

The effects of temporary ischaemia on rat muscle spindles

F. H. DIWAN* AND ALICE MILBURN

Department of Zoology, University of Durham, Science Laboratories, South Road, Durham, DH1 3LE, UK

SUMMARY

Soleus muscle of adult rat is revascularized 5–8 days after sectioning the supplying blood vessels. The temporary ischaemia thus produced results in the rapid concomitant degeneration of extra- and intrafusal muscle fibres along with their nerve terminals and supplying axons.

The basal lamina of all muscle fibres usually remains intact throughout the degenerative phase. Necrotic sarcoplasm is removed by phagocytic cells. Satellite cells survive the temporary ischaemia and give rise to presumptive myoblasts which fill the basal-lamina tubes. These myoblasts fuse to form myotubes which, by the 14th day after devascularization, are maturing into muscle fibres in the absence of any innervation. Within the spindle, nuclear-bag fibres degenerate more rapidly than nuclear-chain fibres. Regeneration proceeds more rapidly within the basal-lamina tubes of the original bag fibres than within those of the chain fibres.

Reinnervation of regenerating extra- and intrafusal fibres begins 21 days after devascularization and is completed some 7 days later, during which time further equatorial differentiation of some reinnervated intrafusal fibres may occur.

Regenerated spindles vary considerably with respect to their innervation and equatorial nucleation. Most contain short, thin, additional muscle fibres as well as those that have regenerated within the basal-lamina tubes of the original fibres.

INTRODUCTION

The capacity of a skeletal muscle to regenerate following injury was first recognized more than a century ago (e.g. Waldayer, 1865), although it was not the subject of intensive study until more recently (see reviews by Carlson, 1973; Reznik, 1973; Carlson & Faulkner, 1983). The first study of the fate of intrafusal muscle fibres in regenerating muscle showed that they fail to regenerate in reinnervated minced-muscle grafts of rat (Zelená & Sabotková, 1971). In contrast to this, both reinnervated and non-reinnervated freely grafted transplanted muscles of rat contain spindles (Carlson & Gutmann, 1975; Schmalbruch, 1977; Rogers & Carlson, 1981; Rogers, 1982).

As a model for the study of muscle fibre degeneration and regeneration, standard muscle grafting has the major disadvantage that the neuromuscular pathways and connections are disrupted at the time of grafting when the nerve is cut; reinnervation of the regenerating graft is thus difficult to control. Because of

* Author's address: Department of Biology, College of Science, University of Basrah, Iraq.

this, techniques were introduced that produced the destruction of whole skeletal muscles *in situ*, without any uncontrollable damage to the nerve supply. It was the application of one such technique, the intramuscular injection of a myotoxic local anaesthetic, that led to the first ultrastructural study of the processes involved in the degeneration and regeneration of mammalian intrafusal muscle fibres (Milburn, 1976). The disadvantage of this technique for the study of spindle regeneration lies in its failure to affect the entire spindle population of an individual muscle following a single intramuscular injection. More recently grafting techniques have been refined and combined with the topographical application of myotoxic local anaesthetics to effect the destruction of entire spindle populations in grafted muscles in which the nerve supply is kept intact (Rogers, 1982).

Temporary ischaemia is known to initiate the degeneration and subsequent regeneration of extrafusal muscle fibres (e.g. Karpati, Carpenter, Melmed & Eisen, 1974), and the cause of the initial degeneration on which the grafted-muscle model and, possibly, the local-anaesthetic model, depend is thought to be ischaemic in origin (Hall-Craggs, 1979). These models are mimicked by devascularization of the muscle (Hall-Craggs, 1978).

The advantage of the ischaemia model, like the nerve-intact graft, is that innervation pathways in the muscle are left intact, although, as with myotoxic local anaesthetics, some temporary peripheral damage to the axons and neuromuscular connections does occur. Reinnervation of the regenerating spindle is therefore likely to be less random, allowing the use of differential denervation techniques to investigate the effects of the various reinnervating axons on intrafusal-fibre regeneration.

This study examined the processes involved in the degeneration and regeneration of rat spindles following temporary ischaemia, produced by the devascularization of soleus muscle. A brief description of this work has been reported elsewhere (Barker, Diwan & Milburn, 1982; Barker & Milburn, 1984).

MATERIALS AND METHODS

Devascularization technique

Adult male Wistar rats (initial weight 200–250 g) were used. Hall-Craggs' (1978) procedure for muscle devascularization was modified to provide optimum conditions for muscle revascularization and reinnervation, as follows.

A 2 cm-long incision was made on the medial surface of the right lower hindlimb of anaesthetized rats, and the soleus muscle exposed. The connective tissue attachments were dissected and the nerve and blood vessels in the neurovascular pedicle carefully separated from one another for a distance of approximately 1 cm from the muscle. All supplying blood vessels were then cut, taking care not to disturb the nerve supply. In addition the blood vessels passing through the tendon were isolated and cut. Any bleeding that ensued was halted by the application of pressure before the wound was sutured, sprayed with plastic dressing and dusted with sulphamethoxypridazine (Lederkyn, Cyanamid Ltd).

Using this technique, all but three or four superficial layers of extrafusal fibres are subjected to temporary ischaemia. Even though the spindles of rat soleus are distributed throughout the muscle (Yellin, 1969), serial transverse sections have shown that the most superficial spindles are

separated from the periphery by several layers of extrafusal fibres. Hall-Craggs' (1978) supplementary lesions, which eliminate the superficial fibres, by ligation of the tendons and soaking the muscle in bupivacaine, were therefore not considered necessary for this study.

Processing of tissue

Normal and experimental soleus muscles were fixed, slightly stretched, in a solution of 0.02 % paraformaldehyde and 2.5 % glutaraldehyde in 0.2 M-cacodylate buffer, pH 7.3. After 30 min, the muscles were cut into small pieces and fixed for a further 90 min in fresh fixative, followed by 2 h postfixation in 0.1 % osmium tetroxide in cacodylate buffer, before dehydration and embedding in Araldite.

Soleus muscles of two animals, killed 4 days and 28 days after muscle devascularization, were processed intact for serial sectioning for light microscopy.

Examination of spindles

Spindles were identified in 1 μ m transverse sections stained with 1 % toluidine blue. Once located, ultrathin sections were examined with the electron microscope at 20–50 μ m intervals along the remaining length of the spindle. The ultrastructure of some spindles (see below) was examined in longitudinal sections. All ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds, 1963).

Number of animals and spindles

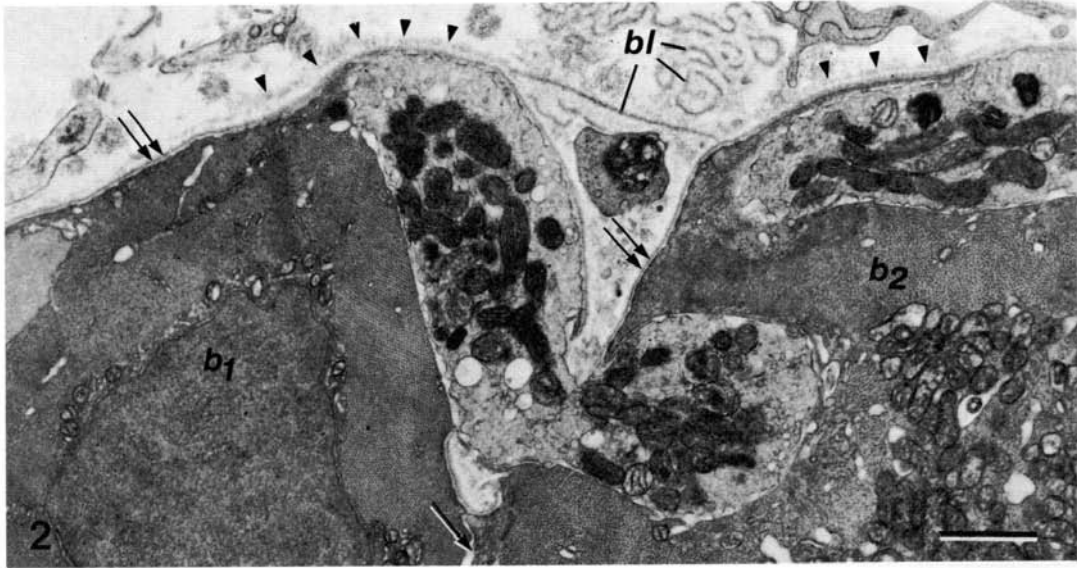
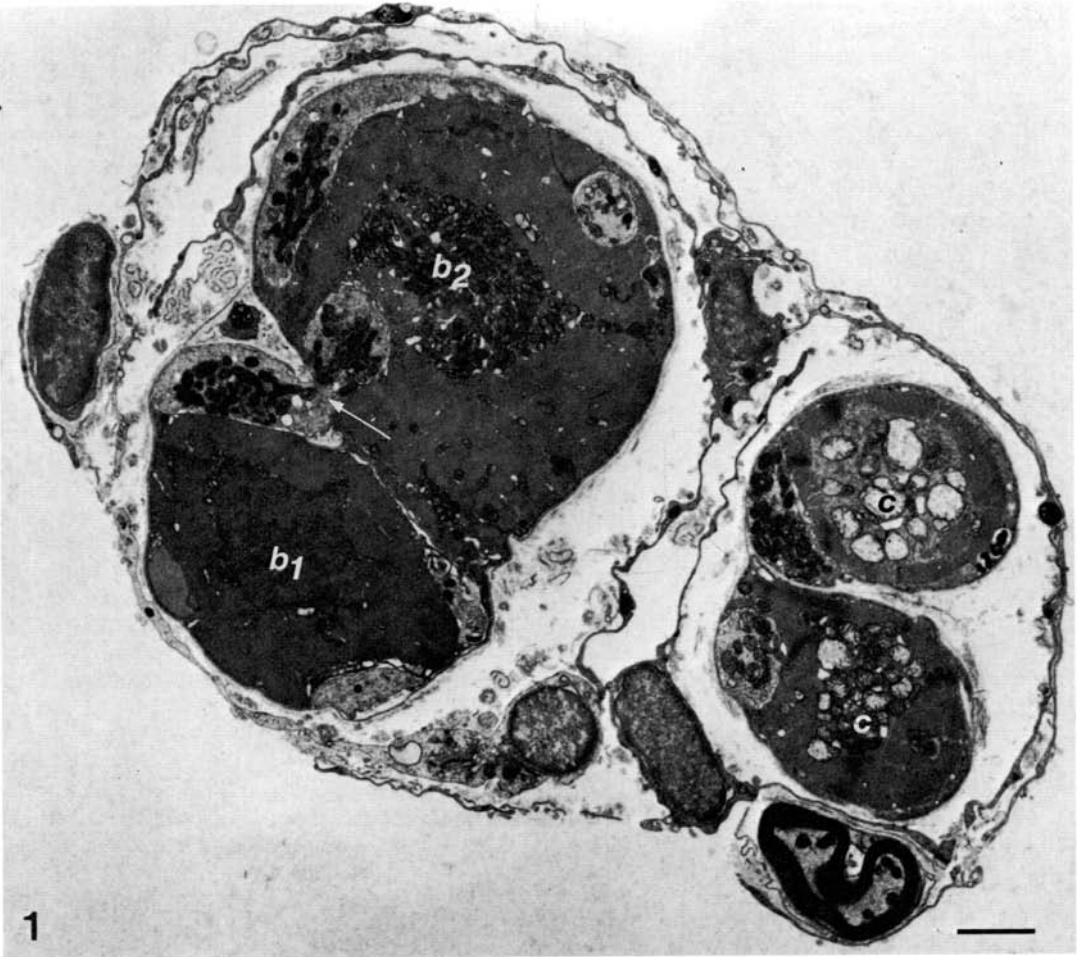
Devascularization of the right soleus muscle was carried out in 49 rats. The experimental muscle from two to five animals was removed at the following postoperative intervals: 30 min; 1, 2, 3, 6 and 12 h; 1, 2, 3, 4, 5, 6, 8, 14, 21, 28, 42, 56, 119, 156, 182 and 364 days. In addition two control soleus muscles were processed for electron microscopic study of the normal spindle. Altogether 64 experimental spindles and 6 normal spindles were examined in transverse and/or longitudinal section. Longitudinal sections of a total of 16 whole or part spindle poles (sps) were examined at the following stages: controls (2sps), 4 day (1sp), 8 day (1sp), 14 day (1sp), 42 day (2sps), 56 day (6sps), 119 day (2sps), 182 day (1sp). In addition, longitudinal sections of 3 spindle equators were examined at the 56-day stage.

RESULTS

Control muscle

Normal soleus spindles usually contain four intrafusal muscle fibres, one bag₁ (b_1), one bag₂ (b_2) and two nuclear-chain (c) fibres (Fig. 1). The b_2 fibre is the thickest and longest fibre; the b_1 fibre is slightly shorter and significantly thinner than the b_2 . The thin, short c fibres usually end within the limits of the capsule.

The association between b_2 and c fibres that is reported to be a common feature of the equatorial region of cat spindles (Barker *et al.* 1976) is not a feature of spindles from rat soleus. In contrast, a close association between the two c fibres, and a more intimate and common association between the two bag fibres, is seen at the polar ends of the myotube region (Figs 1, 2). The b_2 and b_1 fibres share a common basal lamina in this region (Fig. 2) and a satellite cell is usually wedged between the two fibres. Sensory nerve terminals that innervate the b_2 and b_1 fibres in this area are often connected by a band of axoplasm (Fig. 2). Such cross-terminals are not seen between the two associated fibres, although common c sensory terminals may be present. The association between the two c fibres is less intimate than that of the bag fibres, consisting of their enclosure in a separate



compartment of the axial sheath (Fig. 1). Only occasionally are the *c* fibres contained within a common basal lamina. In addition to basal lamina, all intrafusal muscle fibres are surrounded by a thin, discontinuous but discrete reticular lamina (Sanes, Marshall & McMahan, 1978) at the spindle equator (Fig. 2).

