CROSS-SECTIONAL MYOFIBRE AND MYOFIBRIL GROWTH IN IMMOBILIZED DEVELOPING SKELETAL MUSCLE

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SUMMARY

The effect of immobilization on skeletal muscle fibre and myofibril growth in the developing chick latissimus dorsi posterior muscle was investigated. Immobilization, immediately upon hatching, results in a 25% decrease in mean myofibre cross-sectional area within 24 h. Longer periods of immobilization further retard normal myofibre growth until by day 27 after hatching the mean myofibre cross-sectional area was 70% less than normal. The variation in individual myofibre cross-sectional areas (measured as the coefficient of variation, CV = standard deviation/mean x 100) was greater in immobilized muscles. The larger immobilized myofibres showed atypical myofibril and sarcotubular complex organization at the ultrastructural level of observation whereas the smaller immobilized myofibres appeared normal, except for their size. In 1- to 27-day-old chicks, freeing the immobilized muscles for 24 h resulted in myofibril reorganization and sarcotubular proliferation but little myofibre growth. Freeing the immobilized muscles for 72 h resulted in a nearly complete recovery, in chicks up to 18 days post-hatching, of both cross-sectional myofibre area and ultrastructural morphology. Myofibres immobilized for longer than 18 days and subsequently freed for 72 h develop a normal myofibrillar and sarcotubular morphology but remain 23 to 29% smaller in cross-sectional area than myofibres from control muscles.

Normal myofibril growth and organization are severely retarded in the immobilized myofibres. The evidence presented here suggests that sarcomere shortening causes growing myofibrils to rupture. The ruptured myofibrils are filled with proliferating elements of the sarcotubular complex which, in turn, completely subdivide (split) the myofibril.

INTRODUCTION

It is generally agreed that the number of striated muscle fibres in vertebrate muscles remains constant once differentiation is complete. The amount or duration of postnatal muscle differentiation varies among the different vertebrate classes and is apparently dependent upon the relative maturity of the animal at birth (Morpurgo, 1897; Goldspink, 1962; Chiakulas & Pauly, 1965). However, differentiated muscles are able to increase their mass during normal postnatal growth and in response to altered functional demands (MacCallum, 1898; Adams, Denny-Brown & Pearson, 1962; Shear & Goldspink, 1971; Goldspink, 1972; Shear, 1975). In the developing and fully differentiated muscle, the increase in total muscle mass is due to longitudinal and cross-sectional muscle fibre growth and to a lesser extent, to the growth of non-contractile connective tissue elements within the muscle (Goldspink, 1968; Bridge & Allbrook, 1970; Shear & Goldspink, 1971; Williams & Goldspink, 1971; Shear, 1975).
During normal post-hatching development of the chick, the fast-phasic muscle fibres of the latissimus dorsi posterior (PLD) and the slow-tonic muscle fibres of the latissimus dorsi anterior (ALD) increase 100-fold in cross-sectional area. This increase in fibre size is the result of an increase in myofibrillar (contractile) and sarcoplasmic (non-contractile) components of the growing fibre. In young fibres of both types, newly synthesized contractile proteins are assembled into myofilaments (Morkin, 1970) and incorporated into the existing myofilament lattice (Larson, Jenkison & Hudgson, 1970; Larson, Fulthorpe & Hudgson, 1973). The growing myofibrils of the PLD muscle fibres incorporate new contractile filaments, double their size, and then split or divide longitudinally into 2 daughter myofibrils (Shear & Goldspink, 1971). The fibres have a 'Fibrillenstruktur' myofibril morphology with myofibrils of uniform size, each surrounded by a well developed sarcotubular complex (sarcoplasmic reticulum and transverse tubular system) and each I band traversed by a straight and narrow Z disk (Krüger, 1950; Krüger & Günther, 1955; Hess, 1961).

In a recent study of fish myotomal muscle fibres, peripheral myofibrils were found to incorporate new contractile protein at a higher rate and to split more frequently than central myofibrils (Patterson & Goldspink, 1976). In the present investigation, the location of the dividing myofibrils within the growing chick muscle fibre has been investigated. The role of the sarcotubular complex in subdividing the contractile filament mass into discrete myofibrils has also been examined.

The homogenous fast-phasic, Fibrillenstruktur fibre composition of the newly hatched, developing, and adult chick PLD muscle serves as an ideal model with which to study myofibril growth and division. In the present study immobilization was employed to limit whole muscle shortening and hence sarcomere shortening in newly hatched chick myofibres because; (1) the load placed on the myofibres by their skeletal attachments remains unchanged; (2) the myofibres may be fixed at a predetermined resting length; and (3) the trauma caused by denervation and tenotomy is absent. In order to determine whether immobilization had irreversibly limited growth in the developing myofibres, immobilized muscles were freed and light-microscopic and ultrastructural analysis made of whole fibre cross-sectional area and myofibril morphology. A partial account of these results was reported before the Third International Congress on Muscle Diseases (Shear, 1975).

