Regeneration of the Rat Gastrocnemius Muscle from Sibling and Non-sibling Muscle Fragments [1,2]

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ABSTRACT Regeneration of the gastrocnemius muscle from minced transplanted muscle fragments was studied in 46 rats. The jumping complex of muscles (gastrocnemius, soleus and plantaris) was removed from the donor and minced into 1 mm^3 fragments. In one experimental series minced muscle was orthotopically implanted into sibling animals, and in the other series minced muscle was implanted into non-sibling animals of the same strain. During the first week after implantation, regenerative activity in the homotransplanted muscle fragments was intense and did not differ histologically from that occurring in autotransplanted muscle. Starting in the second week, areas of regenerating homotransplanted muscle fibers became infiltrated with small lymphocytes. Despite relatively massive cellular rejection in the regenerates, individual skeletal muscle fibers regenerated to maturity. Regenerates from homotransplanted muscles often attained the same gross form as regenerates from autotransplanted material, but in no case was the amount of muscle fibers in homografted regenerates greater than 50% of that seen in regenerates arising from autografted muscle fragments. About half of the advanced regenerates were reduced to broad bands of connective tissue containing no muscle fibers.

Despite an increasing interest in tissue and organ transplantation, the transplantation of skeletal muscle has received relatively little attention. Early work (Elson, '29) on transplantation of skeletal muscle in the rat showed that autotransplanted muscle was maintained by virtue of a regenerative process which compensated for the partial degenerative loss of the originally transplanted muscle. In contrast, Elson found that homotransplanted muscle not only degenerated, but was massively infiltrated by lymphocytes. Very little regeneration was noted.

Hoja ('57, '58, '59) studied several aspects of skeletal muscle transplantation in rats, mice and rabbits. He (Hoja, '58) noted little difference between the vascularization of pieces of autografted and homografted muscle and concluded that homografts of muscle fail not because of a lack of vascularization, but rather because revascularization provides a ready access to the transplanted muscle by immune cells. Regenerative activity was present, particularly when the pieces of transplanted muscle were small (1–2 mm^3).

Working with inbred mice, Laird and Timmer ('65, '66) reported that transplants of the lateral head of the gastrocnemius muscle were quickly surrounded and infiltrated by lymphocytes, but that following an early degenerative period, regeneration occurred within the transplanted muscle. As late as 30 days after transplantation normal, presumably transplanted striated muscle fibers, were seen within the transplant. These authors also noted early lymphocytic infiltration in regenerating non-transplanted muscle (Laird and Timmer, '65).

Studitsky and Rumyantseva ('64) reported on the regeneration of the entire gastrocnemius muscle in rats from transplanted minced muscle fragments. They removed a gastrocnemius muscle from each of a pair of littermate rats, minced each muscle into 1 mm^3 fragments and orthotopically transplanted fragments from one muscle into the other member of the pair. They found that muscles did regenerate from the transplanted fragments, but that they were smaller than normal. Their report concentrated upon the latter stages

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[2] Reported earlier in abstract form (Carlson, '68a).

of regeneration, and no histological preparations were made before 21 days. The present report deals with the regenerative ability of minced muscle transplanted not only between littermates, but between non-sibling rats of the same strain. Primary attention is given to the histological aspects of muscle regeneration and lymphocytic infiltration of the regenerate during the first month after implantation of the minced muscle.

MATERIALS AND METHODS

In this investigation 46 rats from two strains were used. In one series (36 animals), muscle was exchanged between sibling animals (both sexes) of Wistar rats obtained from Albino Farms, Red Bank, New Jersey. In ten cases muscle was exchanged between non-sibling female Sprague-Dawley rats obtained from Spartan Research Animals, Haslett, Mich. The animals were always operated upon in pairs. After anesthetization with sodium pentobarbital, the jumping complex of muscles (gastrocnemius, soleus and plantaris) was exposed in one leg of each animal. Then each muscle complex was removed and minced into 1 mm^3 fragments as described previously (Carlson, '68). The minced muscle fragments from one animal were then transplanted into the bed of the muscle removed from the other animal of the pair so that each rat received minced muscle fragments from the other. Following implantation of the minced muscle, the biceps femoris muscle was sutured over the proximal half of the implanted fragments, and the skin incision was sutured over that. Postoperatively the animals were maintained on antibiotics (Cosa-Terramycin — Pfizer) in the drinking water for a week. No immuno-suppressive agents were used during the course of these experiments.