This study confirmed that normal rat intrafusal muscle fibres differ with respect to the size and distribution of their mitochondria and sarcotubular systems, as well as the condition of the M-line (Barker *et al.* 1976; Banks, Harker & Stacey, 1977; Kucera, Dorovini-Zis & Engel, 1978). The myofibrils of *b*₁ fibres display M-lines in the extreme extracapsular polar region only (Kucera *et al.* 1978). Bag₂ fibres contain a larger equatorial bag of nuclei (three to four abreast) than *b*₁ fibres (two to three abreast) and lack M-lines at the spindle equator only.

All intrafusal muscle fibres receive terminals from a Ia axon. Every intrafusal muscle fibre also receives at least one motor nerve terminal in one or other of its poles. The neuromuscular junctions of *c* fibres display postjunctional folds and well-developed sole-plates. Two types of motor end-plate were identified in *b*₁ fibres. Those lying within the limits of the capsule lack postjunctional folds but usually display some postjunctional specialization of the sarcoplasm. In contrast, those lying in the extracapsular polar regions have deep, unbranched post-junctional folds and well-developed sole-plates (Kucera *et al.* 1978). All *b*₂ motor end-plates are intracapsular in position, generally lack postjunctional folds and have poorly developed sole-plates.

Experimental muscle

Extrafusal muscle fibres

During the first five days after devascularization, extrafusal muscle fibres of soleus undergo a process of degeneration that begins as early as 30 min with the accumulation of enlarged mitochondria at the periphery of some fibres. At 3 h, disruption and dissolution of the plasmalemma, loss of Z-lines from some myofibrils, nuclear pyknosis and changes in the sarcotubular system are widespread in muscle fibres at the periphery of the muscle.

The invasion of necrotic muscle fibres by phagocytic cells begins as early as 6 h after devascularization, when interstitial inflammation and oedema are widespread. By day 3, phagocytosis of necrotic sarcoplasm is completed in the peripheral extrafusal muscle fibres and the phagocytes withdraw from the

Fig. 1. Low-power electron micrograph of a transverse section of a *normal rat soleus spindle* at the end of the myotube region. Note the association between the large and small bag fibres (*b*₂ and *b*₁) and between the two chain fibres (*c*), which lie in a separate compartment of the axial sheath. The arrow indicates a sensory cross-terminal between the bag fibres. Scale bar, 2 µm.

Fig. 2. High-power electron micrograph of the sensory cross-terminal shown in Fig. 1. Note that the common basal lamina (double arrows) of the bag₁ (*b*₁) and bag₂ (*b*₂) fibres is absent from their apposed surfaces (single arrow). Arrowheads indicate a discontinuous reticular lamina. *bl*, basal lamina profiles in the periaxial space. Scale bar, 1 µm.

preserved basal-lamina tubes of the degenerated fibres. The degeneration of extrafusal muscle fibres lying at the core of the muscle lags behind that of the peripheral layers as in muscle grafts (see Carlson & Faulkner, 1983), but is generally completed by day 5.

In common with other studies of mammalian skeletal muscle regeneration (see Carlson & Faulkner, 1983), the basal lamina of extrafusal muscle fibres survives temporary ischaemia. Disruption of the lamina was observed in only a few fibres, more often at the time of phagocytic invasion than at the earlier stages of degeneration.

The changes that occur at extrafusal motor end-plates following devascularization are similar to those reported after denervation (e.g. Miledi & Slater, 1970). The axon terminals retract from the myoneural junction, become enclosed by Schwann-cell processes and undergo structural changes to form 'honeycomb' profiles. The subneural sarcoplasm degenerates, whereas the junctional basal lamina remains intact and folded, reflecting the pattern of the original post-junctional folds.

Satellite cells survive temporary ischaemia and give rise to presumptive myoblasts within the basal-lamina tubes. The disintegration of the basal lamina in some fibres appears to free satellite cells into the interstitial space. At 3–4 days after devascularization, regenerating extrafusal fibres consist of bundles of myoblasts and nascent myotubes, which, by day 5, have fused to form distinctive myotubes, with central myonuclei. By day 14 these myotubes have differentiated into thin muscle fibres, most of which retain central nuclei. From day 21 onwards, the muscle fibres increase in diameter and most nuclei become subsarcolemmal. Satellite cells are present at all stages.

Small, immature muscle fibres are found in the interstitial space at the later stages of regeneration. They may arise from the division of satellite cells that appeared to migrate from degenerating extrafusal muscle fibres through the disruptions in the basal lamina.

Reinnervation of regenerating extrafusal muscle fibres was first seen at day 21 and is completed about 7 days later. The regeneration of subneural sarcoplasmic specializations, typical of sole-plate sarcoplasm precedes reinnervation of the end-plate, as in regenerating amphibian muscle (Sanes *et al.* 1978; Burden, Sargent & McMahan, 1979; Bader, 1981). Some muscle fibres fail to receive any innervation and show signs of denervation atrophy at the later stages. In this study, no evidence was seen of the later establishment of new ectopic neuromuscular junctions as reported in freely grafted extensor digitorum longus (EDL) muscle of rat (Hansen-Smith, 1983).

Muscle spindles

General. Devascularization of soleus muscle also produces ischaemic effects in muscle spindles. Intrafusal muscle fibres degenerate in the 3 days following devascularization and in the following 2 days, when revascularization occurs, regeneration begins. The nuclear-bag fibres degenerate more rapidly than the

chain fibres, and among the bag fibres the degeneration of b_2 fibres precedes that of the b_1 fibres.

All sensory nerve terminals degenerate completely, whereas the motor nerve terminals retract, withdraw from the intrafusal muscle fibre and degenerate. Necrotic sarcoplasm and axoplasm are removed by phagocytes.

Satellite cells, the capsule and axial-sheath cells and the basal lamina of the intrafusal muscle fibres all survive temporary ischaemia.

Reinnervation of the regenerating intrafusal muscle fibres occurs at 21–28 days after devascularization. Since the regenerating fibres lack many of the characteristics of normal intrafusal muscle fibres, they are designated as ‘thick’ and ‘thin’ fibres, representing fibres that have regenerated within the basal-lamina tubes of the original bag and chain fibres, respectively. The regenerated spindles vary considerably in structure and innervation. Apart from ‘thick’ and ‘thin’ regenerated muscle fibres, most contain short, aberrant fibres, named ‘additional’ fibres.

The main stages in the degeneration and regeneration of intrafusal muscle fibres, and the variation in the structure and sensory innervation of the regenerated spindles are illustrated schematically in Figs 16, 23.

Not all spindles are affected to the same extent at any one particular stage. The following detailed observations are therefore generalized for each stage.

0–3 days. As a general rule, changes in intrafusal muscle fibres in the 3 days following devascularization are similar to those occurring in the extrafusal fibres. The early changes are not related to any particular type of intrafusal muscle fibre, but from 6 h onwards, nuclear-bag fibres degenerate more rapidly than nuclear-chain fibres (Figs 3–6).

Some changes are seen as early as 30 min after devascularization. Mitochondria enlarge and by 2 days only remnants of cristae are seen (Figs 4–6). The rod-like inclusions seen at the earlier stages of mitochondrial degeneration (Stenger, Spiro, Scully & Shannon, 1962; Reznik & Hansen, 1969) have also disintegrated by 2 days.

Changes in the plasmalemma are similar to those described in degenerating extrafusal muscle fibres (Jirmanová & Thesleff, 1972; Karpati *et al.* 1974; Mäkitie & Teräväinen, 1977). Disintegration of the plasmalemma is seen at 3 h in some intrafusal muscle fibres. This initial degeneration is not related to the degree of damage to the myofibrils: the plasmalemma of otherwise well-preserved muscle fibres is sometimes absent, while it may be intact in extensively damaged fibres. By 2 days the plasmalemma is usually absent (Figs 5, 6) or reduced to an occasional strand. In the myotube region of the spindle, where the b_1 and b_2 fibres are closely associated (Fig. 1), dissolution of the apposed plasmalemmae leads to the mixing of the necrotic sarcoplasm of the paired bag fibres.

The basal lamina of the intrafusal muscle fibres usually survives temporary ischaemia (Figs 4–6) and subsequently plays an important role in regeneration. In some intrafusal muscle fibres examined at 3–6 h, the basal lamina had degenerated in some regions, prior to phagocytic invasion. Ruptures in the basal lamina were

