Chapter 3

THE REGENERATION AND TRANSPLANTATION OF ENTIRE SKELETAL MUSCLES IN MAMMALS*

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THE FIRST ACCURATE descriptions of skeletal muscle regeneration were made well over a century ago (Waldeyer, 1865; Weber, 1867; Volkmann, 1893), but during the early years of the twentieth century statements that skeletal muscle does not regenerate crept into the English pathology and surgical literature and have persisted with remarkable tenacity. The modern era of research on muscle regeneration began with the work on restoration of ischemic muscle in rabbits by Le Gros Clark during World War II (Le Gros Clark, 1946; Le Gros Clark and Blomfield, 1945).

Subsequently, two developments were influential in shaping the course of much subsequent work on muscle regeneration. One was the discovery by Mauro (1961) of the muscle satellite cell, a nondescript mononuclear cell located between a skeletal muscle fiber and its surrounding basal lamina. The satellite cell was postulated to be a possible precursor cell for regenerating skeletal muscle. After almost two decades of often acrimonious debate (*see* Mauro et al., 1970, 1979), the satellite has been demonstrated to be a source of myogenic cells in regenerating mammalian muscle (Snow, 1977a, b). Whether or not it is the sole source of myoblasts in regenerating mammalian muscle remains to be determined. There is still no definite information on the source of regenerating muscle cells in amphibian limb regeneration.

The other major development that set the stage for much of the contemporary work in the field was the demonstration by Studitsky (1952, 1959) that entire muscles in birds and mammals can regenerate from minced fragments. The minced muscle model, originally designed to prove the validity of Lepeshinskaya's (1945, 1952) "new cell theory" that cells can arise from a non-cellular "living substance," has proven to be of considerable value in demonstrating the properties of skeletal muscle regenerating as a tissue and an organ. Much of the work on minced muscle regeneration has been summarized by Carlson (1972).

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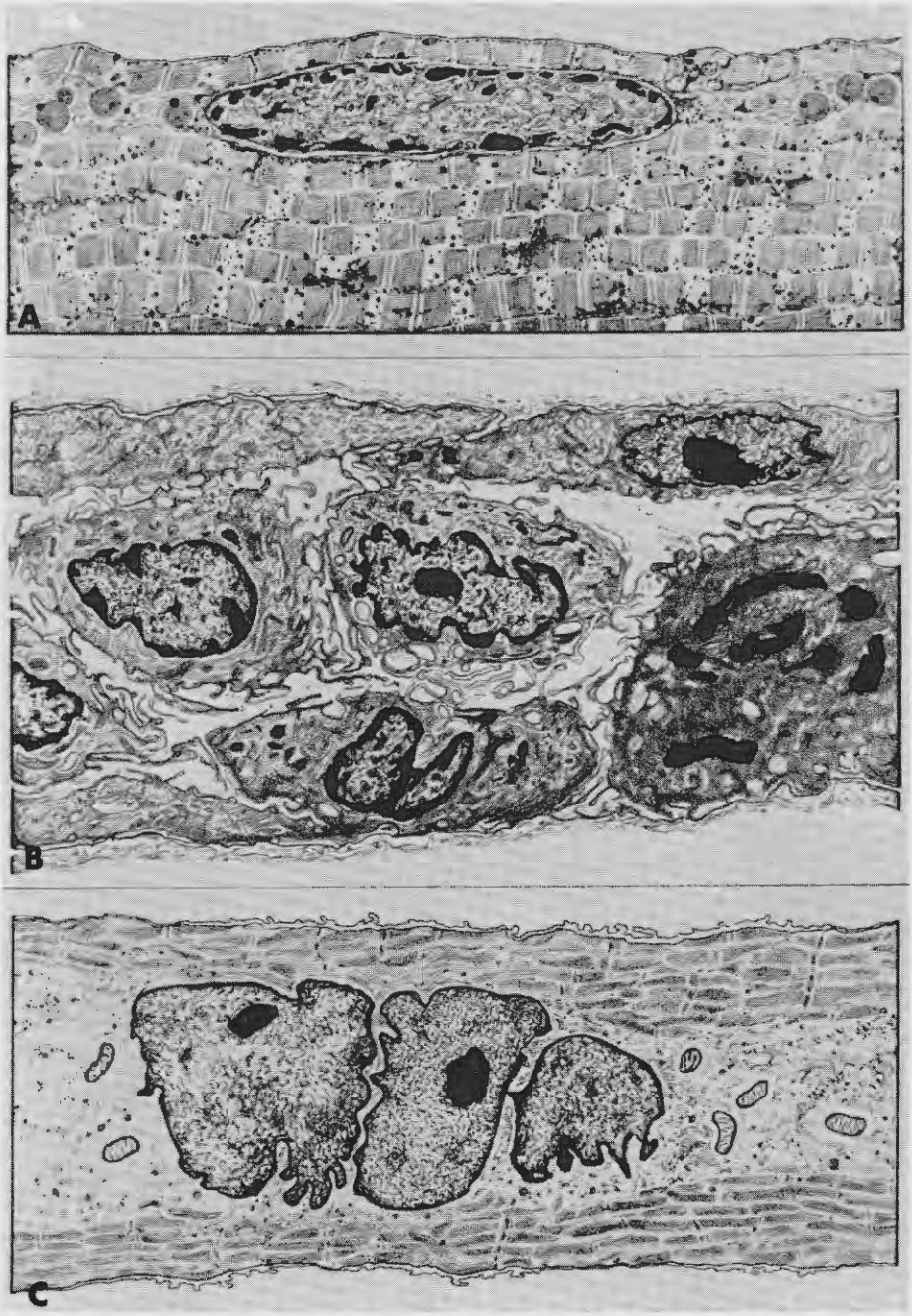

Regeneration of a Muscle Fiber

A typical sequence of degeneration and regeneration of a muscle fiber after ischemic damage is illustrated in Figure 3-1 (Reznik, 1973; Hansen-Smith and Carlson, 1979). Within hours after the onset of ischemia the

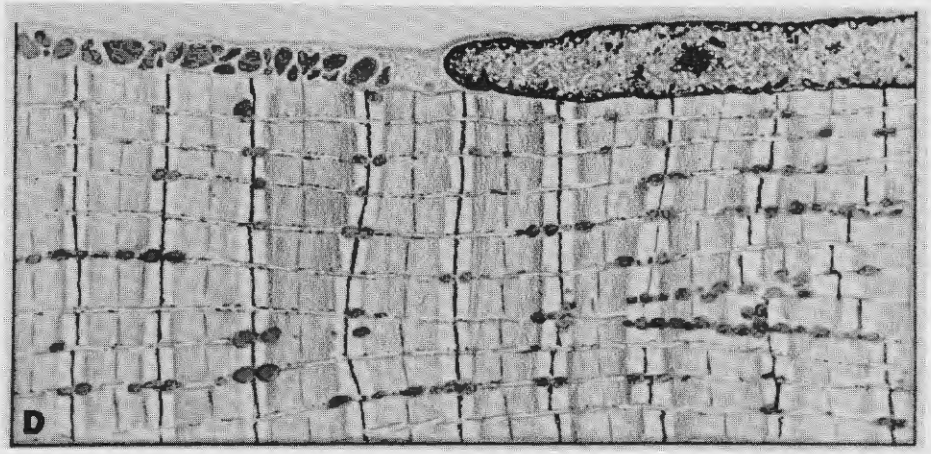

Figure 3-1. Drawings of representative electron micrographs, illustrating major stages in the breakdown and regeneration of a skeletal muscle fiber. *A.* Intrinsic ischemic degeneration, with nuclear pycnosis and break-up of sarcomeres; *B.* Cell-mediated destruction of the original muscle by phagocytic cells (center) and the establishment of spindle-shaped myoblastic cells beneath the original basal lamina (top); *C.* Multinucleated myotube; *D.* Mature regenerated muscle fiber. (From A. H. Foster and B. M. Carlson, *Anesth. Analog., 59*:727-736. 1980. Reprinted by permission of the International Anesthesia Research Society.)

muscle fiber begins to undergo a series of intrinsic degenerative changes. The myonuclei become pycnotic (Fig. 3-1A) although the nuclei of satellite cells retain a viable appearance (Snow, 1977a). Myofibrillar integrity is disrupted by the loss of Z-band material, and the sarcoplasmic reticulum and T-system are broken down. The basal lamina surrounding the degenerating muscle fiber remains intact.

A cell-mediated phase of destruction and removal of the damaged muscle fibers begins as blood vessels regrow into the area of ischemia (Fig. 3-1B). Large numbers of macrophages invade the degenerating muscle fiber and ingest the cytoplasmic debris. Spindle-shaped presumptive myoblastic cells line up beneath the basal lamina of the original muscle fiber after most of the original muscle fiber has been removed. According to the experiments of Snow (1977b), these cells are derived from satellite cells that have survived the period of ischemia.