Materials and Methods

Latissimus dorsi posterior muscles (PLD) from female White Leghorn chickens, ranging in age from 1 to 27 days old (1, 3, 6, 9, 12, 18, 21 and 27 days after hatching), were used in this study. The PLD muscle originates from the tips of the neural spines of the ninth to the eleventh thoracic vertebrae and inserts by means of a short, flat tendon on the medial surface of the proximal portion of the humerus. Whole muscle shortening was restricted by fixing the position of one wing at a predetermined body or rest length, i.e., that length at which maximum isometric tension is obtained. The unpublished observations of Shear & Goldspink show maximum isometric tension for the PLD muscle, in chicks of all ages, to occur with the wings folded next to the body. The position of the fixed wing and hence the length of the immobilized muscle is most important. Studies on whole muscle weight and immobilization indicate the importance of fixing the wing at rest or body length. Muscles immobilized at greater than rest length show...
an initial increase in total dry weight (Thomsen & Luco, 1944; Ferguson, Vaughan & Ward, 1957), while those tenotomized or fixed at a length shorter than rest length lose weight more rapidly (Summers & Hines, 1951; Price, Howes & Blumberg, 1964). Apparently the prolonged maintenance of long sarcomere length results in the serial addition of new sarcomeres to the ends of the existing myofibrils, while shortened sarcomere lengths cause the loss of existing sarcomeres (Williams & Goldspink, 1971). In order to avoid sarcomere degeneration which might confuse an ultrastructural interpretation of myofibril growth, care was taken to immobilize PLD muscles at their proper resting lengths. Immediately upon hatching, the position of one wing was fixed next to the body with a surgical adhesive cast. Thereafter the casts were examined at daily intervals and adjustments made to allow for increased limb development. Newly hatched chicks became adapted to single wing immobilization within the first 90 min after hatching. Chicks with immobilized wings were maintained in battery brooders, separate from non-immobilized birds, and were given food and water ad libitum. Experimental chickens were assigned to 3 groups, according to the experimental designs described below. The chicks were sacrificed by massive intraperitoneal or intravenous injections of tubocurarine chloride, thus blocking myoneural transmission which might otherwise cause the muscle fibres to shorten.

**Immobilized.** One wing of the newly hatched chicks was immobilized and the animals sacrificed 1, 3, 6, 9, 12, 18, 21 and 27 days post-hatching. Both the immobilized and non-immobilized contralateral muscles were removed and prepared for electron microscopy (n = 3, for each age) or used to determine whole muscle dry weight (n = 3, for each age).

**Immobilized-freed.** Animals with one wing immobilized for 3, 6, 9, 15, 18 and 24 days post-hatching and then freed of the casts for 72 h were sacrificed and the PLD muscles from both sides prepared for electron microscopy (n = 3, for each age) or dry weight measurement (n = 3, for each age). Another group of chicks was immobilized for 2, 20 and 26 days post-hatching, freed 24 h and the muscles prepared for electron microscopy (n = 3, for each age) or dry weight measurement (n = 3, for each age). As in the preceding group, the chicks resumed use of the immobilized wing within 2-5 h following removal of the cast.

**Non-immobilized.** A population of non-immobilized animals was maintained as described above and sacrificed 1, 3, 6, 9, 12, 18, 21, and 27 days after hatching. Both the right and left PLD muscles of each chick were removed and prepared for electron microscopy (n = 3, for each age) or dry weight measurement (n = 3, for each age). The fibre cross-sectional area values and whole muscle weights proved too variable, between different birds of the same age, to permit comparisons between non-immobilized and immobilized animals. In this investigation the muscle contralateral to the immobilized side served as a control for each bird.

For electron microscopy, the muscles were exposed, dissected free of connective tissue, and cold (4 °C) phosphate-buffered 2.5% glutaraldehyde fixative (pH 7.2) containing 0.54% glucose was pipetted on to the muscles for 5 to 15 min. The muscles were then tied to an applicator stick, removed, and placed in a larger volume of the same fixative at 4 °C for an additional 2 h. After fixation the mid-belly region of the muscle was minced, washed briefly in cold (4 °C) 0.1 M phosphate buffer and postfixed for 2 h in 1% OsO4 buffered to pH 7.2 with 0.1 M phosphate buffer and containing 0.54% glucose. The muscles were dehydrated in ethanol and embedded in Araldite, CIBA CY 212 (Glauert, 1965). Ultrathin sections showing a silver-grey interference colour were collected on naked grids and stained with uranyl acetate and lead citrate solutions (Reynolds, 1963). The sections were examined with a Philips 300 or Zeiss EM 9S-2 electron microscope and the magnifications calibrated using a carbon replica of 2200 lines/mm diffraction grating. Semithin sections for light microscopy were cut at 1 μm and stained with toluidine blue.

The cross-sectional area of 100 fibres from each muscle were measured directly from electron micrographs using an electronic rolling disk planimeter (Los Angeles Scientific Supply Co., Inc.). Only those fibres showing discrete, punctate thick and thin filaments were considered to represent true transverse sections and to be suitable for fibre cross-sectional area determination. All of the fibres within each randomly selected fasciculus were measured and the mean myofibre cross-sectional area ± the standard error was calculated for each muscle. The coefficient of variation (standard deviation/mean x 100) was calculated for each immobilized, immobilized-freed and non-immobilized contralateral muscle. In this way the distribution and variability of myofibre area, between chicks of different ages, could be compared (Snedecor & Cochran, 1967).

