THE DEVELOPMENT OF CAPILLARIES IN POST-TRAUMATIC REGENERATION OF THE SKELETAL MUSCLE TISSUE OF MAMMALS

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Despite numerous investigations [9, 10, 13, 18], the problem of muscle tissue regeneration in mammals is far from resolution. Up until now, there has been insufficient study done on the processes of regeneration accompanying repair of skeletal muscle fibers. In particular, the character of development of the blood capillaries in the muscle regenerate is almost completely unknown [6].

At the same time, a large number of investigations have been devoted to the regeneration of the capillaries during the rebuilding of other organs [1, 4, 5, 7, 11, 12, 14, 19, 22, 24]. Therefore, each new detail concerning the development of the vascular bed in a muscle regenerate carries doubtless interest.

The purpose of this work was to study the routes of development of the capillaries in regenerating skeletal muscle tissue.

EXPERIMENTAL METHOD

The experiments were carried out on 40 young rabbits, weighing 800-1000 grams, and 60 rats, weighing 160-180 grams. Under sterile conditions, standard muscle injuries were inflicted on all the animals. In the rabbits, an aperture was cut out of the center of the m. tibialis anterior, with a diameter of 5 mm. In the rats, after a longitudinal, linear incision was made in the skin at the external surface of both posterior extremities, using scissors, the m. gastrocnemius was cut transversely in its central portion, up to one half its thickness; in this case, only the edges of the transected muscle fibers were separated.

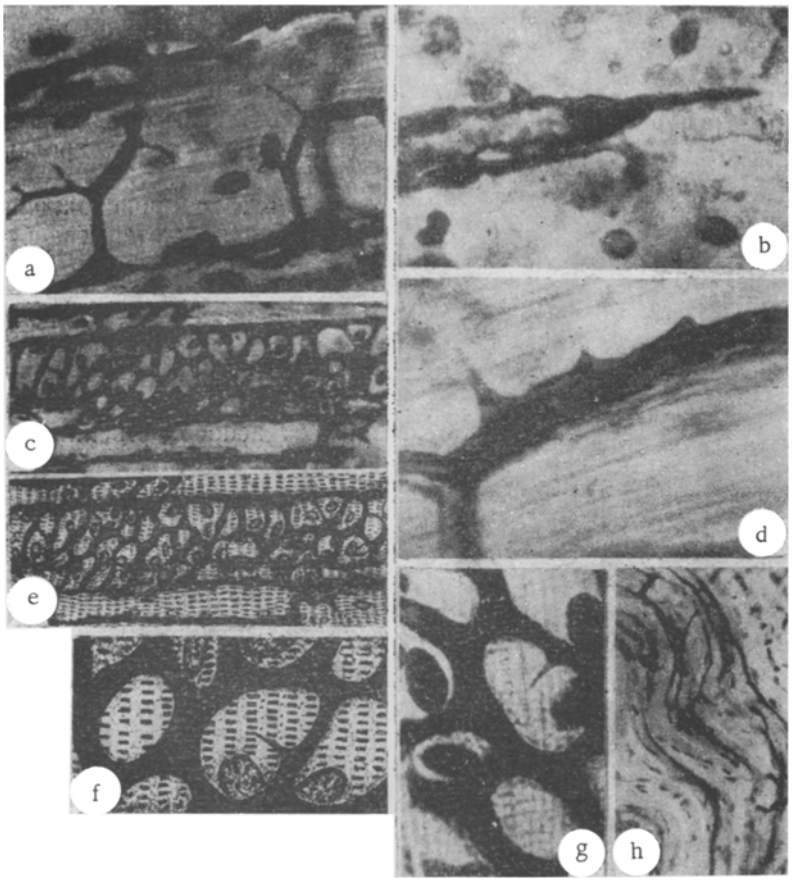
The animals were sacrificed on the 2, 5, 7, 10, 15, 20, and 30th days after the operation: the rabbits by the injection of air into a vein, and the rats, by exposure to chloroform vapor. The material was fixed in a solution of neutral formalin. Sections with a thickness of 50-60 μ were obtained on a quick-freeze microtome, and impregnated with silver according to Bielshowsky-Gross; however, for impregnation of the vessels in regenerating muscle tissue, this method was modified to a significant degree*. The use of the impregnation method made it possible, for the first time, to demonstrate not only blood vessels of varying caliber in the thick muscle sections, but also to illustrate the series of phases in the growth of the newly forming capillaries; this method also made it possible to demonstrate different methods of capillary formation in the very same preparations.

