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REGENERATION OF GASTROCNEMIUS MUSCLE FOLLOWING TRANSPLANTATION IN MICE

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ABSTRACT.—Regeneration of gastrocnem ius muscle following transplantation was studied in mice. It was discovered that there was an initial phase of degeneration of almost all of the original muscle fibres followed by a regeneration of a new population of myotubes within the graft. By about the second week post-grafting, a distinct atrophy and degeneration of regenerated muscle fibres had set in. This process continued unabated so that by about the end of the fourth week there remained only a few regenerating muscle fibres within the graft, while the rest of the area was occupied by connective and adipose tissue elements. These results indicate that mouse gastrocnemius muscle does not possess good regenrative ability following transplantation. Possible reasons for this inability are discussed.

INTRODUCTION

Despite an increasing interest in tissue and organ transplantation, the transplantation of skeletal muscle has received relatively little attention. For a long time all attempts to graft entire mammalian skeletal muscle were unsuccessful (Zhenevskaya et al., 1965; Roy, 1966; Thompson, 1971). Such grafts were either resorbed or underwent a more or less complete fibrosis and degeneration.

During the last two decades, however, successful free autografting of entire mammalian muscles has been achieved in a number of cases (Bosova, 1962; Thompson, 1974; Hakelius, 1974; Carlson and Gutmann, 1975; Mufti et al., 1977). It has been observed that during this process there is an initial phase of degeneration of almost all of the original muscle fibres which is then followed by extensive regeneration of new muscle fibres. One other important observation made in such studies is that the size of the muscle is somehow related to its ability to survive and regenerate in transplanted condition. It has been claimed by Zhenevskaya et al. (1965) and Carlson and Gutmann (1974), that free grafting of muscles weighing more than 2 g in the rat is usually unsuccess ful. However, recently, cat Extensor Digitorum Longus (EDL) muscle, which

weighed more then 3 g, has been successfully transplanted (Faulkner et al., 1976; Mufti et al., 1977; Maxwell et al., 1978). This indicates that relative weight of the muscle with respect to the total body weight of the animal rather than the absolute weight of the muscle is of considerable importance. Such a maximum threshold of w ight and size of the muscle in relation to different animals has thus far not been worked out.

It was with this background information that the present investigation was initiated. The gastrocnemius muscle in mice, which is relatively a heavy and large muscle in relation to the total body weight of the animal, was tried for its ability to survive and regenerate in transplanted condition. This information, along with similar observations to be made in other animals and on some other muscles, should given us a fairly good idea about the maximum size and weight of the muscle which could be safely and successfully transplanted in an individual, including humans.

Materials and Methods

In this series of experiments, 75 male mice belonging to the species *Mus musculus* were used. The average weight of the mice was 32 g.

The surgeries were performed in semi-sterile conditions. The instruments and glassware were boiled and washed with 70% alcohol.

The transplantation of the Gastrocnemius muscle was performed in a manner described for many skeletal muscles (Carlson and Gutmann, 1975; Mufti et al. 1977). Briefly described, this process involves isolation of the muscle by snipping all its neural, vascular and tendinous connections. The muscle is then taken out, weighed and grafted back in its original bed in proper orientation. Both proximal and distal tendons are sutured back but there is no attempt to reattach neural and vascular elements. After grafting of the muscle, the overlying fascia and the skin are sutured with 5-0 silk. The operated leg was then wiped with 5% solution of Acriflavin to avoid infection. The operated animals were then kept singly in clean cages and were administered Tetracycline, in their drinking water for about 3-4 days post-operative.

After various prescribed intervals, the operated animals were again anaesthetized. The muscle grafts were exposed, isolated and removed. These were weighed and fixed in Bouin's fixative for 6-12 hours. These were then processed for histology. Thin paraffin sections (8-10 μm) were cut and stained with Heamatoxylin and Eosin.

RESULTS

Gross Morphological Observations

Morphologically, the graft appeared quite pale and puffy during the first couple of days. By the end of the first week, it had established loose connections with the surrounding tissues as well as with its proximal and distal tendon stumps. It also became moderately vascularized as a result of which its colour turned pinkish red. Another important morphological observation made at this stage was that its overall size had also become reduced. During the second week post-transplantation, the graft had become strap-like but was well vascularized. Its proximal and distal tendinous connections had also become quite firm. However, during the 3rd and 4th week post-grafting, the overall size of the graft became quite reduced. It had taken the form of a thin strap of fibrous tissue with obviously little muscle tissue in it.

