THE SPECIES SPECIFICITY OF ORGAN REGENERATION OF SKELETAL MUSCLES IN MAMMALS

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As a result of extensive research, not only has it been demonstrated that regeneration of certain organs (liver, spleen, kidneys, ovaries, etc.), of complex structure, may take place, but the ways by which they regenerate have also been discovered [2-5, 8]. Less work has been done on the study of the organ regeneration of skeletal muscles, by which we mean the complete or almost complete regeneration of the muscle after its subtotal extirpation. An impetus to the study of the organ regeneration of skeletal muscle was given by the discovery [12] of the wide powers of regeneration of skeletal muscle tissue of rats. It was subsequently shown [9, 10, 14, 15, 16] that the gastrocnemius muscle in rats may regenerate after removal, leaving a stump only 3-4 mm long attached to the tendon after resection.

The discovery of this important phenomenon raised two main problems: 1) is the regeneration of the gastrocnemius muscle after subtotal resection a special case, or are the other muscles of the same animal capable of total (organ) regeneration? 2) Is the phenomenon of total regeneration of the gastrocnemius muscle observed in other mammals after resection, and how far does it extend?

In our earlier research [10] we found that other muscles of the rat besides the gastrocnemius are capable of complete or almost complete regeneration after resection, provided that pieces of muscle tissue are left behind on the tendons.

The object of the present investigation was to examine the ability of the gastrocnemius muscles of other mammals to undergo organ regeneration.