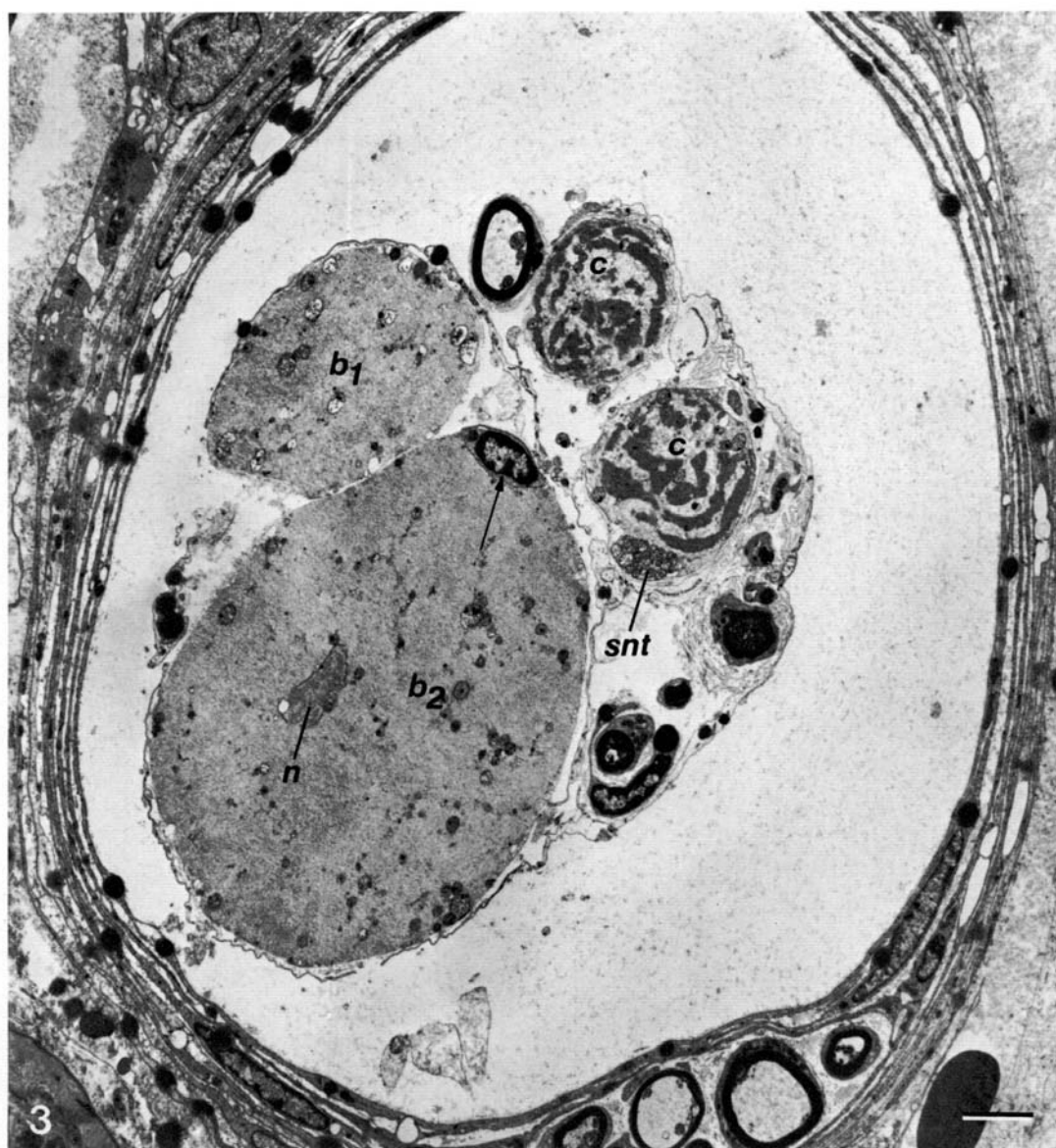


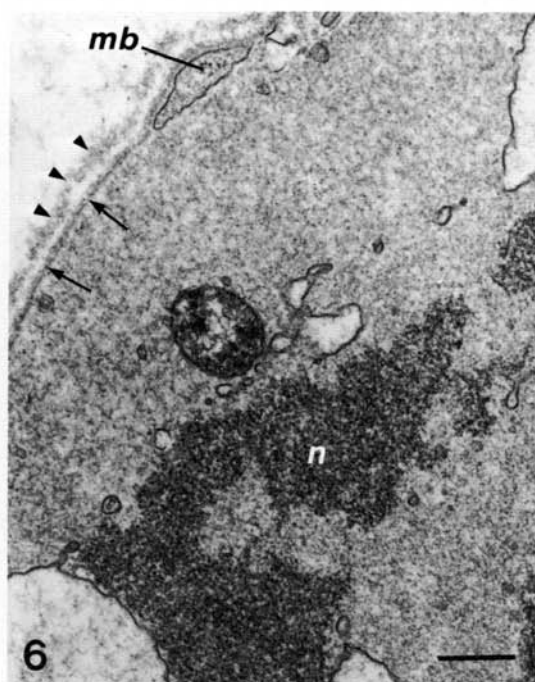
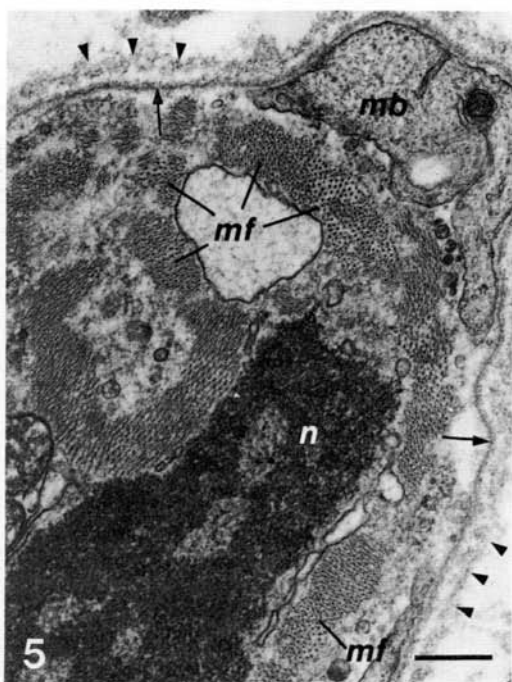
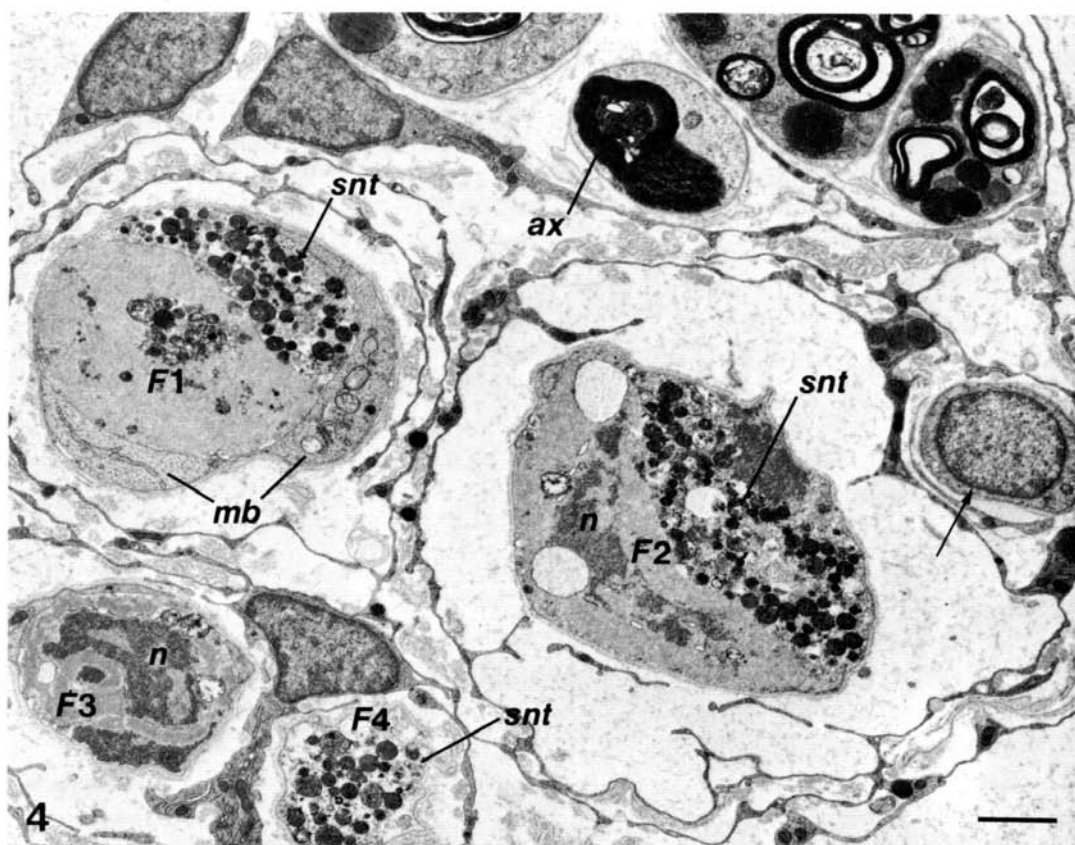
Fig. 3. Low-power electron micrograph of a transverse section through the juxta-equatorial region of a spindle, 12 h after devascularization. Both bag fibres (b_1 and b_2) exhibit hyaline degeneration, whereas myofibrils are still present in the two chain fibres (c), one of which bears a sensory nerve terminal (snt). Compare the pyknotic myonucleus (n) of the b_2 fibre with the normal nucleus of its satellite cell (arrow). Scale bar, 3 μ m.

more commonly seen at 1–2 days and were then probably produced by invading phagocytes. At such rupture sites, satellite cells appeared to be freed from the degenerating intrafusal muscle fibre and undifferentiated cells were occasionally seen in the periaxial space (Fig. 4). The equatorial reticular lamina of the intrafusal muscle fibres appears to be unaffected by temporary ischaemia (Figs 5, 6).

By 6–12 h most intrafusal myonuclei are pyknotic (Fig. 3). By day 2, most myonuclei have degenerated, so that the characteristic bags and chains of nuclei at the spindle equator are lost, and only nuclear remnants are seen (Figs 4–6). At the same time the myofibrils undergo degenerative changes that are generally similar to those described in bupivacaine-treated spindles (Milburn, 1976) and in ischaemic extrafusal muscle fibres (Moore, Ruska & Copenhaver, 1956). At 3 h some myofibrils are disorganized and the Z-lines and I-bands fragmented. A-bands, with or without M-lines, may persist even 2 days after devascularization, particularly in chain fibres (Fig. 5). By 6 h the sarcoplasm and myofibrils of nuclear-bag fibres, and particularly b_1 fibres, have degenerated into an homogeneous hyaline mass (Figs 3, 4, 6) typical of the hyaline degeneration reported in other studies of intrafusal (Milburn, 1976) and extrafusal (Allbrook & Aitken, 1951; Harris, Johnson & Karlsson, 1975; Harris & Johnson, 1978) muscle fibre degeneration and in diseased muscle (see Hudgson & Field, 1973). Bundles of preserved myofilaments are seen in nuclear-chain fibres even at 2 days (Fig. 5), giving the fibres a 'granular' appearance (Moore *et al.* 1956).

The spindle nerve terminals are also affected by temporary ischaemia. Degeneration of sensory nerve terminals is first seen 3 h after devascularization and is widespread by 3 days. The terminal mitochondria dilate, their cristae fragment, and the axoplasm clumps (Figs 4, 7). The disintegration of the junctional axolemma results in the mixing of the necrotic contents of both sensory nerve terminal and intrafusal muscle fibre (Fig. 7). The spindle motor nerve terminals retract and withdraw from the muscle fibres and become invested by Schwann cells. The terminals fill with lipid droplets, autophagic vacuoles and swollen mitochondria, although these changes are not always seen in the first postoperative day (Fig. 8). By the second day, degeneration of some motor nerve terminals is advanced. The contents of the engulfed terminal are converted to a dense granular material, similar to the 'honeycomb' structures described in denervated muscle (Miledi & Slater, 1970). Unlike degenerated extrafusal neuromuscular junctions, the junctional basal lamina at intrafusal motor end-plates frequently degenerates (Fig. 8), and the junctional cleft is occupied by Schwann cells and macrophages.

Within 3–12 h after devascularization, preterminal sensory and motor nerve axons become swollen, and their axoplasm degenerates (Fig. 3), as in mouse muscle following injection of Black Widow spider venom (e.g. Gorio, Hurlbut & Ceccarelli, 1979; Duchon & Queiroz, 1981). By 1–2 days, these changes are also seen in the spindle and intramuscular nerve trunks, where the myelin becomes disorganized (Fig. 4), the degenerated axoplasm forms 'honeycomb' structures, and the Schwann cells hypertrophy and engulf the axon remnants. The perineurial epithelium of the nerve trunks, the spindle capsule and the axial-sheath cells are generally resistant to ischaemia. Some capsule cells appear to hypertrophy, others vacuolate and degenerate, but most are unaffected. The space between the capsule layers is often filled with erythrocytes. The periaxial space is often reduced.



The interstitial spaces of the muscle, and the periaxial space of the spindle are invaded by phagocytic cells. By day 2 these phagocytes have invaded the degenerating intrafusal muscle fibres, where they phagocytose the necrotic sarcoplasm and axoplasm of the sensory nerve terminals, and subsequently leave the basal-lamina tubes of the original fibres. By day 3, phagocytes are seen both within the necrotic muscle fibres and in the periaxial space (Fig. 10).

Both extra- and intrafusal satellite cells survive the temporary ischaemia, and during the early stages of degeneration are clearly distinguishable from the degenerating muscle fibre elements (Fig. 3). By day 2, satellite cells have increased in size and now resemble activated satellite cells (Nichols & Shafiq, 1979) or presumptive myoblasts (Figs 4, 5).

3–6 days. Regeneration of intrafusal muscle fibres has started by day 3 (Figs 9, 10) and is most rapid in the 'thick' fibres, representing the remnants of the original bag fibres. At 3–4 days, the basal laminae of the 'thin' regenerating fibres fill with rows or bundles of myoblasts, formed by the division of activated satellite cells. The lamina of the 'thick' fibres usually contains one or more nascent myotubes (Fig. 10), formed by the fusion of myoblasts. At 5–6 days this profile has changed and the 'thick' fibres have matured into well-formed myotubes or myofibres and the 'thin' fibres into bundles of nascent myotubes with associated myoblasts (Fig. 11).

Bands of Büngner produced by the proliferation of Schwann cells are associated with the remnants of nerve axons (Figs 10, 11), and beneath the basal lamina of the regenerating intrafusal muscle fibres.

6–14 days. From day 6 onwards, phagocytic macrophages are less frequently encountered inside the regenerating fibres but may persist in the periaxial space (Fig. 11). Bands of Büngner are a common feature of the spindle nerve trunk, periaxial space and beneath the basal lamina of the regenerating fibres (Fig. 11).

Fig. 4. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 2 days after devascularization. Degeneration is more advanced in the thick intrafusal fibres (F1 and F2) than in the thin fibre (F3). The lumen of the second thin fibre (F4) is occupied almost exclusively by a degenerating sensory nerve terminal (*snt*). The equatorial nuclei (*n*) are pyknotic. Two presumptive myoblasts (*mb*) are associated with F1. Note the degenerating nerve axon (*ax*) and other sensory nerve terminals (*snt*). Arrow indicates an undifferentiated cell. Scale bar, 2 μ m.

Fig. 5. High-power electron micrograph of a transverse section through a chain fibre (serial section of F3 in Fig. 4), 2 days after devascularization. The intact basal lamina (arrows) is surrounded by an additional sheath (arrowheads) of reticular lamina. The plasmalemma of the muscle fibre has degenerated. The nuclear membrane surrounding the pyknotic nucleus (*n*) consists of dilated membrane-bound vesicles. In contrast to these degenerative changes, the architecture of several myofibrils (e.g. *mf*) is well-preserved. *mb*, myoblast. Scale bar, 0.5 μ m.

Fig. 6. High-power electron micrograph of a transverse section through a bag fibre (serial section of F2 in Fig. 4), 2 days after devascularization. Note the absence of myofibrils from the hyaline sarcoplasm. Other labels and scale bar as in Fig. 5.

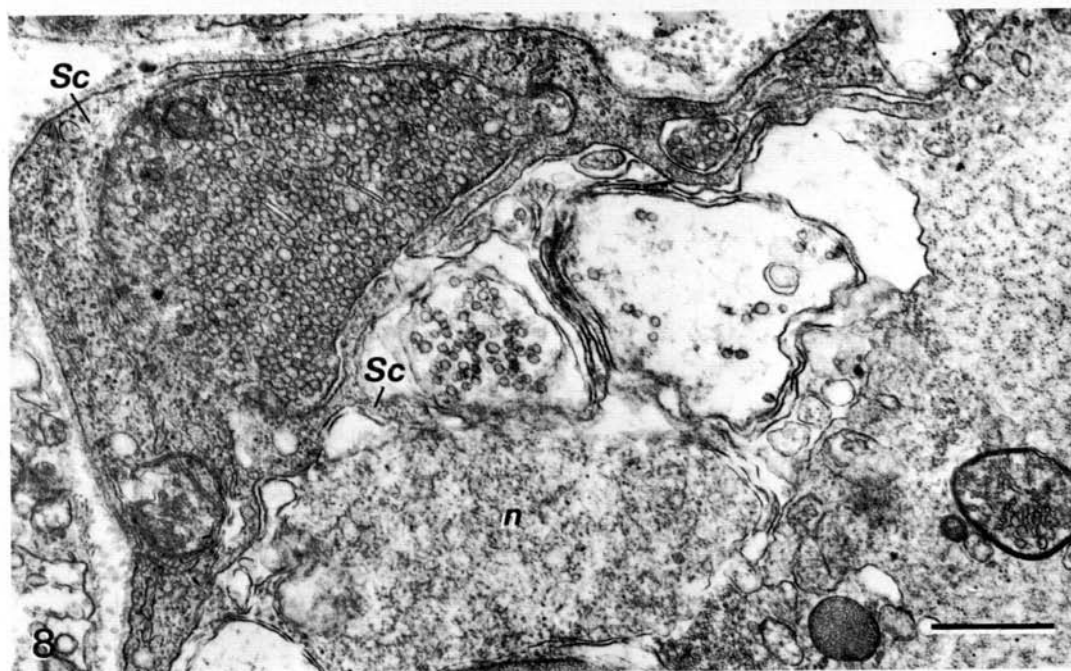
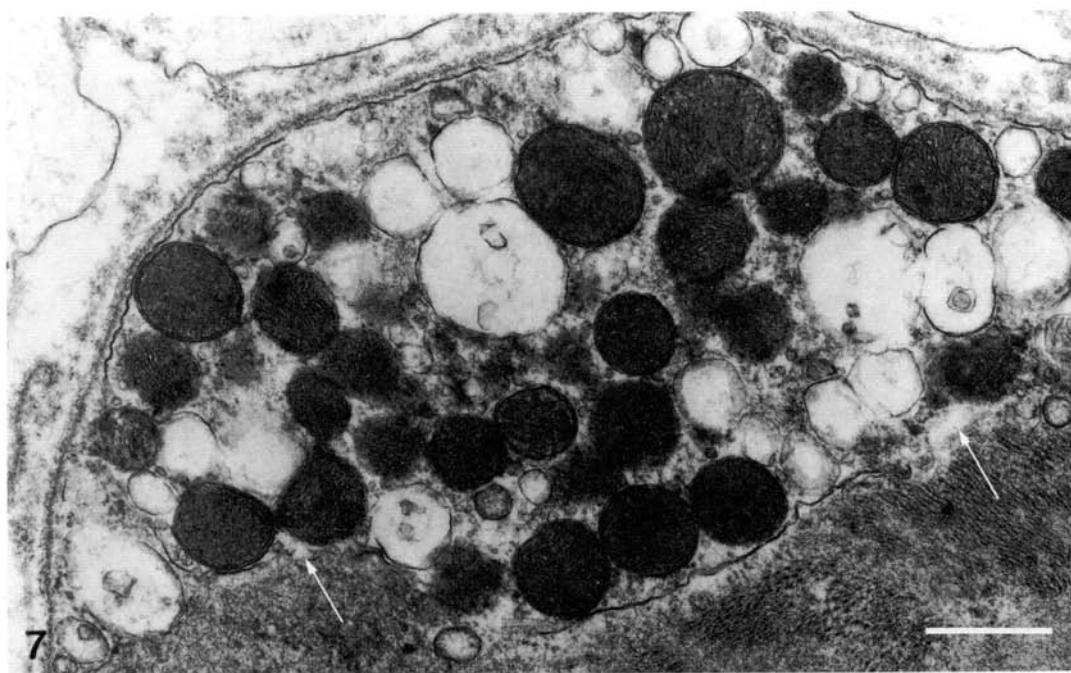


Fig. 7. High-power electron micrograph of a degenerating sensory nerve terminal, 6 h after devascularization. Note the clumped axoplasm containing dense swollen mitochondria with vesicular and granular contents. In places (arrows) the junctional axolemma is absent. Scale bar, 0.5 μ m.

