THE INFLUENCE OF INFLAMMATION ON THE REGENERATION OF MUSCLE IN EXPERIMENTS WITH MINCED MUSCLE TISSUE

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The method of restoration of muscles by means of autotransplantation of minced muscle tissue, suggested by A. N. Studitskii [12, 13] and developed by a group of his co-workers [3-6, 9-11], has attracted increasingly the attention of research workers and has begun to be applied in several laboratories. In a series of papers [1, 2, 8, 15], the positive results of transplantation of minced muscle tissue in mammals and even in man are described. Meanwhile, in the investigations of Klika and Doskocil [16], homotransplantation of ground embryonic muscle tissue to the site of damage to a muscle had no very obvious effect; this led these authors to have doubts regarding the use of transplanted material in the regeneration of muscle tissue.

G. M. Litver and N. N. Dampel' [7] have expressed themselves even more categorically, asserting that minced muscle tissue, grafted at the site of an excised muscle, takes no part in its regeneration, but simply provokes an inflammatory reaction, as a result of which it undergoes absorption. These conclusions, in our view, are due to failure to observe the details of the operative technique which we have developed. In a special article, devoted to the restoration of muscle by means of grafting with minced muscle tissue [14], the method of the operation was described in detail and attention was drawn to the necessary conditions for successful regeneration of muscle. One of these essential conditions is the sterility of the operation, preventing suppuration, which sharply inhibits the regeneration of muscle. A no less essential condition for the successful regeneration of muscle by means of this method is the proper mincing of the muscle tissue. Drying of the material, overheating it, or other factors leading to necrosis of the grafted tissue will also cause a more intensive inflammatory reaction.

The present research was undertaken in order to show the importance of inflammation as a condition preventing the process of regeneration in experiments with minced muscle tissue.

METHODS

Two series of experiments were performed on white rats aged 2½-3 months. In the first series, the gastrocnemius, soleus, and plantaris muscles of the animals were excised by division at the level of the tendons. The neurovascular bundle was preserved. Minced muscle tissue, taken from the lateral head of the gastrocnemius muscle, was placed in the bed of the excised muscles. The whole operation was performed in strictly sterile conditions. In the second series of experiments, the animals underwent the same operation, but without aseptic precautions. In order to intensify the inflammatory process and to stimulate suppuration, balls of cotton wool, 0.5-0.8 mm in diameter, were mixed with the minced muscle tissue. Fixation was in Zenker's fluid and in 12% neutral formalin, 10, 21, 30, and 60 days after operation. Sections were stained with azocarmine by Heidenhain's method, with Regaud's iron-hematoxylin, and with hematoxylin and counterstaining by Mallory's method. Part of the material was treated with silver by the Bielschowsky-Gros-Lavrent'ev method.

RESULTS

A comparative analysis of the results obtained in the two series of experiments clearly demonstrates the role of inflammation in the regeneration of muscle from minced muscle tissue. In many animals, in the second series of experiments, on naked-eye examination of the regenerating muscles suppuration was found. The results given in the table show the change in weight of the regenerating muscles in the process of their development. In the first stage of regeneration – 10 days after autotransplantation – the weight of the regenerating muscles showed little difference in the two series of experiments; the mean weight of the regenerate in the presence of experimental inflammation was 324 mg, and in sterile conditions 333 mg. By the end of the 3rd week, however, the weight of the regenerates in the series with experi-

Change in Weight of the Regenerating Muscles in Experiments with Minced Muscle Tissue

Serial no.	In sterile conditions		Accompanied by experimental inflammation	
	Time after operation, in days	Weight of regenerate, in mg	Time after operation, in days	Weight of regenerate, in mg
1	10	320	10	220
2	10	300	10	420
3	10	300	10	180
4	10	450	10	350
5	10	340	10	450
6	10	300	—	—
7	10	320	—	—
		Mean 333		Mean 324
1	21	170	21	150
2	21	100	21	60
3	21	190	21	180
4	21	150	21	90
5	21	150	21	70
		Mean 152		Mean 110
1	30	250	30	60
2	30	200	30	120
3	30	170	30	190
4	30	180	30	130
5	30	270	—	—
		Mean 214		Mean 125
1	60	310	60	170
2	60	270	60	150
3	60	300	60	120
4	60	200	60	60
5	60	290	60	190
6	60	310	60	160
7	60	300	60	170
8	60	380	60	120
9	60	310	60	170
10	60	180	60	150
11	60	400	60	110
12	60	300	—	—
13	60	460	—	—
14	60	350	—	—
15	60	370	—	—
		Mean 315		Mean 142

mental inflammation began to fall appreciably behind that in the control series (110 mg in the series with inflammation and 152 mg in the control series); 2 months after the operation, the regenerates of the control series weighed more than twice those obtained in the series with inflammation (315 mg in the control series and 142 mg in the series with experimental inflammation).

