ULTRASTRUCTURAL AND CYTOCHEMICAL FEATURES OF MAMMALIAN SKELETAL MUSCLE FIBRES FOLLOWING DENERVATION

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SUMMARY

After denervation, the usual cytochemical criteria for identifying fibre types in mammalian skeletal muscle are lost, and thus ultrastructural analysis becomes essential. The width of the Z-line is a valuable criterion for the identification of fibre types in normal muscle, and the differences in width remain apparent even after denervation. In the red portion of the normal rat semitendinosus muscle, there is a mixture of red, intermediate and white fibres. The red fibre has the highest mitochondrial content and the widest Z-line, whereas the white fibre has the lowest mitochondrial content and the narrowest Z-line (about half as wide as that of the red fibre). The intermediate fibre is intermediate in both characteristics. At 14 days after denervation, there is a shift toward a more homogeneous population of fibres, which are rich in mitochondria, but which have an ultrastructural appearance distinct from that of normal fibres. Most of the fibres resemble normal red or intermediate fibres, but lack the characteristic subsarcolemmal aggregations of mitochondria. There are also some fibres which exhibit severe myofibrillar disruption. These have narrow Z-lines, and are therefore classified as white fibres. This suggests that there is a preferential alteration of white fibres, and that their degradation may be responsible for the apparent increase in the proportion of red or intermediate fibres at this stage of denervation. In these disrupted fibres the sarcoplasmic membrane systems lose their usual organization, and triads become aligned parallel to the longitudinal axis of the myofibrils. All fibres, therefore, are, to some extent, altered by denervation, though the changes differ according to the type of fibre.

In normal fibres, ribosomes are sparse, but following denervation they are abundant, particularly at the periphery. This is a general response to removal of the nerve supply, and does not appear to be related to fibre type; but it is more apparent in red and intermediate fibres, which possess an extensive superficial sarcoplasm. Conspicuous aggregations of large mitochondria normally occur in the subsarcolemmal region of these fibres, but after denervation, there are, instead, massive accumulations of free ribosomes and a few cisternae of rough-surfaced endoplasmic reticulum. Our observations suggest, moreover, that this concentration of protein-synthetic machinery corresponds to a spread in the sensitivity to acetylcholine along the surface of the fibres.

INTRODUCTION

Observations on the ultrastructural changes in skeletal muscle which follow removal of the nerve supply are conflicting. It has been reported, for example, that the mitochondrial content of frog muscle increases following denervation (Muscatello, Margreth & Aloisi, 1965). Under similar circumstances, however, there is apparently a

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decrease in mitochondria in muscles of the rat (Pellegrino & Franzini, 1963; Miledi & Slater, 1968) and of the pigeon (Muscatello & Patriarca, 1968). In the chicken, the mitochondrial response is related to the particular muscle denervated; that is, mitochondria may decrease in size and number or may not be altered at all (Jirmanová & Zelená, 1970). Cytochemical observations, likewise, suggest that in skeletal muscles of the rat there is either an increase (Bajusz, 1964; Feng & Lu, 1965) or a decrease (Nachmias & Padykula, 1958; Feng & Lu, 1965) in the mitochondrial content of muscle fibres following denervation. These contradictory findings arise, in part, because of differences in the species of animal studied; they may be related also to the interval of time following denervation, or the age of the animal. In addition, there has been little recognition of the fact that skeletal muscles tend to be composed of different types of fibres. It is important that the entire population of fibres be examined when analysing the effects of experimental alteration.

