ORGANIC REGENERATION OF SKELETAL MUSCLES IN RATS

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There has been hardly any research on the capacity of various muscles in mammals for organic regeneration. Only a very few investigations have been concerned with this question. According to A. N. Studitskii [3], regeneration of a rat's gastrocnemius muscle after its resection is impossible without a minced tissue transplant. It was then shown [2], however, that a rat's gastrocnemius muscle, after resection, can regenerate from small, 3-4 mm stumps left on the tendon.

The purpose of this work was to determine whether the ability to regenerate after almost total resection is, in the rat, a faculty peculiar to the gastrocnemius muscle, or whether the other muscles of the rat are also capable of organic regeneration.

METHODS

The experiments were performed on 160 male white rats weighing 200-320 g each. We studied the capacity of the following muscles for organic regeneration: 1) biceps brachii; 2) extensor carpi radialis longus; 3) flexor carpi ulnaris; 4) gastrocnemius; 5) tibialis anterior.

The regeneration of each muscle with and without the use of minced muscular tissue was studied. One should mention that when these muscles had been totally excised, and no muscle stumps were left on their tendons (as checked by magnifying glass), regeneration was not observed in a single case, regardless of whether or not minced muscular tissue had been implanted in the muscle bed. Ruza [5] obtained similar results in experiments implanting minced muscular tissue in the bed of a resected biceps brachii muscle. The method and the data obtained from the results of our experiments with the gastrocnemius muscle are described in [2].

The operations were performed under ether anesthesia in aseptic conditions, the rules of surgery being carefully observed. The extremity on which the operation was performed was wrapped in sterile gauze, and the entire animal, except for the head, was wrapped in a thick layer of cotton wool. A spacious cage was provided for each animal after its operation; the rats were fed a full-value, vitamin-rich diet. Any animal in which pyosis was observed was excluded from the experiment.

The rats were sacrificed at different times - from 16 to 145 days after the operation. The muscles were dissected under a binocular lens. Histological sections were prepared from the regenerated muscles in order to establish the presence of muscle fibers microscopically. All the data obtained were documented with photographs, fixed anatomical preparations and drawings.

RESULTS

In rats, resection of the biceps brachii is rather difficult because of its complex topographical relations with other muscles [1].

In our experiments, resections of the biceps brachii were done as follows: 1) with the rat lying on its back, a diagonal incision in the skin was made along inner volar surface of the shoulder from the head of the humerus to the articulation of the elbow; 2) the same incision was continued through the superficial and muscular fascia; 3) the superficial and deep pectoral muscles and the clavicular and acromial portions of the deltoid muscle were then sectioned at the head of the humerus in order to gain access to the biceps brachii; 4) the two heads of the biceps brachii were separated with a blunt instrument without disturbing the important neurovascular bundle located in this region, and the biceps brachii was excised at its attachment sites in such a way that small fragments [2-4 mm] of muscle were left at the transition between muscle and tendon; after the operation, there was a distance of about 1.5 cm between the tendon ends of the muscle left at the attachment sites and the pieces of muscular tissue (see figure, a); 5) a continuous catgut suture was applied to the dissected muscles, the fascia and panniculus adiposus, and the skin was sutured with silk in the usual fashion.

The degree of muscular regeneration was determined on the 30th, 45th, 50th, 65th and 95th post-operative days.

On the 30th post-operative day, the excised muscle was found to be totally replaced by regenerated muscular tissue growing in at the proximal end in two heads which then joined.

On the 95th day, the new muscle had the two-headed, fusiform shape and characteristic location of the biceps brachii [see figure, b]. Its weight and thickness approximated the normal. The nerves innervating it had grown into the belly of the muscle.

It is considerably easier to excise the extensor carpi radialis longus than it is to resect the biceps brachii; it is easier to gain access to the former muscle, and there is less danger of injuring the adjacent muscles. With the rat lying on its back, a straight incision was made in the skin and panniculus adiposus along the outer volar surface of the forearm from the articulation of the elbow to the wrist; then the skin and the panniculus adiposus were parted above the muscle, and the edges of the wound were pulled apart with blunt hooks to expose the muscle [when the fascia had been dissected]. The muscle was excised at its proximal end by a transverse section made in such a way that a 2-4 mm stump of muscle remained on the tendon [see figure, c]. Then the distal end of the muscle was laterally transected at a point proximal to the place where its tendon passes beneath the tendon of the abductor pollicis longus, leaving a small, 2-3 mm stump of muscle. The skin and muscles were sutured as in the preceding operation.

The animals were sacrificed 15, 30, 35 and 50 days after the operation. We observed some degree of muscular regeneration at all these dates. On the 15th day, the muscle was oblong in shape, and the belly had not yet formed. On the 30th-35th day, the muscle had acquired the pronounced belly and the tendons characteristic of its normal form [see figure, d]. The figure, e, shows the histological structure. The picture in the case of the other regenerated muscles was analogous.