The outward appearance of the regenerates differed not only in their size but also in their shape and color (Fig. 1). Regenerates after 2 months under sterile conditions had the appearance of a muscular organ, dark pink or red in color (dark on a light background); in the series with stimulation of suppurative inflammation, thin bands of a white or yellowish hue were obtained (light on a dark background). Microscopic examination showed differences in the course of the regenerative process in the two series of experiments.

Ten days after operation, in the first series, carried out in sterile conditions, the regenerating muscles were in the stage of transition from the myo-

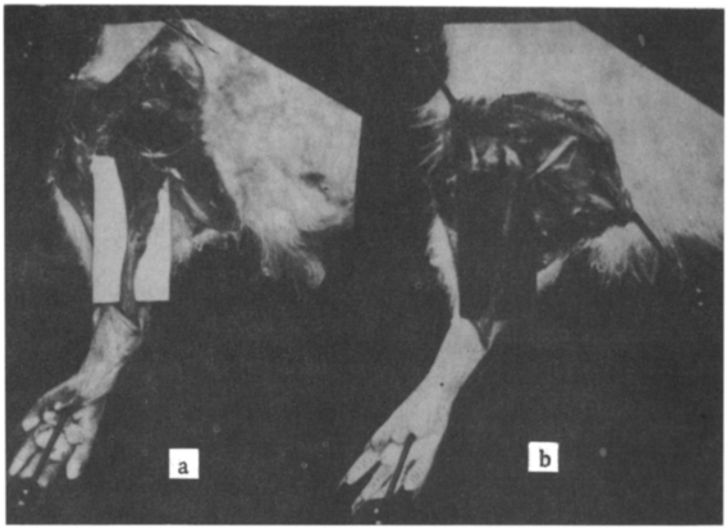

Fig. 1. Regenerates 2 months after autotransplantation of minced muscle tissue. a) In sterile conditions; b) accompanied by experimental inflammation.

blastic phase of development into the myosymplastic phase. The transplanted material was now undergoing transformation, the inflammatory reaction was feebly developed and a change to a proliferative process was apparent. The regenerating muscle was found to contain streams of myoblasts and bundles of muscle tubules, some having longitudinal fibrils, and crossing the regenerate in various directions. Those parts of the grafted muscle tissue which had not undergone progressive change were disappearing, and were almost completely absorbed, isolated fragments being seen here and there. Numerous small blood vessels penetrated into the thickness of the regenerating muscle. The commoner cells to be found in the blood and connective tissue filling the spaces in the areas where the myoblasts and muscle tubules were less compact, were lymphocytes, monocytes, and polyblasts of histogenous and hematogenous origin, with nuclei of a round or bean-shaped appearance. Fibroblasts and very fine, delicate collagen fibers could be seen.

In the second series, accompanied by experimental inflammation, the regenerating muscles contained extensive areas of unabsorbed, highly necrotic fragments of transplanted muscle tissue, mixed with cotton wool fibers. These areas were infiltrated with an enormous number of leukocytes, which surrounded them with a continuous leukocytic barrier. Here were to be found neutrophils with segmented and annulated nuclei, lymphocytes, monocytes, and numerous polyblasts, of various shapes and sizes, with compact, round nuclei. There were a few blood vessels in the regenerating muscle. In places, a young connective tissue had developed, containing fibroblasts and thin collagen fibers. Sometimes a thin layer of connective tissue surrounded areas with remains of necrotic portions of muscle fibers. A repair reaction in the muscle tissue was apparent only at its proximal end, in the remnant of the excised muscle; here were found a small number of myoblasts and muscle tubules. The latter turned back upon themselves on meeting fragments of necrotic muscle tissue, surrounded with a connective tissue capsule, in their path.