In the series of transplants between sibling Wistar rats, at least one regenerate per day was studied grossly and histologically for the first 17 days, and at irregular intervals up to 42 days. In the series of transplants between non-sibling Sprague-Dawley rats ten regenerates were studied at intervals between 7 and 38 days following implantation. All regenerates were fixed in Bouin's, serially sectioned at 7 μ and stained with either Ehrlich's hematoxylin and eosin or with Heidenhain's aniline blue stain for connective tissue.

RESULTS

Regeneration of the gastrocnemius from autotransplanted muscle fragments

Both the gross and histological course of muscle regeneration from autotransplanted muscle fragments have been previously described (Studitsky, '59; Carlson, '68), but to facilitate comparison with the present experiments, the important features are summarized here.

For the first day or two following implantation, the muscle fragments are loosely adherent to one another. Initially the minced muscle is roughly molded into the shape of the normal muscle by pressures of the surrounding tissues. Starting at three or four days, individual muscle fragments can no longer be seen at the periphery of the implanted mass, but in the central area of the implant the gross appearance of the muscle fragments differs little from that of freshly implanted muscle. By the fourth or fifth day, new connective tissue is proliferating in the area of the excised Achilles tendon. The distal region of the regenerating mass progressively becomes thinner and more tendon-like. From four to ten days the diameter of the proximal part of the regenerate gradually decreases due to the continuing degeneration of the originally implanted muscle. At this time the mass of old muscle which is lost by degeneration is greater than the mass of new muscle fibers being formed by regenerative processes. After the second week the diameter of the proximal part of the regenerate gradually increases due to the increasing diameter of the regenerating muscle fibers.

Microscopically, an early (3–7 day) regenerate can be divided into three zones. (1) The central zone contains the originally implanted muscle fragments. In this area there is no microscopic evidence of regenerative activity. Sarcoplasmic degeneration is seen, and the number of stainable nuclei associated with the muscle fibers is very low. Mononuclear inflammatory cells may be present among the muscle fragments. (2) Peripheral to and sur-

rounding the former area is a transitional zone in which the first histological evidence of muscle regeneration is seen. Here, cuffs of basophilic myoblastic cells appear under the basement membranes of the degenerating muscle fibers. Within the basophilic cuffs extensive sarcoplasmic degeneration occurs. This is normally associated with the presence of macrophages. (3) The outer regenerating zone is characterized by the complete absence of the originally implanted muscle fibers. Instead, it is occupied by various stages of regenerating muscle as well as newly forming connective tissue. In general, there is a gradient of maturity in early minced muscle regenerates so that the most advanced stages of regeneration of individual muscle fibers are seen at the periphery and the least advanced at the center.

During the middle of the second week, an architectural organization of the regenerating muscle fibers occurs. In earlier stages, the newly formed myotubes are oriented almost randomly throughout the regenerate. Their initial orientation within the regenerate seems to be determined largely by that of the originally implanted muscle fragments. As the myotubes mature, they begin to align themselves parallel to the longitudinal axis of the regenerate. By the time cross striations appear, the muscle fibers are almost invariably oriented along the lines of tension in the regenerate. Further development of the regenerating muscle consists of a peripheral migration of the nuclei and an increase in fiber diameter. The connective tissue of the regenerate also matures with respect to the increasing diameter of collagen fibers and the morphology of the fibroblasts.

Experimental series — Gross observations

During the first week, regenerates from sibling muscle fragments are indistinguishable from those arising after autotransplantation of minced muscle. This observation applies not only to the size and consistency of the regenerates, but also to the pattern of superficial vascularization. The seven-day regenerate from non-sibling muscle was also identical to autotransplanted controls. By the end of the second week, regenerates in both experimental series were usually, but not always slightly smaller than autografted regenerates. Beyond the third week, both sibling and nonsibling regenerates were invariably smaller than the average regenerate arising from autografted muscle. In these experiments, close to half of the regenerates three weeks and older were reduced to little more than a dense white band of connective tissue without any obvious muscle. The other regenerates were characterized by areas of grossly recognizable muscle in the proximal regions.

A typical older regenerate (27 days) is illustrated in figure 1. In this particular regenerate most of the actual muscular tissue had been destroyed by the immunologic defense mechanisms of the host and what remains is primarily the regenerated connective tissue stroma, upon which the muscle fibers would normally rest in a regenerate formed from autografted muscle fragments.