Histological Observations

Histologically, it was observed that degeration of the muscle fibres within the graft had set in quite early (Fig. 1). The destruction of the original muscle fibres was especially pronounced in the peripheral areas of the graft as is typical in such systems. Intense macrophage activity was quite apparent in such areas. By the 5th day post-grafting, one could easily identify few highly basophilic, spindle-shaped myoblasts within the peripheral zone of muscle degeneration (Fig. 2). Such myoblasts were mostly located within the endomysial tubes. By the end of the first week, most of the original muscle fibres had undergone complete degeneration and elimination and their place had been taken up by many differentiating myotubes. At this stage, a gradient of muscle degeneration and regeneration could be made out from the peripheral to the central zone of the graft.

During the second week post-transplantation, the regenerating muscle fibres had developed to a considerable extent (Figs. 3 and 4). They had acquired distinct cross and longitudinal striations. There were, however, two other features within the graft which were interesting to note at this stage. Firstly, there was a gradual increase in the amount of connective and adipose tissue within the graft. These features signified the beginning of the processes of degeneration and atrophy of the graft. (Fig. 5). Secondly, there were also

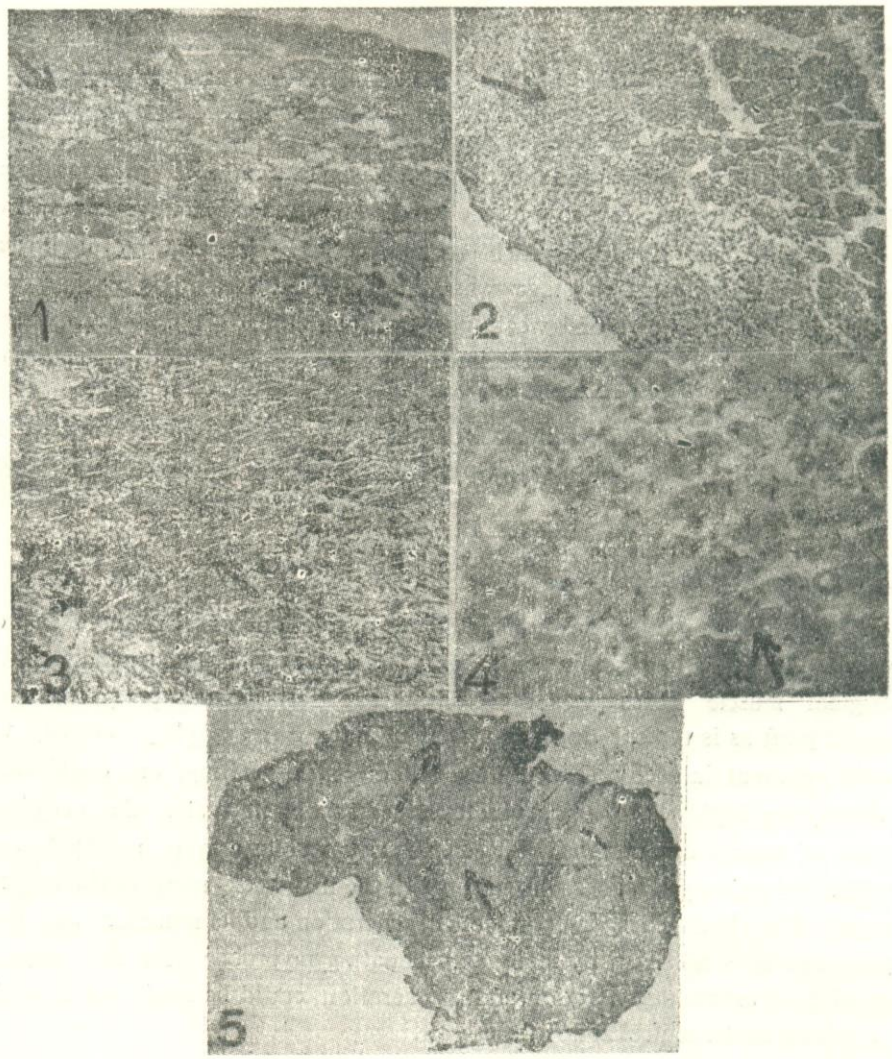

FIG. 1. A portion of 3-day regenerate. Most of the muscle fibres in the peripheral area have undergone degeneration (arrow). ×80.