It has long been known that the fibres of an individual mammalian skeletal muscle vary in their microscopic appearance (Ranvier, 1874; Grützner, 1884; Knoll, 1891), but procedures for the histochemical demonstration of enzymic activity have revealed an even more striking difference among fibres (Padykula, 1952; Ogata, 1958; Dubowitz & Pearse, 1960; Engel, 1962; Padykula & Gauthier, 1963; Guth & Samaha, 1969). This heterogeneity of skeletal muscle can explain some of the apparent discrepancies among experimental findings. Thus, in the rat, for example, some fibres which are normally rich in mitochondria appear to survive denervation (Bajusz, 1964). It has, on the other hand, been suggested that those fibres which have a low mitochondrial content become richer in mitochondria, while those which have a high mitochondrial content lose their mitochondria (Feng & Lu, 1965). The pattern of distribution of mitochondria within individual fibres may also be altered, so that concentration at the periphery in particular may decrease after denervation (Nachmias & Padykula, 1958). The present study is concerned primarily with the changes which occur in the semitendinosus muscle of the rat at 14 days following denervation. This muscle normally consists of 3 major types of fibres (Gauthier, 1969). After removal of the nerve supply, there is a shift toward a more homogeneous population of fibres. The response is selective, but each of the 3 fibre types displays a distinctive change in its ultrastructural appearance.

MATERIALS AND METHODS

Surgical procedure

Adult male albino rats, approximately 90 days old, were anaesthetized lightly with ether. The belly region of the semitendinosus muscle was exposed ventrally by blunt dissection. The branch of the sciatic nerve, which innervates the red (anterior) portion of the muscle in the belly region, was carefully separated from the accompanying vasculature, and approximately 1 cm of the nerve, close to its contact with the muscle, was removed. The animals were sacrificed at 14 days after surgery. Normal male rats, 90–120 days old, were used as control animals.

Preparation of tissue

All animals were killed with chloroform, and the musculature of the upper hind limb was exposed ventrally. The gracilis posticus was cut and retracted to gain access to the semiten-
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dinosus. A flat wooden splint was tied to the muscle in the middle of the belly region, and a segment, about 1 cm in length, was cut beyond the ligatures through the entire thickness of the muscle. Tied specimens were frozen in dry ice and 95 % ethanol at −70 °C for 10 min. For electron microscopy, a thin strip (approximately 1 mm) of muscle was separated from the anterior border of the muscle. The strip was tied to a wooden toothpick, and then severed beyond the ligatures and transferred to cold fixative.

CytocJiemical procedures

Transverse sections (5-10 μm) of frozen muscle were cut in a cryostat and placed on glass slides. Succinic dehydrogenase activity was localized using nitro blue tetrazolium as substrate, according to the method of Nachlas et al. (1957). Sections were incubated for 20 min at 37 °C.

To demonstrate cytoplasmic basophilia, equivalent frozen sections were fixed for 10 min in acetic acid–ethanol–formol (Serra’s fixative) at room temperature, and then stained with eosin–methylene blue at pH 4-1 for 3 h. Control sections were treated with ribonuclease before staining.

Carbohydrate (glycogen) was demonstrated in frozen sections cut sequentially to those stained with eosin–methylene blue. The sections were fixed for 10 min in picric acid–ethanol–formol (Rossum’s fixative) at 0-5 °C, and then stained by the periodic acid–Schiff (PAS) reaction. Equivalent sections were digested with diastase to assure identification of stained carbohydrate as glycogen.

Electron microscopy

Thin strips of muscle were fixed in cold (0–5 °C) 6-25 % glutaraldehyde buffered with 0-1 M sodium cacodylate at pH 7-4 (Sabatini, Bensch & Barrnett, 1963) and postfixed in 2% un-buffered osmium tetroxide. The strips of muscle were cut into 1-mm cubes and stained with 1 % uranyl acetate before dehydration, and then dehydrated blocks were embedded in Epon. Thin sections were cut on a Porter-Blum MT-2 ultramicrotome, using a diamond knife. They were mounted on 200-mesh copper grids, stained for 2 min with 3 % uranyl acetate and for 10 min with lead hydroxide (Karnovsky, 1961), and then examined with a Siemens Elmiskop I A.

