J. exp. Biol. **115**, 55–68 (1985)
Printed in Great Britain © *The Company of Biologists Limited 1985*

ADAPTABILITY OF ULTRASTRUCTURE IN THE MAMMALIAN MUSCLE

BY BRENDA R. EISENBERG

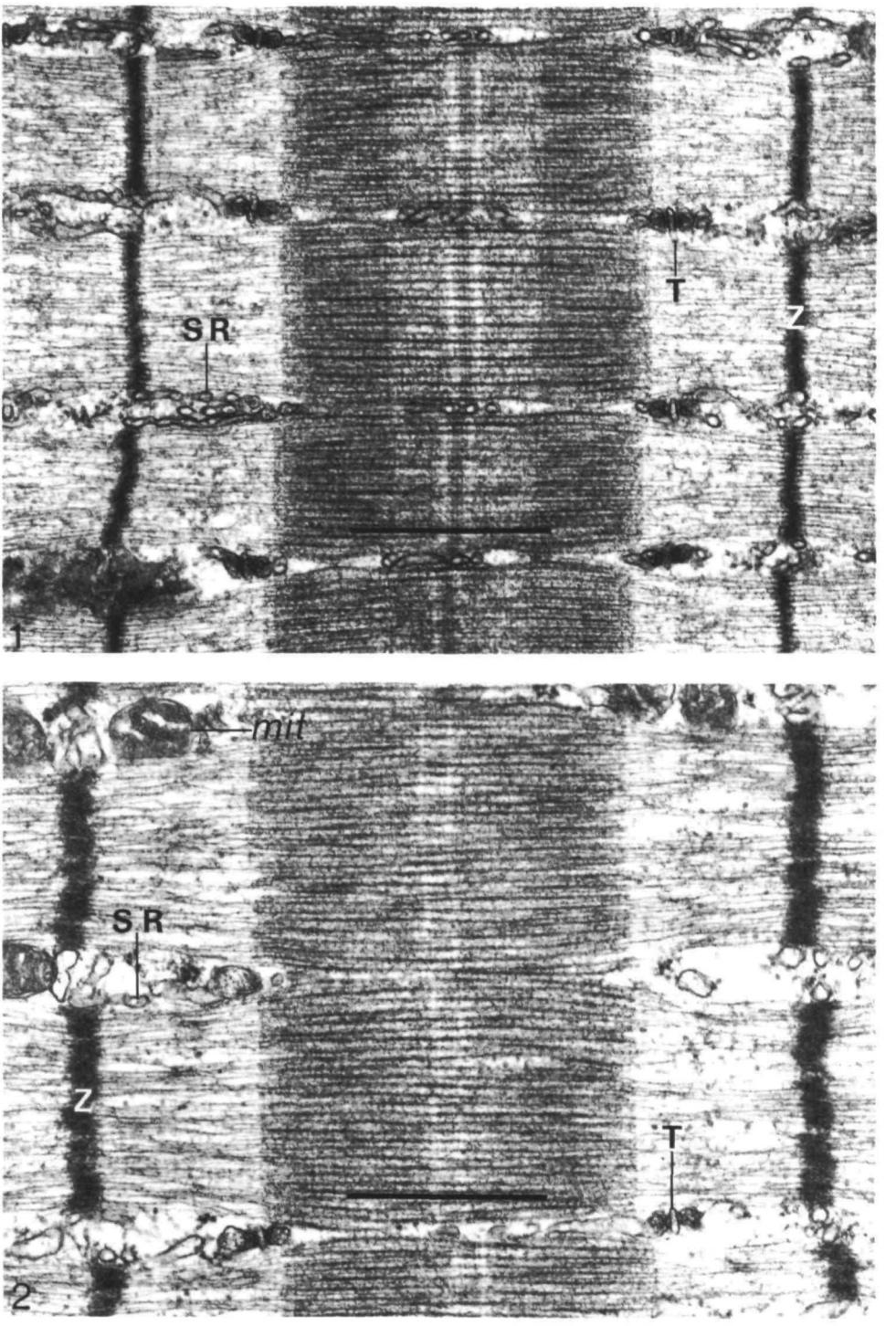
Department of Physiology, Rush Medical College, 1750 West Harrison St, Chicago, IL 60612, U.S.A.

SUMMARY

All the skeletal muscle fibres taken from an adult mammal do not look alike. The structural differences are a result of adaptations which allow gradations in mechanical output to be achieved. The anatomy is described and the amounts of the subcellular components are measured by stereological techniques from electron micrographs. A population of normal, adult fibres is classified by the Z-line width, by the amounts of the mitochondria, T-system and terminal cisternae (TC), and by the isoforms of contractile proteins present. Classification of fibres by some of these ultrastructural components gives clusters named fast-twitch and slow-twitch types, but classification by other components gives a continuum of overlapping properties. Transformation from the fast- to the slow-twitch type or *vice versa* follows a specific alteration in the use of the fibre. The mechanical demand on the fibre is modified by changing the frequency of stimulation in the nerve with an implanted electrode. The time course of the changes in subcellular composition in the fibre during adaptation is followed for many weeks. Changes in the membrane systems begin within hours and are complete in days. Changes in the contractile proteins and metabolic systems begin in days and are complete in weeks. During these transitional phases of adaptation the fibres have an unusual complement of components never seen in a normal adult fibre. Extreme alterations, such as myofibril disassembly or supranormal amounts of mitochondria also result during some adaptive transitions. The aberrant appearance in the transitional fibres may be a result of doing the required mechanical work with a less than optimal set of proteins. At the end of the fibre type transformation, the fibre ultrastructure is indistinguishable from normal.

Modern cell biology is concerned with the ways by which the cell is able to produce specific proteins by the regulation of gene expression. This is often studied in developing tissues where a predetermined sequence of changes occurs involving an orderly progression from the embryonic to the adult forms of the protein. However, there are also naturally occurring changes which take place in adulthood and these give the animal the evolutionary advantage of surviving transient changes in the adult environment. Cardiac and skeletal muscles show an example of adult adaptation at the

Key words: Fibre-type, myofibrils, adaptation, sarcoplasmic reticulum.