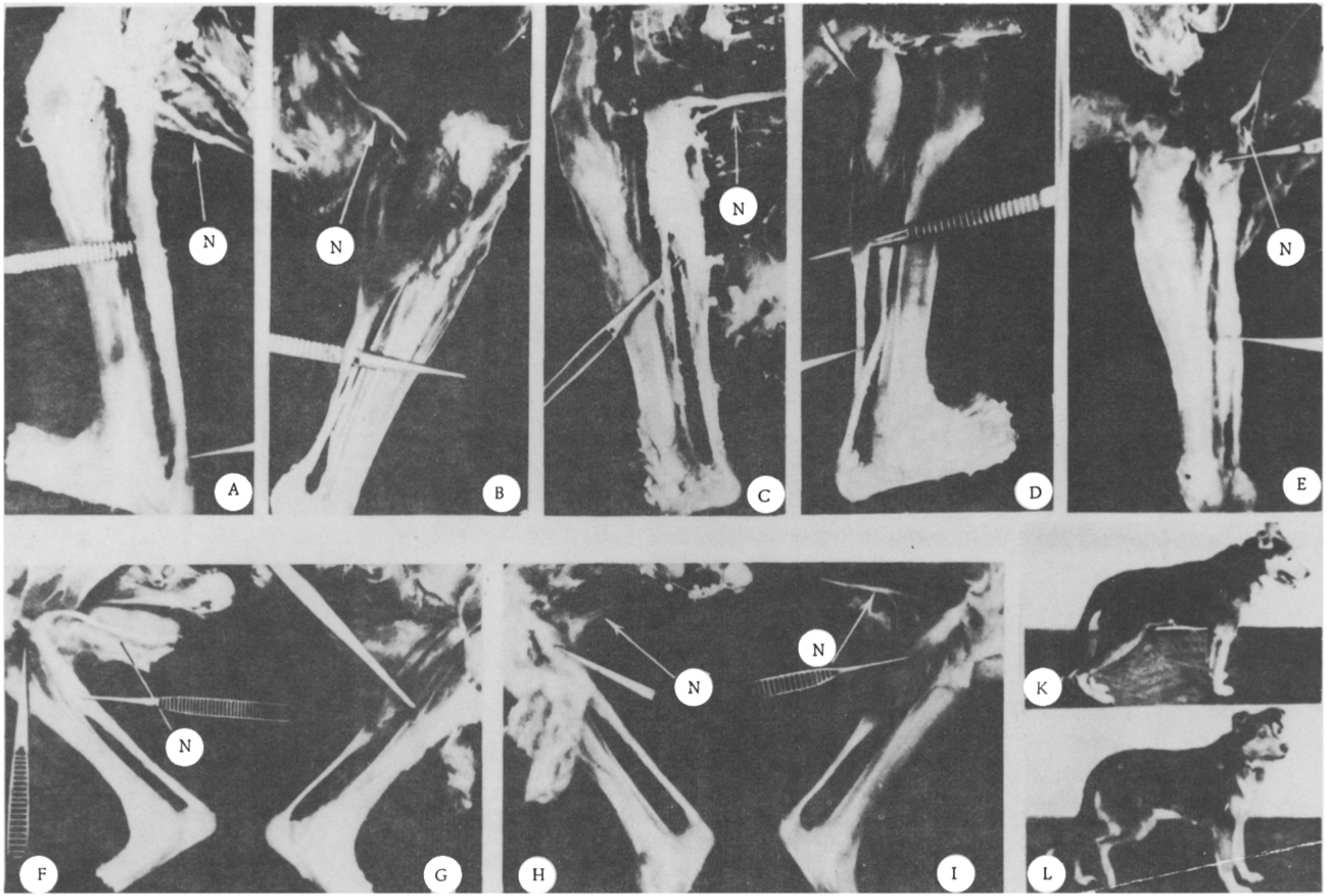
EXPERIMENTAL METHOD

The investigations were conducted on adult dogs and puppies aged from 2.5 to 4 months. We used dogs after rats for the experiments because by the character of their metabolism, their feeding pattern and many other biological features, they show a great similarity to man [13].

The triceps muscle of the leg in dogs consists of only the two heads of the gastrocnemius muscle. The soleus muscle is absent and is partially replaced by the flexor digitorum sublimis [7]. The lateral head of the gastrocnemius muscle is attached by a short, wide tendon to the lateral epicondyle of the femur, while the medial head is also attached by a wide tendon to the medial epicondyle of the same bone. After uniting to form the belly of the muscle, the two heads terminate in a common tendon at the calcanean tuberosity.

In its proximal part the gastrocnemius muscle is covered posteriorly and laterally by the distal portion of the biceps femoris muscle, and on its medial aspect by the distal portion of the semitendinosus, semimembranosus, and gracilis muscles. Anteriorly to the gastrocnemius muscle lies the flexor digitorum sublimis. This arises from the femur in the region of the lateral condyle. The well developed belly of this muscle, covered by the gastrocnemius muscle, changes in the middle of the leg to a thin tendon. It winds around the tendo Achillis, crosses the apex of the calcanean tuberosity, and descends along the metatarsus.

All these muscles are invested by the fascia cruris for the extent of two-thirds of the leg; this fascia thins out toward the tendo Achillis to such an extent that the latter, together with the tendon of flexor digitorum sublimis, can be seen clearly through it.



Dissections of the operated and control limbs of the dog No. 1: A) experiment; B) control (unoperated limb);dog No. 2: C) experiment; D) control; dog No. 3: E) experiment; puppy No. 5: F) experiment; G) control; puppy No. 8: H) experiment; I) control; N) peroneal nerve; K) photograph of puppy No. 8 in a stationary position; L) during movement.

All the operations were carried out under morphine-ether anesthesia, in strictly aseptic conditions.

In the first series of experiments (3 adult dogs and 3 puppies), the field of the operation was painted with benzene, alcohol, and iodine, and the skin was divided on the lateral surface of the lower third of the right thigh and leg. The tissues were divided in layers along the whole length of the incision, and the gastrocnemius muscle and the flexor digitorum sublimis were exposed.

The gastrocnemius was divided at the point where it joins the tendo Achillis, and proximally at the level of entry of the tibial nerve into the muscle, leaving stumps of the lateral and medial heads 1.0-1.5 cm in length (in the puppies the stumps were 0.5-1.0 cm long). In two adult dogs muscle stumps were left attached to the tendo Achillis, 1 cm long. The flexor digitorum sublimis was removed, completely detached from the lateral condyle of the femur, and its tendon divided at the level of the calcanean tuberosity. In the bed of the excised gastrocemius muscle was buried a thin layer of minced muscle tissue taken from the belly of the same muscle. In the course of this procedure strict asepsis was observed and the buried material was protected against drying, overheating, and cooling.