On the 21st day, the regenerates obtained in the first series of experiments contained thin, cross-straited muscle fibers and muscle tubules, forming a plexus; muscle cells sometimes lay in compact bundles, and in other places were arranged less closely together. In these areas could be seen blood vessels, close to which were found isolated, small fat cells. The muscle fibers and tubules were interspersed with fine collagen fibers, which were entwined between them. Only very rarely could isolated fragments of grafted muscle fibers be found in the regenerates, which had not undergone progressive development.

In the second series, the regenerates appeared to consist mainly of connective tissue; in places this tissue was loose and contained many cells, mainly fibroblasts. In other areas were dense bundles of collagen fibers. Here and there the regenerate was penetrated by blood vessels. Islands of necrotic fragments of transplanted muscle tissue were found in the regenerates, infiltrated by many leukocytes of various forms, polyblasts, and macrophages. Here also were occasional fibroblasts and very fine collagen fibers. A noteworthy feature was the appearance, close to the blood vessels, of numerous groups of large fat cells, round or polygonal in shape, with small, compact, unsegmented nuclei containing coarse granules, staining a yellow color with the azan method. The regenerates contained a very small number of newly formed muscle cells – myoblasts, muscle tubules, and young, slender, cross-striated muscle fibers with large nuclei.

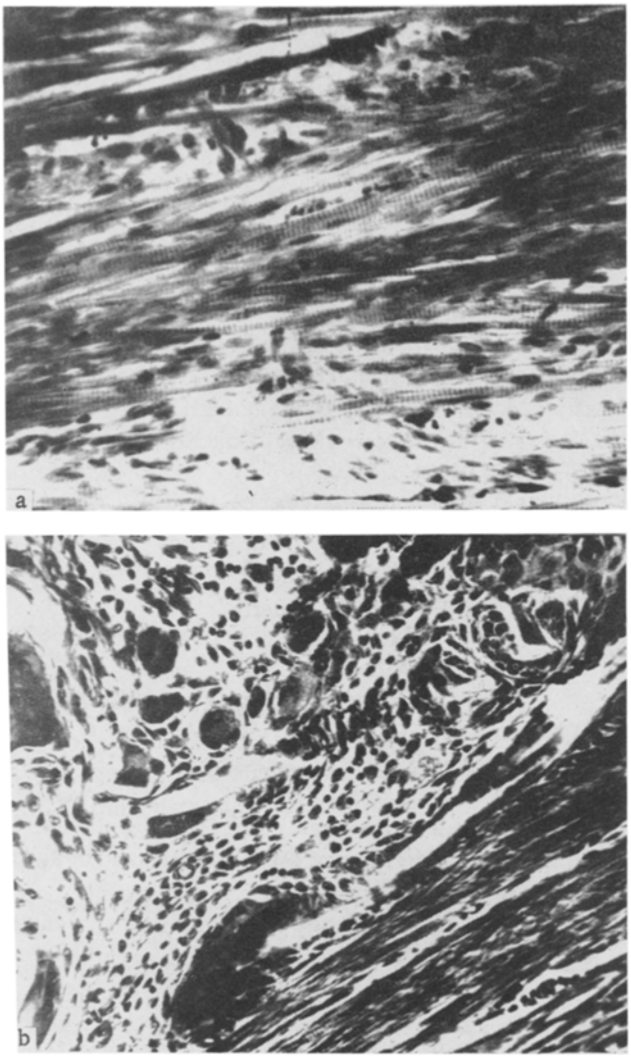

Fig. 2. Structure of the regenerating muscles 21 days after autotransplantation of minced muscle tissue into the bed of the excised muscles. Microphotograph. a) In sterile conditions (cross-striated muscle fibers differentiated; b) accompanied by experiment inflammation (area containing necrotic fragments of muscle fibers, infiltrated with leukocytes, polyblasts, and macrophages; below, on the right—fibrous connective tissue).

Thirty days after operation, the regenerates obtained under sterile conditions (first series) consisted almost entirely of interwoven and completely differentiated muscle fibers, larger in diameter than at the time of the previous examination. Signs that the repair reaction was not yet complete were found in the presence of numerous chains of muscle nuclei. The bundles of muscle fibers were separated by small connective tissue septa.