Figs 1, 2

molecular level. A sudden drastic change in the physiological requirement of the kind of mechanical output of the cell is met at first by using the existing contractile proteins in a non-optimal, perhaps energetically inefficient way. Within a period of a few days the cell adjusts the gene expression so that a different and better suited protein is produced instead. This protein exchange can sometimes be accomplished without a catastrophic interruption of function which would end in destruction of the animal. The heart beats and pumps blood throughout the protein exchange.

Changes which require alteration of the contractile protein composition are not a commonly occurring natural event in skeletal muscle. We are normally more concerned with the changes that involve merely an alteration in the amounts of the subcellular components rather than a change in kind. For example, strength training or immobilization regulates fibre hypertrophy and atrophy, and endurance training regulates metabolic content (Salmons & Henriksson, 1981). There are thus complex interactions possible between several regulatory systems which determine the final appearance and performance of any one muscle fibre.

In the normal adult mammal, the activity patterns in the motor nerve are restricted to a limited repertoire and therefore there is not an infinite variety of performance demanded from the muscle fibres. As the activity pattern largely determines the composition of the fibre it follows that the fibre composition is necessarily also limited to restricted types (Burke & Edgerton, 1975). Thus the normal skeletal muscle fibres can be classified by their composition into types. In some cases the structure within the fibre can differ in the extent of the organelles and in other cases in the kind of molecule used to build the organelles.

The first problem in the study of the anatomy within mammalian skeletal muscle was to find an objective definition of the fibre types rather than the use of qualitative descriptions of the subcellular organelles and the arbitrary assignment to a type. Electron microscopy is one of the few methods which views more than one system at a time. I combined stereological techniques (Weibel, 1979) to estimate the amount of the organelles from many fibres and used a multivariate discriminant analysis to allocate them into types. A series of papers gave structural data for these systems in normal muscle (Eisenberg, Kuda & Peter, 1974; Eisenberg & Kuda, 1975, 1976; Eisenberg, 1983). I found that in many cellular components there was a broad, continuous distribution in the quantitative amount of the organelle present rather than a very narrow, limited amount. Classification could not be made from some of the components as claimed previously but it was possible to classify fibres into types by combinations of several systems and by assays which depended on the discrete molecular isoforms found in the contractile proteins. These many systems work

Fig. 1. Electron micrograph of longitudinal section of normal fast-twitch skeletal muscle from the guinea pig white vastus lateralis muscle, showing narrow Z-bands, few mitochondria and extensive sarcoplasmic reticulum (SR) and T-system. (From Eisenberg & Kuda, 1975.) Scale bar, 1 μm.

Fig. 2. Longitudinal section from normal slow-twitch soleus muscle of guinea pig showing wider Z-bands, more mitochondria (*mit*) and sparser SR and T-systems than fast-twitch muscle. (From Eisenberg, Kuda & Peter, 1974.) Scale bar, 1 μm.

together to produce ranges in the properties of the mechanical twitch, the time course, output of force and resistance to fatigue.

I will illustrate the major ultrastructural differences by micrographs of a fast-twitch and a slow-twitch guinea pig muscle selected to represent the typical properties (Figs 1, 2). The quantitative distribution of each organelle within a muscle fibre is related to its own physiological function. The functional role of the membrane systems of muscle fibres is to regulate the speed and duration of the contraction and perhaps to control the peak twitch tension. The T-system carries inward the electrical signal which activates the inner fibrils; then the terminal cisternae release calcium to initiate contraction. Fast-twitch fibres have a T-system that is about twice as extensive as that of slow-twitch fibres of the same species. The membrane area of the T-system has the most functional significance because the action potential travels over this surface, which can be estimated by surface density and length of T-luminal axis per

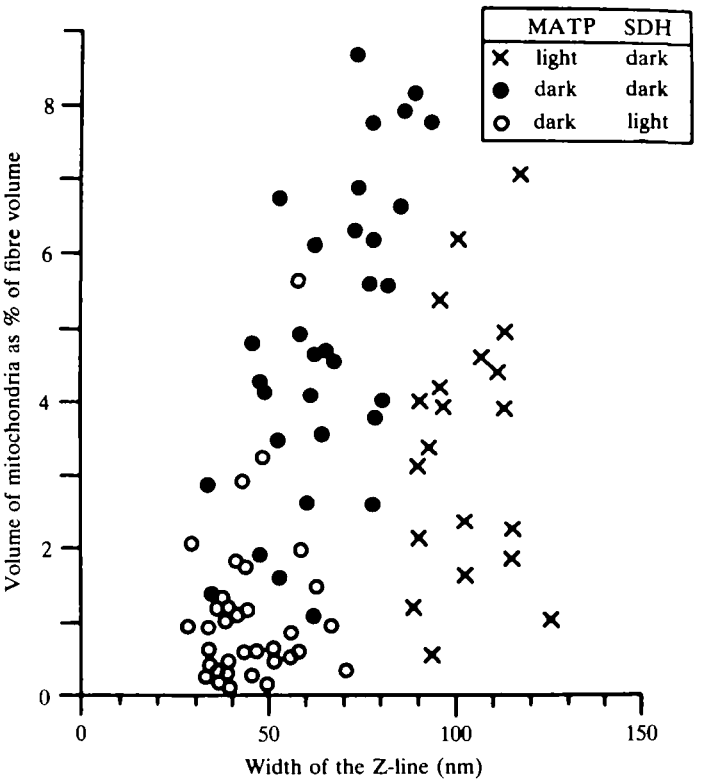
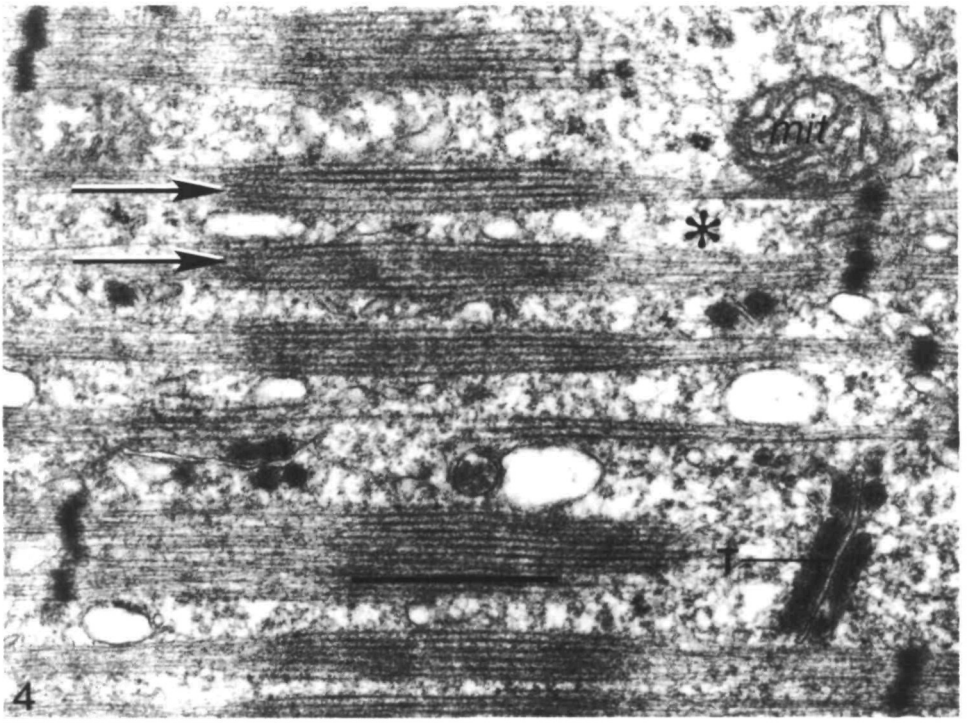