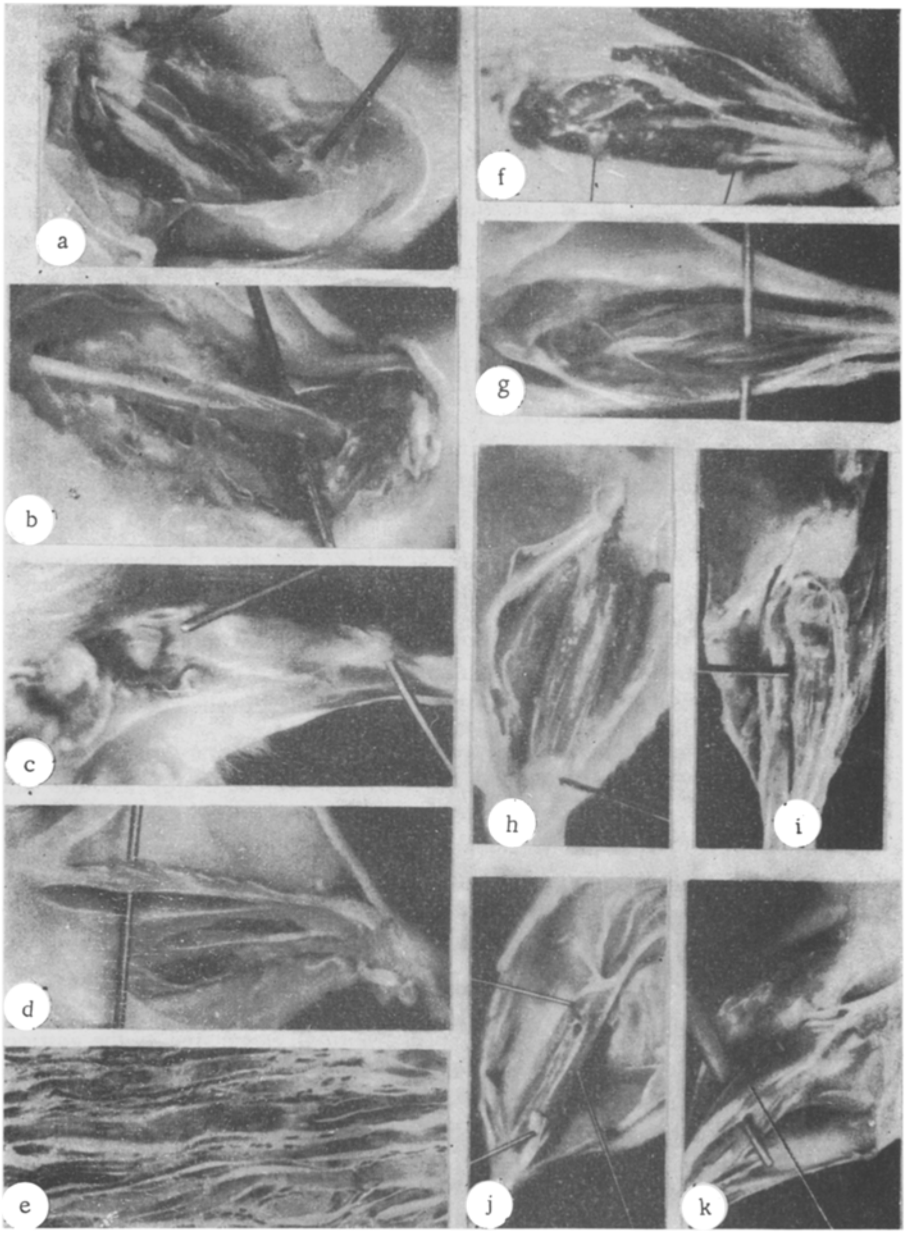
The flexor carpi ulnaris was excised as follows: a straight incision was made in the skin and subcutaneous cellular tissue along the inner volar aspect of the forearm from the articulation of the elbow to the wrist. The muscle was laterally transected at its attachment sites in such a way that 2-3 mm stumps of muscle were left on the tendons [see figure, f]. The integuments of the forearm were then sutured. On the 65th post-operative day, the regenerated muscle had the shape, attachment sites and location typical of this muscle, but was somewhat smaller than the normal muscle [see figure, g].

In rats, the gastrocnemius muscle is part of the saltatory complex of muscles; this complex is made up of the triceps surae and the plantaris muscles of the lower leg. The triceps surae has three heads, two of which form the gastrocnemius muscle, while the third is the soleus muscle.

The plantaris muscle lies slightly inwards of the medial head of the gastrocnemius. The whole saltatory complex is covered by fascia and overlying muscles. The saltatory complex is covered for some distance on the external aspect of the lower leg by the posterior part of the biceps femoris, which is attached to the lateral surface of the tibial crest; from the medial aspect, the complex is covered [also for some distance] by the posterior portion of the semitendinosus and the posterior semimembranosus muscle, the tendons of which are attached to the lower medial surface of the tibial crest. For almost two thirds of the lower leg, all these muscles are invested with fascia, which thins out towards the Achilles tendon so that the latter is clearly apparent beneath it. Dissection of the overlying muscles and fascia exposes the whole gastrocnemius muscle. The tendon of the plantaris muscle lies alongside of and medial to the Achilles tendon.