After removal of the cytoplasmic debris and departure of the macrophages, the myoblasts fuse to form long myotubes, with chains of central nuclei (Fig. 3-1C). The nucleoli of the myotubes are very prominent, and these cells actively synthesize contractile proteins, which first become assembled into myofibrils at the periphery of the myotube. The myotube is still surrounded by the basal lamina of the original muscle fiber, but often newly formed basal lamina material can be seen within the redundant folds of the original basal lamina.

As the myotube continues its maturation, the nuclei move away from the central chain and become settled at the surface, after which the myotube can be called a muscle fiber. By this stage contractile filaments fill the entire muscle fiber. Final structural and functional differentiation of the muscle fiber into fast and slow types does not occur until after the reestablishment of neuromuscular contact. Satellite cells are found in mature regenerated muscle.

Models of Muscle Regeneration

Skeletal muscle regeneration can be elicited experimentally by a variety of means. Most of the methods have particular advantages or disadvantages, and the choice of the method should be based upon the aspect of muscle regeneration that is to be studied.

Simple Transection

Transection of a muscle is a relatively simple procedure and, as an experimental model, it duplicates a condition seen after many types of surgical incisions. The transection model is, however, one of the more difficult ones to evaluate. Except for the local area of trauma, this is essentially a nonischemic lesion, but following the complete transection of limb muscles, relatively massive ischemia may be seen in the distal segment. In nonischemic areas, transected muscle commonly regenerates by the continuous mode (Hall-Craggs, 1974a; Hudgson and Field, 1973). A number of aspects of continuous muscle regeneration remain poorly understood, including the origin of the myonuclei. Some investigators (Elyakova, 1972) feel that original myonuclei, which survive this nonischemic lesion, may either migrate into the budding end of the regenerating muscle fiber or break off from the muscle fiber and form myoblasts. Although the site of transection commonly fills in with dense connective tissue, muscle fibers do regenerate through the plane of transection, and they may reconnect with muscle fibers on the other side of the lesion.

Applications of Heat or Cold or Radiant Energy

Degenerative changes, followed by regeneration, occur after exposure of a muscle to local heat or cold (Price et al., 1964), often by means of contact with a metal bar of the desired temperature. Although considerable muscle damage can be produced, it is difficult to control the exact amount of damage. The variability lessens the usefulness of this model. An unusual means of producing the degeneration and regeneration of extraocular muscles is exposure to the radiant energy from incandescent lights (O'Steen et al., 1975).

Crush Lesions

Either local or complete damage of a muscle can be produced by crushing. Several mechanical devices have been designed for the produc-

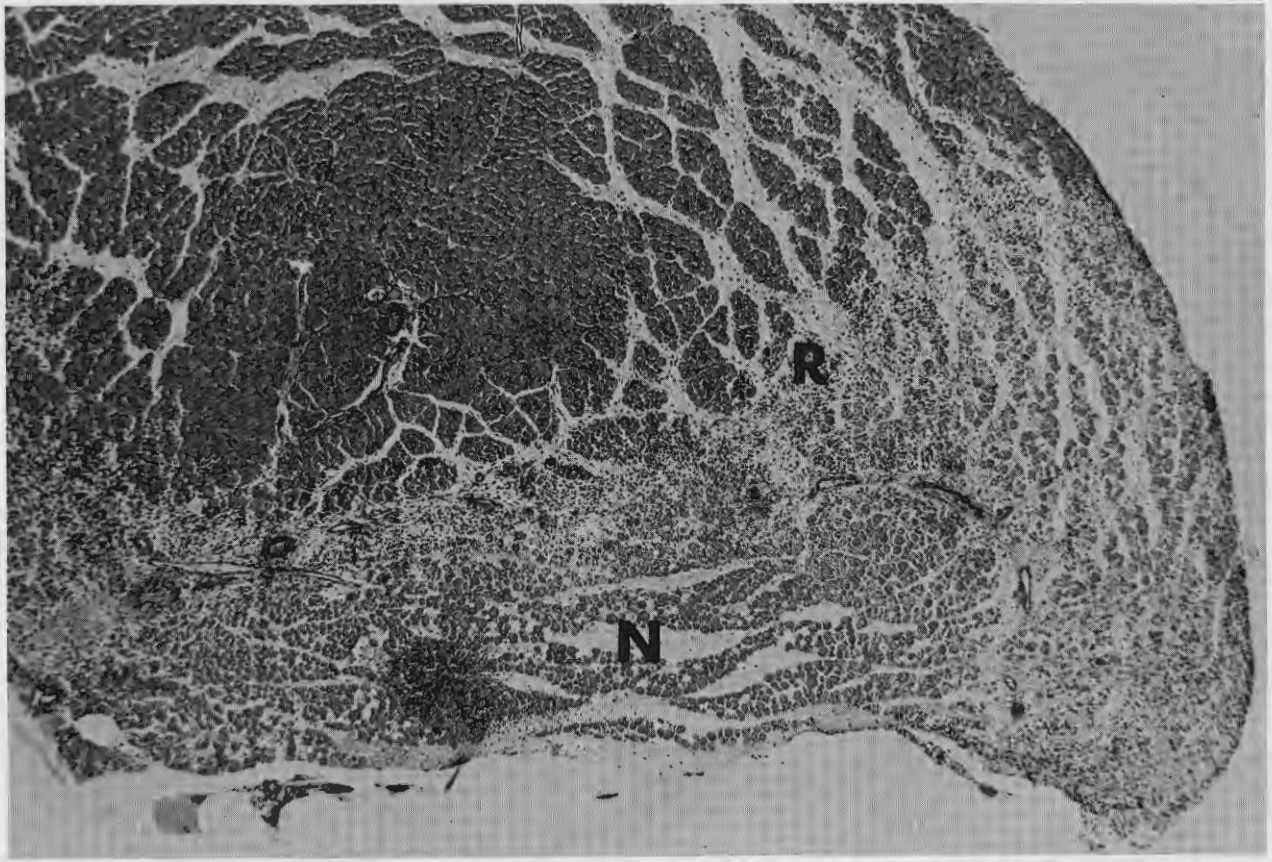

Figure 3-2. Tibialis anterior muscle of the rat two days after the muscle was injected with 200μl of dibucaine. The lower part of the muscle section contains longer muscles of necrotic (N) and regenerating (R) muscle fibers. H & E stain. (From A. H. Foster and B. M. Carlson, *Anesth. Analog.*, 1980, in press.)

tion of uniform local crush lesions (Allbrook et al., 1966; Järvinen and Sorvari, 1975), but simply pinching with a hemostat is an effective way of crushing a thin muscle. For some relatively inaccessible muscles, such as the levator ani, overlapping crushes with a hemostat is a good way to produce essentially complete destruction of the muscle fibers and yet retain the integrity of the nerve and blood supply (Gutmann and Carlson, 1978).

Local Anesthetics

When they were originally tested for histotoxic effects, most of the common local anesthetics were considered to exert few ill effects on skeletal muscle except for some degree of muscle atrophy, which was sometimes considered to be of neurogenic origin (Pizzolato and Mannheimer, 1958; Libelius et al., 1970). It now appears that the atrophy reported was actually unrecognized muscle regeneration and that many of the commonly used local anesthetics are moderately to highly mytoxic in laboratory animals (Fig. 3-2). The best studied of the myotoxic local anesthetics is Marcaine® (bupivacaine), which is so myotoxic that it is commonly injected into muscles to produce massive damage and regeneration (Benoit and Belt, 1979; Jirmanová and Thesleff, 1972; Hall-Craggs, 1974; Carlson, 1976).

Ischemia

Since the early studies of Le Gros Clark (1946), temporary ischemia has been used as an effective means of stimulating the breakdown and repair of skeletal muscle. Temporary ischemia is a particularly effective means of stimulating muscle regeneration in larger animals with easily manipulable blood vessels. Recently Hall-Craggs (1978) has described a technique for producing complete ischemia in a rat muscle.