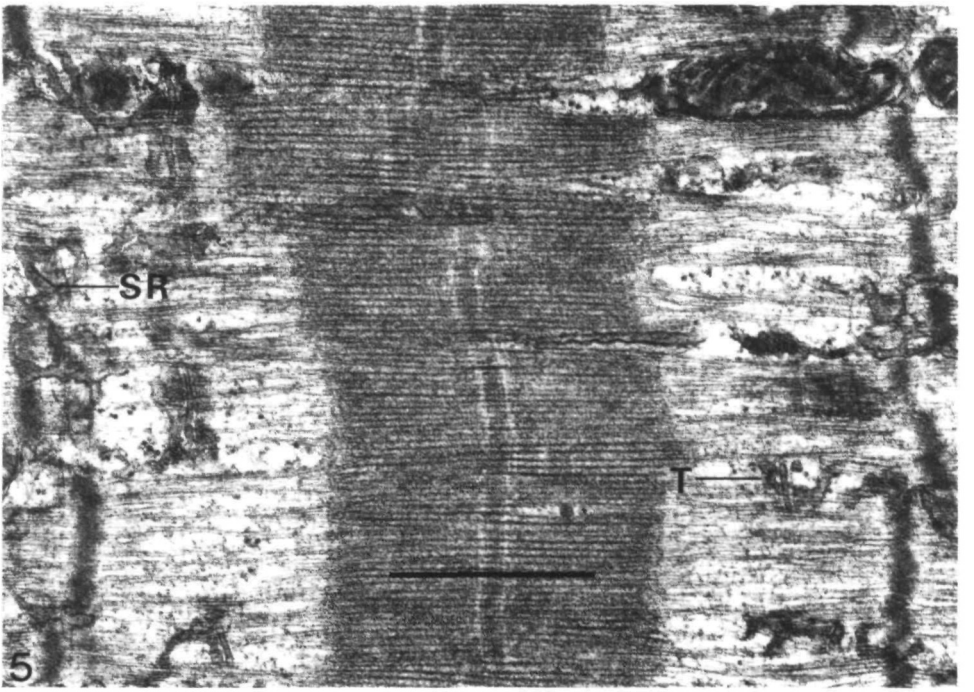
Fig. 3. Scattergram of Z-line width *vs* mitochondrial volume measured from electron micrographs of medial gastrocnemius guinea pig muscle fibres that had been frozen, thawed and then fixed. Serial cryostat sections were used to determine histochemical stains of myofibrillar ATP (MATP) and succinic dehydrogenase (SDH) of each fibre to give the conventional fibre types: ×, slow-twitch, oxidative; ●, fast-twitch, oxidative, glycolytic; and ○, fast-twitch, glycolytic. The three types form one large cluster that can be separated into three subclusters only by reference to histochemical profile of the fibre. (From Eisenberg & Kuda, 1977, reprinted from *J. Histochem. Cytochem.* Copyright 1977 by The Histochemical Society, Inc.)

unit volume of muscle fibre (quantitative values reviewed by Eisenberg, 1983). Fast-twitch fibres also have a more extensive (TC) system than do slow-twitch fibres and the volume fraction of the TC is related to histochemical type and contraction time (Kugelberg & Thornell, 1983). The T and TC systems cooperate functionally and the amounts of both systems within a given fibre are highly correlated (Eisenberg & Kuda, 1976). Thus the amount of membrane controlling the release of the calcium stores is high in fast fibres and low in slow fibres. Between the terminal cisternae runs the longitudinal sarcoplasmic reticulum (SR) which is densely covered by Ca^{2+}-ATPase pump sites. These reaccumulate the calcium into the SR. However, surprisingly, in guinea pig muscle the surface area of the SR was the same in the fast- and slow-twitch fibres even though one might have expected faster fibres would have higher amounts of pumping membranes. The fact that the terminal cisternae and T-systems do differ in the fast and slow fibre types but that the SR does not perhaps implies that the calcium release dominates contractile speed but that the relaxation speed is governed by the absolute amount of calcium present in the sarcoplasm.