Under certain conditions, the plantaris muscle shifts laterally after excision of the gastrocnemius and can, by hypertrophying, simulate regeneration of the gastrocnemius. The plantaris muscle was therefore excised as well as the gastrocnemius in the experiments with the latter.

The gastrocnemius muscle was excised while the rat was lying on its stomach. The skin and subcutaneous cellular tissue were incised along the inner aspect of the lower leg and the calcaneus to the middle of the thigh, exposing the fascia cruris covering the gastrocnemius muscle and the distal parts of the biceps femoris and semimembranosus muscles, which reach part way down the proximal portion of the lower leg. The latter were dissected along the center line of the lower leg from the Achilles tendon to the entry place of the peroneal nerve and drawn aside with a blunt instrument.



Organic regeneration of skeletal muscle in rats (explanation in text).

To excise the gastrocnemius muscle, we transected it at the distal end at the point where the Achilles tendon passes into the muscular tissue, leaving a 3-4 mm stump of muscle on the tendon, and at the proximal end, at the entry place of the tibial nerve, leaving 2 stumps (medial and lateral heads) measuring 3-4 mm each. The tibial nerve and the vascular bundle were left intact. Then the soleus and plantaris muscles were resected at the tendons (leaving no muscle stumps). In this operation, therefore, the whole saltatory complex of muscles was removed. In this way, we excluded the possibility of later mistaking a hypertrophied plantaris muscle for a regenerated gastrocnemius. The overlying muscles and fascia which had been dissected were sutured with a continuous catgut suture, and the skin was sewn with an interrupted silk suture. The animals were sacrificed for anatomical study 16, 35, 60, 70, 90 and 145 days after the operation.

A different degree of gastrocnemius muscle regeneration was observed at each of these times. On the 16th day, the regenerating muscle was merely a thin, light pink band of muscular tissue; the muscle was still thin on the 35th day, but had acquired the characteristic, two-headed shape.

To remove the tibialis anterior muscle, a 2.5 cm incision was made in the skin along the lateral edge of this muscle. The fascia cruris was exposed along this line. After the skin, fatty tissue and fascia cruris had been drawn aside with a blunt instrument, the fascia of the tibialis anterior was dissected along the center line of the muscle. A blunt instrument was then used to separate the middle part of the tibialis anterior from the adjoining tissues; the muscle was then excised at the proximal end, at the point where the anterior tibial artery (muscular branch) enters the muscle, leaving a 2-3 mm stump and at the distal end where the tendon passes into the muscle, leaving 2-3 mm muscle stumps (see figure, h). The animals were sacrificed after 42, 52 and 65 days.

The figure, i, shows a regenerated tibialis anterior muscle 42 days after the operation. It was somewhat smaller than the excised muscle, but typical in shape and location.

In our experiments with these muscles, regeneration did not occur in every experiment. There was a certain percentage of failure. The biceps brachii regenerated in approximately 77% of the cases, the gastrocnemius and flexor carpi ulnaris in 70%, the extensor carpi radialis longus 65% and the tibialis anterior in 52% of the cases.

The failure of the muscles to totally regenerate was sometimes due to injury of the fascia of the adjacent muscles. This created conditions in which the tendons of the excised muscles could grow into the adjoining muscle or its connective tissue, and in this case, regeneration of the resected muscle did not occur. The regenerative process was also distrubed by thrombosis or injury of the vessels supplying the tissue in the region of the wound. A high percent of failure occurred in the experiments with simultaneous resection of the gastrocnemius and plantaris muscles (whole saltatory complex) of both legs. As we have already mentioned, the gastrocnemius muscle often does not regenerate if the plantaris muscle is not excised along with the triceps surae. If the plantaris muscle is left, it shifts laterally and merges at its proximal end with the stumps of the two heads of the gastrocnemius muscle and at its distal end, with the stumps on the Achilles tendon, which causes it to shift still further towards the center line of the leg. The plantar muscle therefore begins to occupy the position normally occupied by the gastrocnemius. The further hypertrophy of this muscle, which seems to be a result of its increased function, makes it very similar to a regenerated gastrocnemius muscle. In such cases, however, we found that anatomical analysis of the muscle's topography and the character of the tendon and attachment site at its distal end made it possible to establish definitely that the plantaris muscle had shifted and hypertrophied (see figure, j and k).

Therefore, our observations demonstrated that regeneration after almost total excision is not a faculty peculiar to the gastrocnemius muscle, since the other muscles of a rat's extremities are also capable of organic (total) regeneration after their excision if even small stumps of muscle are left on the ends of their tendons. In the light of the data obtained in this work and in our previous investigations [2], the use of rats' muscles to prove the significance of transplanted minced muscular tissue in muscle regeneration is hardly sound, since a number of rat's muscles are capable of organic regeneration without the aid of minced muscular tissue.

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