The main vessels and nerves were not injured during the operation. Hemorrhage was arrested by careful ligation of the vessels. Catgut sutures were inserted into the muscles covering the injured area, and the facia and skin were sutured with silk. The wound was covered by a sterile dressing, which was kept in place about 6 hr.

The operative technique in the second series of experiments (3 adult dogs and 3 puppies) differed only in the fact that no minced muscle tissue was transplanted.

As a rule the postoperative course was smooth, and if edema or the onset of inflammatory changes was observed in any of the animals they were given antibiotics. The dogs received a diet adequate in protein and vitamins, and the puppies had their daily ration supplemented by one egg for a period of 3 months. The animals were not immobilized and so no limitation of their movements developed.

Anatomical investigations were carried out on 2 experimental dogs 3 months after the operation, on 5 dogs after 5 months, on 2 after 7 months, on 1 after 11 months, and on 2 after 1 year. Some of the material was studied histologically.

Soon after the operation the dogs were able to stand and bear weight on their injured limb, in which position they were no different from animals which had not undergone the operation; disturbances of limb function appeared only in movement.

EXPERIMENTAL RESULTS

Since the character of the process of regeneration in all dogs after the operation was the same, we shall confine ourselves to describing the results of experiments on five animals.

First series of experiments. In adult dog No. 1, weighing 16.5 kg, sacrificed 5 months after the operation, the gastrocnemius muscle was resected so as to leave a stump at the proximal end, and the flexor digitorum sublimis was excised completely. Minced muscle tissue was placed in the bed of the excised gastrocnemius muscle. The dog was sacrificed 5 months later. Dissection (See figure, A and B) showed that the tendo Achillis and the tendon of flexor digitorum sublimis, enveloped in loose connective tissue, had grown toward the stumps of the gastrocnemius muscle, covered with connective tissue. Laterally, they had fused with the biceps femoris muscle. Both tendons were grossly thickened throughout their length, and in their proximal portion they were remarkably widened. The peroneal nerve passed between the stumps of the gastrocnemius muscle.

Neither with the naked eye nor under the microscope could we see traces of muscle tissue in any portion of the regenerating muscle (except in the stump). We could see only the typical development of regenerating tendon [1] and cells with bundles of coarse collagen fibers.

The result of the experiment on adult dog No. 2, weighing 16.8 kg, sacrificed 8 months after operation (first series) is illustrated in the figure, C and D. The only difference from the previous case was that the regenerating tendon of the flexor digitorum communis reached the firm band of the tendo Achillis and merged with it at the level of the middle of the leg. Profusely developed loose connective tissue formed wide, interwoven, thin layers, by means of which the tendo Achillis and the tendon of flexor digitorum were joined to the stumps of the gastrocnemius muscle and with nearly all the muscles directly covering the gastrocnemius posteriorly, medially, and laterally. In this dog, as in dog No. 1, we could not detect the presence of regenerating muscle tissue.

Second series of experiments. In the adult dog No. 3, weighing 18 kg, sacrificed 11 months after operation, the gastrocnemius muscle and the flexor digitorum sublimis were removed, leaving the stumps of the heads of the gastrocnemius. At dissection the regenerating tendo Achillis was observed in the form of a firm, thick, tendinous band which fused in its distal part, 5 cm from the calcanean tuberosity, with the regenerating tendon of the flexor digitorum sublimis. The stumps of the gastrocnemius muscle were pointed at the site of section, and covered with dense fascia. The regenerating tendons appeared as bands of irregular thickness, reaching the stumps of the gastrocnemius muscle, and joined to them by loose connective tissue (see figure, E). Cellular tissue filled the whole space between the regenerating organ and the muscles covering it. The peroneal nerve passed between the stumps of the excised gastrocnemius muscle. No muscle fibers could be seen in the regenerating organ either with the naked eye or microscopically.

In two adult dogs (Nos. 6 and 9) in the first series of experiments the regenerating tendo Achillis was not joined to the stumps of the gastrocnemius muscle but grew into the substance of the semitendinosus muscle and was firmly attached by means of strongly developed loose connective tissue to the fascia of this muscle. The stumps of the gastrocnemius muscle were tapering and adherent to the surrounding tissues.