Mincing

The technique that produces the greatest damage and organizational disruption to a muscle is mincing (Studitsky et al., 1956; Carlson, 1968). Minced muscle regeneration is obtained by completely removing a muscle, chopping it into 1 mm^3 pieces with scissors and implanting the fragments back into the bed of the removed muscle or into another heterotopic site (Fig. 3-3). The muscle fragments are not only severely mechanically damaged but are also ischemic because the blood supply has been completely disrupted. As blood vessels regenerate into the mince, the muscle fragments break down along a centripetal gradient. After the destructive phase, new muscle fibers regenerate within the basal laminae of the muscle fragments. Ultimately a new muscle is produced (Fig. 3-4). The minced muscle model is a useful experimental tool because it can be used to demonstrate the organizational capabilities of regenerating muscle under

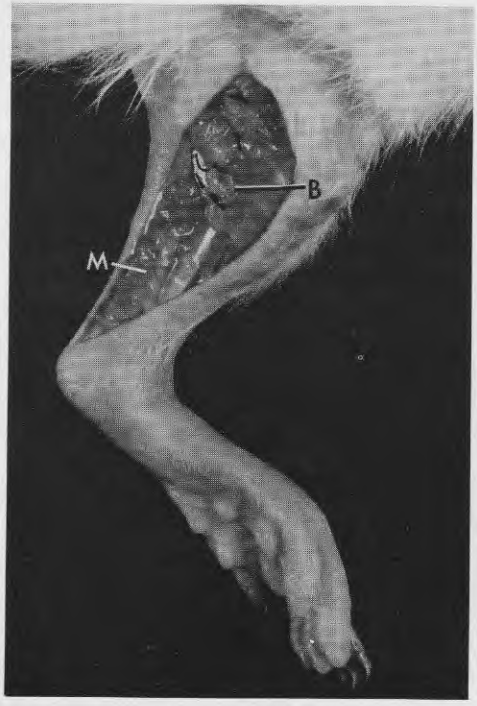

Figure 3-3. The minced gastrocnemius muscle of the rat at the time of the operation. M = minced muscle; B = overlying biceps femoris muscle sutured in place. (From B. M. Carlson, *J. Morph., 125*:447, 1968. Reprinted by permission of Alan R. Liso, Inc.)

Figure 3-4. Typical minced muscle regenerate in the rat. The gastrocnemius, plantaris, and soleus muscles were removed, minced, and implanted sixty-eight days previously. (From B. M. Carlson, *The Regeneration of Minced Muscles,* 1972. Courtesy of S. Karger, Basel.)

conditions of maximal disturbance to the gross and internal architecture of the muscle.

Free Grafting

Free grafting consists of the complete removal of a muscle from its bed (severing of all neural, vascular, and tendinous connections) and replacing it back into its own bed (orthotopic graft) or into the bed of another muscle (heterotopic graft). This technique was first successfully used by Studitsky and Bosova (1960) in rats and was independently reported by Thompson (1971) to be successful in dogs and humans. In many respects the development of a free muscle graft is similar to that of a mince, except that in a free graft the internal architecture of the muscle is preserved. Because of this, a free graft typically possesses a thin rim of muscle fibers that have survived the grafting procedure, probably through diffusion of oxygen and nutrients. The remaining muscle fibers of the graft degenerate and regener-

ate in a manner similar to that of a minced muscle regenerate. At present, free grafting appears to be the method of choice for obtaining relatively large amounts of functioning muscle in both experimental animals (Carlson, 1978; Gutmann, 1978) and humans (Thompson, 1974).

Factors Controlling Morphogenesis of Regenerating Muscles

It is now well established that in mammalian muscle regeneration mechanical factors can account almost entirely for gross morphogenesis and the internal architectural pattern of muscle fibers. Because of the great degree of initial destruction, the minced muscle model of regeneration has proven to be very useful in the analysis of morphogenetic factors (Carlson, 1972). Immediately after reimplantation a minced muscle consists of a chaotic mass of randomly oriented muscle fragments. Within hours of implantation these fragments become loosely bonded together by fibrin into the shape of the muscle bed into which they are placed. There are no changes in internal architecture during the first few days, during which much of the degeneration and early regeneration have begun.

In a minced muscle or a muscle graft in the rat, the first tenuous connections between the regenerate and the tendon stump of the muscle bed begin to form four or five days postoperatively, and tension exerted throughout the newly regenerating tendons exerts a profound effect upon the internal architecture of the regenerating muscle, causing realignment of the muscle fibers in a manner appropriate to the mechanical environment to which the graft is subjected. The external form of the muscle, as well, corresponds to tension by elongating, whereas in the absence of tension it rounds up.

The effect of tension upon the shape of regenerating muscle is well illustrated by two experiments. One consists of implanting pieces of minced muscle beneath the abdominal skin of a rat. In the absence of linear tension, the regenerating muscle rounds up into a button-shaped mass (Fig. 3-5), in which the muscle fibers are distributed in a seemingly random fashion in three dimensions. When mechanical tension is artifically applied to the regenerating mince (Fig. 3-6), the muscle becomes elongated along the direction of tension (Fig. 3-7). Similarly, if most of the gastrocnemius muscle of a rat is removed except for a 3-4 mm proximal stump, the Achilles tendon frequently regenerates proximally, making contact with the muscle stump. When this occurs, the muscular remnant elongates considerably (to up to 26 mm; Fig. 3-8), whereas in the absence of a connection with the regenerating tendon the muscle stump does not elongate but becomes attached to the underlying tissues (Litver et al., 1961; Carlson, 1974).

One morphogenetic phenomenon associated with minced muscle regeneration (rat gastrocnemius) is the formation of well-organized nodules

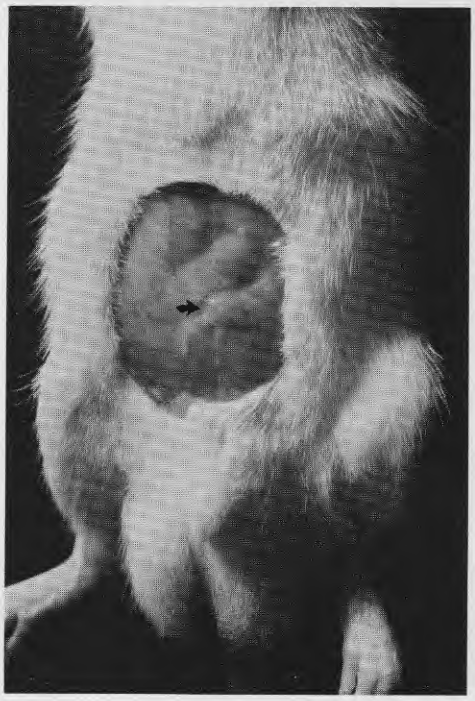

Figure 3-5. Fourteen day minced muscle regenerate in the rat. Half of a gastrocnemius was minced and freely implanted beneath the abdominal skin. The regenerating muscle (arrow) has rounded up into a button-shaped mass. (From B. M. Carlson, *The Regeneration of Minced Muscles,* 1972. Courtesy of S. Karger, Basel.)

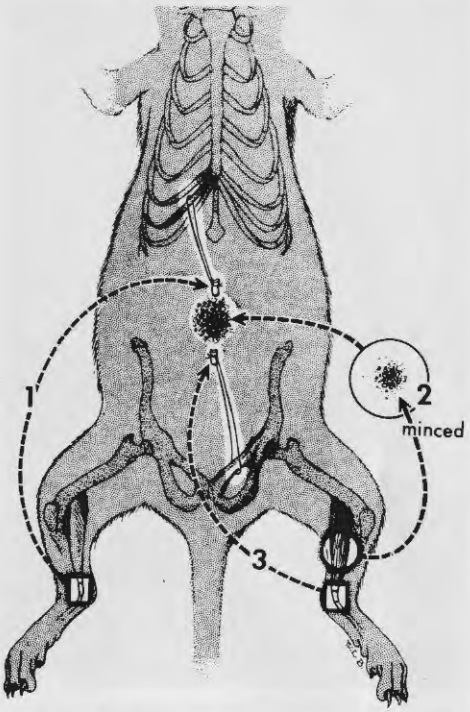

Figure 3-6. Design for applying directed tension to a regenerating minced muscle. The gastrocnemius muscle is minced and implanted beneath the abdominal skin (2). A segment of one Achilles tendon is implanted beneath the skin and attached by a suture to a rib (1). A segment of the other Achilles tendon is similarly implanted, but attached by a suture to a pubic ramus (3). Within days, the tendon stumps become attached to the implanted muscle fragments, and as the animal grows, the tendon stumps are pulled apart, thus creating continuous tension upon the regenerating minced muscle. (From B. M. Carlson, *The Regeneration of Minced Muscles,* 1972. Courtesy of S. Karger, Basel.)

of cartilage and bone (Fig. 3-9) in a high percentage of minced gastrocnemius regenerates (Carlson, 1972). It is noteworthy that a high degree of organization of these bony nodules, including the formation of structures resembling epiphyseal plates, is generated in regenerating muscles lacking most of the attributes of the morphogenetic field activity associated with the regenerating amphibian limb. It appears that the control of mor-