The next physiological system is the metabolic system, which mainly supplies the energy to maintain contraction and is energized directly by ATP. The organelles involved in oxidative and glycolytic metabolism are the mitochondria, glycogen granules and lipid droplets. The blood system supplies the muscle cell with the necessary oxygen and other nutrients. Fibres with a high oxidative capacity have a high mitochondrial content, a rich capillary supply and a red colour. The low oxidative (and high glycolytic) fibres have a low oxidative capacity, a sparse blood supply and a whiter colour. In human muscle a bimodal distribution allows easy separation into two populations of high-oxidative (red) and low-oxidative (white) fibres. However, in most mammals there are intermediate fibres with a pinkish colour (e.g. in guinea pig the distributions are far more complicated and many of the fibres belong to an intermediate category). The mitochondrial content does not correlate in any way with the speed of an individual twitch, but it has been correlated with resistance to fatigue or with the frequency and duration of a burst of twitches. The mitochondrial content is readily altered by increased demands on the muscle. Mitochondrial enzymes can be assessed histochemically on frozen sections by succinic dehydrogenase (SDH) and stereologically in the electron micrographs by estimates of the volume fraction of the mitochondria.

The final system to be used physiologically is the contractile machinery of the actin and myosin filaments, which slide past each other to produce a mechanical twitch. The filaments are attached to the Z- and M-bands which do show fibre-type distinctions in the electron micrographs and we used Z-band width in our morphometric analysis. The contractile filaments of fast and slow muscles show no apparent differences in electron micrographs. The molecular differences in the myosin isoforms have not yet been identified in whole tissue as they have with the electron microscope. However, histochemical separation with light microscopy has been successful. The relationship of the myosin ATPase activity to the fibre type has long been demonstrated (Barany, 1967) and fast fibres split ATP at higher rates than do slow fibres. In skeletal muscle, the enzymatic ability of the myosin isoform to use





Figs 4, 5

ATP is differentially affected after incubation in solutions of altered pH. This pH sensitivity is a useful tool for selective inhibition of only one isoform while the other is spared (Dubowitz & Brooke, 1973). Acid preincubation allows histochemical separation of the two fast subtypes of skeletal muscle fibres and alkaline preincubation allows the slow type to be separated.

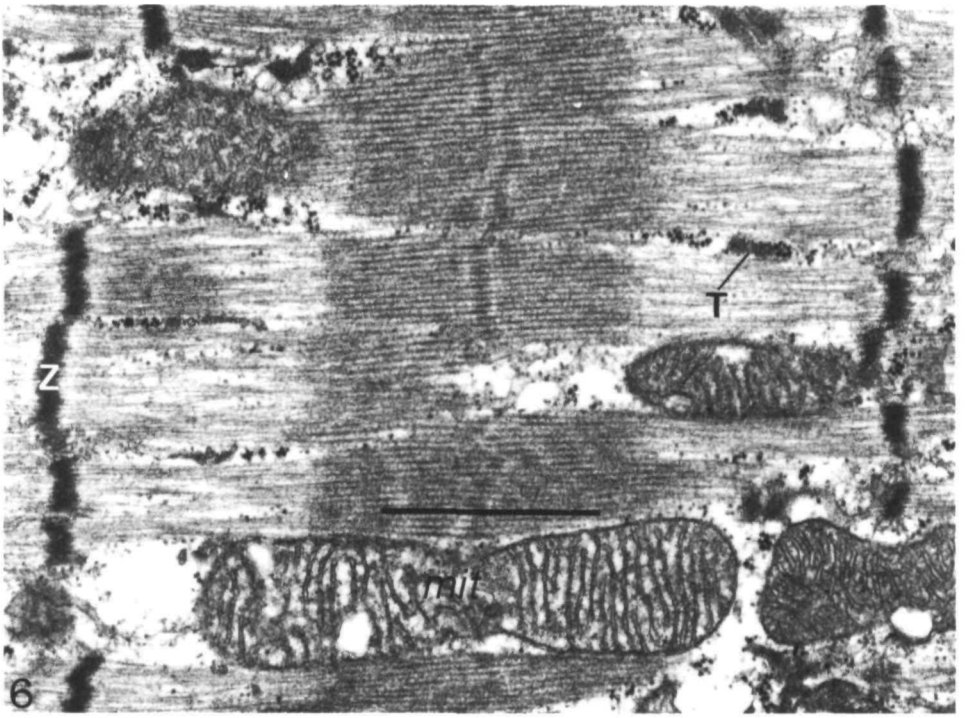
Fibres in serial frozen sections were analysed by ATPase, SDH and quantitative electron microscopy of all elements within many fibres. The distribution of fibre properties assessed in several ways at once is shown in Fig. 3. Clusters of fibres into types can be formed but clearly there is the ability to show a continuum of overlapping properties. We found from this histochemical study (Eisenberg & Kuda, 1977) and the earlier morphometric studies (see review by Eisenberg, 1983) that the width of the Z-band was correlated with the ATPase fibre-typing and the set of contractile protein isoforms. With these anatomical methods well defined, we can proceed to look at the way in which a muscle adapts to altered physiological demands.