RESULTS

The differential response to denervation

The semitendinosus muscle of the rat consists of 2 longitudinal bands, one red (anterior) and one white (posterior). Normally, the red portion of the muscle is composed of a mixture of red (52 %), intermediate (40 %), and white (9 %) fibres (Gauthier, 1969). Red fibres have relatively small cross-sectional dimensions, but they are rich in large mitochondria with abundant cristae, especially at the periphery of the fibres (Figs. 1, 3); they also have wide Z-lines (compare Figs. 3 and 7). White fibres have large cross-sectional dimensions, a low mitochondrial content, and very narrow Z-lines (Figs. 1, 7). Preliminary measurements indicate that the Z-line of the red fibre is consistently about twice as wide as that of the white fibre (approximately 80 and 40 nm, respectively). In intermediate fibres, all 3 characteristics are intermediate between those of red and white fibres; these fibres resemble red fibres closely, particularly in the content and distribution of mitochondria (Fig. 1), but the Z-line is clearly narrower than in the red fibre.

At 14 days after removal of the nerve supply to the red portion of the muscle, cytological features are altered, and the alteration is confined to the red portion. That is, there is no apparent change in the white region, which has a separate nerve supply.
Because the usual criteria for identification of fibre types are lost following denervation, ultrastructural analysis becomes essential for classification. Differences in the width of the Z-line remain even after 14 days, and this variation in width provides an important criterion for identification at the ultrastructural level.

Succinic dehydrogenase activity was used to demonstrate mitochondrial content at the light-microscopic level. After denervation, there is a definite shift toward a more homogeneous population of fibres, which resemble red or intermediate fibres. Most of the fibres have a relatively small diameter, and their mitochondrial content is high (Fig. 2). However, the characteristic subsarcolemmal aggregations of mitochondria (Fig. 3) are absent (Fig. 4). Instead, the subsarcolemmal sarcoplasm contains massive accumulations of small electron-opaque particles and only a few small mitochondria (Fig. 4). There occur, in addition, profiles of narrow cisternae of rough-surfaced endoplasmic reticulum (Figs. 5, 6). The particles which are free in the sarcoplasm resemble those which are attached to the membranes of these profiles (Fig. 6), and thus are believed to be ribosomes (see below). Scattered filaments, occasional myelin figures, and a few lysosomes may also be present in this region of the sarcoplasm, but otherwise there are no apparent changes. In the interior of the fibres, myofibrils have the usual banding pattern and alignment observed in mammalian skeletal muscle fibres in general, and large mitochondria form longitudinal interfibrillar rows, which are characteristic of red and intermediate fibres in particular. By direct comparison with normal fibres, dimensions of the Z-lines were estimated. The relatively wide Z-lines further indicate that these denervated fibres are red and intermediate fibres.

There are present, in the denervated muscle, some fibres which exhibit severe myofibrillar disruption (Fig. 8). These fibres have Z-lines which are comparable in width to those of normal white fibres, and therefore they are classified as white fibres. They have, in addition, very few mitochondria, even in the interior, which is also a characteristic feature of normal white fibres (Fig. 7). These fibres most likely correspond to the few fibres with very low mitochondrial content, which can be distinguished with the light microscope as well (Fig. 2). Sarcomeres are usually disorganized (Fig. 8). Although transverse striations are evident, they tend to be less distinct and less orderly than in normal muscle fibres. Disruption of Z-lines is so conspicuous that their profiles often appear to be 'smeared' in an irregular fashion along the longitudinal axis of the myofibrils; in many instances they are not even visible, and only A- and I-bands are apparent (Fig. 8). The sarcoplasmic membrane systems are altered also. Triads, in particular, which are normally aligned transverse to the longitudinal axis of the myofibrils (Fig. 9) are now more or less parallel to the longitudinal axis (Figs. 10, 11). The form and distribution of the other components of the sarcoplasmic reticulum are, as a result, not easily discerned. This altered arrangement of triads is not necessarily a consequence of myofibrillar disorganization, since longitudinal triads may occur in regions where myofibrils are intact. In addition, occasional lysosomes are present in these disrupted fibres. The disrupted fibres have, in common with the more intact mitochondria-rich fibres, an abundance of small electron-opaque particles, especially in the subsarcolemmal sarcoplasm (Fig. 8).
Cytochemical analysis of the sarcoplasm