EXPERIMENTAL RESULTS

On the 2nd day after the operation, in the tissues around the site of the defect, we could see numerous extravasates, accumulations of polymorphonuclear cell elements predominantly located between the mucle fibers. In the zone of direct injury, there were destructive changes in the connective tissue, muscle fibers, and capillaries, and processes of resorption of the necrotic masses. At a distance from the wound, we observed the typical signs of muscle tissue irritation. In the vascular network, this was expressed by an increase in the argentophilia of the vessels.

On the 5th day after the operation, the wound was completely cleared of necrotic masses, and the development of connective tissue had begun along its periphery. Traces of the destruction of vessels and muscle fibers were observed

*A detailed report on the method of impregnating vessels in thick muscle sections will be published in a special work.



Capillary development in regenerating skeletal muscle tissue at different intervals in the experiment. b) on the 7th day: growing tip of a capillary in the region of direct injury to the muscle (obj. 90, ocul. 15); e) 10th day: phases in the formation of endothelial offshoots in the zone of reactive changes (obj. 90, ocul. 15); g and a) 20th day: subsequent stages in the development of the offshoots; f) schema (obj. 90, ocul. 10 and 7); c) 20th day: fine-looped network of capillaries in the zone of reactive changes; d) schema (obj. 40, ocul. 7); h) 30th day: relative arrangement of the muscle fibers and the newly formed capillaries (obj. 10, ocul. 10).

only in the distal areas of the wound region, where this and subsequent regenerative processes lagged in time behind the corresponding changes in the proximal region. In the proximal areas of the wound, intense growth of muscle bundles, extending to the myosimplasts, occurred from fragments of muscle fibers that had been injured but retained their viability.

Vasoformative processes arose primarily in those areas of the wound where, during the period of destructive changes, disruption of the capillaries and muscle fibers was least manifest.

Growth of new vessels (4th-5th day after the operation) was first noted from the viable capillaries located in the lateral edges of the wound, directly adjacent to the region of injury. The capillary offshoots appeared like pointed, hollow cones, whose walls consisted of a single layer of endothelial cells (see figure, b).

On the 7th day after the operation, a considerable portion of the wound was already filled in with loosely arranged connective tissue elements and young vessels, considerably leading the growth of muscle tubules and myosimplasts in the region of the defect. The regenerating capillaries appeared as wide, slightly curved, branching tubules, the lumens of which often contained regular blood elements. The diameter of the offshoots reached 18-20 micra, which exceeded the diameter of the preexisting capillaries by 4-4½ times. The walls of the newly formed capillaries remained a single layer.

On the 10-15th day after the operation, the central region of the defect was filled with loose connective tissue and capillaries, a portion of which underwent reversion and wasted away, and a portion which were converted into arterioles. Capillaries which did not lead into anastomoses passed into the layers of connective tissue, separating the muscle tubules and the young muscle fibers that were beginning to form.