The results obtained by resecting the gastrocnemius muscle in the puppies, in the conditions of both the first and second series of experiments, were similar to those observed under similar conditions in adult dogs.

In the figure, F, G, we illustrate the result of an experiment on puppy No. 5, aged 2 months, sacrificed 5 months after the operation (second series of experiments). The tendo Achillis regenerated and reached the stumps of the resected gastrocnemius muscle. It had the appearance of a comparatively thick band, widened in its proximal part, and intimately attached by abundantly developed loose connective tissue to the stumps of the gastrocnemius muscle and to the distal segments of the semitendinosus and gracilis muscles. No muscle tissue could be observed in any part of the regenerating organ (verified by microscopic examination).

Similar results were also obtained in puppy No. 8, aged 2 months (see figure, H, I), in the first series of experiments, sacrificed 5 months after the operation. The bed of the excised muscle was filled with minced muscle tissue, but this in no way modified the end results of the regeneration process by comparison with the results of the operation on puppy No. 5.

From the point of view of function, the operated limb differed considerably from normal, although the regeneration of the tendo Achillis and its attachment to the stumps of the gastrocnemius and neighboring muscles compensated to some extent for the resected gastrocnemius muscle. When standing, the dog simply touched the ground with the operated limb, and flexed the ankle only very little. During movement, however, the animal lifted the leg and foot off the ground and actually walked on three limbs, sometimes using the operated limb for support (figure, K, L).

It may be concluded from the analysis of our results and comparison between them and the findings reported in the literature that in dogs, irrespective of whether minced muscle tissue is transplanted into the bed of the resected muscle or whether it is left empty, no true regeneration of the gastrocnemius muscle took place. At the same time, under the same experimental conditions (resection of the muscle and leaving stumps at the ends of the tendons), regeneration does occur in rats [9, 10, 15, 16]. It appears that certain skeletal muscles in rabbits, notably the biceps brachii muscle, also possess the power of organ regeneration [12].

In ordinary conditions the skeletal muscles of guinea pigs [11] are incapable of organ regeneration, which may take place, however, in special conditions (for example in hyperthyroidism). In our experimental conditions, organ regeneration of the skeletal muscles did not take place in dogs.

We are not concerned here with the question of tissue regeneration in dogs, which has been reported to occur in these animals [12]. Of greater importance is the problem of the regeneration of the muscles of these animals as a complete organ.

Until the 1940's, the erroneous idea was accepted that the skeletal muscle tissue of mammals was incapable of regeneration. In the last decades it has been proved that the muscles of some mammals possess a high regeneration potential. However, the ability of mammalian muscles to undergo regeneration has been overestimated without adequate reason [12].

Within the limits of the systematic categories of the various groups of animals, irrespective of the degree of intensity of their metabolic processes, forms may be found with highly developed and with diminished regenerative powers.

It appears that this principle holds good for the organ regeneration of the skeletal muscles in mammals: However, this does not avert the need for a careful study of the conditions affecting the regeneration of skeletal muscle tissue in those mammals possessing a diminished capacity for post-traumatic regeneration.

SUMMARY

The capacity for organ regeneration was studied in 12 dogs on m. gastrocnemius. In the first experimental series on 3 adult dogs and 3 puppies, m. gastrocnemius was excised with retention of the stumps of the head ranging from 0.5 to 1.5 cm in size, while flexor digitorum sublimis was excised completely. In the second series of experiments, staged on the same number of dogs, minced muscular tissue was placed into the bed of the removed muscles. Follow-up observations ranged from 5 to 12 months. No regeneration of gastrocnemius occurred either in the first or in the second series of experiments. Tendo Achillis regenerated, up to the stumps, and adhered to them and the surrounding muscles.

Thus, no organ regeneration was observed in dog gastrocnemius, as distinct from rats [10, 12]. This points to the species differences in the capacity of the muscles of various mammals for regeneration. These differences should be taken into consideration so as not to overestimate this capacity.

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