Fig. 8. High-power electron micrograph of a transverse section through an intrafusal motor end-plate, 12 h after devascularization. The motor nerve terminals do not exhibit degenerative changes, but are engulfed by Schwann-cell processes (Sc). Basal lamina is absent from the junction. n, pyknotic sole-plate nucleus. Scale bar, 0.5 μ m.

By day 14, regenerated intrafusal muscle fibres are of a similar diameter and at the same stage of regeneration in most regions (Fig. 12), although 'thick' and 'thin' fibres are still distinguishable in some areas (Fig. 13). M-lines were identified in the myofibrils of all of the regenerating intrafusal myotubes examined in longitudinal section at the 6-, 8- and 14-day stage.

New basal lamina forms only in those areas where the regenerated sarcoplasm is widely separated from the old lamina. Occasionally, two regenerating muscle fibres were seen to fuse for a short distance before separating again. There are no bags or chains of nuclei in the regenerated muscle fibres at the spindle equator.

From day 14 onwards, most regenerating spindles contain short, aberrant muscle fibres in addition to those that have regenerated within the basal lamina of the original bag and chain fibres (Figs 12, 13).

21 days. At day 21, the irregularly shaped intrafusal muscle fibres are packed with myofibrils that are separated by a well-developed sarcotubular system (Figs 14, 15), and some intrafusal myonuclei have migrated to the periphery of the fibre (Fig. 15). Bags and chains of nuclei are still absent from the spindle equator (Fig. 14).

Additional fibres are a common feature of the spindle equatorial region at this stage (Fig. 14) and some receive terminals from reinnervating sensory nerve axons (Fig. 14). These terminals are sparse and not all regenerated muscle fibres are innervated.

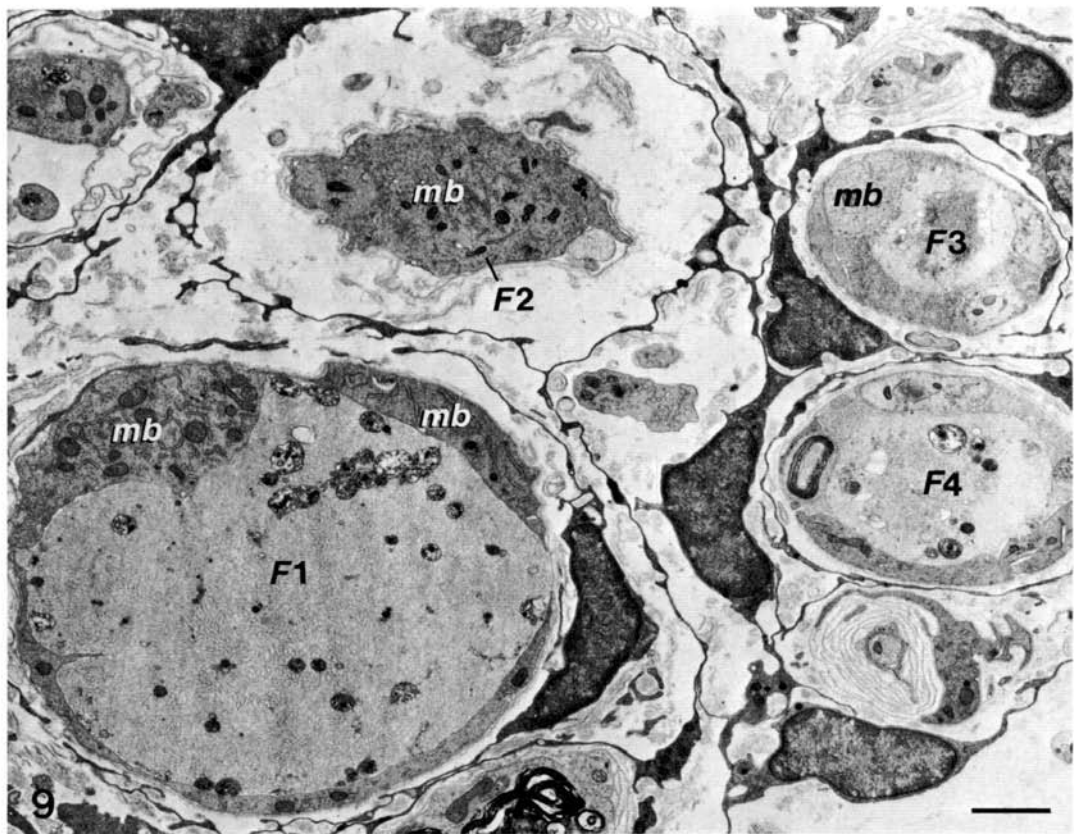
Reinnervation by motor nerve axons is also occasionally seen at this stage, but is more widespread by 28 days.

28–182 days. Reinnervation of regenerating intra- and extrafusal muscle fibres occurs between 21 and 28 days after muscle devascularization. Spindles examined at 42–182 days did not show any additional regenerative changes compared with those examined at day 28. It was therefore concluded that all spindles examined at 28–182 days (18 in all) had reached their maximum degree of restoration.

The regenerated spindles can be classified into four groups on the basis of their innervation and equatorial nucleation: these are summarized schematically in Fig. 23.

Group 1. The three spindles of this group appeared normal (Figs 17, 23A). Sensory nerve terminals are distributed to all intrafusal muscle fibres, which can be classified as b_1 , b_2 or c on the basis of their equatorial nucleation, diameter and M-line differences. Motor nerve terminals are distributed to all intrafusal muscle fibres. 'Additional' muscle fibres are also present in this group of spindles.

Group 2. In this group of three spindles, sensory nerve terminals are distributed to all intrafusal muscle fibres, which have the ultrastructural features of b_2 , b_1 , and c fibres, with the exception of deficiencies in the equatorial nucleation of the thicker fibres (Figs 18, 23B). Motor nerve terminals are distributed to all intrafusal muscle fibres and 'additional' muscle fibres are present.



Group 3. In this group of eight spindles, the intrafusal muscle fibre types cannot be clearly identified (Figs 19, 23C). They are classified as 'thick' and 'thin' fibres and probably represent, respectively, the bag and chain fibres of the original spindle. Sensory nerve terminals are mostly confined to the 'thin' fibres (Fig. 19), which contain poorly developed equatorial chains of nuclei. Apart from deficiencies in their sensory innervation, the 'thick' fibres also lack equatorial nuclei, but exhibit variations in the condition of the M-line, characteristic of normal bag fibres.

Motor nerve terminals are distributed to all intrafusal muscle fibres. Those that innervate the 'thick' intrafusal muscle fibres that lack any sensory innervation have particularly well-developed postjunctional sole-plates. The spindles of this group also contain 'additional' muscle fibres.

Group 4. The four spindles of this group lacked both sensory innervation and equatorial nucleation. Two subgroups, each of two spindles, were recognized on the basis of differences in their motor innervation (Fig. 23D,E).

The polar regions of spindles belonging to subgroup A are innervated by a few motor nerve axons which end in well-developed terminals. Free nerve endings are associated with the cells of the spindle capsule. The intrafusal muscle fibres are thicker than normal (Fig. 20) and all exhibit distinct M-lines throughout their length. The hypertrophy of the regenerated fibres obliterates the periaxial space at the spindle equator. The capsule tightly encloses the axial bundle, which frequently contains 'additional' muscle fibres (Fig. 20), some of which also receive motor nerve terminals.

Although the spindles of subgroup B contain more nerve axons in their equatorial (Fig. 21) and polar (Fig. 22) regions than those of subgroup A, their intrafusal muscle fibres are devoid of any innervation. Preterminal axons and free axon terminals are commonly seen in all regions of the capsule, periaxial space and axial sheath. Each of the two spindles contains two intrafusal muscle fibres (Figs 21, 22), as well as 'additional' muscle fibres (Fig. 22). The intrafusal muscle fibres are thin and exhibit signs of denervation atrophy seen in intrafusal muscle fibres as early as 21 days after denervation (Diwan, 1983). These include streaming and disintegration of Z-lines and general disorganization of the myofibrils (F2, Fig. 21). At the spindle equator the capsule is flattened and the periaxial space reduced (Fig. 21). At the intracapsular polar regions, the intrafusal muscle fibres are surrounded by large amounts of collagen and elastic fibrils.

Fig. 9. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 3 days after devascularization. Hyaline degeneration is evident in fibres F1, F3, and F4. Myoblasts (*mb*) are present in three of the fibres, F1, F2 and F3. Note the absence of sensory nerve terminals. Scale bar, 2 μ m.

Fig. 10. Low-power electron micrograph of a transverse section through the polar region of the same spindle as in Fig. 9. All four fibres (F1–F4) show signs of regeneration only. Arrow points to phagocyte in periaxial space; double arrow to bands of Büngner in spindle nerve trunk. Scale bar, 2 μ m.

'Additional' muscle fibres. Several short, thin, additional muscle fibres were first seen in spindles 14 days after devascularization (Figs 12, 13). At the spindle equator they are usually associated with the regenerating original fibres and the axial-sheath cells. Some are also found in the periaxial space and others between the capsule layers (Fig. 12), where their delineating basal lamina is continuous