In an effort to identify the nature of the small subsarcolemmal particles which are so conspicuous following denervation, sections of normal and denervated muscle were stained with eosin–methylene blue to demonstrate basophilia, which might be expected at sites containing large numbers of ribosomes. In addition, glycogen was localized in serial sections by the periodic acid–Schiff (PAS) reaction. Normal skeletal muscle fibres in the semitendinosus exhibit no obvious cytoplasmic basophilia (Fig. 12). The effectiveness of the staining procedure is demonstrated by the basophilia of nuclei and by the metachromasia of the cytoplasm of mast cells present between the muscle fibres (Fig. 12). In contrast, fibres in the denervated muscle possess a distinct peripheral ‘rim’ of basophilic material (Fig. 13), which corresponds in position to the peripheral accumulation of particles visible with the electron microscope; and this rim is absent in sections treated with ribonuclease. There is, however, no equivalent preferential distribution of PAS-positive material at the periphery of serial sections of the same fibres (Fig. 14). PAS-positive material is distributed, in varying amounts, throughout the interior of the fibres, where glycogen might be expected. It is concluded, therefore, that the massive subsarcolemmal accumulations of particles reflect, at least quantitatively, ribosomes rather than particulate glycogen. Consistent with these observations is the fact that the absence of peripheral basophilia in normal skeletal muscle fibres corresponds to an overall lack of particles in the subsarcolemmal sarcoplasm of normal fibres. Conspicuous accumulations of ribosomes and rough-surfaced endoplasmic reticulum in normal fibres are present only in the immediate vicinity of the neuromuscular junction (see Discussion). No attempt has been made, in this study, to determine the relationship between glycogen content or basophilia and fibre type. The observations suggest, in fact, that accumulations of subsarcolemmal ribosomes are a characteristic response to denervation in all 3 fibre types.

DISCUSSION

Selective effects of denervation on fibre types

Because most mammalian skeletal muscles consist of mixtures of different types of fibres it is essential that the total population of fibres be evaluated with the light microscope before attempting to analyse the ultrastructural effects of experimental alteration. Examination with the electron microscope, on the other hand, is essential, since the usual criteria for identifying fibre types may be lost. Decreased heterogeneity following denervation is not a new observation. There is, in general, a loss in the ability to distinguish fibre types in skeletal muscles of the rat, based on mitochondrial enzymic activity (Bajusz, 1964; Feng & Lu, 1965); fibres rich in mitochondria (comparable to red or intermediate fibres) predominate. Using myofibrillar ATPase activity as a criterion for identification, there is a preferential atrophy of ‘type II’ fibres (equivalent to white fibres) in skeletal muscles of the cat, guinea-pig, and in man (Engel, Brooke & Nelson, 1966; Karpati & Engel, 1968a, b; Guth, Dempsey & Cooper, 1971). Experimental manipulations which cause disuse of a muscle without loss of
innervation (tenotomy, for example) give rise to selective atrophy of 'type I' fibres (probably equivalent to red or intermediate fibres) (Engel et al. 1966). Analogous observations have been made on human muscle in certain pathological conditions (see Engel, 1970). Where the disease is neurological in origin, for example, there is a characteristic atrophy of white fibres (Edström, 1970a; Buchthal, Schmalbruch & Kamieniecka, 1971), while red fibres tend to show signs of atrophy after injuries to a ligament that result in dysfunction (Edström, 1970b). The specificity of the neurological lesion or the removal of the nerve supply is consistent with the view that the effects of denervation are not the result of consequent disuse of a muscle following removal of its nerve supply, but rather are related to some trophic influence of the nerve itself (Guth, 1968; Gutmann, 1969).