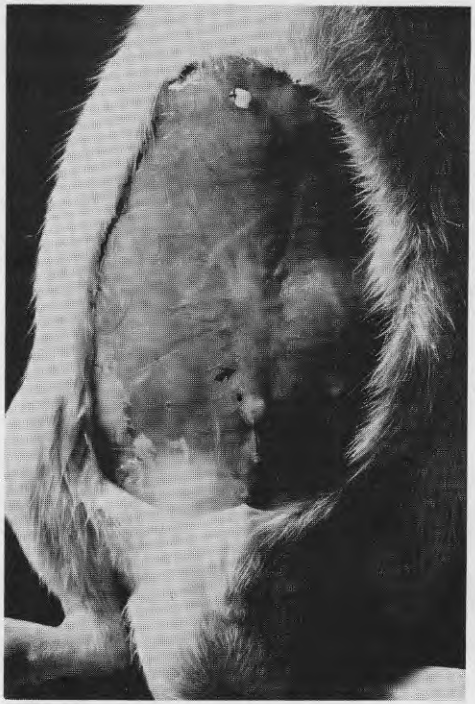

Figure 3-7. Fourteen-day minced muscle regenerate in the rat (see design in Fig. 3-6). The posterior tendon implant (attached to the black suture) has made a connection with the implanted minced muscle and with time has pulled a portion of the regenerating minced muscle into a tongue-like band (arrow). The anterior tendon implant (top of figure) pulled away from the muscle implant before connections could be established. Note the lack of elongation of that portion of the regenerate. This regenerate has not been dissected, and adhesions of connective tissue can be seen radiating from the edges of the regenerate. Compare with Figure 3-5. (From B. M. Carlson, *The Regeneration of Minced Muscles,* 1972. Courtesy of S. Karger, Basel.)

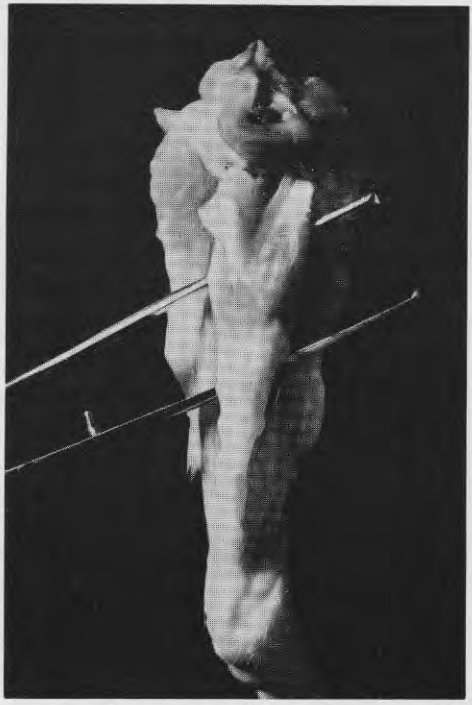

Figure 3-8. A fifty-five day gastrocnemius muscle that had arisen from 3-4 mm proximal stumps. The musclar part of this regenerate is 14 mm long. The distal two-thirds of the regenerate is composed of connective tissue, which had originated by proximal regeneration from the Achilles tendon. (From B. M. Carlson, *Experientia, 30:*275, 1974. Reprinted by permission of Birkhäuser Verlag, Basel.)

phogenesis of these skeletal elements in mammals is subject to control factors about which we are almost completely ignorant.

Development of Function in Regenerating Muscle

From the teleological point of view, the restoration of functional mass in an effective morphological package is the goal of muscle regeneration.

Many aspects of the recovery of function recapitulate quite closely the ontogenetic pattern of development. Studying the minced gastrocnemius muscle of the rat, Carlson and Gutmann (1972) found that the regenerating muscle first contracted to direct stimulation seven or eight days after mincing (Fig. 3-10). Initially, the speed of contraction (e.g. time to peak tension) is very slow, resembling that of newborn muscle. The regenerate progressively contracts more rapidly until by forty days the time to peak tension has returned to almost normal levels.

Much of the developmental speeding of contraction of regenerating muscle occurs before functional neuromuscular connections are established; during the early slow period, the immature regenerating muscle

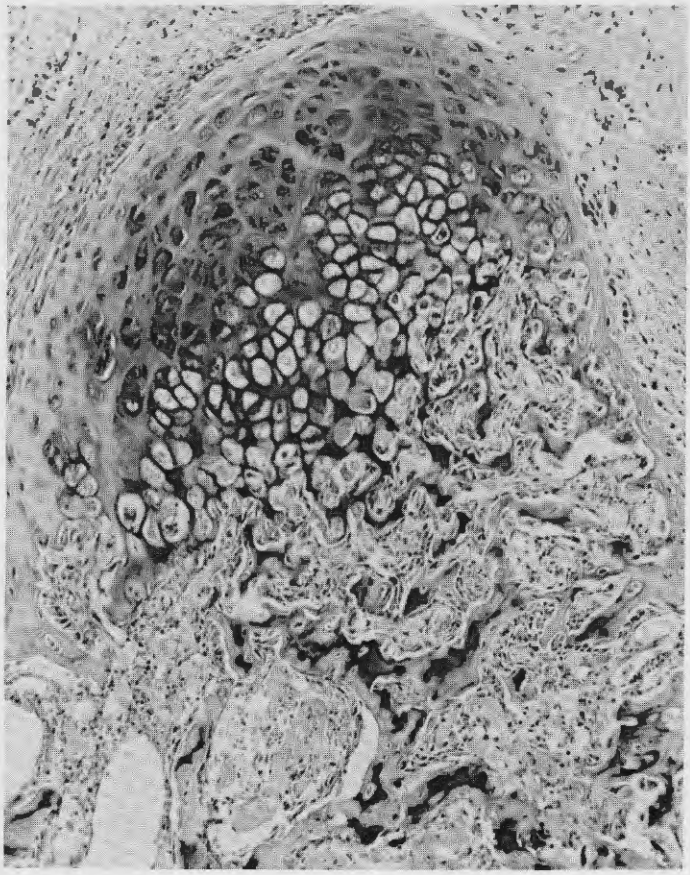

Figure 3-9. A portion of a typical nodule of cartilage and bone found in the distal part of a thirty-two day minced gastrocnemius muscle in the rat. The lower part of the nodule is composed of spongy bone. The sequence of ossification is very similar to that seen in an epiphyseal plate. H & E. (From B. M. Carlson, *The Regeneration of Minced Muscles*, 1972. Courtesy of S. Karger, Basel.)

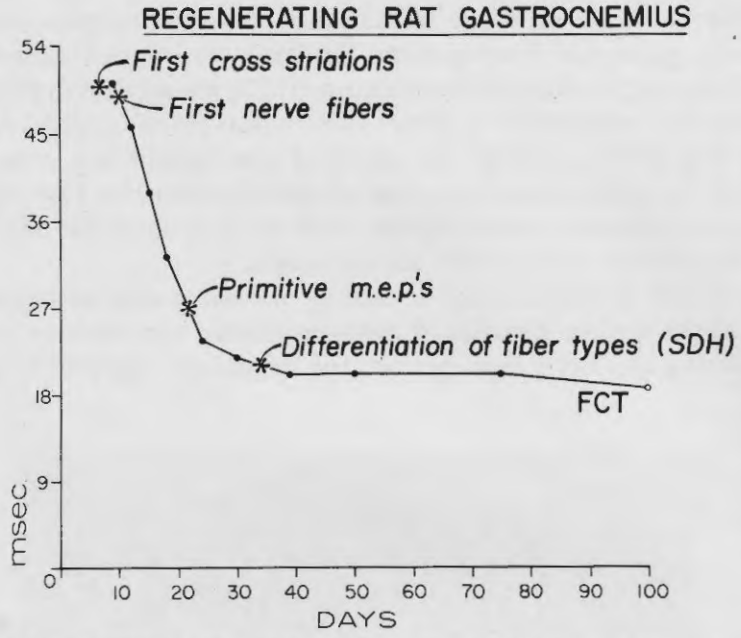

Figure 3-10. Correlation between the development of full contraction time (FCT) and morphological events in the regenerating minced gastrocnemius muscle of the rat.

fibers are characterized by uniformly dark histochemical staining for myosin ATPase activity (Carlson and Gutmann, 1974b). After functional reinnervation has occurred and the speed of contraction has stabilized to normal fast levels, the muscle fibers stain heterogeneously for histochemical ATPase activity, although the muscle fibers are arranged in "type groups" (Karpati and Engel, 1968) rather than the normal checkerboard pattern. Although the time parameters of a contraction in a regenerating minced muscle in the rat return quite closely to normal, the tension generated by the regenerate falls far short (10-20%) of normal, reflecting the reduced mass and increased amount of connective tissue. Minced muscle regenerates in the mouse demonstrate a much better functional recovery (Salafsky, 1971).