On the 15-20th day, in the tissue zone surrounding the site of direct trauma (the zone of reactive changes), where prior to this time the blood vessels differed by their increased argentophilia, the initial phases of the development of new capillaries were observed. However, this process differed from the one just described. First on the capillaries running along the muscle fibers, and then in the anastomoses between them, there arose coniform protrusions of protoplasm with wide bases (see figure, d). The tips of these formations gradually extended, changing into thin, protoplasmic filaments (see figure, g, f_1, a). Some of these filaments contained a nucleus. At this stage, according to our observations, the development of the vessel could take two routes: either the offshoot connected with similar protoplasmic extensions, growing from the vessel which passed along the opposite edge of the muscle fiber, in which case the arch that was formed gradually thickened and canalized, subsequently acquiring a cellular strucutre, or a lumen formed even before connection of the offshoot with the other capillary. However, even in this case the distal portion of the branch remained without a lumen until it connected with the capillary lying opposite it.

Thus, formation of the capillaries in the region of the direct muscle tissue defect differed from the means by which they grew in the zone of reactive changes.

By the 20th day after the operation, the wound region had gradually filled with young muscle fibers and the capillaries which accompanied them. Contact between them became closer. Individual capillaries directly adjoined a series of fibers. In the zone of reactive changes, the capillary network was reconstructed. A fine-meshed network developed around each muscle fiber (see figure, c d_1). The form of each mesh was round or polygonal; in the intact muscle, as we know, the capillaries create a network in the form of rectangles, extending along the muscle fibers. The diamter of the formed meshes did not exceed 15-20 micra; in this case, the diameters of the capillaries forming the network were equal to 6-5.5 micra, and their walls consisted of one layer of endothelial cells.

This reconstruction of the vascular network, accompanied by an increase in the number of capillaries per unit volume of muscle tissue, enables an acceleration in the metabolic processes, both in the region of direct injury, and in the zone surrounding it.

On the 30th day after the operation, the region of the defect was filled with disorientedly arranged muscle fibers, with layers of connective tissue between them. Here the newly formed capillaries accompanied the muscle fibers, repeating their numerous and diverse curves (see figure, h). Nevertheless, the network of vessels in the regenerate was insufficiently developed, and its elements were arranged in three planes, due in large measure, to the chaotic growth of the young muscle fibers. We did not observe the close contact between the muscle fibers and capillaries which exists normally in intact muscle. The large-meshed network of capillaries here encompassed several muscle fibers simultaneously, while in the intact muscle, each fiber is enveloped individually. The density of the network in the zone of reactive changes decreased, but the meshes in it were markedly smaller than normal. At this time, the number of anastomoses between parallel running capillaries also decreased markedly.

Analyzing the obtained data, we deemed it possible to advance certain hypotheses on the means of formation of new blood capillaries, and their trophic role.

As we know, several hypotheses exist on the origin of capillaries, but two viewpoints are most widely held. Adherents of the first [1, 2, 7, 14-17, 22, 23, 24] believe that new capillaries arise from preexisting ones by means of the formation of thin protoplasmic extensions, which connect with capillaries lying nearly; then, these extensions canalize and acquire a cellular structure.

According to the other concept [8, 12, 19, 20], in the formation of the newly generated capillaries there is direct participation of the branch cells which enter into the composition of the young connective tissue or the mesenchymal syncytium. Thus, the question of the development of capillaries remains insufficiently elucidated [4, 5, 11, 12].

In investigating the growth and development of the of the blood vessels in the brain, it was shown that, under varying conditions, regenerating capillaries easily change their form of growth [4, 5]. Under the conditions of our experiment, attention was also drawn to the dependence of the process of formation and growth of the capillaries on the conditions that arose in the different areas of the developing muscle regenerate. Thus, with development of the vessels directly in the region of the wound, where there were a large number of cell elements of immature, loose connective

tissue, the walls of the developing capillaries had a cellular structure even in the first stages of their construction. In the areas where the connective tissue was completely formed, and there was almost a complete absence of young elements (the zone of reactive changes), the growth of the capillaries occurred through the formation of protoplasmic offshoots.