The shift toward a homogeneous population of fibres could represent a transformation of one fibre type to another or a preferential loss of a particular fibre type. The exact mechanism is difficult to evaluate with the light microscope alone, since the criteria used to identify a given fibre may themselves become changed. Since characteristic differences in Z-line width remain after removal of the nerve supply, this seemed to provide a reasonably stable criterion for identification with the electron microscope. The present study has demonstrated that myofibrillar disruption following denervation is most prevalent in fibres with very narrow Z-lines (white fibres), which suggests a preferential degradation of this fibre type in particular. This degradation, therefore, is most likely responsible for the apparent increase in red or intermediate fibres under the conditions of this study. Because of the small percentage of white fibres normally present, however, the sampling problem inherent in electron microscopy is enhanced. Therefore it is difficult to rule out the possibility that some intact white fibres might be present but not observed in the denervated muscle.

The presumed survival of red or intermediate fibres is poorly understood. The phenomenon of selective atrophy may reflect an intrinsic difference in the muscle fibres themselves, or in the motoneurons which serve them. It has been suggested that the red fibre is less dependent on the nerve supply than is the white fibre (Bajusz, 1964). It has also been suggested that certain muscle proteins can be maintained without a 'neurotrophic influence' (Guth et al. 1971). Our observations have indicated that those fibres which predominate after denervation have a distinctive ultrastructural appearance; that is, they are not completely resistant. Cytochemical differences among motoneurons have been demonstrated (Campa & Engel, 1970); and physiological differences among motor units (Edström & Kugelberg, 1968; Burke, Levine & Zajac, 1971) indicate that a given type of motoneuron supplies one type of muscle fibre. In addition, the size of the neuromuscular junction is directly proportional to the diameter of the muscle fibre (Nystrom, 1968). Ultrastructural differences in the neuromuscular junctions of red, intermediate and white fibres also indicate that there are differences, not only among muscle fibres, but also among their motoneurons (Padykula & Gauthier, 1970). It has been demonstrated, furthermore, that the length of the distal stump which remains early after denervation is an important factor in the subsequent changes in physiological and ultrastructural properties of the neuromuscular junction in the rat (Miledi & Slater, 1970; Harris & Thesleff, 1972); yet differences in physio-
logical characteristics (miniature end-plate potentials) among fibre types persist even after axonal degeneration in the frog (Miledi & Stefani, 1970). Therefore, differences among muscle fibres may reflect differences in the corresponding motoneurons, but the differences may exist even after the motoneurons have been transected.

While the exact basis of ultrastructural changes following denervation remains unclear, the selective nature of the response among fibres in a heterogeneous population may account for some of the apparent discrepancies among reported ultrastructural observations on denervated skeletal muscle.

Implications of increased protein synthesis

In general, reports of an increase in RNA, DNA, or protein synthesis in denervated skeletal muscle (Gutmann et al. 1966; Zak, Grove & Rabinowitz, 1969; Bowman & Martin, 1970) have been concerned with a special phenomenon of hypertrophy, which occurs early (about 3 days) after denervation in the diaphragm in particular, and this involves an increase in the size of the component muscle fibres (Sola & Martin, 1953; Feng & Lu, 1965; Miledi & Slater, 1969). In the absence of hypertrophy, the existence of machinery for protein synthesis might be responsible for the formation of hydrolytic enzymes, which are known to increase following denervation (Pollack & Bird, 1968). Alternatively, it has been suggested that denervated skeletal muscle fibres are in a state of dedifferentiation, and that the presence of ribosomes among myofibrils reflects an increased formation of organelles such as mitochondria or sarcoplasmic reticulum (Muscatello et al. 1965; Muscatello & Patriarca, 1968). The present study suggests that denervated fibres are similar, in some respects, to undifferentiated fibres. Longitudinal orientation of triads, for example, is characteristic, not only of denervated muscle, but also of developing muscle (Walker & Schrodt, 1968; Schiaffino & Margreth, 1969; Edge, 1970); and the absence of subsarcolemmal aggregations of mitochondria is observed during postnatal development (Gauthier, 1970) as well as following denervation. None of these explanations seems adequate, however, to account for the massive accumulations of ribosomes, particularly in the subsarcolemmal regions of denervated fibres.