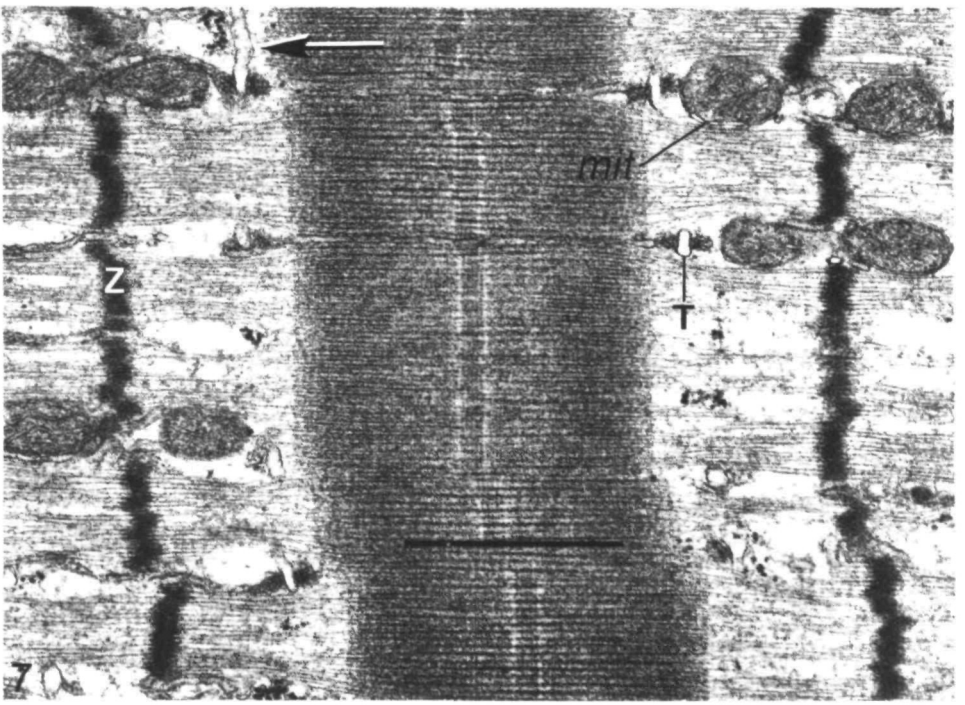
The great diversity in the anatomical composition within mammalian skeletal muscle led to the rejection of the concept of a rigid, immutable, fibre type in favour of the concept of plasticity. Several books and reviews cover this extensive area of research (Salmons & Henriksson, 1981; Pette, 1980; Jolesz & Sreter, 1981). Muscle fibres of the fast-twitch type are normally activated by brief bursts of high-frequency impulse activity. Using the technique of chronic electrical stimulation of the motor nerve, this 'fast' pattern may be overlaid with continuous low-frequency activity at 10 Hz. Under these conditions fast-twitch fibres gradually acquire the physiological and biochemical characteristics of fibres of the slow-twitch type. The mechanisms by which this fibre plasticity are accomplished can be followed ultrastructurally and the time course of fibre transformation studied (Salmons, Gale & Sreter, 1978; Eisenberg & Salmons, 1981). The implantable stimulator could then be switched off, leaving the muscle under the influence of its normal, phasic pattern of activity. The transformed slow fibres then recover their original fast morphologial characteristics under these conditions (Eisenberg, Brown & Salmons, 1984). In both fast-to-slow and slow-to-fast directions the membrane systems regulating the cell calcium begin to change first, followed by the contractile proteins. The metabolic system is quadrupled above normal during the fast-to-slow transformation at the time when the fibre gives slow twitches with fast isomyosin composition. We concluded that this inappropriate use of the fast crossbridge wastes energy.

A few hours after initiation of nerve stimulation at 10 Hz the fast tibialis anterior (TA) muscle began to adapt with longitudinal SR swelling in many fibres. By 5–12 days there was a generalized reduction in the amount of T-system and terminal

Fig. 4. Rabbit tibialis anterior (TA) muscle stimulated at 10 Hz for 9 days. Narrow myofibrils (arrows) separated by abundant amorphous material (asterisk), few mitochondria (*mit*) and scant SR and T-systems. (From Eisenberg & Salmons, 1981.) Scale bar, 1 μm.

Fig. 5. Electron micrograph of longitudinal section of a rabbit tibialis anterior (TA) muscle fibre after 6 weeks' indirect stimulation and 2 weeks' recovery. T-system (T) and SR returning towards fast fibre levels. Myofibrils are well organized. (From Eisenberg, Brown & Salmons, 1984.) Scale bar, 1 μm.





Figs 6, 7

cisternae. Mitochondrial content increased throughout this early period, both in the subsarcolemmal region and in the fibre core. There was evidence of myofibrillar disassembly and the Z-band was interrupted into short irregular segments (Fig. 4). There was gross muscle atrophy in fibre size and weight at this time. After 2 weeks the majority of fibres had a reduced T-SR system, a high mitochondrial content and a normal fibrillar structure with a thickened Z-band. At the same time interval of about 2 weeks of recovery towards the fast type (Fig. 5), the SR and the T-system were increased in extent, but the T-tubule profiles were not always associated with terminal cisternae. The mitochondrial content was variable. There was no noticeable change in myofibrillar structure even though considerable fibre hypertrophy and weight gain were taking place at this time. The Z-band had a decreased width in some fibres.

