RESTORATION OF INNERVATION OF REGRAFTED DOGS' LIMBS

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Complete recovery of the peripheral innervation is found in the late stages after regrafting of the hind limb in dogs in uncomplicated cases. Motor end-plates with normal structure appear in the muscles of the thigh, leg, and foot of the regrafted limb. The innervation of the blood vessels and skin of the regrafted limbs is similar to that of the control limbs.

Morphological changes taking place in regrafted limbs of experimental animals are important, not only from the theoretical aspect, but also for practical purposes because regrafting has recently begun to be used in traumatology and reconstructive surgery [1, 6, 7].

Data concerning regeneration of the peripheral nervous system in regrafted hind limbs of dogs are given below. Operations on the animals were performed in the Department of Experimental Traumatology and Orthopedics of the Central Research Institute of Traumatology and Orthopedics by A. G. Lapchinskii and collaborators, and the histological investigations were carried out by O. N. Timofeeva.

EXPERIMENTAL

Regrafting of the hind limb after amputation at the level of the middle third of the thigh was performed on the following dogs: 1) Slavka, operation on March 19, 1954, survived 9 years 7 months after operation; 2) Galeta, operation on December 25, 1956, survived for 6 years 3 months; 3) Dzhessi, operation on September 28, 1961, survived 1 year 11 months; 4) Belogrudka, operation on December 13, 1961, survived 5 years 6 months; 5) Snegurka, operation on June 20, 1962, survived 1 year 7 months; 6) Omega, operation on January 26, 1967, is still alive 2 years later; 7) Dzhek, operation on October 19, 1967, is still alive after over 1 year.

The regrafting operations of these dogs were performed under different conditions and the circulation in the amputated limb was interrupted for different periods. For instance, in the dog Slavka, regrafting was carried out after a nonsterile traumatic amputation, in Galeta after preservation of the isolated limb for 25 h in a refrigerator with an artificial circulation, in Snegurka after traumatic amputation with comminution of the bone into four fragments, and in Dzhessi, Omega, and Dzhek soon after sterile amputation. The technique of the amputation operation, of storage of the limb and regrafting, and the method of suturing the vessels and nerves were described previously [2-5]. The causes of death of the experimental animals were not directly connected with the previous limb regrafting operation. For example, Dzhessi died from accidental injury, Belogrudka from pneumonia,and Galeta from cystitis and uremia. All the dogs made active use of their regrafted limb.

Material for histological study was taken as biopsy or autopsy. The skin and muscles were exercised at the level of attachment of the regrafted hind limbs (middle third of the thigh), and also above and below this level, in the region of the upper third of the leg and foot. Tissues were fixed in 15-20% formalin. Frozen

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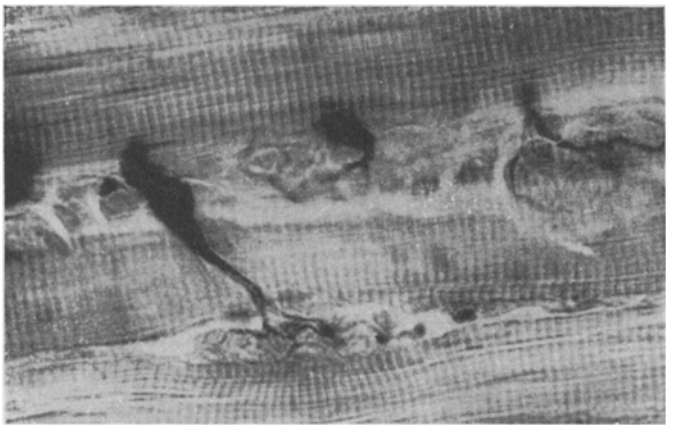

Fig. 1. Dog Slavka, 9 years 7 months after nonsterile traumatic amputation of hind limb. Motor end-plates in gastrocnemius muscle of regrafted limb. Campos, 100 ×. Photomicrograph.