FIG. 2. Portion of a 5-day implant, showing many myoblasts and myotubes towards distal and peripheral side (arrow). ×80.

FIG. 3. A portion of 10 day regenerate showing the myotubes (arrow). ×80.

FIG. 4. Higher magnification of previous photograph. Note well developed myotubes (arrow). ×320.

FIG. 5. Longitudinal section of a 15 day implant showing extensive fibrosis. Note also extensive adipose tissue (arrow). ×20.

indications of degeneration within the regenerated muscle fibres as well. These indications were in the form of greater eosinophilia and dissolution of sarcoplasm within these fibres as well as pycnosis of their nuclei (Fig. 6).

By the end of the third week following transplantation, the most important observation was an obvious degeneration and atrophy of the muscle fibres within the graft. Almost all of the regenerated muscle fibres were seen affected and showed typical degenerative changes both in the form of sarcoplasmic dissolution and vacuolation and mycnuclear clumping and pycnosis (Figs. 7 and 8). The nuclear configuration (disc-shaped) and their stacking together is quite typical of a denervation-induced atrophy of the muscle fibres (Mufti, 1977). There were indications that some of the muscle fibres may have even undergone a complete degeneration and their place having been taken by the spreading connective and adipose tissue (Fig. 9). This conclusion could be drawn by virtue of the fact that both these tissues had now spread quite widly within the graft. There were, however, still present a few original muscle fibres which had not yet undergone complete degeneration. These were present mostly towards the distal side of the graft. Some of the regenerating myotubes also survived, especially towards the periphery of the graft.

By the end of the fourth week following transplantation, most of the regenerated muscle fibres had been eliminated (Fig. 10). Their place had been occupied by dense connective and adipose tissue, as observed previously. There had also been a tremendous increase in the tendon formation within the graft. A few of the regenerated muscle fibres still survived, present mostly towards the peripheral region of the graft. The graft as a whole presented poor picture of muscle regeneration. It is obvious from these observations that muscle atrophy and necrosis would continue till the whole graft may take the form of a band of nothing but dense fibrous tissue with only a few regenerated muscle fibres within it.

Discussion

The results obtained in the present study indicate that mouse gastrocnemius muscle does not undergo successful long term transplantation. Although there is definitely observed an initial phase of muscle regeneration within the transplant, there occurs extensive fibrosis within the muscle resulting into a practically non-viable graft. Studitsky and Besova (1960) and