Experimental series — Microscopic observations

Series I. Muscle exchanged between sibling rats. The earliest stages of regeneration are histologically indistinguishable from those occurring after the autotransplantation of muscle fragments. During the first day fibrin clots and localized infiltrates of polymorphonuclear leukocytes are characteristically found among the degenerating muscle fibers. By the third day regenerative activity has begun at the periphery of the implanted muscle fragments. Early stages of both regenerating muscle (including a few myotubes) and connective tissue are present. Most of the central area is occupied by the originally implanted muscle fragments. These exhibit little evidence of regenerative activity. By the end of the first week, regenerative activity is intense and is equal in amount and in distribution to that of regenerates arising from autografted fragments. Occasional small lymphocytes are seen but at this time they are also seen in normal regenerating muscle.

Figure 2 illustrates a portion of a typical early (6 day) regenerate. It illustrates the three zones of regenerative activity which were described more fully in the

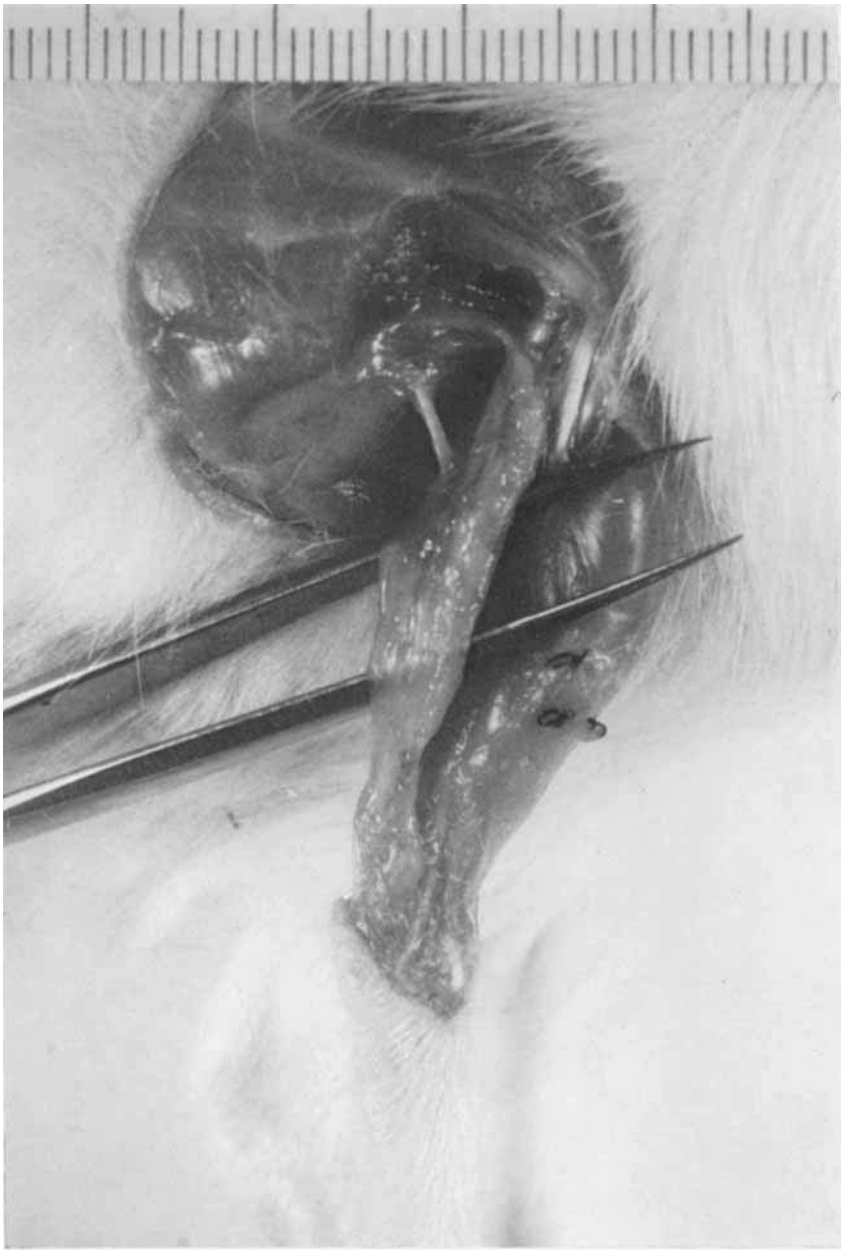

Fig. 1 Twenty-seven-day muscle regenerate from sibling muscle. This regenerate is quite thin, and most of its mass is composed of connective tissue.