Our observations suggest that increased ribosomes may be concerned with increased synthesis of a protein receptor substance. This possibility is supported by physiological observations. Normally, skeletal muscle fibres are sensitive to acetyl choline only at the neuromuscular junctions, but after denervation there is a spread in sensitivity along the entire length of the fibres (Axelsson & Thesleff, 1959; Miledi, 1960); and this spread is inhibited by compounds which block protein synthesis (Grampp, Harris & Thesleff, 1972). Also, skeletal muscle fibres normally contain very few ribosomes; the majority of small electron-opaque particles are glycogen (Galavazi, 1971). Ribosomes are abundant, however, together with cisternae of rough-surfaced endoplasmic reticulum, in the immediate vicinity of the neuromuscular junction (Padykula & Gauthier, 1970); and it is only after denervation that they accumulate in large numbers along the entire surface of the fibres. The spread in sensitivity to acetyl choline in the tenuissimus muscle of the cat (Axelsson & Thesleff, 1959) corresponds, moreover, in time (14 days) to the accumulation of ribosomes observed in the
semitendinosus muscle of the rat in the present study. Our hypothesis is further supported by a recent study of the soleus and extensor digitorum longus muscles of the rat (Lømo & Rosenthal, 1972). Either nerve impulse blockade or denervation causes an increased sensitivity to acetyl choline 'as early as 3 days', particularly in the soleus. Although the present study has emphasized the 14-day stage of denervation, we have examined earlier time intervals as well. There is a definite aggregation of subsarcolemmal ribosomes in fibres of the rat semitendinosus at 3 days and also at 2 days following denervation, which suggests that accumulation of ribosomes accompanies, or possibly even precedes, the onset of acetyl choline sensitivity.

In addition, muscle fibres of the rat diaphragm are sensitive to acetyl choline along their entire length at birth (Diamond & Miledi, 1962). In this laboratory we have observed a corresponding abundance of subsarcolemmal ribosomes in the fibres of the rat diaphragm at birth (Ruderman, 1972). It has been demonstrated, furthermore, that undifferentiated muscle fibres in tissue culture are sensitive to acetyl choline along their entire surfaces (Fambrough & Rash, 1971; Robbins & Yonezawa, 1971), and that inhibition of protein synthesis prevents the development of 'supersensitivity' in vitro (Fambrough, 1970).

Finally, recent studies using neuromuscular blocking agents have shown that denervation results in the extension of the binding of certain neurotoxins beyond the usual binding site at the neuromuscular junctions (Truog & Waser, 1970; Creese, Taylor & Case, 1971; Miledi & Potter, 1971).

The above findings are consistent with a spread in the distribution of a receptor substance, which, at the neuromuscular junctions of normal fibres, is composed, at least in part, of protein (De Robertis, 1971). The morphological counterpart, namely a spread in the aggregation of ribosomes and rough-surfaced endoplasmic reticulum beyond the neuromuscular junction, could provide the machinery for the synthesis of this protein. It has, in fact, been suggested that the increased sensitivity to acetyl choline following denervation reflects, not an increase in sensitivity of the existing receptor, but rather an 'increase in size of the receptor area' (Axelsson & Thesleff, 1959) or a 'proliferation of receptors' (Miledi, 1960).