According to our data, in the initial development of the muscle regenerate, the basic trophic role is played by the vessels of the granulation tissue, which lead the growth of the myosimplasts [4, 6, 10]. But in the period of greatest development and differentiation of the muscle fibers (from the 15th to the 25th day after the operation), the number of vessels decreases sharply. In addition, at this time there is still not sufficiently close contact between the growing muscle fibers of the regenerate and their accompanying capillaries.

Apparently, the trophic role of these capillaries is not adequate to ensure nourishment of the young muscle fibers, and, beginning with the 15th day after the operation, there develops a supplementary trophic system in the form of a dense vascular network, which grows around the muscle fibers that are located in direct proximity to the site of trauma.

By the 25th-30th day, when the contact between the capillaries and the restored muscle fibers of the regenerate has been gradually established (supplementary anastomoses have appeared, series of fibers have been individually encompassed by the vessels, etc.), the capillary network in the zone of reactive changes of the muscle tissue around the regenerate undergoes reversion.

SUMMARY

A study was made of the ways of the capillary development in the regenerating skeletal muscle tissue. A standard muscle injury was inflicted on rabbits and rats. Capillaries were mainly detected by impregnation. At the beginning of the muscular regenerate development, the main trophic role is played by the vessels in the granulation tissue. Later, around the muscles fibers adjacent to the site of the trauma, an additional trophic system - a thick network of capillaries developed differently and depended on the local condition of the regeneration area.

LITERATURE CITED

1. A. Golubew, Arch. mikr. Anat., 1869, Bd. 5, S. 49.
2. A. A. Zavarain, Essays on the Evolutional Histology of Blood and Connective Tissue [in Russian]. M., 1947, No. 2-3.
3. A. A. Zavarzin and S. I. Shchelkunov, Handbook of Histology [in Russian]. L., 1954.
4. B. N. Klosovskii, The Development of Capillaries of the Brain [in Russian]. M., 1949.
5. B. N. Klosovskii, Blood Circulation in the Brain [in Russian]. M., 1951.
6. V. V. Lavrenko, Arkh. anat., 1959, No. 5, p. 55.
7. A. Maximow, Beitr. path. Anat., 1902, Suppl. 5.
8. M. N. Rinchino, Trudy Kursk. med. in-ta, 1948, Vol. 2, No. 2, p. 41.
9. A. N. Studitskii, Experimental Muscle Surgery [in Russian]. M., 1959.
10. A. N. Studitskii and Z. P. Ignat'eva, Regeneration of Muscles in Higher Mammals [in Russian]. M., 1961.
11. N. G. Khlopin, General Biological and Experimental Bases of Histology [in Russian]. L., 1946.
12. S. I. Shchelkunov, Arkh. anat., 1937, Vol. 17, No. 1, p. 6.
13. R. D. Adams, D. Denny-Brown, and C. M. Pearson, Diseases of Muscle. New York, 1954.
14. J. Arnold, Arch. path. Anat., 1871, Bd. 53, S. 70.
15. E. R. Clark and E. L. Clark, Am. J. Anat., 1932, Vol. 49, p. 441.
16. E. R. Clark and E. L. Clark, Am. J. Anat., 1934, Vol. 55, p. 407.
17. E. R. Clark and E. L. Clark, Am. J. Anat., 1937, Vol. 60, p. 253.
18. H. M. Hines and J. D. Thomson, Am. J. phys. Med., 1956, Vol. 35, p. 35.
19. M. J. Jolli, C. R. Seances Soc. Biol., 1906, Vol. 28, p. 146.
20. Kolliker, Ann. sci. natur., 1846, Vol. 6, S. 91.
21. A. Krogh, The Anatomy and Physiology of Capillaries, New Haven, 1924.
22. Ch. Rouget, Arch. Physiol. norm., 1873, N. 6, p. 603.
23. J. C. Sandison, Am. J. Anat., 1928, Vol. 41, p. 475.
24. S. Stricker, S. B. Akad. Wiss. Wien, 1865, S. 379.