In recent years most work on contractile properties of regenerating muscle has been performed on free grafts (Carlson and Gutmann, 1975b; Faulkner et al., 1980). The analysis of contractile properties of free grafts is complicated by the contribution to the overall contraction of the muscle by the thin rim of surviving muscle fibers around the periphery of the graft. The surviving muscle fibers can, however, be eliminated by briefly soaking the muscle in Marcaine at the time of surgery; then an essentially pure population of regenerating muscle fibers can be obtained (Carlson and Gutmann, 1976). For physiological studies, Marcaine-treated free grafts are preferable to minced muscle regenerates, at least in the rat.

The Reinnervation of Regenerating Muscle

Studies on muscle-nerve interrelationships during regeneration first concentrated upon the reactions of regenerating muscle to the presence or absence of functional innervation. Although research on the regenerating muscle fiber as an end organ continues, particularly with respect to trophic effects of the nerve, there has been a recent increase in activity of research on mechanisms of innervation of regenerating skeletal muscle.

Response of Regenerating Muscle to Innervation

There are several strategies for investigating the effects of nerve upon regenerating muscle. One, a basically descriptive approach, is to analyze by morphological, chemical, and physiological approaches the changes in regenerating muscles after neuromuscular connections have been established. Another approach is to isolate the regenerating muscle from a nerve supply and observe the extent to which the regenerating muscle can develop in the absence of nerves. A third approach is to provide the regenerating muscle a nerve different from the type that originally supplied it (e.g. introducing a fast nerve into a slow muscle and vice versa).

Both descriptive studies (Allbrook and Aitken, 1951; Zhenevskaya, 1962; Carlson et al., 1979) and denervation experiments (Betz and Reznik, 1964; Zhenevskaya, 1974) have demonstrated that the degeneration and early regeneration (the establishment of a population of myoblasts and their fusion into myotubes) of muscle can occur in the absence of nerves. Denervation studies have demonstrated species specific variation in the reactions of post-fusion stages of regenerating muscle fibers to the absence of nerves. Regenerating muscle fibers in both the frog (Hsu, 1971) and the mouse (Mufti, 1977) undergo pronounced atrophy and degeneration, leading to their almost complete disappearance by about three weeks after the initiation of regeneration. In the rat, noninnervated regenerating muscle fibers become cross-striated and slowly increase in diameter during the first three or four weeks, but thereafter they undergo a gradual atrophy (Mong, 1977). Noninnervated muscle regenerates in the rat do not develop heterogeneous populations of muscle fibers (myosin ATPase staining), and the contractile properties remain intermediate between those of a fast and a slow muscle (Carlson and Gutmann, 1976). These results of denervation experiments are in agreement with data on the development of free muscle grafts and minced muscle regenerates, in which the maturation of histochemical and contractile properties is completed a week or two after the establishment of functional neuromuscular connections (Fig. 3-10). The early work of Zhenevskaya (1960) showed that the motor component of the nerve is the critical one for the maintenance of regenerating skeletal muscle.

Cross-transplantation studies of fast muscles into the beds of slow mus-

cles and vice versa (Gutmann and Carlson, 1975) have demonstrated the almost complete conversion of a slow muscle into a fast regenerate, but the incomplete conversion of a fast muscle regenerating with slow innervation (Fig. 3-11). The conversion of cross-transplanted regenerating muscles is somewhat better than the conversion of cross-innervated, nonregenerating muscles (Close, 1969). Cross-transplantation of the minced anterior (slow) and posterior (fast) latissimus dorsi muscles of the chicken results in a conversion of fiber types to a pattern characteristic of the host site (Gordon and Vrbová, 1975) even though the same muscles do not undergo conversion upon cross-innervation (Hník et al., 1967). The results of studies involving the transplantation of dystrophic muscles into normal hosts or normal muscles into dystrophic hosts to test the myogenic versus neurogenic hypotheses of muscular dystrophy (Salafsky, 1971; Cosmos, 1973; Neerunjun and Dubowitz, 1974) have not provided easily interpretable results.

In summary, there is substantial agreement in the literature that the early stages of muscle regeneration, including the fusion of myoblasts into myotubes, are independent of innervation. The presence of motor innervation is required for the final stages of functional differentiation and the maintenance of regenerating muscle fibers.

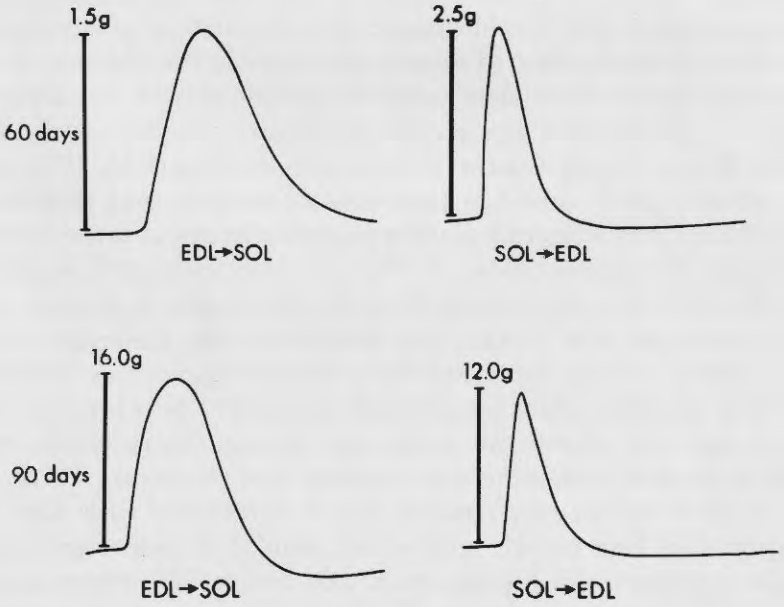

Figure 3-11. Twitch curves showing the transformation of contractile properties of the fast extensor digitorum longus (EDL) muscle cross-transplanted into the bed of the slow soleus (SOL) muscle (left) and the soleus muscle grafted into the bed of the EDL (right). (From B. M. Carlson and E. Gutmann, *Experientia, 30:*1292-1294, 1974. Reprinted by permission of Birkhäuser Verlag, Basel.)

Mechanisms of Reinnervation of Regenerating Skeletal Muscle

There are some pronounced differences between the innervation of embryonic skeletal muscle fibers and the reinnervation of regenerating skeletal muscle. In regeneration, the new muscle fibers regenerate within the confines of the persisting basal lamina that surrounded the original muscle fiber. Acetylcholinesterase activity persists in muscle long after the original muscle fibers have degenerated (Carlson et al., 1979), and it has been shown in frogs that cholinesterase is associated with the original basal lamina in the area of the former neuromuscular junction (McMahan et al., 1978). In many systems of muscle regeneration, muscle fibers are not contacted by nerves until they are in a far more advanced state of differentiation than are embryonic muscle cells.

Evidence from two experimental model systems strongly suggest that the original motor end plate region and more specifically the basal laminae in this area are of great importance in the establishment of new neuromuscular junctions in regenerating muscle. In free muscle grafts of the rat, regenerating nerve fibers enter the graft midway during the second week and form neuromuscular junctions late in the third or early in the fourth week (Carlson et al., 1979). If the zone of original motor end plates is surgically removed from a grafted muscle, nerves enter the graft at the normal time, but the vast majority of them retract from the graft without establishing junctions with the muscle fibers.

The role of the original basal lamina, particularly in the region of the original myoneural junction, has been demonstrated in regenerating frog muscle by McMahan and his colleagues (Marshall et al., 1977; Sanes et al., 1978), who have shown that even in the absence of muscle fibers within the basal laminae, ingrowing axons settle down on the original synaptic sites of the basal lamina. This work, coupled with the recent demonstration of specific antigens concentrated at the synaptic sites of the basal laminae of the muscle fibers (Sanes and Hall, 1979), points to some very specific recognition factors that are involved in the reinnervation of regenerating muscle.

The Regeneration of Muscle Spindles

Muscle spindles are known to degenerate and regenerate after exposure to Marcaine (Milburn, 1976), but their appearance in entire muscle regenerates is variable. Muscle spindles do not reform in minced muscle regenerates (Zelená and Sobotková, 1971; Carlson, 1972), but they have been seen in free muscle grafts in rats (Fig. 3-12; Carlson and Gutmann, 1975a) and cats (Hakelius et al., 1975). The number of muscle spindles regenerated in free grafts does not approach normal values. The reason for this is not understood, but it is possible that some of the spindles have undergone irreversible degenerative changes.