summary of regeneration from autotransplanted muscle fragments. Figure 3 provides an indication of the extent of regenerative activity in the peripheral zone of a five-day regenerate. Although the number of mononuclear leukocytes in this photomicrograph is slightly greater than normal, almost none are the characteristic small lymphocytes which predominate in later stages. The myotubes themselves appear

perfectly normal. Small (less than 10 cells) to moderately extensive (50–100 cells/section) pockets of small lymphocytes are seen in association with the regenerating muscle fibers early in the second week (fig. 4). From this stage on a rather characteristic pattern of lymphocytic infiltration is established. Lymphocytic infiltration of the remaining minced muscle fragments in the central part of the regenerate is never observed. In fact, this region remains remarkably free of any invading cells. The bands of newly formed dense connective tissue are seldom associated with appreciable numbers of small lymphocytes. Pockets of lymphocytes are normally closely associated with the regenerating muscle fibers themselves, but often relatively large areas of regenerating muscle (up to 1 mm^2) remain essentially free from such cellular infiltrates during this period. Another common cellular phenomenon which is seen beginning in the second week is the presence of multinucleated giant cells in isolated pockets throughout the regenerates. Such cells are seldom seen in regenerates arising from autografted muscle fragments.

Architectural reorganization of the newly formed muscle fibers within the regenerate occurs in homograft as well as in autograft regenerates. Figure 5 depicts the central region of an 11-day regenerate and illustrates a typical example of this phenomenon. To the left of the photomicrograph are bands of striated muscle fibers oriented parallel to the lines of tension in the muscle. In the center are earlier regenerative stages of muscle fibers (identified by the rows of nuclei) which have not yet become aligned parallel to the older fibers. The amount of lymphocytic infiltration is typical of this stage of regeneration.

Individual regenerating muscle fibers mature according to the same morphological and temporal pattern as those in autografted regenerates, but the microscopic structure of the regenerating muscle as a whole begins to differ greatly from autografted regenerates after the middle of the second week. The primary qualitative difference is the presence of increasing amounts of lymphocytic infiltration, especially among the regenerating muscle fibers. The other major difference lies in the amount of muscle relative to the amount of connective tissue. The mass of connective tissue in regenerates arising from sibling muscle fragments is about the same as that seen in autograft regenerates. The mass of muscle, however, decreases from normal (i.e., autograft) levels at the beginning of the second week to less than half of that level by the third week. In no regenerate over 21 days did muscle fibers comprise more than 25% of the entire regenerate. Figure 6 illustrates some regenerated muscle fibers 27 days after implantation. Their morphology is quite similar to normal muscle fibers except for their slightly smaller diameter. They are associated, however, with rather dense lymphocytic infiltrates.

Regenerates over four weeks old vary considerably in morphology. About 50% are composed entirely of parallel bands of dense fibrous connective tissue without any muscle fibers whereas the other regenerates contain varying amounts (10–25%) of muscle fibers which are normally associated with pockets of small lymphocytes. Transplants were made between all possible combinations of sex with respect to donor and recipient, but no differences in the extent of cellular rejection were noted.

Series II. Muscle exchanged between non-sibling rats of the same strain. The course of regeneration is basically the same as that reported above except that the amount of lymphocytic infiltration is greatly increased. Larger numbers of multinucleated giant cells are also seen. Dense masses of small lymphocytes surround regenerating muscle fibers as early as seven days (fig. 7). The numbers continue to increase, and in later stages the muscle fibers often appear to be engulfed in extremely dense lymphocytic masses. Nevertheless maturation of individual muscle fibers continues. Figure 8 shows some well developed regenerated muscle fibers in a 32-day regenerate. They are partially surrounded by an extremely dense mass of small lymphocytes. In some cases relatively extensive regeneration of muscle occurred. This is illustrated in figure 9, which was taken from the muscular part of a 38-day regenerate.