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REFERENCES


Denervated skeletal muscle fibres


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Fig. 1. Normal semitendinosus. Succinic dehydrogenase. In this transverse section, 3 fibre types are apparent. Red fibres (r) have the smallest diameter and the highest mitochondrial content, especially at the periphery. White fibres (w) have the largest diameter and the lowest mitochondrial content. Intermediate fibres (i) have characteristics between the two. ×400.

Fig. 2. Denervated semitendinosus. Succinic dehydrogenase. Most fibres are small in diameter and rich in mitochondria; however, peripheral accumulations of mitochondria are not conspicuous. ×400.
Fig. 3. Normal red fibre. This electron micrograph shows the periphery of the fibre and includes a portion of a nucleus (n). Numerous large mitochondria (mt) with closely packed cristae are aggregated in this region. These, together with the wide Z-line (Z), permit identification of this fibre type. × 14,220.

Fig. 4. Denervated red fibre. In the nuclear region (n) of this fibre the characteristic aggregations of mitochondria are absent. Instead, there is a massive accumulation of small electron-opaque particles and only a few small mitochondria (mt). Otherwise the fibre appears to be intact. × 14,220.
Fig. 5. Denervated red fibre. Abundant small electron-opaque particles and a few profiles of rough-surfaced endoplasmic reticulum (arrows) are present in the peripheral sarcoplasm near the nucleus (n). \( \times 22500 \).

Fig. 6. Denervated red fibre. Profiles of rough-surfaced endoplasmic reticulum (arrows) are seen to advantage in the subsarcolemmal sarcoplasm. Comparison of dimensions suggests that free particles as well as those attached to membranes are ribosomes (see text). Myelin figures are apparent also, especially at the left of the micrograph. \( \times 32500 \).
Denervated skeletal muscle fibres
Fig. 7. Normal white fibre. Even in the region of the nucleus (n), mitochondria are absent. The overall low mitochondrial content and narrow Z-line (Z) are characteristic of this fibre type. × 14,220.

Fig. 8. Denervated white fibre, nuclear region (n). Numerous ribosomes and a few lysosomes (arrow) are present beneath the sarcolemma. Myofibrils are highly disrupted. Organization of sarcomeres is indistinct, and Z-lines tend to be 'smearred' along the longitudinal axis of the myofibrils. × 14,220.
Denervated skeletal muscle fibres
Fig. 9. Normal white fibre. The characteristic arrangement of the sarcoplasmic membrane systems on either side of the Z-line (Z) is apparent. Two triads (arrows) are aligned transverse to the longitudinal axis of the myofibrils, close to the A-I junctions. ×38700.

Fig. 10. Denervated white fibre. Orientation of triads (arrows) is oblique to or perpendicular to the usual direction. Compare with the equivalent region of the normal fibre in Fig. 9. ×38700.

Fig. 11. Denervated white fibre. Portions of 3 triads (arrows) illustrate the altered alignment of the sarcoplasmic membrane systems. Triads are more or less parallel to the longitudinal axis of the myofibrils. ×38700.
Fig. 12. Normal semitendinosus. Eosin-methylene blue. Photographed using a Kodak no. 25 red filter; basophilic areas (stained with methylene blue) appear dark in the micrograph. The muscle fibres are, for the most part, not stained with methylene blue; only the nuclei are stained. The intensely basophilic cytoplasm of a mast cell (upper right) indicates the effectiveness of the staining procedure. × 640.

Fig. 13. Denervated semitendinosus. Eosin-methylene blue. Most of the fibres possess a distinct peripheral 'rim' of basophilic material. × 640.

Fig. 14. Denervated semitendinosus. PAS. Section is serial to that in Fig. 13. There is no preferential distribution of PAS-positive material at the periphery of the fibres, that is, at sites corresponding to the basophilic regions in the serial sections of the same fibres. PAS-positive material is present, in varying amounts, in the interior of the fibres, where glycogen is known to be present. × 640.
Denervated skeletal muscle fibres