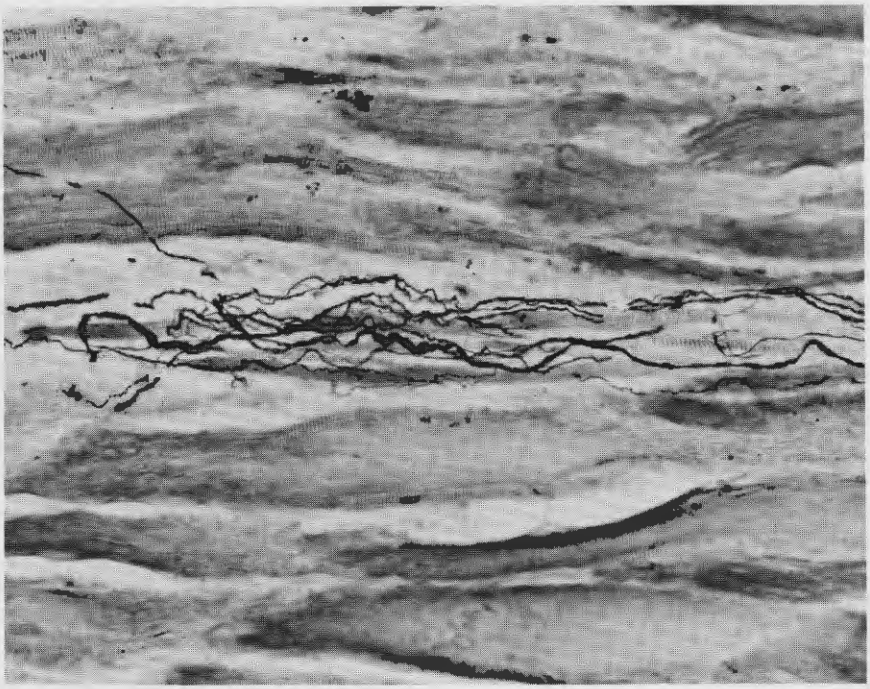

Figure 3-12. Regenerated muscle spindle within a freely grafted extensor digitorum longus muscle in the rat. Silver-stained nerve fibers (black) form a network around the small intrafusal muscle fibers of the spindle.

In normal embryonic development the formation of muscle spindles is induced by sensory nerve fibers growing into the muscle during the late fetal period (Zelena, 1957). The relationship between nerves and the regeneration of muscle spindles was tested by grafting muscles into denervated limbs of rats (Rogers and Carlson, 1980). Numbers of spindles equal to that found in reinnervated grafts were found, demonstrating that nerves are not required for their regeneration.

The critical factors in the regeneration of muscle spindles are still incompletely understood, but it appears that satellite cells present within the original spindles serve as the source of the myoblasts that form the new intrafusal fibers. It seems quite likely that the spindle capsule is a critical factor in the regeneration of muscle spindles and that it serves as a scaffolding for the organization of the new intrafusal fibers. Regenerating muscle spindles become reinnervated (Fig. 3-12), but the extent to which the pattern of innervation is normal has not yet been determined. There is some electrophysiological evidence for the return of sensory function in muscle grafts (Rogers and Quick, unpublished), but the sensory function cannot be definitely assigned to spindles or tendon organs.

Free Grafting of Entire Muscles

The free grafting of skeletal muscles is not only an experimental laboratory model of muscle regeneration, but it is also used clinically in the treatment of some conditions characterized by neuromuscular deficiency. Because of its potential clinical relevance, some of the major features in the development of a free muscle graft will be described here. Most of the experimental material will be based upon work conducted on the rat.

Free muscle grafts follow a characteristic pattern of degeneration and regeneration (Fig. 3-13). Within hours after grafting, the muscle becomes

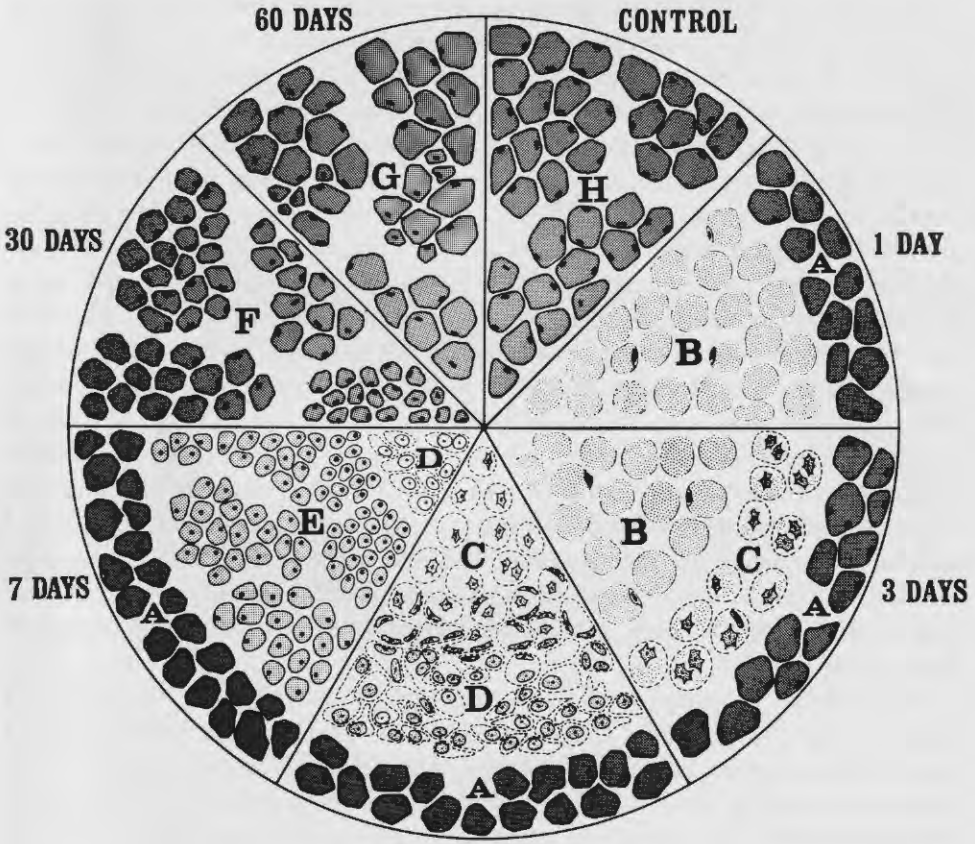

Figure 3-13. Schematic diagram of the postoperative course of a freely grafted extensor digitorum longus muscle in the rat. Days indicated are days after grafting. A. Peripheral surviving muscle fibers. B. Muscle fibers in a state of ischemic necrosis. C. Ischemic muscle fibers being broken down by flood-borne phagocytes. D. Early regenerating muscle cells within the basal laminae of original muscle fibers. E. Early regenerating myotubes and muscle fibers. F., G. Later stages of muscle fiber regeneration. (From F. M. Hansen-Smith and B. M. Carlson, *J. Neurol. Sci., 41*:149, 1979. Reprinted by permission of Elsevier/ North-Holland Biomedical-Press of Amsterdam.)

subdivided into two zones: a large central ischemic core and a thin peripheral rim of surviving muscle fibers that are apparently able to be maintained through diffusion. Within the ischemic muscle fibers, the myonuclei become pyknotic and die within hours, whereas satellite cells survive for a longer period (Snow, 1977a; Carlson et al., 1979; Hansen-Smith and Carlson, 1979). The length of time that satellite cells can survive in ischemic muscle is not known. Within the zone of ischemia the muscle fibers undergo a certain degree of intrinsic degeneration and then remain in that condition until they become invaded by phagocytic cells brought in by the ingrowing blood supply.

Immediately after being grafted, the muscle is completely divorced from the general circulation, and the blood vessels within the graft degenerate as well. Within twenty-four hours the original capillaries have disappeared, and the larger vessels are themselves ischemic (Hansen-Smith et al., 1980). Within a couple of days new blood vessels begin to grow into the graft from multiple foci around the periphery. The ingrowing vessels, at first sinusoidal in nature, grow past the surviving peripheral muscle fibers and begin to penetrate the central ischemic region. Macrophages, brought in with the new blood vessels, penetrate the basal laminae of the ischemic muscle fibers and remove the myofibrils and other sarcoplasmic debris, leaving in their wake the original basal laminae of the muscle fibers, which then contain a population of spindle-shaped myogenic cells and residual phagocytic cells (Fig. 3-13, three days). The wave of phagocytic removal of ischemic muscle proceeds inward in pace with the progressive penetration of the graft by the blood vessels, some of which make secondary connections with portions of larger degenerated original vessels. As removal of sarcoplasmic debris within a muscle basal lamina nears completion, the regeneration of new muscle begins, with the activation of the myogenic cells into myoblasts and their subsequent fusion to form myotubes.