DISCUSSION

The morphological data presented here confirm the reports of others (Hoja, '58, '59; Studitsky, '64; Studitsky and Rumyantseva, '64; Studitsky and Zhenevskaya, '67) that normal mammalian skeletal muscle fibers can fully regenerate even in an immunologically hostile environment. The results of these experiments show that following the homotransplantation of minced muscle in rats, considerably greater regenerative activity occurs than had been previously described (Elson, '29). Laird and Timmer ('65) reported regenerative activity in whole transplanted muscles of normal mice. When they transplanted the lateral head of the gastrocnemius muscle of a normal mouse into a dystrophic host, some initial regeneration was noted, but degenerative changes soon predominated and the normal muscle was replaced by fat and connective tissue.

There appears to be nothing in the early humoral environment of the host which is inimicable to the early regenerative process. This is supported by the normal amounts of muscle regeneration during the first week in these experiments and also by the work of O'Steen ('63) who obtained early stages of regeneration of human muscle fragments in diffusion chambers implanted into mice.

The pattern of lymphocytic infiltration of the regenerating transplanted muscle fragments is consistent and quite characteristic. In these experiments none of the originally implanted muscle fragments were surrounded by lymphocytes even though some persisted as long as eight days. This lack of cellular rejection is correlated with the complete absence of a direct blood supply to the old muscle fragments (Carlson, unpublished vascular injection studies). It may mean that any remaining antigenicity of these fragments is not carried to the small lymphocytes already present in the regenerate or that the lymphocytes do not or can not respond to the antigenic stimulus. The connective tissue components of the regenerates seldom contain more than occasional lymphocytes. This may be due to either the low antigenicity of collagen or to the fact that much of the connective tissue in the regenerates is actually of host rather than transplant origin (Carlson, unpublished experiments).

The presence of multinucleated giant cells in these experiments is worthy of comment. Such cells are rarely seen after autotransplantation of muscle fragments. They are present as isolated cells or in groups of two or three in grafts of sibling muscle, and they appear in even greater numbers (occasionally in groups of 8–10) in non-sibling transplants. Hoja ('57) has noted their presence in homografts of rabbit muscle. In cases of minced frog muscles transplanted into rats (Carlson, unpublished), giant cells are scattered singly or in groups throughout the degenerating regions of the implant. In the case of muscle transplantation, the number and distribution of giant cells seems to provide a fairly reliable indicator of tissue incompatibility.

ACKNOWLEDGMENTS

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PLATE 1

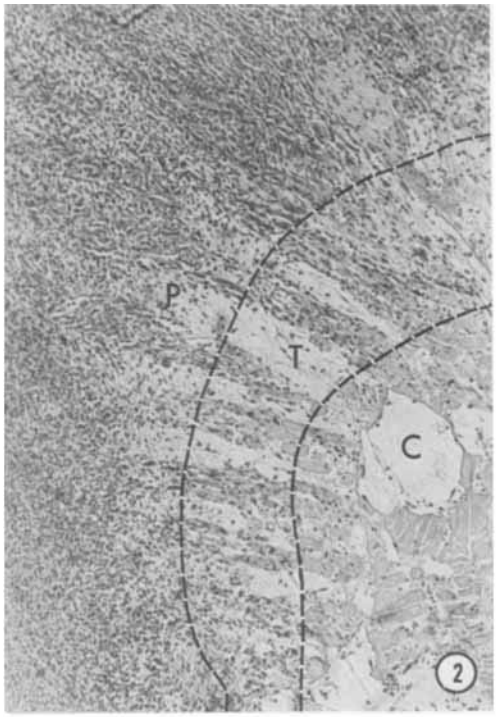
EXPLANATION OF FIGURES

2 Six-day regenerate from sibling muscle fragments. The center (c) of the regenerate contains originally implanted muscle fragments. In this regenerate the transitional area (t) from originally implanted fragments to the peripheral zone (p) of regeneration assumes an almost radial configuration. H & E. × 210.

3 Five-day regenerate from sibling muscle fragments. This is a tangential section through the peripheral zone of early regeneration. Regenerative activity is intense. All muscular elements in this photomicrograph are newly regenerated. The number of mononuclear cells is slightly greater than most, but not all, normal regenerates at this stage. H & E. × 210.

4 Eight-day regenerate from sibling muscle fragments. Early regenerated muscle fibers with cross striations are becoming infiltrated by small pockets of lymphocytes. H & E. × 400.