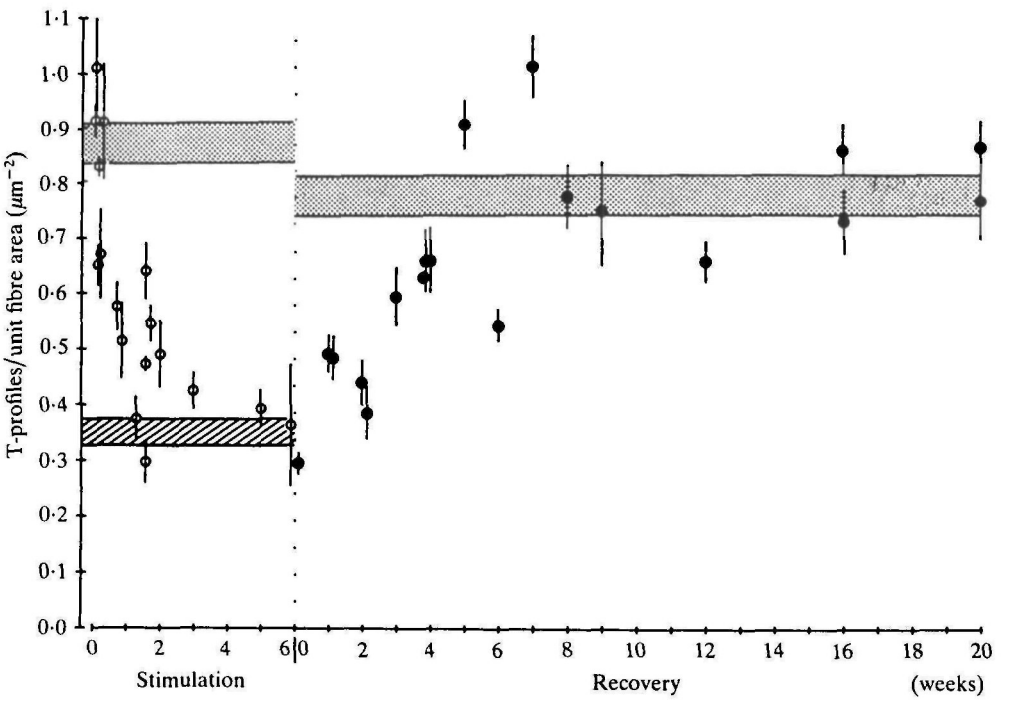


Fig. 8. Time course of changes in extent of T-system during stimulation (open circles) and recovery (filled circles). Each plot represents mean ± S.E. of measurements from approximately 10 fibres in the left tibialis anterior muscle of one animal. Shaded areas, mean ± S.E. of control data from contralateral fast (stippled) or slow (cross-hatched) muscles. Data corresponding to period of stimulation were derived from a previous study (Eisenberg & Salmons, 1981). T-system had regained its original extent about 4 weeks after cessation of stimulation. (From Eisenberg, Brown & Salmons, 1984.)

Fig. 6. Tibialis anterior muscle stimulated for 5 weeks. Structure of fibrils similar to that of normal soleus fibre, with wide Z-bands (Z), few T-profiles (T), and supranormal content of mitochondria (*mit*). Scale bar, 1 μm.

Fig. 7. Tibialis anterior muscle after 6 weeks' stimulation and 4 weeks' recovery. Transitional appearance: numerous T-profiles, some without adjacent terminal cisternae (arrow), but many mitochondria (*mit*), and wide Z-bands. (Eisenberg & Salmons, 1984.) Scale bar, 1 μm.

Fast muscle fibres which had been stimulated for more than 3 weeks were difficult to distinguish from normal slow soleus fibres except that the mitochondrial content was greater (Fig. 6). When chronic stimulation was ended the fibres regained the appearance of fast-twitch muscles of normal fibre size. During the stage of 3–6 weeks of recovery many of the fibres showed a mixture of features of both fast and slow fibres. It was possible to find a wide Z-band and high mitochondrial content in combination with an extensive T-SR system (Fig. 7). After 6 weeks of recovery fibres had regained an appearance typical of the fast-twitch muscle fibre type.

Morphometric parameters from electron micrographs can be plotted as a function of the time course of adaptation. The starting point for recovery was based on data from 5- to 7-week stimulated muscles. The number of T-tubules per unit fibre area (Q_t/A_f) was used an an index of the amount of T-luminal length in unit fibre volume (see Eisenberg & Salmons, 1981). The time course for the fast-to-slow and then slow-to-fast recovery is shown in Fig. 8. During the first week of recovery there was a rapid growth of the T-system and after 4 weeks the T-system was as extensive as that of the control fast-twitch muscle fibres. By this stage, the T-system per unit area of fibre had increased from 0·33 to 0·80 μm^{-2} and the fibre cross sectional area itself by 60 %. The absolute increase in the mount of the T-system was therefore approximately four-fold by the end of the recovery process.

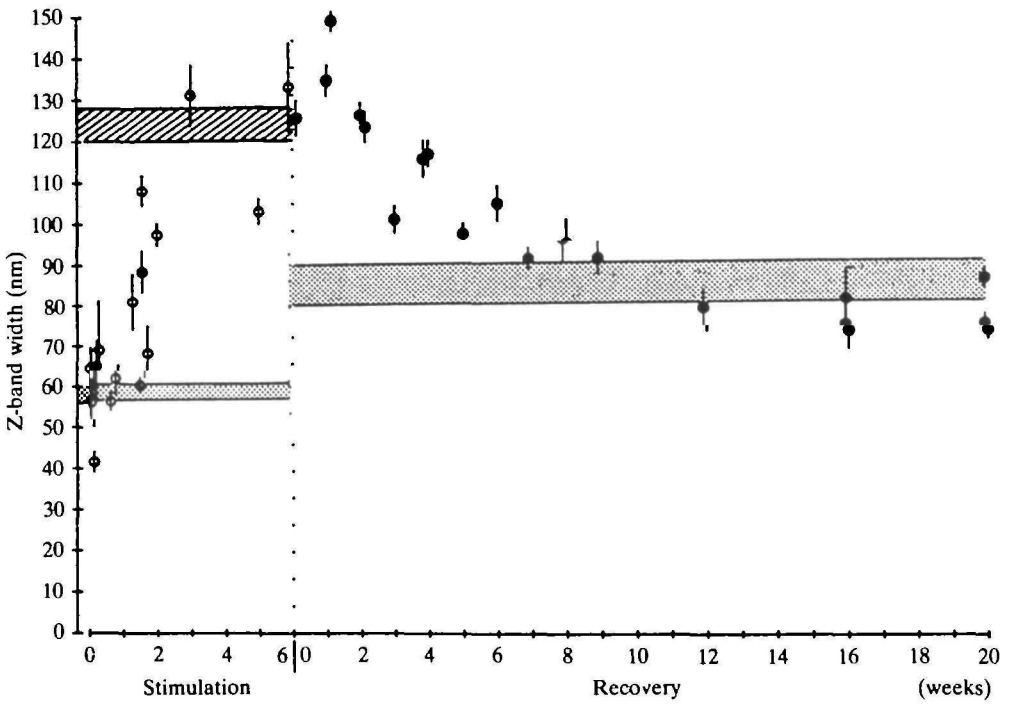


Fig. 9. Time course of changes in Z-band width during stimulation (open circles) and recovery (filled circles). Details as for Fig. 8. Z-band showed dimensions typical of fast muscle after 7 weeks' recovery. (From Eisenberg, Brown & Salmons, 1984.)

In both fast-to-slow (Heilmann & Pette, 1979) and slow-to-fast transformations the membrane systems were the most rapidly changing organelles. Physiological recordings of isometric twitch contractions during the transformation processes showed that times to peak tension and half relaxation could partly be explained by changes in the isoform of myosin. Initially, however, alterations in the time course of activation could also have arisen from transient calcium changes in the sarcoplasm. The transient calcium distribution within a muscle depends in a complex way on the regulatory proteins and the membrane systems that have been calculated for a given set of parameters by computer simulations (Gillis, Thomason, Lefevre & Kretsinger, 1982). Changes in the kinetics of calcium release, binding and reaccumulation could have resulted not only from the observed addition of membrane area, but also from a change in the density of pump sites in the SR.