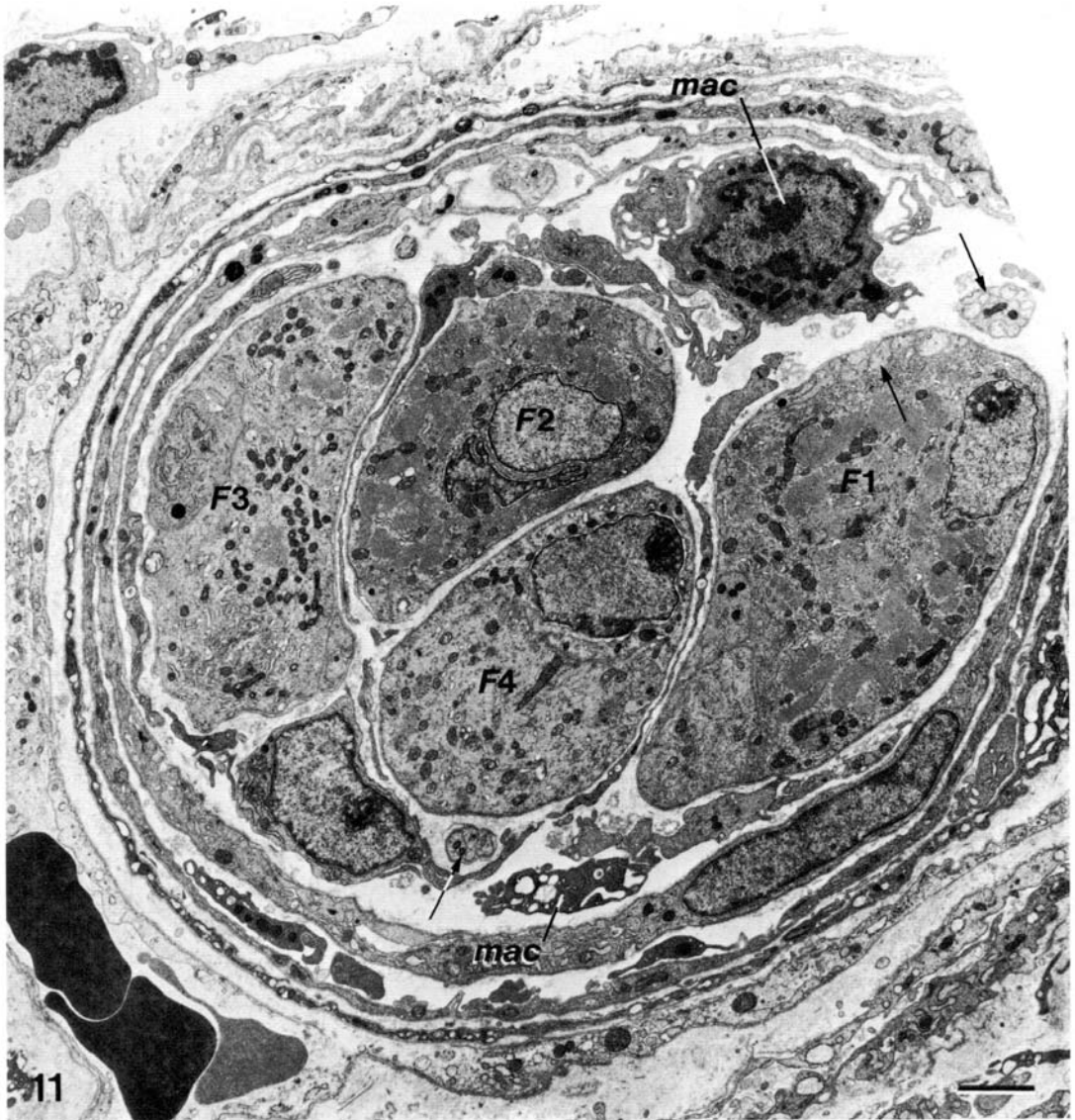
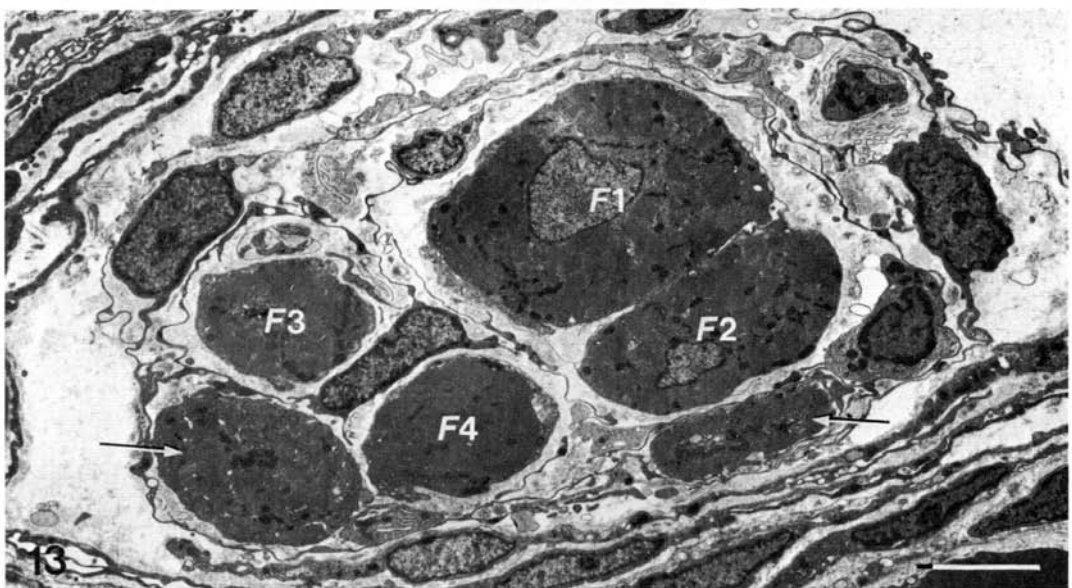
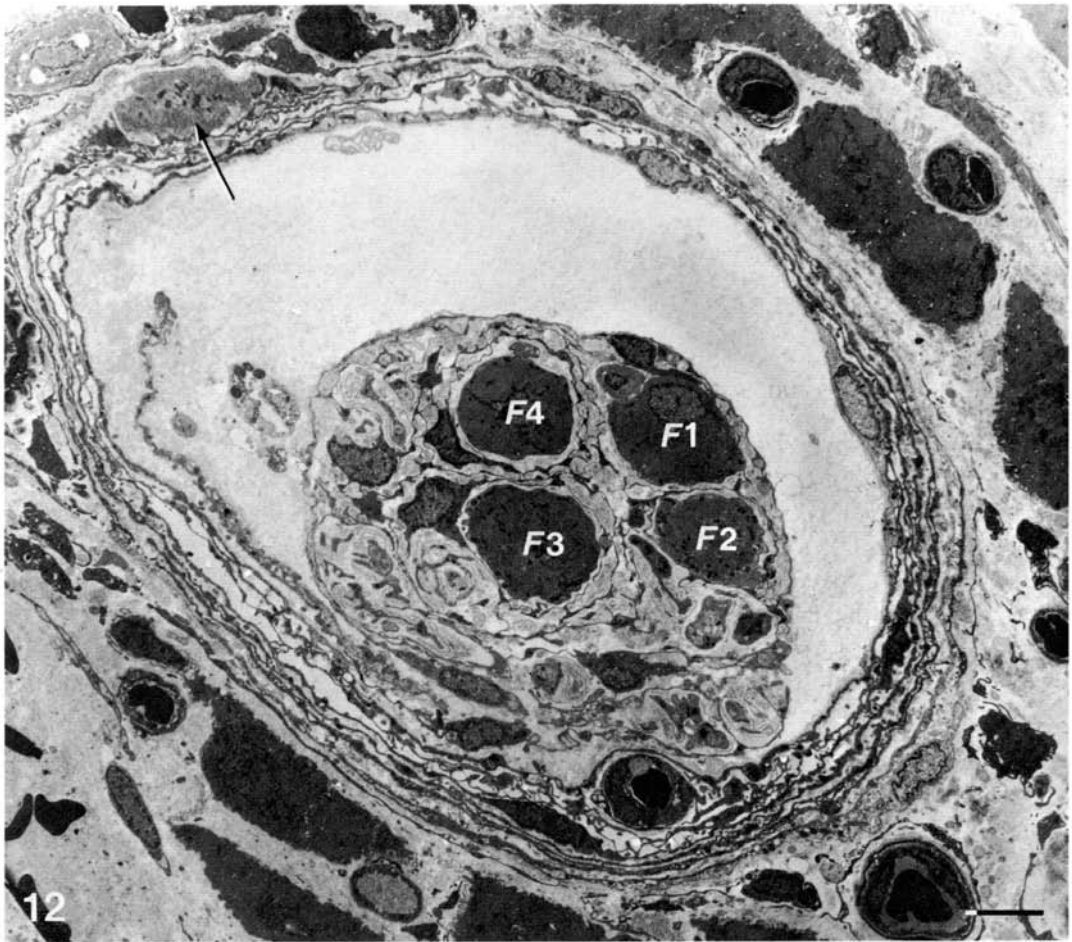
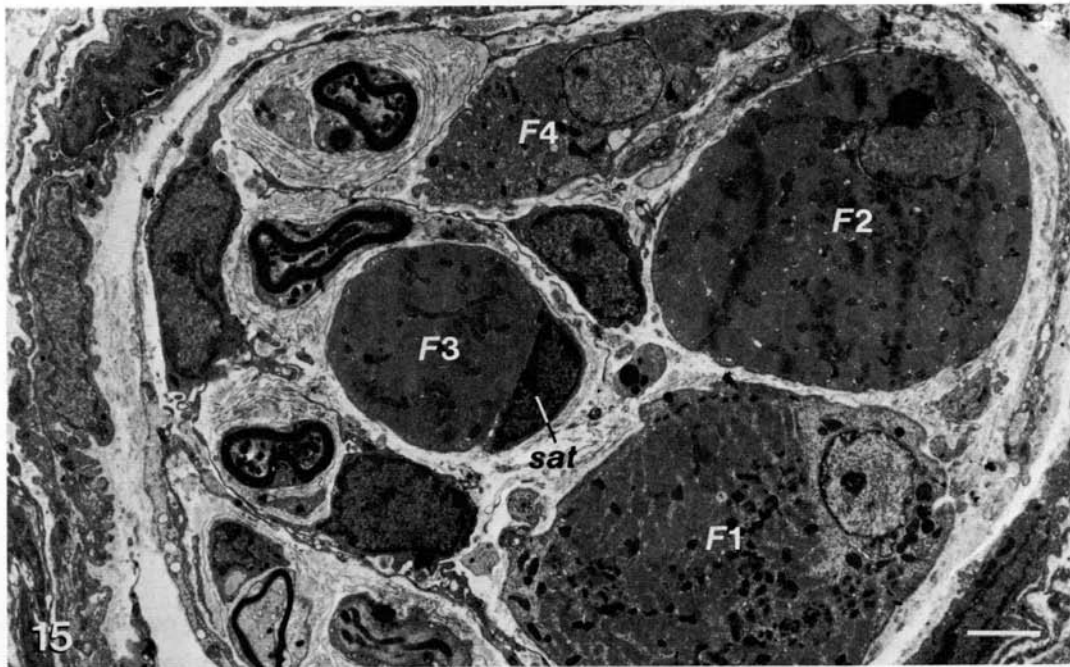
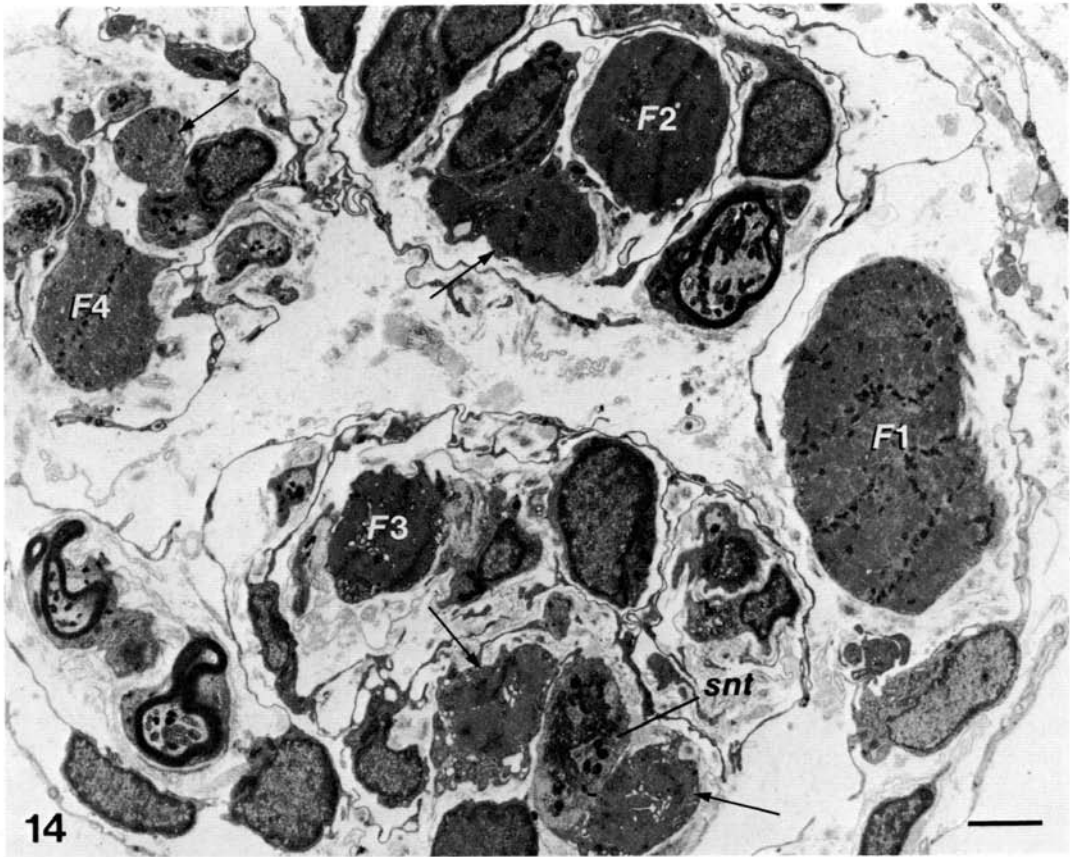


Fig. 11. Low-power electron micrograph of a transverse section through the intracapsular polar region of a spindle 6 days after devascularization. All four fibres (F1–F4) are of a similar diameter, but are at different stages of regeneration. F1 and F2 are composed of well-developed myotubes. F3 and F4 are composed of nascent myotubes and myoblasts. *mac*, macrophages in periaxial space. Arrows indicate bands of Büngner. Scale bar, 2 μ m.



Figs 12, 13. For legend see p. 241



with that of the capsule cells. Those associated with the axial sheath usually lack a basal lamina (Fig. 13). 'Additional' fibres located at the spindle pole are usually thicker and longer than those at the equator.

By day 21 some 'additional' fibres have matured into myofibres of a similar diameter to the regenerated original fibres (Fig. 20). Others are poorly developed, particularly at the spindle equator (Fig. 14).

From day 28 onwards, most spindles contain 'additional' fibres, some of which contain well-developed myofibrils that are undergoing fragmentation (Fig. 14), as noted in some regenerated extra- and intrafusal muscle fibres. Others appear as fragments scattered in the axial bundle. Some are short, unicellular structures resembling myoblasts and are enclosed by axial-sheath cells. Most additional fibres lack innervation, but some that are associated with the regenerated original fibres receive sensory nerve terminals (Fig. 14).

Various abnormalities were noted in the structure of regenerated intrafusal muscle fibres. Some original and 'additional' fibres are enclosed by a common basal lamina. Others are laterally fused or appear as fragments of one fibre. The common basal lamina may also contain satellite cells, Schwann cells, sensory nerve terminals or myelinated axons.

Some regenerated muscle fibres, that lack innervation, also exhibit signs of denervation atrophy.

DISCUSSION

General effects of temporary ischaemia on the muscle spindle

Previous studies of spindle degeneration and regeneration, whether induced by myotoxic agents (Milburn, 1976), or by muscle grafting (Schmalbruch, 1977; Rogers & Carlson, 1981; Rogers, 1982), have laid down a broad pattern for these processes. This study has confirmed that temporary ischaemia produces a rapid concomitant degeneration of intra- and extrafusal muscle fibres and their nerve terminals. The removal of necrotic sarcoplasm by phagocytes coincides with the

Fig. 12. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 14 days after devascularization. The axial bundle consists of four intrafusal fibres (F1–F4) of similar diameters and at a similar stage of regeneration. Arrow indicates an 'additional' fibre between the capsule layers. Scale bar, 5 μ m.