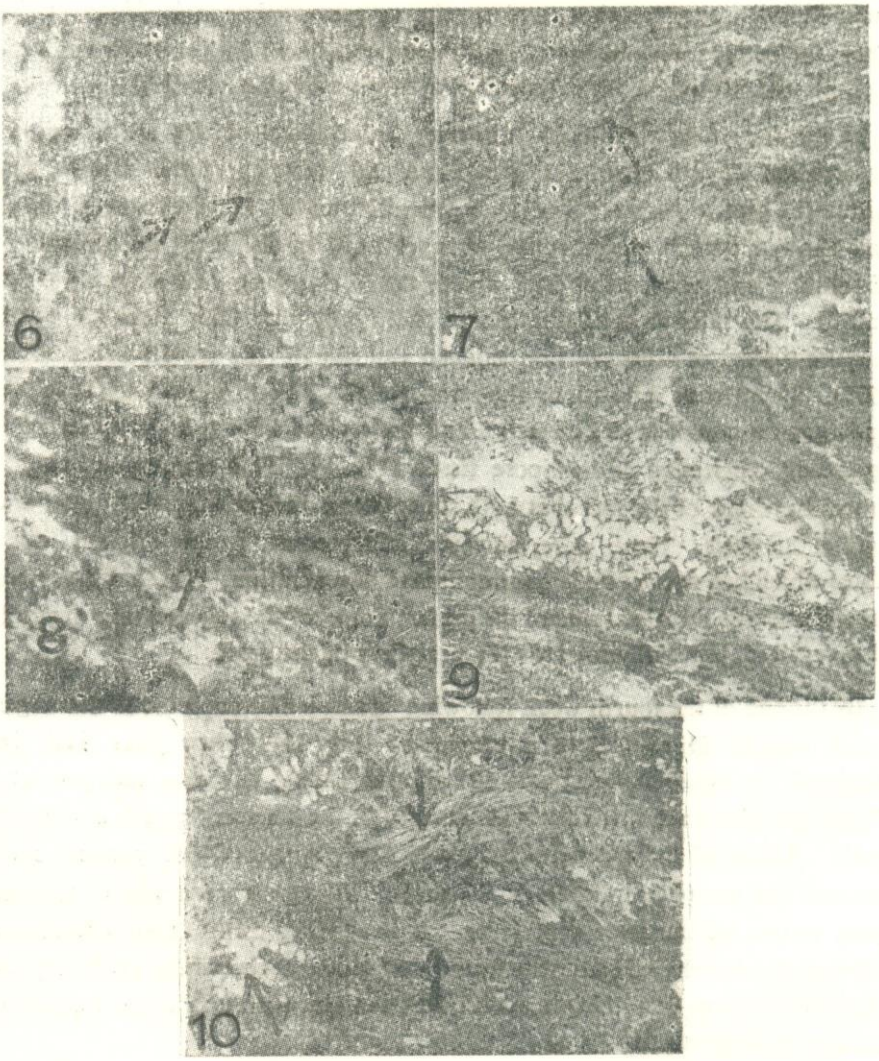

FIG. 6. A higher magnification of a portion of a 15-day implant. Note myctubular degeneration in the form of sarcolysis (arrow) and nuclear clumping (double arrow). ×320

FIG. 7. A portion of an 18 day implant. Note atrophy and degeneration of many of the regenerating fibres in the form of nuclear pycnosis and stacking (arrow). ×80.

FIG. 8. Higher magnification of degenerating and atrophying muscle fibres present within a 18-day implant. Note nuclear clumping (arrow). ×320.

FIG. 9. A portion of 18-day regenerate. Note that almost all of the myotubes are affected within the implant. Note also the elaboration of extensive connective and adipose tissue (arrow). ×80.

FIG. 10. A portion of a 30-day regenerate. Note that most of the muscle fibres have undergone atrophy and degeneration and most of the space has been occupied by collagenous and adipose tissue (arrow). ×320

Bosova (1962) had also reported unsuccessful transplantation of rat gastrocnemius muscle, when this muscle was transplanted without the pre-denervation treatment. These authors studies long term transplants and reported a more or less complete fibrosis of the muscle resulting into the formation of a thin band-like structure full of connective and adipose tissue. Carlson (1972) also observed that in the free autografts of the normal gastrocnemius muscle in rat, there was an almost complete destruction of the implanted muscle fibres so that the whole space was occupied by a dense mass of connective and collagenous tissue. He further pointed out that there was 'seldom any evidence of muscle regeneration seen". In the present study, however, there is an initial phase of sound muscle regeneration within the transplant, which lasts for almost two weeks, before degenerative phase sets in. This initial phase of muscle regeneration, in the form of myoblast formation and myotube differentiation, is quite similar in many respects to what has been described in many other recent intact muscle transplantation studies, such as the ones involving rat soleus and EDL muscle (Carlson and Gutmann, 1975) and cat EDL muscle (Mufti et al., 1977). Similarly, mouse EDL also showed good regeneration following transplantation (Hironaka and Miyata, 1975) The occurrence, in the present study, of considerable muscle regeneration within the gastrocnemius muscle is quite interesting.

One of the foremost reasons given for the lack of muscle regeneration following transplantation of the rat gastrocnemius muscle was cited to be the large size of the muscle. It was generally observed that muscle weighing more than 2g did not undergo successful transplantation and regeneration (Carlson and Gutmann, 1974). Due to the large size of the muscle, the rate and amount of the reinnervation and revascularization was considered to be too slow to facilitate regeneration. However, in a recent study, cat EDL muscles, which weighed more than 3-4 g, were successfully transplanted with or without pre-denervation treatment (Faulkner et al., 1976; Mufti et al., 1977). This observation threw doubts over the notion held previously that an absolute weight of more than 2 g of the muscle renders it incapable of undergoing successful transplantation. It seemed that the relative weight of the muscle in relation to the total body weight of the animal rather than an absolute weight may be pertinent to its survival and regeneration following transplantation. The results obtained in the present study tend to support this contention. Mouse gastrocnemius muscle, which had and average weight of

0.715 g, although showed an initial phase of muscle regeneration, could not undergo a successful transplantation, and eventually underwent extensive fibrosis and degeneration.