5 Eleven-day regenerate from sibling muscle fragments. Regenerated mature muscle fibers (left) and connective tissue fibers (right) are oriented parallel to the longitudinal lines of tension of the muscle. Less mature muscle fibers (center) have still not assumed a parallel orientation. Areas of slight lymphocytic infiltration may be seen throughout the regenerating muscle. Note the relatively small numbers of lymphocytes in the connective tissue at the right side. H & E. × 340.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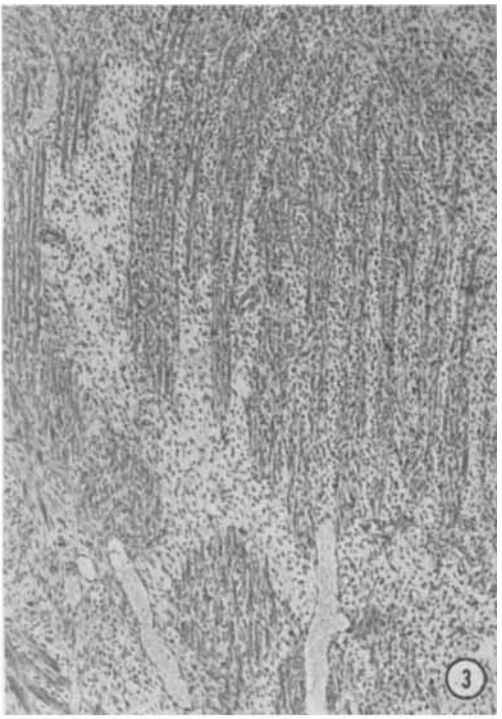
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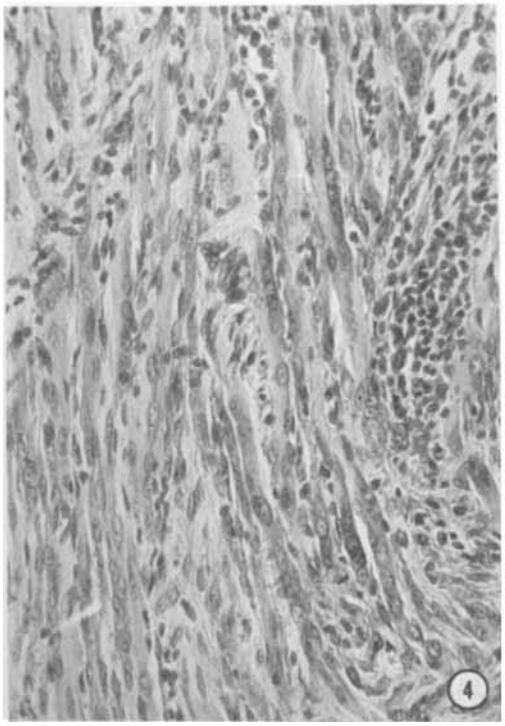
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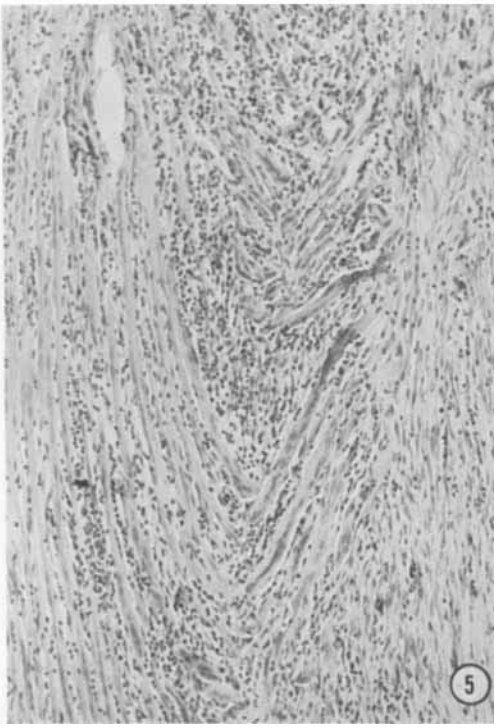
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PLATE 2