In the presence of experimental inflammation (second series of experiments) the regenerates consisted of connective tissue — either fibrous, forming compact bundles of relatively thick collagen fibers, or loose, containing fibroblasts, fat cells, and collections of mast cells. Only occasional cross-striated muscle fibers were present in the regenerate, mainly at its proximal end. In the center of the regenerates could clearly be seen necrotic fragments of transplanted muscle fibers, surrounded by cells of various types from the purulent exudate.

We were especially interested in the examination of the regenerated muscles 60 days after operation, as representing the final result of the process of repair. At this time, we examined the largest number of animals (see Table). The regenerates were stained by the silver method, to show nerve endings, as well as by the usual histological methods.

In the first series the regenerates consisted of interwoven, differentiated muscle fibers; these were still larger in diameter, showed obvious cross-striation and often contained long chains of nuclei. At the proximal end and in the center of the regenerate were seen a few fine septa of collagen fibers, which merged at the distal end with the tendo Achillis. Here and there were found small areas of loose connective tissue containing blood vessels and fat cells; very occasionally solitary, small fat cells were seen, which had lost their granularity. The regenerated muscle was richly supplied with nerve cells. A well-developed plexus of nerve trunks and single nerve fibers penetrated into the thickness of the regenerate, most of which had a formed myelin sheath. The nerve fibers terminated in motor end-plates in varying stages of maturity. They showed great variation in the form, the number, and the degree of branching of their endings and in the number of nuclei of the end-plate. The terminal branches ended in fairly large loops, such as are characteristic of regenerating motor end-plates.

In the second series, the regenerates consisted of bands of fibrous connective tissue, between the bundles of which, occasional cross-striated muscle fibers could here and there be found. In the areas of looser connective tissue there were blood vessels, fibroblasts, cells with annulated nuclei, and a still considerable number of fat cells. In some regenerates at this time, remains of necrotic fragments of the transplanted tissue could be seen, mingled with traces of cotton wool fibers. These areas were filled with infiltrations of small cells, among which could be seen many degenerating cells. In the region of the newly formed muscle fibers were seen regenerating nerve fibers, some of which had a myelin sheath, although the majority were bare axis-cylinders. These had simple endings in the form of "buttons," glomeruli, spirals or primitive motor end-plates. More mature motor end-plates were only very rarely observed.

From the foregoing it can be concluded that the character of the inflammatory process is of great importance for the regeneration of muscle from minced muscle tissue.

In the series of experiments carried out in strictly sterile conditions, the greater part of the transplanted material underwent radical reorganization, resulting in the development of muscle cells and the formation of a typical muscle organ;. this was differentiated into striped muscle fibers, interspersed with a small amount of connective tissue which merged into the tendon at the distal end of the muscle. The newly formed muscle was richly supplied with nerves and equipped with mature motor end-plates.

In the presence of suppurative inflammation, the processes of progressive development of the particles of transplanted material and formation of muscle and nerve cells were inhibited, the absorption of necrotic fragments of the minced muscle fibers was long delayed, and the productive development of connective tissues was stimulated. The regenerate formed as a result of the inflammatory process was no more than a connective tissue reproduction, incorporating only a small number of striped muscle fibers, supplied with very primitive motor nerve endings.

It must therefore be recognized that, besides tension and intact neuromuscular connections, an essential condition for the successful and perfect restoration of whole muscles by the method of autotransplantation of minced muscle tissue is the performance of the operation in conditions excluding the development of a suppurative inflammatory process.

SUMMARY

Experiments (2 series) were conducted on white rats. The significance of the characterof inflammatory process for the restoration of the muscles was demonstrated by the method of autotransplantation of minced muscular tissue. Only isolated fragments of material transplanted in place of removed muscles of the posterior extremity underwent narcosis and resolutions the greater part of the minced muscular tissue appeared to be viable and underwent radical reconstruction, bringing about the development of myoblasts, muscle tubes, and differentiated cross-striated muscle fibers supplied with nerve elements. A typical muscular organ was formed as a result of restorative process. Necrosis of minced muscular fibers was intensified in the presence of purulent inflammation; their progressive development and the resolution of necrotic particles became sharply retarded and the productive development of connective tissue, resulting in the formation of connective tissue reproduction at the site of the transplant, was stimulated

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