Fig. 13. Low-power electron micrograph of a transverse section through the juxtaequatorial region of the same spindle as in Fig. 12. Arrows indicate 'additional' fibres between the layers of the axial sheath. These particular fibres do not extend into the equatorial region. Scale bar, 5 μ m.

Fig. 14. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 21 days after devascularization. The intrafusal fibres (F1–F4) are irregular in shape and difficult to distinguish from 'additional' fibres (arrows) in a single section. A sensory nerve terminal (*snt*) is shared by two 'additional' fibres, one of which has a fragmented appearance. Scale bar, 3 μ m.

Fig. 15. Low-power electron micrograph through the intracapsular polar region of the same spindle as in Fig. 14. Fibres F1 and F4 lack any kind of innervation. Note the absence of 'additional' fibres. *sat*, satellite cell. Scale bar, 3 μ m.

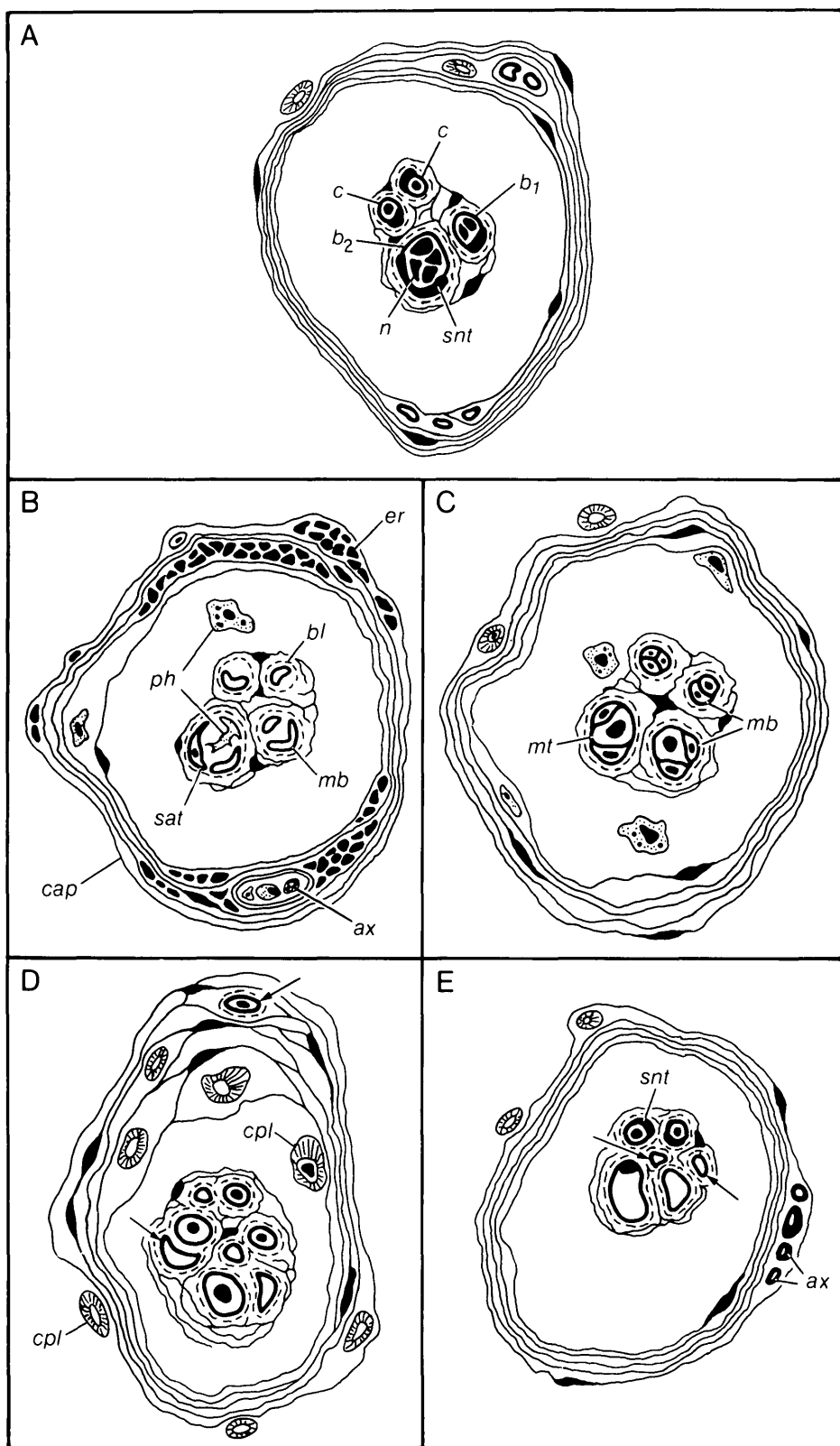


Fig. 16. For legend see p. 244



Figs 17, 18. For legend see p. 244

onset of a regenerative process instigated by the divisions of satellite cells. The presumptive myoblasts so formed fuse within the preserved basal-lamina tubes to form myotubes and myofibres in the absence of nerve terminals. Reinnervation of regenerating intrafusal muscle fibres leads to some equatorial differentiation, but this is incomplete in most spindles.

In addition to confirming these basic processes, this study has revealed additional aspects that are significant for the understanding of spindle regeneration.

Firstly, nuclear-bag fibres degenerate and regenerate more rapidly than nuclear-chain fibres. The reasons for this difference are not clear. Although it correlates well with the difference in the size and distribution of mitochondria between the

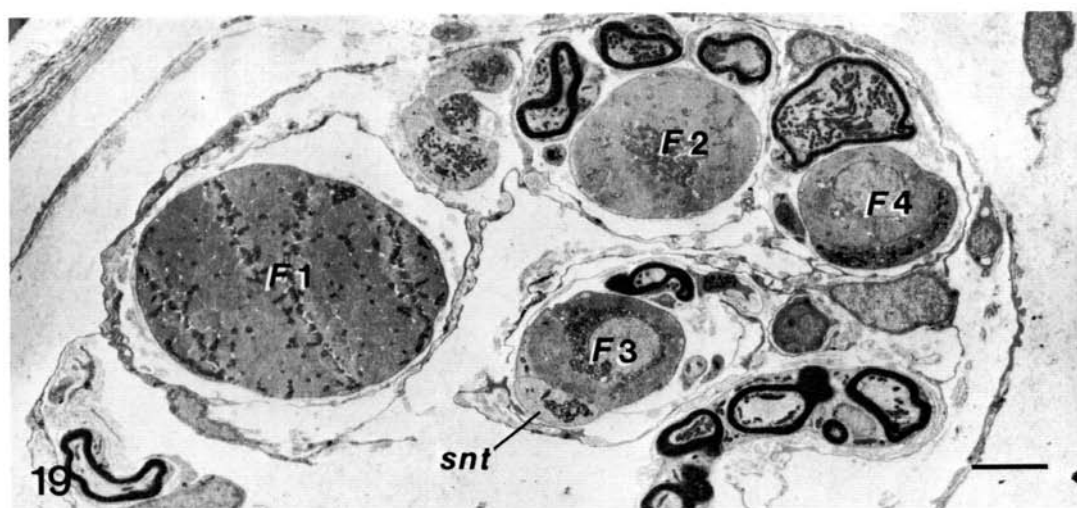
Fig. 16. (A–E) Schematic diagrams based on a number of transverse sections through the equatorial regions of rat soleus spindles, illustrating the effects of temporary ischaemia at various times following devascularization. (A) *Normal spindle*: the bag₂ (b₂) fibre is thicker than the bag₁ (b₁) fibre and contains more equatorial nuclei (n). Sensory nerve terminals (snt) are distributed to both bag and chain (c) fibres. (B) *0–3 days*: the sarcoplasm and plasmalemma of all intrafusal muscle fibres degenerate and necrotic debris is removed by phagocytes (ph). The preserved basal-lamina tubes (bl) contain satellite cells (sat) and presumptive myoblasts (mb). Sensory nerve terminals and nerve axons (ax) degenerate. The capsule (cap) is packed with erythrocytes (er). (C) *3–5 days*: regeneration proceeds more rapidly in the basal-lamina tubes of the original bag fibres, which contain myotubes (mt) and myoblasts (mb), than in those of the chain fibres, which contain myoblasts only. (D) *5–21 days*: regenerated intrafusal muscle fibres (nucleated) mature into muscle fibres with central nuclei, in the absence of innervation. Bags and chains of nuclei are absent. ‘Additional’ muscle fibres are located within the axial bundle (one arrowed) and between the capsule layers (arrow). Capillaries (cpl) have increased in number. (E) *21–28 days*: sensory nerve terminals (snt) of reinnervating myelinated axons (ax) are mostly distributed to the thin regenerated intrafusal muscle fibres, which contain central nuclei. The thicker fibres receive few terminals and lack equatorial nuclei (Group 3 regenerated spindle). Arrows point to ‘additional’ muscle fibres.

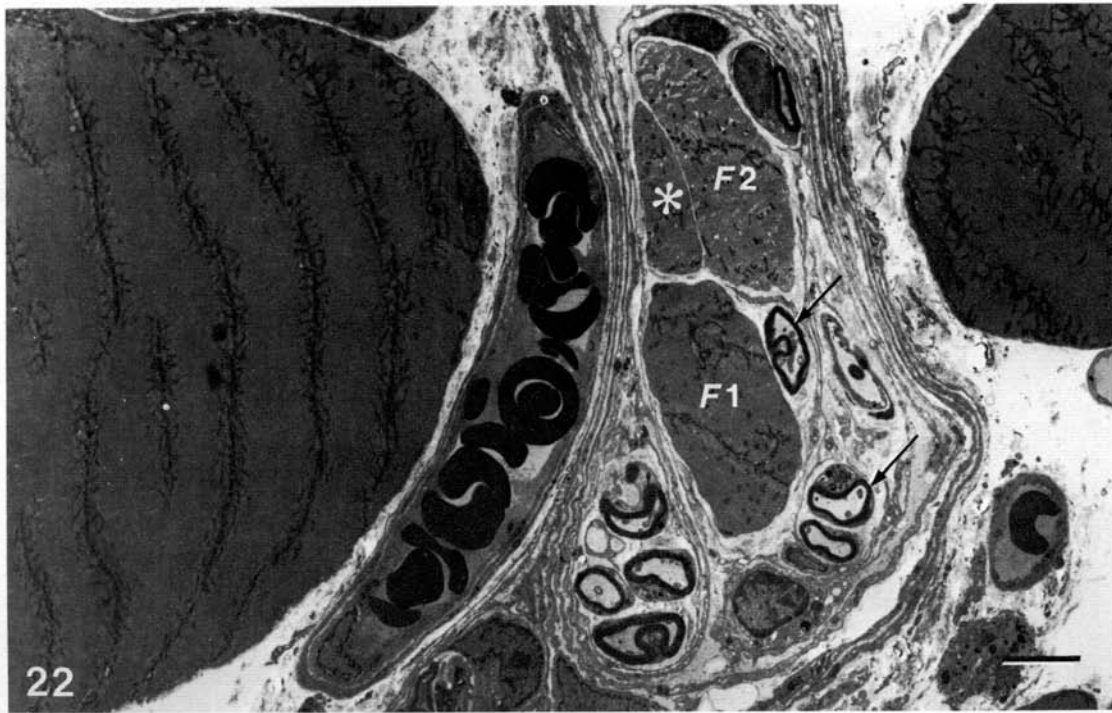
Fig. 17. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 28 days after revascularization. Note the fully differentiated bag₂ (b₂), bag₁ (b₁) and chain (c) fibres and their extensive sensory reinnervation (snt). This is an example of a Group 1 regenerated spindle. Scale bar, 5 µm.

Fig. 18. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 28 days after devascularization. Note the presence of sensory nerve terminals (snt). Serial sectioning of the equatorial region showed that the chain (c) fibres are fully differentiated with respect to their equatorial nuclei, whereas the bag (b₁ and b₂) fibres are deficient. This is an example of a Group 2 regenerated spindle. Scale bar, 5 µm.

Fig. 19. Low-power electron micrograph of a transverse section through the equatorial region of a spindle 28 days after devascularization. Sensory nerve terminals (snt) are confined to the ‘thin’ fibres (F3 and F4), which have poorly developed chains of nuclei. The ‘thick’ fibres (F1 and F2) lack equatorial nucleation. This is an example of a Group 3 regenerated spindle. Scale bar, 5 µm.

Fig. 20. Low-power electron micrograph of a transverse section through the equator of a spindle 28 days after devascularization. The regenerated ‘original’ fibres (F1, F2 and F3) are thicker than normal intrafusal fibres and lack both sensory nerve terminals and equatorial nucleation. The fibres indicated with an asterisk (*) are ‘additional’, newly formed fibres which do not extend the full length of the spindle. Note the reduced periaxial space. This is an example of a Group 4A spindle. Scale bar, 5 µm.





various types of intrafusal muscle fibre (larger and more numerous in chain than in bag fibres), there is no evidence from extrafusal studies to show that slow oxidative extrafusal muscle fibres are more resistant to myotoxic lesions than their faster glycolytic neighbours. Indeed, myotoxic snake venom toxins leave fast glycolytic fibres undamaged in rat EDL muscle (Harris *et al.* 1975), as well as the intrafusal fibres of the muscle spindle. The overall effect of this difference in the rates of degeneration of bag and chain fibres is that the onset of regeneration of the presumptive bag fibres precedes that of the thinner chains, and this is probably correlated with the difference in the rate of removal of necrotic sarcoplasm (Carlson, Hansen-Smith & Magon, 1979). This produces a gradient of regeneration amongst the various presumptive intrafusal muscle fibres, prior to their reinnervation and may account for the overall preponderance of sensory nerve terminals on the thinner regenerated fibres.