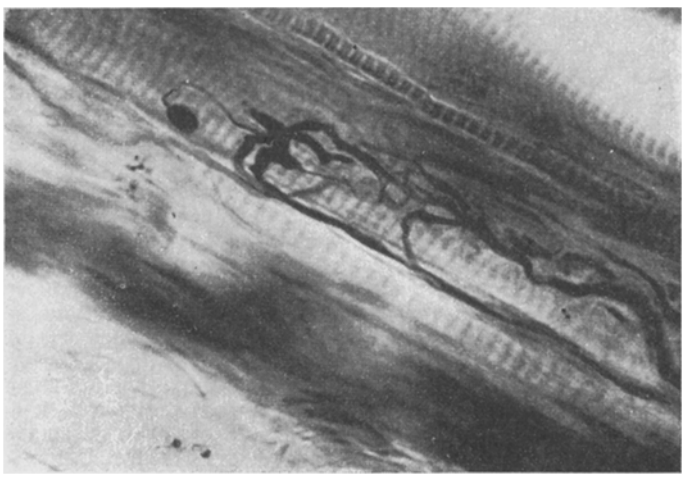

Fig. 2. Dog Snegurka, 1 year 7 months after traumatic amputation and regrafting of hind limb. Excessive innervation of thigh muscles of regrafted limb close to point of attachment. Bulb of growth. Campos, 900 ×. Photomicrograph.

sections were impregnated with silver nitrate by Campos's method. Some material was stained by Spielmeyer's method and with Sudan-3 and Sudan black, and also by various general histological methods.

EXPERIMENTAL RESULTS

Observations showed that changes in the peripheral nervous system in the regrafted hind limbs were similar to those of degeneration and regeneration of peripheral nerves after simple division without regrafting [8, 9]. In the early periods after regrafting (material obtained from other dogs surviving for a few days to one month after regrafting) axons of nerves of the regrafted limb showed increased argentophilia and were swollen: varicose thickenings appeared along their course. This was followed by complete fragmentation of the axons, amounting in some cases to the appearance of fine, dust-like granules. These changes were accompanied by changes in the myelin sheath of the nerve fibers characteristic of Wallerian degeneration, sudanophilia, the formation of myelin globules, and so on. Typical Buengner's bands formed at the site of degeneration of nerve trunks. Destructive changes in the nerves of the regrafted limbs and Buengner's bands were observed sometimes by the writers in later stages after the operation (for example, in the dogs Snegurka, Dzhessi, and Dzhek) against the background of complete restoration of the peripheral innervation.

Ingrowth of axons from the central end of the divided nerves into Buengner's bands of the regrafted limb began quite early. For instance, numerous thin regenerating axons, with bulbs of growth on their ends, were found by examination of biopsy material obtained from the dog Snegurka 42 days after regrafting.

Dogs which survived for long periods after regrafting of the hind limb actively used it (they ran almost without a limp, and when standing on the hind limbs bore weight on the regrafted limb). It was therefore decided to examine the state of the motor endings in the muscles of the grafted limb. Examination showed that most motor end-plates in the late stages after regrafting were normal in structure (Fig. 1). However, near the place of suture between the amputation stump and the regrafted limb, hypertrophied motor end-plates were seen. Nerve endings of this type were hardly ever seen in the muscles of the leg and foot. Pycnotic motor end-plates were much less common, and were located mainly in the zone next to the region of attachment of the amputated limb. The region of the postoperative scar was characterized by excessive innervation. In some dogs (Snegurka, Belogrudka) neuromatous growths of nerve fibers were found: the axons radiated fanwise and separated into neurofibrils, and numerous collaterals, growth bulbs of different sizes, and Perroncito's figures were formed. The thigh muscles immediately distal to the point of attachment of the amputated limb also were hyperinnervated (Fig. 2). It was in this region that the motor end-plates showed the greatest polymorphism.

Many nerve fibers spread along the course of the blood vessels of the regrafted limb, and their distribution in the wall of the vessels throughout the leg and foot was indistinguishable from that of nerves in the walls of blood vessels of the contralateral limb.

The distribution of small nerve trunks and single fibers in the dermis of the leg and foot of the regrafted and control limbs was identical. Branches of the thin nerve fibers, having lost their myelin sheath, approached the epithelium and formed a subepithelial plexus.

In the late stages after grafting, the innervation of the regrafted limb thus showed no significant difference from that of the control limb.

Comparison of these observations of restoration of the peripheral innervation of the regrafted limb with clinical and physiological data [5] showed that the degree of perfection of this process depends on several factors, but mainly on the operative technique and on the presence of postoperative complications. In principle, regeneration takes place in the same way after surgical and after traumatic amputation of the limbs.

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