged muscle fibers formed motor endings of typical structure (Fig. 3), and terminal structures resembling the motor endings observable at various stages of embryogenesis.

Besides the changes described above, near the tendinous attachments of the leg muscles of the homografted limb, neuromuscular spindles were observed. Numerous nerve trunks and single nerve fibers were found in the papillary and reticular layers of the dermis.

The material studied shows that after homografting processes similar in principle to those of regeneration of the peripheral nervous system after experimental division of nerve trunks [15] or after retransplantation of a limb [1, 7-13], take place. Not only regeneration of nerve trunks, but also innervation of the skin and restoration (structually, at least) of specialized nerve structures such as motor end-plates, took place. The observations showed that the formation of myoneural synapses took place by a gradual increase in complexity such as is observed in ontogenesis and phylogenesis [6]. One of the most important features of regrafted and homografted limbs is their initial absolute denervation, developing after their removal from the body. It is more complete than under any other experimental conditions. Nerve fibers in the grafted limb composing nerve trunks of all calibers, with motor and sensory endings in all situations and of all types, undergo Wallerian degeneration and total destruction. During regeneration of the peripheral nervous system in homografted limbs of dogs, the recipient's axons invade the foreign graft tissues, reinnervating them, the recipient's nerve fibers growing along within the donor's Beungner's bands. During the formation of motor end-plates, the arborization of the terminal fiber belong to the recipient, while the sarcoplasmic and nuclear component of the end-plates probably belongs to the donor. Nevertheless, this has no significant effect on the morphogenesis of the myoneural synapses or on their final structure.

Regeneration of the peripheral nervous system after homografting is a new problem which calls for further special systematic study.

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