The presence of the initial phase of muscle regeneration, as found in the present study, had apparently not been recorded in the case of rat gastrocnemius muscle. Unfortunately in both cases where it was tried (Bosova, 1962; Carlson and Gutmann, 1974) only long term observations were made. It may be possible that even in rat gastrocnemius muscle, there may have been present an initial phase of muscle regeneration following transplantation with an eventual necrosis as seen in the present study. A more careful study of the initial stages of rat gastrocnemius muscle regeneration following transplantation are in order to further comment on the presence of an early muscle regenerative response in the mouse gastrocnemius muscle. It may, however, be pointed out here that muscle regeneration in the form of myotubes differentiation, has been reported even in a completely denervated condition (Mufti, 1977; Mong, 1977). It is thus quite possible that in a situation like the present series of experiments, a considerable degree of muscle regeneration can take place during the initial period following transplantation, inspite of complete denervation and avascularity. After going through such an initial phase of muscle regeneration, proper innervation and vascularization have been seen to be vital for further growth and maturation of the differentiating muscle fibres (Hsu 1974; Mufti, 1977; Mong, 1977). In a denervated condition, regenerated myotubes undergo atrophy and are eventually eliminated. Interestingly enough, the pattern of atrophy and degeneration of the regenerated myotubes encountered in the present study, is very similar to the one seen in muscle regeneration studies in a denervated condition. The myonuclear clumping, their disc-shaped appearence and pycnosis as well as sarcoplasm eosinophilia and dissolution are all indications of myotubular degeneration as seen in a transplanted muscle in a denervated condition (Mufti, 1977; Mong, 1977).

Although in the resent study there was no direct attempt to eliminate the nerve supply, it seems that adequate innervation could not develop in relation to the muscle transplant, especially towards its more interior regions. There was survival of quite a few of the regenerated muscle fibres towards the periphery showing thereby that perhaps there was adequate innervation developed at this site to ensure growth and maturation of the muscle fibres. Apparently,

HSU, L., 1974. The role of nerves in the regeneration of minced skeletal muscle in adult anurans. *Anat. Rec.*, **179** : 119-136.

MAXWELL, L.C., FAULKNER, J.A., MUFTI, S.A. AND TUROWSKI, A.M., 1978. The free autografting of entire lmb muscles in the cat : Histochemistry and Biochemistry. *J. App. Physiol.*, **44** : 431-438.

MONG, F.S.F., 1977. Histological and histochemical studies on the nervous influence on minced muscle regeneration of triceps surae of the rat. *J. Morphol.*, **151** : 451-462.

MUFTI, S. A., 1977. Regeneration following denervation of minced gastrocnemius muscle in mice. *J. Neurol. Sci.*, **33** : 251-266.

MUFTI, S.A., CARLSON, B.M., MAXWELL, L.C. AND FAULKNER, J.A., 1977. The free autografting of entire limb muscles in the cat : Morphology. *Anat. Rec.*, **188:** 417-430.

ROY, P.R., 1966. Behaviour of a free autogenous muscle graft into the skeletal muscles of the dog. *J. Exp. Med. Sci.*, **9** : 78-

STUDITSKY, A.N. AND BOSOVA, N.N., 1960. Development of atrophic muscular tissue in transplantation in place of mechanically damaged muscles. *Arkh. Anat. Gist. Embriol.*, **39** : 18-32.

THOMPSON, N., 1971. Autogenous free grafts of skeletal muscle. A preliminary experimental and clinical study. *Plast. Reconst. Surg.*, **48** : 11-27.

ZHENEVSKAYA, R.P., RUMYANTSEVA, O.N., NOVOSELOVA, I.L. AND PROSHLYAKOVA, E.V., 1965. Regenerative processes in a transplant of untreated muscle of young rats. *Zhur. Obsch . Biol.*, **26** : 569-576.