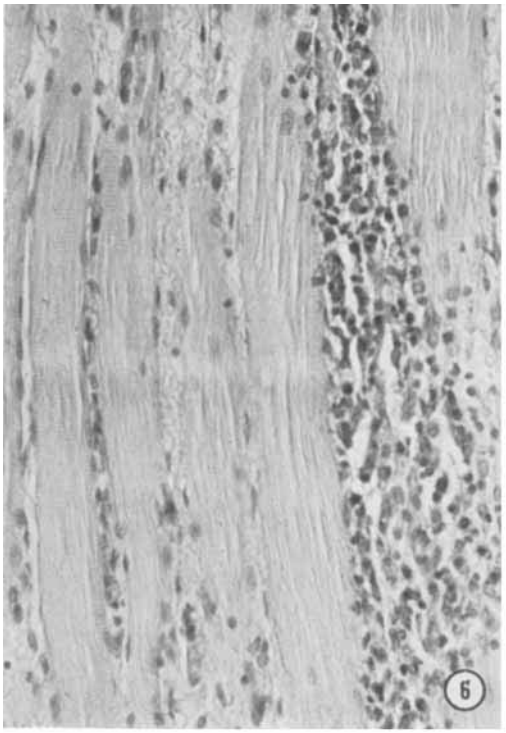
EXPLANATION OF FIGURES

6 Twenty-seven-day regenerate from sibling muscle fragments. The morphology of the regenerated muscle fibers is close to normal despite the proximity of pockets of lymphocytes in the vicinity. H & E. × 800.

7 Seven-day regenerate from non-sibling muscle fragments. Relatively intense infiltration of regenerating muscle fibers. Heidenhain's aniline blue. × 340.

8 Thirty-two-day regenerate from non-sibling muscle fragments. Regenerated muscle fibers with cross striations in the midst of extremely dense mass of small lymphocytes. H & E. × 800.

9 Thirty-eight-day regenerate from non-sibling muscle fragments. Low power view showing relatively large amounts of regenerated muscle fibers and areas of intense lymphocytic infiltration. H & E. × 106.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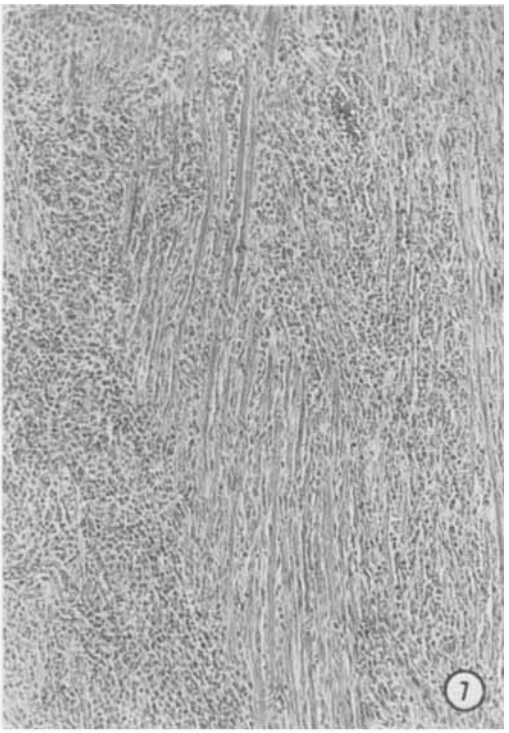
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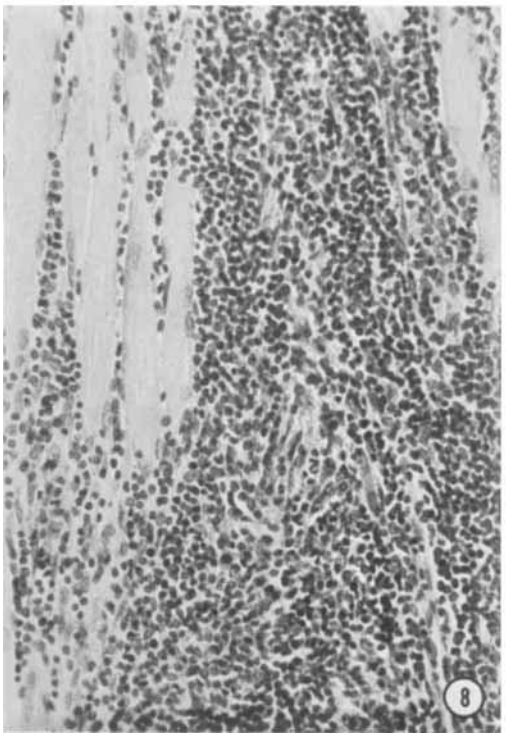
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