The cellular activity in early muscle grafts is reflected in their wet weights (Fig. 3-14). Early after grafting, the graft weighs more than its normal counterpart because of edema. The edema is likely brought on by increased osmotic pressure due to the presence of breakdown products within the graft. The weight of a graft falls precipitously during the period of phagocytic removal of the ischemic muscle fibers. After that time it gradually increases with the regeneration of new muscle fibers, but a typical muscle graft attains only one-third to one-half the weight of a normal muscle.

By the time the entire thickness of the graft has been vascularized (Fig. 3-13, seven days), the graft consists of the peripheral rim of surviving muscle fibers (by this time decreased in size because of the denervation) and a central zone of regenerating muscle fibers in the late myotube or

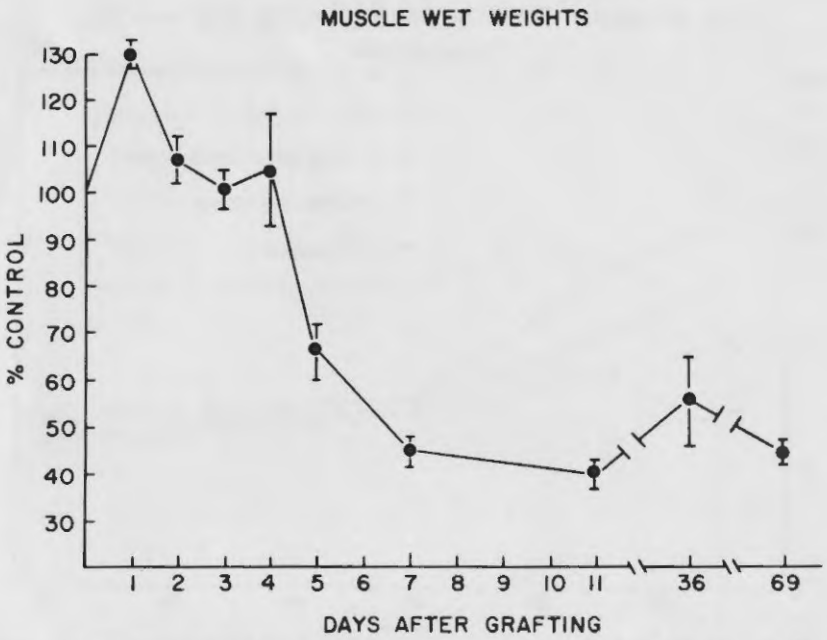

Figure 3-14. Wet weights of free grafts of the extensor digitorum longus muscle in the rat, expressed as percent of weight of control muscle. (From K. R. Wagner, B. M. Carlson, and S. R. Max, *J. Neurol. Sci., 34*:373, 1977. Reprinted by permission of Elsevier/North-Holland Biomedical-Press of Amsterdam.)

early cross-striated stage. Regenerating nerve fibers have just started to enter the graft, but contact has not yet been made with the regenerating muscle fibers. From this stage, development of the graft is highlighted by the intrinsic differentiation of the muscle fibers, their receiving functional innervation and then the nerve-dependent final stages of differentiation of the muscle fiber into heterogeneous populations of structurally, chemically, and functionally distinct types. In standard muscle grafts reinnervation is typically incomplete, but details of the fate and integration of nondirectly innervated muscle fibers into the functional mass of the graft are not well understood. Little is also known about functional maturation of the new vasculature of a muscle graft. Recent work on grafted cat muscles suggests that the resting blood flow of early grafts is higher tban normal and that the regulation of blood flow is less effective than it is in normal muscle (Faulkner, unpublished). The structural correlates of these functional observations have not been examined.

The contractile properties of free muscle grafts are complex because a graft is composed of two different populations of muscle fibers — surviving original muscle fibers and newly regenerating ones — with quite different contractile properties. The pattern of time-to-peak contraction times differs with different models of muscle grafting (Fig. 3-15). A stan-

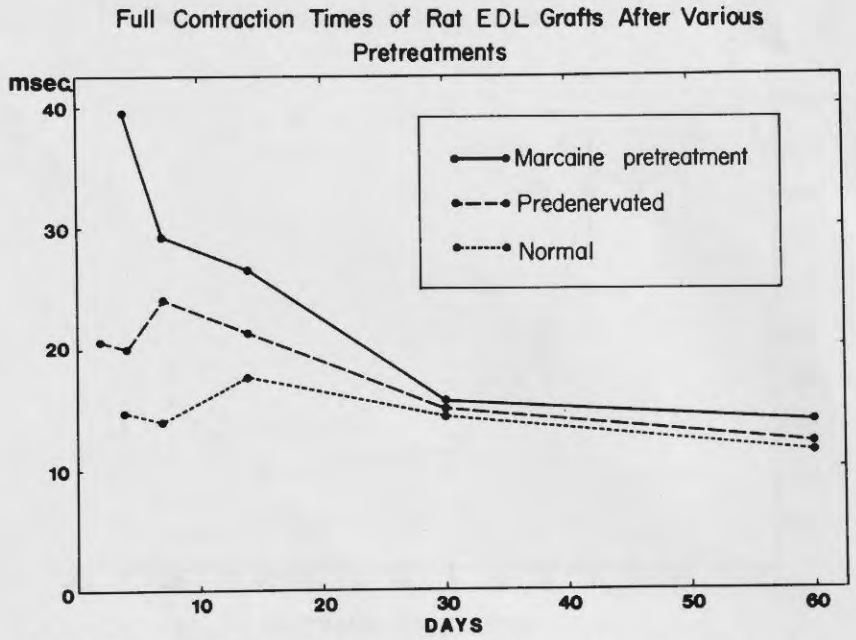

Figure 3-15. Full contraction times (time-to-peak tension) of rat muscle grafts after various pretreatments. (From B. M. Carlson, F. M. Hansen-Smith, and D. K. Magon, *in* A. Mauro and R. Bischoff (eds.), *Muscle Regeneration,* 1979. Courtesy of Raven Press, New York.)

dard muscle graft does not contract during the earliest post-grafting period. The earliest contractions are due entirely to the contribution of the surviving muscle fibers. As a result the contraction times of earlier grafts are not much different from those of a normal muscle. Within a few days the regeneration muscle fibers acquire the ability to contract, and their weak contribution of slowly contracting muscle fibers causes the overall speed of contraction of the graft to become slower. Then, as the regenerating muscle fibers mature and functional reinnervation occurs, the speed of contraction of the graft progressively approaches normal values. If a muscle has been predenervated for a couple weeks prior to grafting, the contractile characteristics of early grafts are altered because of the denervation. This causes the survival of a larger number of muscle fibers, which possess the contractile properties of dennervated, rather than normal, muscle. The first change is the earlier appearance of contractile ability of the graft. This has been correlated with changes in the resting membrane potential of the surviving denervated muscle fibers within the graft (Vyskočil et al., 1974). In addition to the earlier onset of contraction, the surviving muscle fibers in grafts of predenervated muscle contract more slowly than normal, as is characteristic of denervated fast muscle fibers (Gutmann et al., 1972).

The later phases in the development of contraction times in predenervated grafts are similar to those of standard grafts. If all of the muscle fibers of the graft are caused to degenerate by treating the muscle with Marcaine, the contraction times of the graft reflect those of a pure population of regenerating muscle fibers.

The contractile tension of muscle grafts is relatively low in both rats and cats, averaging somewhat over one-third that of the normal muscle (Carlson and Gutmann, 1975b; Faulkner et al., 1980). The twitch-tetanus ratios are low in immature regenerates, but they approach normal values as the grafts mature. Muscle grafts in the cat become fatigued to repeated stimulation more rapidly than normal muscles (Fig. 3-16).