In the fast-to-slow transformation the width of the Z-band increased rapidly in a few days (Fig. 9), and in the slow-to-fast direction significant narrowing of the Z-band was not seen until about 3 weeks after the cessation of stimulation (Eisenberg *et al.* 1984). During the fast-to-slow transformation, the mitochondrial content reached higher levels than in either the control fast or slow muscles, and fell to typical slow muscle levels after 6 weeks of stimulation (Fig. 10). The volume fraction of

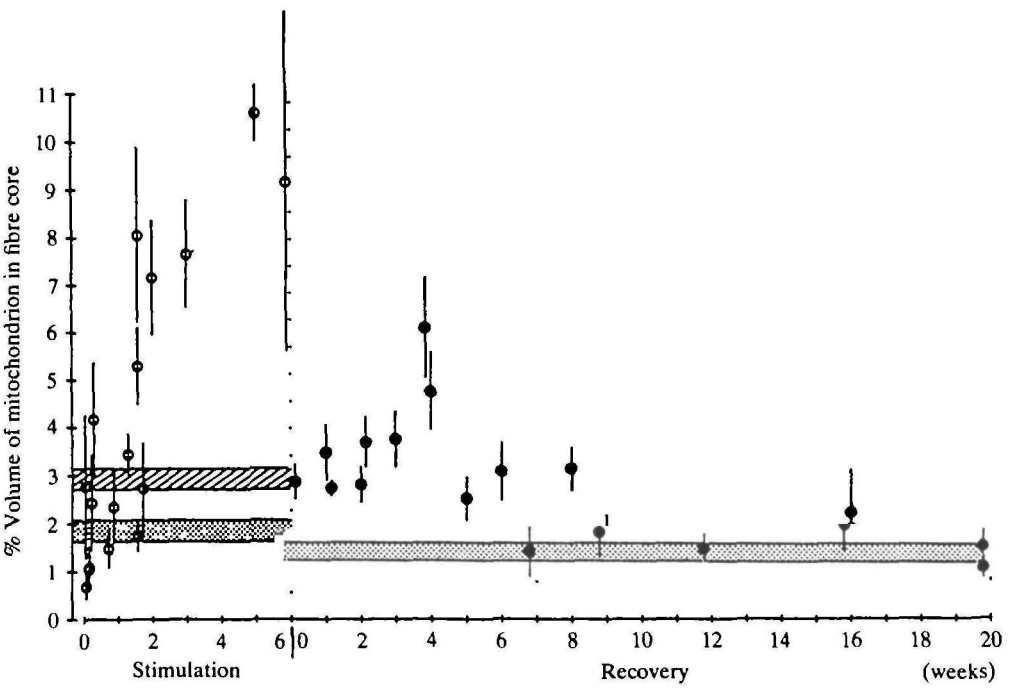


Fig. 10. Time course of changes in mitochondrial volume density during stimulation (open circles) and recovery (filled circles). Details as for Fig. 8. Recovery was complete after 9 weeks. Note absence of sustained increase during course of recovery. (From Eisenberg, Brown & Salmons, 1984.)

mitochondria did not make a comparable overshoot during recovery even after compensation for fibre hypertrophy was considered. There was a rapid reduction in mitochondrial content at 0–3 weeks of the slow-to-fast recovery.

The economical performance of sustained work requires the slow isoform of myosin, with its lower rate of crossbridge cycling (Goldspink, 1975, 1983; Alpert, Mulieri & Litten, 1983). During the fast-to-slow transformation the speed of contraction begins to alter before the conversion of myosin isoforms is complete (Salmons & Vrbová, 1969; Pette, Muller, Leisner & Vrbová, 1976; Brown, Salmons & Whalen, 1983). During this period we observed a supranormal mitochondrial content within the fibre when the utilization of ATP was high because the fast bridges were cycling inefficiently (Eisenberg & Salmons, 1981). After about 6 weeks of chronic stimulation, when the isomyosin was 50% converted (Brown *et al.* 1983) the mitochondrial content declined to a new level, not much higher than that of control slow muscle. During the slow-to-fast transformation, the energy demands have been removed, so that the energetically less efficient faster myosin is reinstated under conditions in which overall ATP consumption was very much reduced and the metabolic stimulus removed.

The net balance between protein synthesis and protein degradation determines fibre size. In fast-to-slow transformation the net protein balance is negative and the fibre atrophies, and in slow-to-fast transformation it is positive and the fibre hypertrophies. Fibre atrophy occurred over the period in which the fibres had their highest mitochondrial content and were using fast cycling contractile proteins to maintain forces. These fibres must have a rapid rate of oxygen consumption and the oxygen partial pressure would be greatly reduced as it diffuses to the innermost myofibrils. The low level of oxygen at the centre of the fibre might eventually limit function and in fact the mitochondrial content is lowest in the centre of fibres (Hoppeler *et al.* 1981). It is possible that oxygen deprivation is involved in myofibril survival.

During fibre transformation of the contractile proteins, many other cellular processes are affected. The membrane systems and metabolic systems are altered in quantity but not in kind. The contractile machinery is being altered in kind, with the isomyosin form being particularly significant. This transformation must involve suppression of genes of 'old' proteins, perhaps it also involves an increase in the rate of degradation of the 'old' proteins. Transformation also requires switching on genes to make 'new' proteins. All these processes must be triggered and controlled by the mechanical function of the fibre. The mechanism of fibre-type transformation is as yet unknown. And there may be more than one way the fibre can transform. The task is now to apply biologically relevant challenges to a muscle and to monitor biochemical and structural changes relevant to the eventual physiological adaptation. A major problem in skeletal muscle research is that the population of fibres is mixed and therefore the staging of the transformation varies from one fibre to the next within the same muscle. This makes control of the experimental manipulation and interpretation of data quite complex. In skeletal muscle it takes over 8 weeks to convert all the myosin to the 'new' kind. The skeletal muscle is not an ideal system for the study of the

fundamental mechanisms involved in adaptation. The nerve is able to deliver a very large variety of signals by packaging bursts of activity into different frequencies and durations. Therefore it is not obvious what kind of complex mechanical activity is the main signal for transformation.