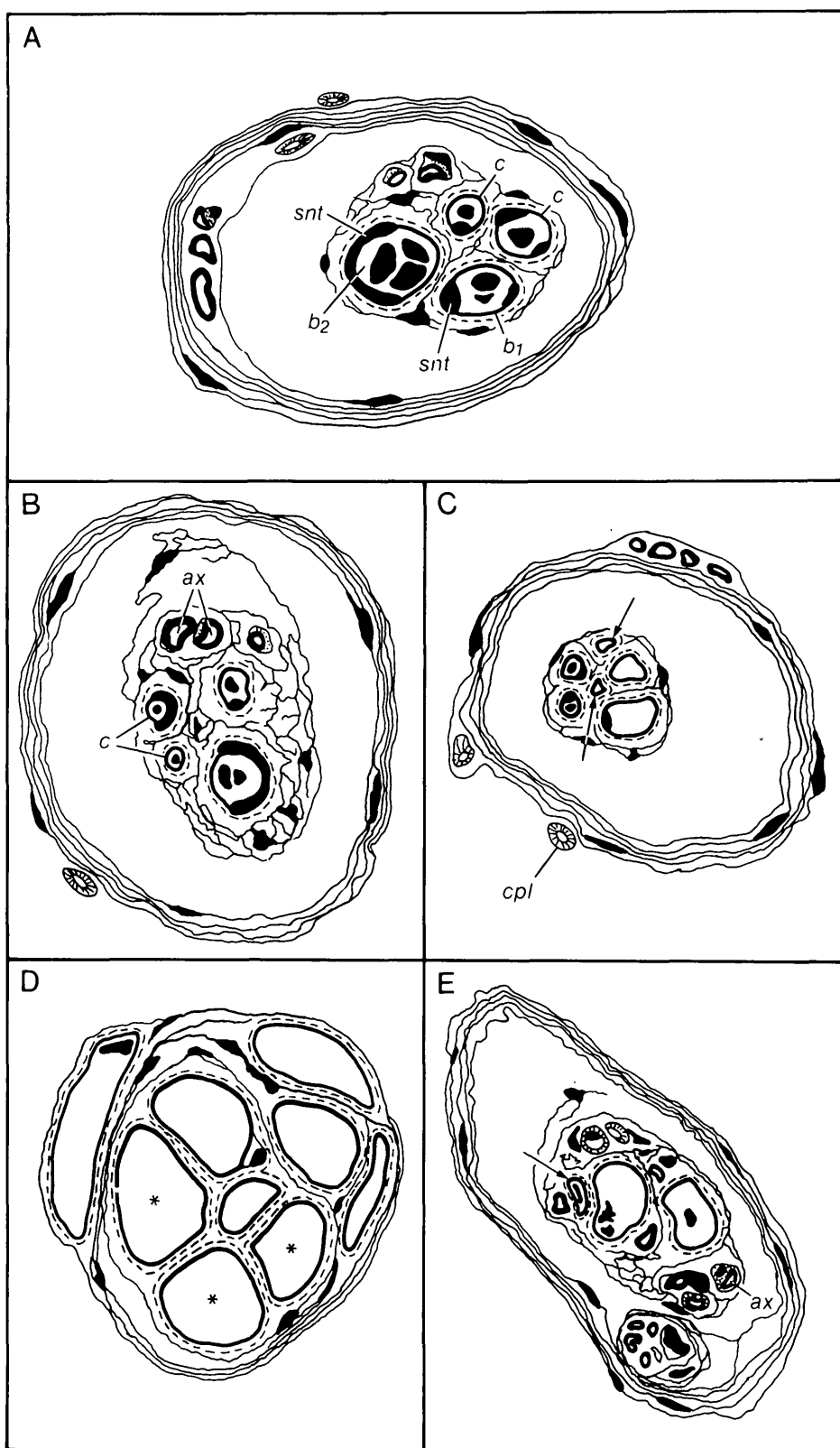
Secondly, the close association between the nuclear-bag fibres, reported here as a common feature of normal rat spindles, produces abnormalities in their regeneration, so that regenerated 'thick' intrafusal muscle fibres appear to branch. Branching is a common feature of regenerated extrafusal muscle fibres (Walton & Adams, 1956; Schmalbruch, 1976, 1979; Ontell, Hughes & Bourke, 1982), and may stem from a similar close apposition between the original muscle fibres.

Thirdly, although the basal lamina generally survives ischaemia, in some spindles examined at 3–6 h it had disintegrated prior to phagocytic invasion. Basal-lamina degeneration is a common feature of spindles in transplanted pigeon muscle and may permit the *de novo* formation of spindles in some heterotopic grafts (Walro, Hikida & Hather, 1984). The loss of basal lamina from degenerating muscle fibres would affect the pattern of regeneration and could lead to fibre loss, as noted in some regenerated spindles in this study. It may also lead to the liberation of satellite cells into the interstitial or periaxial space. The divisions of satellite cells in their new ectopic position may be one of the sources of the 'additional' muscle fibres commonly seen in regenerated spindles in this study. In support of this, Bischoff (1979) has shown that some migrating cells liberated from muscle fibres *in vitro* may fuse to form myotubes, and Lipton & Schultz (1979) have suggested that satellite cells can migrate over short distances.

The location of these 'additional' fibres in regenerated spindles is probably closely related to the way in which they form. Those associated with the regenerated original fibres are also seen in spindles from muscle grafts (Schmalbruch, 1977, 1979), and probably arise from regenerating muscle cells, within the basal

Fig. 21. Low-power electron micrograph of a transverse section through the equator of a spindle 28 days after devascularization. Only two regenerated intrafusal fibres (F1 and F2) are present; both lack sensory innervation and equatorial nuclei. Note the reduced periaxial space. This is an example of a Group 4B regenerated spindle. Scale bar, 5 μ m.

Fig. 22. Low-power electron micrograph of a transverse section through the intracapsular polar region of the same spindle as in Fig. 21. An 'additional' fibre (*) is present in this region. Although there are several myelinated nerve fibres present (two indicated by arrows) none innervates the original (F1 and F2) or 'additional' fibres. Scale bar, 5 μ m.



lamina of the original fibres, that have failed to fuse with other myoblasts or myotubes during the normal regeneration process. Their subsequent investment in a separate sheath of basal lamina, and their separation from the main regenerating fibre would give rise to short 'thin' 'additional' fibres.

No conclusive evidence was found of regenerating intrafusal muscle fibres splitting into multiple fibres, as reported in extrafusal regenerates (Hall-Craggs & Lawrence, 1969, 1970), other than in obviously atrophic fibres.

The origin of other 'additional' fibres, some of which lack basal lamina, is much more speculative. Some may arise from the divisions of migrant satellite cells, released by the degeneration of the basal lamina of the original fibres. Other studies (Teräväinen, 1970; Konigsberg, Lipton & Konigsberg, 1975; Schultz, 1978) support the proposal that satellite cells may migrate from degenerating muscle fibres, and the random location and distribution of this group of 'additional' fibres may reflect the random dispersal of these cells.

Those 'additional' fibres enclosed by the basal lamina of the capsule or axial-sheath cells may also arise from migrant satellite cells or by the redifferentiation of a circulating endomysial or perivascular cell (e.g. Bateson, Woodrow & Sloper, 1967; Partridge & Sloper, 1977).

The structure of the regenerated spindles

Spindles that have regenerated following temporary ischaemia are here classified into one of four groups according to the extent of their sensory and motor reinnervation and their equatorial and ultrastructural differentiation (Fig. 23).

In her study of spindle regeneration in rat muscle grafts, Rogers (1982) reported variations in the structure of spindles from standard, nerve-intact and non-reinnervated grafts. Those in non-reinnervated and standard grafts lack sensory innervation and equatorial nucleation, as in autografted rat soleus muscle (Schmalbruch, 1977). Some muscle fibres in spindles from standard grafts receive motor nerve terminals.

Fig. 23. (A–E) Schematic diagrams based on actual transverse sections through the equatorial regions of the five profiles presented by regenerated rat spindles. (A) *Group 1*: fully restored spindle with the characteristic features of a normal spindle. Sensory nerve terminals (*snt*) are distributed to all intrafusal muscle fibres, which have the ultrastructure of bag₂ (*b*₂), bag₁ (*b*₁) and chain (*c*) fibres. (B) *Group 2*: sensory nerve terminals are distributed to all fibres. Regenerated chain (*c*) fibres have normal equatorial nucleation. Regenerated 'bag' fibres have reduced bags of nuclei. (C) *Group 3*: sensory nerve terminals are mostly confined to the 'thin' intrafusal muscle fibres, which have abnormal chains of nuclei. The 'thick' fibres lack equatorial nucleation. Arrows point to 'additional' fibres. *cpl*, capillary. (D) *Subgroup 4A*: sensory nerve terminals are absent from both the three regenerated 'original' fibres (asterisks), which are reinnervated exclusively by motor axons, and from the six 'additional' fibres. Note the reduced periaxial space. (E) *Subgroup 4B*: sensory and motor nerve terminals are absent from all intrafusal muscle fibres of this subgroup, although numerous axons (*ax*) are present. Arrow points to an 'additional' fibre.

In nerve-intact grafts, Rogers found typical equatorial nucleation in only 9–15 % of the regenerated intrafusal muscle fibres, and this equatorial differentiation is closely correlated with the presence of sensory nerve terminals. All other fibres lack equatorial nuclei, like those in the enucleated spindles from bupivacaine-treated muscles of rat (Milburn, 1976).

Any attempts to correlate the results of these various studies into spindle regeneration must have regard for the technique used to initiate degeneration, the muscle used and the experimental design for spindle investigation. The technique used in this study to produce temporary ischaemia is similar in its effects to the procedure used by Rogers to produce nerve-intact grafts. As in Rogers' investigation a proportion of the regenerated intrafusal muscle fibres displayed typical equatorial nucleation. The relatively higher proportion noted in this study may be due to several causes. It is conceivable that a soleus spindle lying in the most superficial fascicles might escape temporary ischaemia. This would seem most unlikely as all 48 spindles examined at the 30-min to 21-day postoperative period showed signs of damage, and all regenerated spindles containing nucleated intrafusal muscle fibres also contained additional fibres.

The basis of Rogers' investigation into the equatorial differentiation of regenerated spindles was a light-microscope examination of serial sections of grafted muscles, supplemented by the electron-microscope observation of random sections, which may have resulted in equatorial nuclei being missed in some spindles. In support of this, it is significant that Rogers does not report the presence of 'additional' fibres in spindles from muscle grafts, or enucleated intrafusal muscle fibres that receive sensory nerve terminals.

On the other hand, the difference in the extent of equatorial differentiation may be a true reflection of variations in the sensory reinnervation of the spindles of the different muscles, and may illustrate the role that spindle distribution has on the restoration of structure.

The Group 4A spindles identified in this study equate well with some of the spindles identified in nerve-intact and standard grafts (Rogers, 1982). Group 4B spindles are similar to some reported in non-reinnervated grafts (Schmalbruch, 1977; Rogers, 1982), although neither of the muscle-graft studies reported intrafusal muscle fibre loss or a reduction in the periaxial space.

The wide range of spindle structure described in this study, particularly when compared with spindle regenerates in muscle grafts and bupivacaine-treated muscles, indicates that not only is spindle recovery far from complete under optimal conditions for regeneration, but also that a proportion of spindles contain atrophic or grossly abnormal fibres.

The role of innervation and spindle location in the restoration of spindle structure

Differences between regenerated intrafusal muscle fibres are possibly the result of an interaction of three factors, namely the time of arrival of reinnervating sensory and motor axons, the regenerative state of the muscle fibres at the time of

reinnervation, and the location of the spindle in the muscle (Diwan, 1983; Barker & Milburn, 1984).

There appears to be a specific period during intrafusal muscle fibre regeneration after which the Ia axon is unable to exercise an influence on further differentiation (see also Zelená & Sabotková, 1971; Rogers, 1982). Bag fibres degenerate and regenerate more rapidly than chain fibres so that when sensory axons arrive at the regenerating spindle, regeneration of the 'thick' muscle fibres may still be more advanced than that of the 'thin' fibres. Therefore the Ia axon may exert a greater influence on regenerating 'thin' fibres than 'thick' fibres.

The complete restoration of equatorial nucleation requires all of the intrafusal muscle fibres to be at an appropriate stage of regeneration when the Ia axon reinnervates (Group 1 spindles). If its arrival is delayed, the axon may be unable to influence further equatorial differentiation of some or all of the muscle fibres, even if terminals are established (Group 2 and 3 spindles). Further delay prevents sensory innervation, as in Group 4 spindles, and may lead to the formation of atypical spindles.

Because of the nature of revascularization, muscle fibres are subjected to varying periods of ischaemia following devascularization and in muscle grafts (Hansen-Smith, Carlson & Irwin, 1980). Muscle fibres located at the central core are subjected to longer periods of ischaemia than those at the periphery. This may lead to satellite-cell death and an ensuing loss of spindles from some muscle grafts (Rogers & Carlson, 1981).

Phagocytosis of necrotic fibres and regeneration begins at the muscle periphery, in line with the pattern of revascularization (Hansen-Smith *et al.* 1980), and results in a gradient of decreasing maturity amongst the regenerating fibres from the periphery to the core. The spindle population is also likely to be affected by this gradient.

The mode of nerve ingrowth following temporary ischaemia requires further study. However, Allbrook & Aitken (1951) reported that extrafusal motor endplates are not visible until 21 days after devascularization and doubted any further recovery after 28 days. If the regenerating spindles of an individual muscle are reinnervated together, then the internal (between 'thick' and 'thin' fibres) and external (between periphery and core) regeneration gradients may be the major factors in determining the variations in intrafusal muscle fibre regeneration. On the other hand if reinnervation of the spindle population is asynchronous, then the timing and pattern of muscle reinnervation will be another factor in determining these differences.