Clinical Applications for Free Muscle Grafting

Since its introduction to clinical practice in 1971 (Thompson, 1971), free muscle grafting has been employed in the treatment of several varieties of neuromuscular deficit (Thompson, 1974; Hakelins, 1974). The most com-

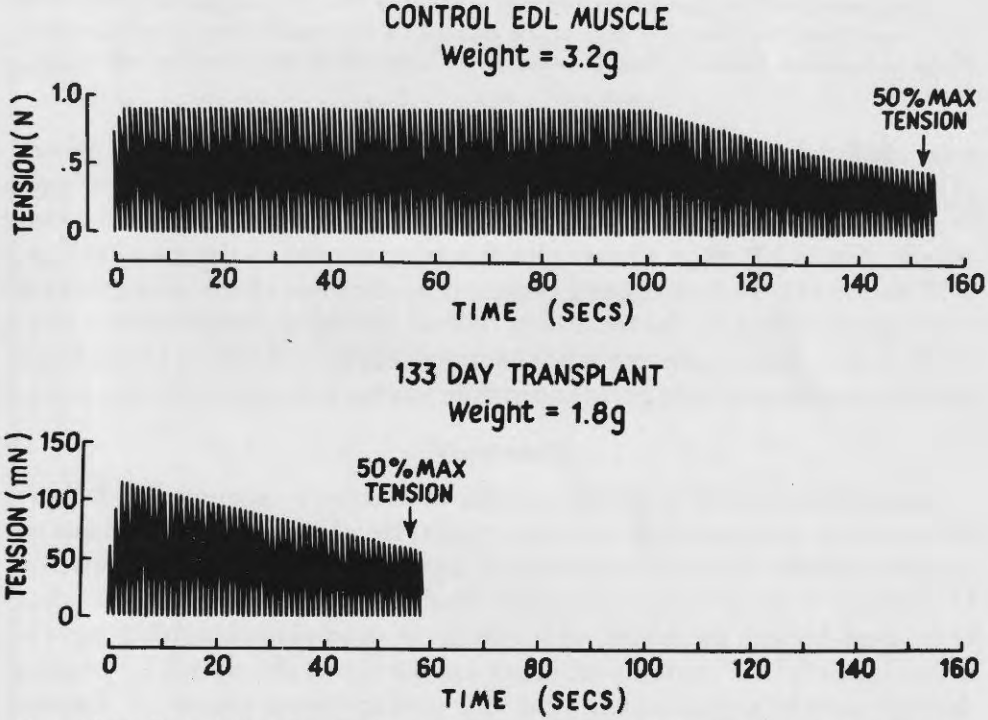

Figure 3-16. Fatigability of grafted versus control extensor digitorum longus muscles in the cat. When stimulated for 200 nms/s at 70 Hz, the maximum isometric tetanic tension in the graft declined to 50 percent of maximum more quickly than did that of the control muscle. (From J. A. Faulkner et al., *Am. J. Physiol., 238:*C120, 1980. Reprinted by permission of the American Physiological Society.)

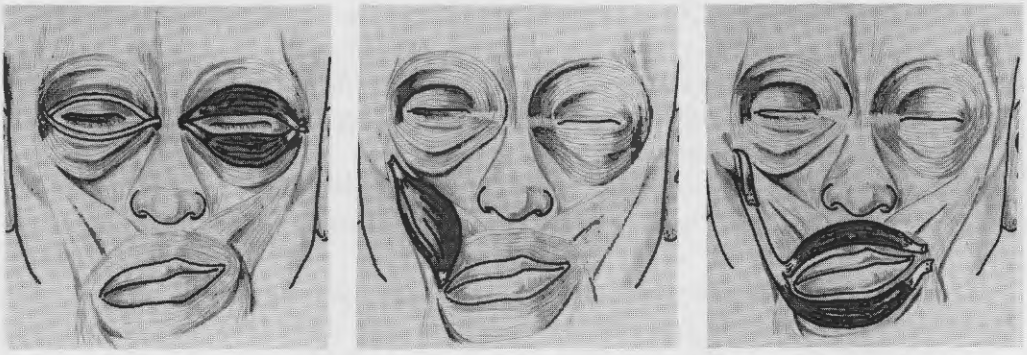

Figure 3-17. Muscle transplantation used to treat various problems related to facial paralysis. *A.* Split muscle grafts placed over normal orbicularis oculi muscle; crossed tendons are passed through the nose and attached to the eyelids on the paralyzed side of the face. The graft is reinnervated by sprouting from the underlying normal orbicularis oculi muscle, resulting in the graft contracting synchronously with the normal muscle. The pull on the tendons of the graft closes the eyelids on the paralyzed side of the face. *B.* Muscle graft placed in contact with a partially denervated muscle to assist in elevating the corner of the mouth. *C.* Split muscle graft placed on the orbicularis oris muscle and a tendon leading to the zygomatic arch on the denervated side of the face. The graft is innervated by nerve fibers sprouting from the right half of the orbicularis oris muscle, which is innervated. (From L. Hakelius, *Scand. J. Plast. Reconstr. Surg., 8*:220, 1974. Reprinted by permission.)

mon application of muscle grafting is in the treatment of facial palsies (Thompson, 1977; Bogdasarian and Olson, 1979). Techniques have been developed to compensate for loss of function of the orbicularis oculi muscle (Fig. 3-17) or to elevate the drooping corner of the mouth (Figs. 3-17 and 3-18). The next most common application of the free grafting technique has been in the treatment of anal sphincter incompetence (Fig. 3-19). Free muscle grafting has not been successful in the limb, where large muscles are involved and great contractile strength is required of the graft.

Summary

Mammalian skeletal muscle is capable of extensive regeneration following a variety of types of injury. One model by which massive amounts of muscle regeneration can be obtained is the free grafting of entire muscles. In addition to its utility in laboratory studies, muscle transplantation has been used for the treatment of a variety of conditions involving neuromuscular deficit in man. Little is yet known about the details of human skeletal muscle regeneration, and the postoperative course of human muscle grafts remains poorly understood.

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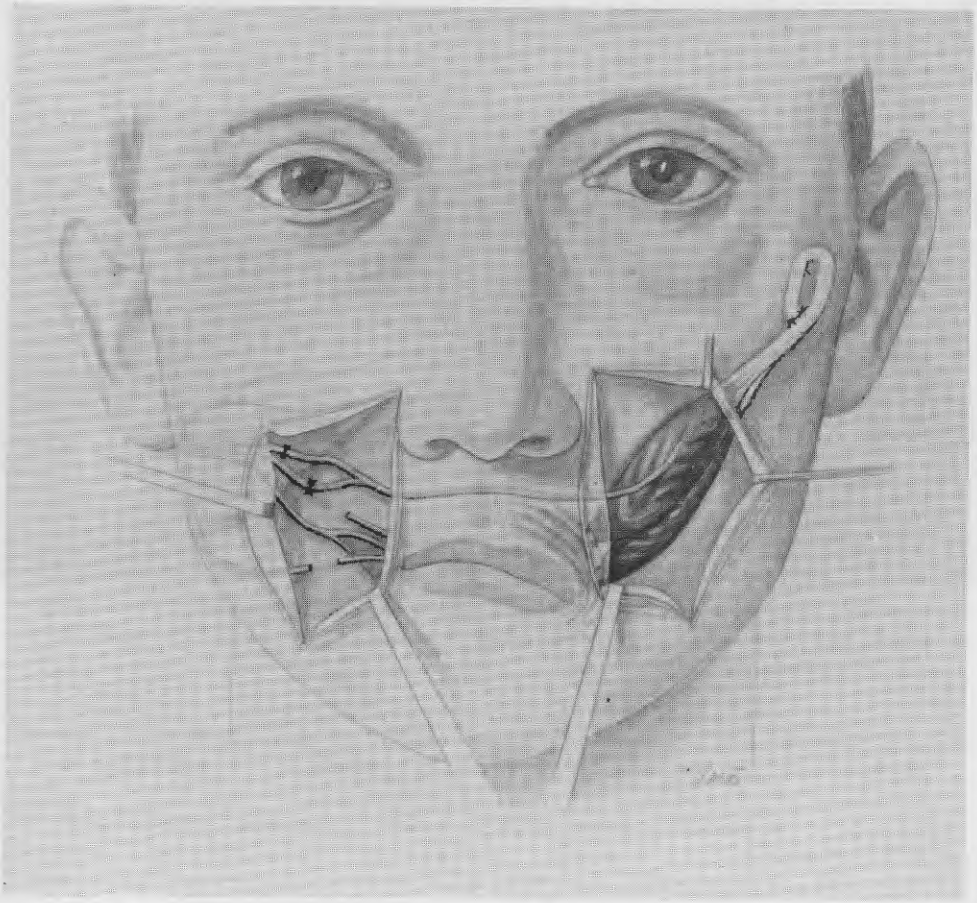

Figure 3-18. Drawing illustrating the technique of placing a sural nerve graft across from the innervated side of the face to the paralyzed side and, after nerve fibers have regenerated through the nerve graft, placing a muscle graft between the corner of the mouth and the zygomatic arch. (From G. Freilinger, *Plast. Reconstr. Surg., 56:*44, 1975. Courtesy of Prof. G. Freilinger.)

Figure 3-19. Drawing illustrating one means of free-grafting to restore deficient anal sphincter function. (From L. Hakelius et al., *J. Ped. Surg.*, *13*:77, 1978. Reprinted by permission of Grune & Stratton, Inc.)

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