This research was supported by the American Heart Association. The technical assistance of Ms Darlene Bruner is gratefully acknowledged.

REFERENCES

ALPERT, N. R., MULIERI, L. A. & LITTEN, R. Z. (1983). Isoenzyme contribution to economy of contraction and relaxation in normal and hypertrophied hearts. In *Cardiac Adaptation to Hemodynamic Overload, Training and Stress*, (eds R. Jacob, R. Gülch & G. Kissling), pp. 147–157. Darmstadt: Steinkopff Verlag.

BARANY, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. *J. gen. Physiol.* **50**, 197–216.

BROWN, W. E., SALMONS, S. & WHALEN, R.G. (1983). The sequential replacement of myosin subunit isoforms during muscle type transformation induced by long term electrical stimulation. *J. biol. Chem.* **258**, 14686–14692.

BURKE, R. E. & EDGERTON, V. R. (1975). Motor unit properties and selective involvement in movement. *Ex. Sports Sci. Rev.* **3**, 31–81.

DUBOWITZ, V., & BROOKE, M. H. *Muscle Biopsy; a Modern Approach*. London: Saunders.

EISENBERG, B. R. (1983). Quantitative ultrastructure of mammalian skeletal muscle. In *Handbook of Physiology: Section 10, Skeletal Muscle*, (eds L. D. Peachey & R. H. Adrian), Chapter 3, pp. 73–112. Baltimore, MD: Williams & Wilkins.

EISENBERG, B. R., BROWN, J. M. C. & SALMONS, S. (1984). Restoration of fast muscle characteristics following cessation of chronic stimulation: the ultrastructure of slow-to-fast transformation. *Cell Tissue Res.* **238**, 221–230.

EISENBERG, B. R. & KUDA, A. M. (1975). Stereological analysis of mammalian skeletal muscle. II. White vastus muscle of the adult guinea pig. *J. Ultrastruct. Res.* **51**, 176–187.

EISENBERG, B. R. & KUDA, A. M. (1976). Discrimination between fiber populations in mammalian skeletal muscle by using ultrastructural parameters. *J. Ultrastruct. Res.* **54**, 76–88.

EISENBERG, B. R. & KUDA, A. M. (1977). Retrieval of cryostat sections for comparison of histochemistry and quantitative electron microscopy in a muscle fiber. *J. Histochem. Cytochem.* **25**, 1169–1177.

EISENBERG, B. R., KUDA, A. M. & PETER, J. B. (1974). Stereological analysis of mammalian skeletal muscle. I. Soleus muscle of the adult guinea pig. *J. Cell Biol.* **60**, 732–754.

EISENBERG, B. R. & SALMONS, S. (1981). The reorganization of subcellular structure in muscle undergoing fast-to-slow type transformation: a stereological study. *Cell Tissue Res.* **220**, 449–471.

GILLIS, J. M., THOMASON, D., LEFEVRE, J. & KRETSINGER, R. H. (1982). Parvalbumins and muscle relaxation: a computer simulation study. *J Musc. Res. Cell Motil.* **3**, 377–398.

GOLDSPINK, G. (1975). Biochemical energetics for fast and slow muscles. In *Comparative Physiology: Functional Aspects of Structural Materials*, (eds L. Bolis, S. H. P. Maddrell & K. Schmidt-Nielsen). Amsterdam: Elsevier.

GOLDSPINK, G. (1983). Alteration of myofibril size and structure during growth, exercise, and changes in environmental temperature. In *Handbook of Physiology: Section 10, Skeletal Muscle*, (eds L. D. Peachey & R. H. Adrian), pp. 539–555. Baltimore, MD: Williams & Wilkins.

HEILMANN, C. & Pette, D. (1979). Molecular transformations in sarcoplasmic reticulum of fast-twitch muscle by electro-stimulation. *Eur. J. Biochem.* **93**, 437–446.

HOPPELER, H., MATHIEU, O., KRAUER, R., CLAASSEN, H., ARMSTRONG, R. B. & WEIBEL, E. R. (1981). Design of the mammalian respiratory system. VI. Distribution of mitochondria and capillaries in various muscles. *Respir. Physiol.* **44**, 87–111.

JOLESZ, F. & SRETER, F. A. (1981). Development, innervation, and activity-pattern induced changes in skeletal muscle. *A. Rev. Physiol.* **43**, 531–552.

KUGELBERG, E. & THORNELL, L.-E. (1983). Contraction time, histochemical type, and terminal cisternae volume of rat motor units. *Muscle Nerve* **6**, 149–153.

PETTE, D. (1980). *Plasticity of Muscle*. Berlin: Walter de Gruyter.

PETTE, D., MULLER, W., LEISNER, E. & VRBOVÁ, G. (1976). Time dependent effects on contractile properties, fibre population, myosin light chains and enzymes of energy metabolism in intermittently and continuously stimulated fast twitch muscles of the rabbit. *Pflügers Arch. ges. Physiol.* **364**, 103–112.

SALMONS, S., GALE, D. R. & SRETER, F. A. (1978). Ultrastructural aspects of the transformation of muscle fibre type by long term stimulation: changes in Z discs and mitochondria. *J. Anat.* **127**, 17–31.

SALMONS, S. & HENRIKSSON, J. (1981). The adaptive response of skeletal muscle to increased use. *Muscle Nerve* **4**, 94–105.

SALMONS, S. & VRBOVÁ, G. (1969). The influence of activity on some characteristics of mammalian fast and slow muscles. *J. Physiol., Lond.* **201**, 535–549.

WEIBEL, E. R. (1979). *Stereological Methods*. I. *Practical Methods for Biological Morphometry*. New York: Academic Press.