General implications of the pattern of spindle regeneration

The essential prerequisite for the development of mammalian muscle spindles is the establishment of contacts between sensory and motor nerve axons and a foetal primary myotube. Additional intrafusal myotubes then assemble and differentiate under the influence of these terminals, each acquiring basal lamina, and are

enclosed by a capsule and axial sheath (see review by Barker & Milburn, 1984). This contrasts markedly with their regeneration, which requires the preservation of a framework, consisting of axonal pathways, capsule and the basal lamina and satellite cells of the original intrafusal muscle fibres. Disruption of these elements in minced-muscle grafts (Zelená & Sabotková, 1971; Carlson, 1972) prevents spindle regeneration.

This difference in the pattern of spindle development and regeneration is not apparent in other vertebrates (Mackenson-Dean, Hikida & Frangowlakis, 1981; Hikida, Walro & Miller, 1984; Walro *et al.* 1984), where spindles are reported to develop *de novo* in muscles that lack spindles transplanted to the site of muscles that contain spindles. This *de novo* generation is thought to arise from the innervation of regenerating muscle cells, of unknown origin, by sensory nerves. Degeneration of pigeon muscle spindles in devascularized muscles is accompanied by the extensive loss of capsule cells and intrafusal basal lamina. The regenerating muscle cells may stem from migrant satellite cells freed by the loss of basal lamina from extrafusal muscle fibres, as noted in this study. *De novo* generation of spindles is not a feature of rat regenerates, and this may be linked to the faster time course of regeneration in rat compared to pigeon. It may be that spindles can be induced to differentiate *de novo* in mammalian muscle under certain conditions, such as prolonged ischaemia.

Little is known of the pattern of spindle regeneration in higher mammals (Mufti, Carlson, Maxwell & Faulkner, 1977) or of the functional recovery of stretch receptors in muscle regenerates, other than the deficiencies reported in rat (Quick & Rogers, 1983). These are areas that clearly require detailed investigation if the full potential of muscle transplantation is to be realized.

We wish to thank Professor D. Barker and Dr M. J. Stacey for their support and advice. The skilled technical assistance of Mr P. Hunter, Mrs C. Richardson and Mr D. Hutchinson is gratefully acknowledged. This research was supported by a grant from the Ministry of Higher Education and Scientific Research, Iraq.

REFERENCES

- ALLBROOK, D. B. & AITKEN, J. T. (1951). Reinnervation of striated muscle after acute ischemia. *J. Anat.* **85**, 367–390.
- BADER, D. (1981). Density and distribution of α -bungarotoxin-binding sites in postsynaptic structures of regenerated rat skeletal muscle. *J. Cell Biol.* **88**, 338–345.
- BANKS, R. W., HARKER, D. W. & STACEY, M. J. (1977). A study of mammalian intrafusal muscle fibres using a combined histochemical and ultrastructural technique. *J. Anat.* **123**, 783–796.
- BARKER, D., BANKS, R. W., HARKER, D. W., MILBURN, A. & STACEY, M. J. (1976). Studies of the histochemistry, ultrastructure, motor innervation and regeneration of mammalian intrafusal muscle fibres. In *Progress in Brain Research*, vol. **44**, *Understanding the Stretch Reflex* (ed. S. Homma), pp. 67–88. Elsevier/North Holland: Biomedical Press.
- BARKER, D., DIWAN, F. & MILBURN, A. (1982). Effects of ischaemia on rat muscle spindles. *J. Physiol.* **327**, 27P.
- BARKER, D. & MILBURN, A. (1984). Development and regeneration of mammalian muscle spindles. *Sci. Prog. Oxf.* **69**, 45–64.
- BATESON, R. G., WOODROW, D. F. & SLOPER, J. L. (1967). Circulating cell as a source of myoblasts in regenerating injured mammalian skeletal muscle. *Nature, Lond.* **213**, 1035–1036.

- BISCHOFF, R. (1979). Tissue culture studies on the origin of myogenic cells during muscle regeneration in the rat. In *Muscle Regeneration* (ed. A. Mauro *et al.*), pp. 13–29. New York: Raven Press.
- BURDEN, S. J., SARGENT, P. B. & MCMAHAN, U. J. (1979). Acetylcholine receptors in regenerating muscle accumulate at original synaptic sites in the absence of the nerve. *J. Cell Biol.* **82**, 412–425.
- CARLSON, B. M. (1972). The regeneration of minced muscle. *Monographs in Developmental Biology*, vol. 4. Basel: S. Karger AG.
- CARLSON, B. M. (1973). The regeneration of skeletal muscle - A review. *Am. J. Anat.* **137**, 119–150.
- CARLSON, B. M. & FAULKNER, J. A. (1983). The regeneration of skeletal muscle fibres following injury: a review. *Med. Sci. Sport Exercise* **15**, 187–198.
- CARLSON, B. M. & GUTMANN, E. (1975). Regeneration in free grafts of normal and denervated muscle in the rat: Morphology and histochemistry. *Anat. Rec.* **183**, 47–62.
- CARLSON, B. M., HANSEN-SMITH, F. M. & MAGON, D. K. (1979). The life history of a free muscle graft. In *Muscle Regeneration* (ed. A. Mauro *et al.*), pp. 493–507. New York: Raven Press.
- DIWAN, F. H. (1983). The effects of ischaemia and denervation on mammalian muscle spindles. Ph.D. thesis: University of Durham, England.
- DUCHEN, L. W. & QUEIROZ, L. S. (1981). Degeneration and regeneration of sensory nerve terminals in muscle spindles: effects of *Latrodectus* spider venoms. *J. Physiol.* **319**, 35P.
- GORIO, A., HURLBUT, W. P. & CECCARELLI, B. (1978). Acetylcholine compartments in mouse diaphragm. Comparison of the effects of Black Widow spider venom, electrical stimulation, and high concentrations of potassium. *J. Cell Biol.* **78**, 716–733.
- HALL-CRAGGS, E. C. B. (1978). Ischaemic muscle as a model of regeneration. *Expl Neurol.* **60**, 393–399.
- HALL-CRAGGS, E. C. B. (1979). The free autogenous muscle transplant. In *Muscle Regeneration* (ed. A. Mauro *et al.*), pp. 541–546. New York: Raven Press.
- HALL-CRAGGS, E. C. B. & LAWRENCE, C. A. (1969). Longitudinal fibre division in rat skeletal muscle. *J. Physiol.* **202**, 76P.
- HALL-CRAGGS, E. C. B. & LAWRENCE, C. A. (1970). Longitudinal fibre division in skeletal muscle: A light- and electron-microscopic study. *Z. Zellforsch. mikrosk. Anat.* **109**, 481–494.
- HANSEN-SMITH, F. M. (1983). Development and innervation of soleplates in the freely grafted extensor digitorum longus (EDL) muscle in the rat. *Anat. Rec.* **207**, 55–67.
- HANSEN-SMITH, F. M., CARLSON, B. M. & IRWIN, K. L. (1980). Revascularization of the freely grafted extensor digitorum longus muscle in the rat. *Am. J. Anat.* **158**, 65–82.
- HARRIS, J. B. & JOHNSON, M. A. (1978). Further observations on the pathological responses of rat skeletal muscle to toxins isolated from the venom of the Australian Tiger Snake, *Notechis scutatus scutatus*. *Clin. exp. Pharmac. Physiol.* **5**, 587–600.
- HARRIS, J. B., JOHNSON, M. A. & KARLSSON, E. (1975). Pathological responses of rat skeletal muscle to a single subcutaneous injection of a toxin isolated from the venom of the Australian tiger snake, *Notechis scutatus scutatus*. *Clin. exp. Pharmac. Physiol.* **2**, 383–404.
- HIKIDA, R. S., WALRO, J. M. & MILLER, T. W. (1984). Ischaemic degeneration of the avian muscle spindle. *Acta neuropath. (Berl.)* **63**, 3–12.
- HUDGSON, P. & FIELD, E. J. (1973). Regeneration of muscle. In *The Structure and Function of Muscle*, vol. 2, Part 2 (ed. G. H. Bourne), pp. 312–363. New York & London: Academic Press.
- JIRMANOVÁ, J. & THESLEFF, S. (1972). Ultrastructural study of experimental muscle degeneration and regeneration in the adult rat. *Z. Zellforsch. mikrosk. Anat.* **133**, 77–97.
- KARPATI, G., CARPENTER, S., MELMED, C. & EISEN, A. A. (1974). Experimental ischaemic myopathy. *J. neurol. Sci.* **23**, 129–161.
- KONIGSBERG, U. R., LIPTON, B. H. & KONIGSBERG, I. R. (1975). The regenerative response of single mature muscle fibres isolated *in vitro*. *Devl Biol.* **45**, 260–275.
- KUCERA, J., DOROVINI-ZIS, K. & ENGEL, W. K. (1978). Histochemistry of rat intrafusal muscle fibres and their motor innervation. *J. Histochem. Cytochem.* **26**, 973–988.
- LIPTON, B. H. & SCHULTZ, E. (1979). Developmental fate of skeletal muscle satellite cells. *Science* **205**, 1292–1294.
- MACKENSON-DEAN, C. A., HIKIDA, R. S. & FRANGOWLAKIS, T. M. (1981). Formation of muscle spindles in regenerated avian muscle grafts. *Cell Tissue Res.* **217**, 37–41.

- MÄKITTIE, J. & TERÄVÄINEN, H. (1977). Ultrastructure of striated muscle of the rat after temporary ischaemia. *Acta neuropath. (Berl.)* **37**, 237–245.
- MILBURN, A. (1976). The effect of the local anaesthetic bupivacaine on the muscle spindle of rat. *J. Neurocytol.* **5**, 425–446.
- MILEDI, R. & SLATER, C. R. (1970). On the degeneration of rat neuromuscular junctions after nerve section. *J. Physiol.* **207**, 507–528.
- MOORE, D. H., RUSKA, H. & COPENHAVER, W. M. (1956). Electron microscopic and histochemical observations of muscle degeneration after tourniquet. *J. biophys. biochem. Cytol.* **2**, 755–763.
- MUFTI, S. A., CARLSON, B. M., MAXWELL, L. C. & FAULKNER, J. A. (1977). The free autografting of entire limb muscles in the cat: Morphology. *Anat. Rec.* **188**, 417–430.
- NICHOLS, J. R. & SHAFIQ, S. A. (1979). Morphological changes in satellite cells in regenerating pectoralis muscle of the chicken. In *Muscle Regeneration* (ed. A. Mauro *et al.*), pp. 239–247. New York: Raven Press.
- ONTELL, M., HUGHES, D. & BOURKE, D. (1982). Secondary myogenesis of normal muscle produces abnormal myotubes. *Anat. Rec.* **204**, 199–207.
- PARTRIDGE, T. A. & SLOPER, J. C. (1977). A host contribution to the regeneration of muscle grafts. *J. neurol. Sci.* **33**, 425–435.
- QUICK, D. C. & ROGERS, S. L. (1983). Stretch receptors in regenerated rat muscle. *Neuroscience* **6**, 851–859.
- REYNOLDS, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J. Cell Biol.* **17**, 208–212.
- REZNIK, M. (1973). Current concepts of skeletal muscle regeneration. In *The Striated Muscle* (ed. C. M. Pearson & F. K. Mostofi), pp. 185–225. Baltimore: Williams & Wilkins.
- REZNIK, M. & HANSEN, J. L. (1969). Mitochondria in degenerating and regenerating skeletal muscle. *Archs Path.* **87**, 601–608.
- ROGERS, S. L. (1982). Muscle spindle formation and differentiation in regenerating rat muscle grafts. *Devl Biol.* **94**, 265–283.
- ROGERS, S. L. & CARLSON, B. M. (1981). A quantitative assessment of muscle spindle formation in reinnervated and non-reinnervated grafts of the rat extensor digitorum longus muscle. *Neuroscience* **6**, 87–94.
- SANES, J. R., MARSHALL, L. M. & McMAHAN, U. J. (1978). Reinnervation of muscle fibre basal lamina after removal of myofibres. *J. Cell Biol.* **78**, 176–198.
- SCHMALBRUCH, H. (1976). Muscle fibre splitting and regeneration in diseased human muscle. *Neuropathol. appl. Neurobiol.* **2**, 3–19.
- SCHMALBRUCH, H. (1977). Regeneration of soleus muscles of rat autografted *in toto* as studied by electron microscopy. *Cell Tissue Res.* **177**, 159–180.
- SCHMALBRUCH, H. (1979). Manifestations of regenerations in myopathic muscles. In *Muscle Regeneration* (ed. A. Mauro *et al.*), pp. 201–213. New York: Raven Press.
- SCHULTZ, E. (1978). Changes in the satellite cells of growing muscle following denervation. *Anat. Rec.* **190**, 299–312.
- STENGER, R. J., SPIRO, D., SCULLY, R. E. & SHANNON, J. M. (1962). Ultrastructural and physiologic alterations in ischemic skeletal muscle. *Am. J. Path.* **60**, 1–19.
- TERÄVÄINEN, H. (1970). Satellite cells of striated muscle after compression injury so slight as not to cause degeneration of the muscle fibres. *Z. Zellforsch. mikrosk. Anat.* **103**, 320–327.
- WALDAYER, W. (1865). Über die Veränderungen der quergestreiften Muskeln bei der Entzündung und dem Typhusprozess, sowie über die Regeneration derselben nach Substanzdefecten. *Virchows Arch. path. Anat. Physiol.* **34**, 473–514.
- WALRO, J. M., HIKIDA, R. S. & HATHER, B. M. (1984). Quantification and origin of spindles in orthotopic and heterotopic grafts of avian muscles. *Cell Tissue Res.* **235**, 515–519.
- WALTON, J. N. & ADAMS, R. D. (1956). The response of the normal, the denervated and the dystrophic muscle-cell to injury. *J. Path. Bact.* **72**, 273–298.
- YELLIN, H. (1969). A histochemical study of muscle spindles and their relationship to extrafusal fibre type in the cat. *Am. J. Anat.* **125**, 31–46.
- ZELENÁ, J. & SABOTKOVÁ, M. (1971). Absence of muscle spindles in regenerated muscles of the rat. *Physiologia bohemoslov.* **20**, 433–440.