In order to determine whether a small change in mean myofibre size results in a measurable
change in whole muscle weight, the dry weights were calculated for each of the groups described above. The muscles were removed, weighed, dried for 2 h at 55 °C, dessicated for 24 h and reweighed.

RESULTS

Immobilization of the PLD muscle resulted in a reduction of mean myofibre cross-sectional growth in chicks of all age groups. Representative histograms showing the distribution of myofibre area in immobilized and non-immobilized muscles are shown in Figs. 2A, B; 3A, C. In Fig. 1, the mean myofibre cross-sectional area for each immobilized muscle is shown as a per cent of the mean cross-sectional area of the non-immobilized contralateral muscle. Owing to the small transverse size of many fibres (less than 5 μm diameter), and the difficulty in distinguishing muscle cells from myoblastic, satellite and fibroblast cells, all of the cross-sectional area measurements were taken directly from electron micrographs.

Even after only 1 day of immobilization, the mean myofibre area ranged from 19 to 22% less than corresponding contralateral muscles (Figs. 1, 2A). After 3 days the
mean myofibre area was 27 to 31% less for immobilized muscles (Figs. 1, 2B). Between 3 and 12 days of post-hatching immobilization, there was a slow but steady reduction in mean myofibre area until by day 12 the mean myofibre area was 34-40% less in immobilized muscles (Figs. 1, 3A). By day 21, the mean myofibre area declined to 60-66% of the corresponding contralateral muscles (Figs. 1, 3C), and by day 27 the immobilized fibres were 70-72% less than their controls.

Considerably greater variation in myofibre cross-sectional area was seen in immobilized muscles between 1 and 12 days post-hatching than in corresponding control myofibres (Figs. 2A, B; 3A). The coefficient of variation (CV) was 17-25% greater in immobilized myofibres through day 12 post-hatching and 6-15% greater in myofibres immobilized for 18, 21 and 27 days than in myofibres of corresponding control muscles. After 1 day of immobilization the muscles weigh 8-14% less than contralateral muscles and by 27 days the immobilized muscles weigh 27-35% less than their controls. In many of the immobilized muscles a pronounced proliferation of the perimysial connective tissue was observed, accounting in part for the less dramatic loss in total dry weight.

Freeing immobilized muscles for 72 h resulted in a surprising recovery of myofibre area in 6- to 18-day-old chicks (Figs. 1, 3B). In these immobilized-freed, 72 h muscles, the mean myofibre areas were only 1-6% less than in corresponding control muscles. Freeing the muscles for 72 h reduced the variability in cross-sectional myofibre area in the 6- to 18-day-old chicks, i.e., as compared to continuous immobilization. The immobilized-freed, 72-h myofibres show CVs 2-5% greater than their controls. In 21- to 27-day-old chicks, the myofibres of muscles immobilized and then freed for 72 h were 23-29% smaller in cross-sectional area than their corresponding control myofibres and their CVs ranged from 6 to 19% greater than CVs for the contralateral muscles (Figs. 1, 3B). The immobilized-freed, 72-h muscles weigh 2-3% less than corresponding control muscles through day 18 post-hatching and 10-13% less in the 21- and 27-day-old animals.
Fig. 3. Representative histograms demonstrating myofibre cross-sectional area distributions in immobilized (shaded, narrow lines) and control (unshaded, heavy lines) PLD muscles: A, 12 days post-hatching; c, 21 days post-hatching. Representative histograms demonstrating myofibre cross-sectional area distributions in immobilized-freed 72-h (shaded, narrow lines) and control (unshaded, heavy lines) PLD muscles: b, 12 days post-hatching; d, 21 days post-hatching.

Fig. 4. Transverse section of an untreated PLD myofibre from a 21-day-old chick. The myofibrils are discrete and well separated from one another throughout the fibre cross-section and at all levels of the sarcomere. × 6400.

Fig. 5. Transverse section of a small PLD myofibre immobilized for 21 days post-hatching. Although the myofibrillar and sarcotubular organization (arrows) appear normal, many of the fibres are very much smaller in cross-sectional area than controls. × 21 800.

Fig. 6. Transverse section of a large PLD myofibre immobilized for 21 days post-hatching. The myofibrils are not well separated and the sarcotubular complex is poorly developed. × 21 800.

Fig. 7. Transverse section of a large PLD myofibre immobilized for 21 days post-hatching. The M line is clearly visible indicating that groups of adjacent thick filaments remain in longitudinal register. × 116 000.
Muscles immobilized for 2, 20 and 26 days post-hatching and freed for 24 h had mean myofibre cross-sectional areas of 20–65% less than their corresponding controls (Fig. 1). As in the continually immobilized muscles, the CVs ranged between 8 and 28% greater than corresponding control values. Whole muscle dry weights ranged from 5 to 30% less for immobilized-freed, 24-h muscles than control muscles, the greatest differences occurring in the 21- and 27-day-old chicks (20–25% and 23–30% respectively).

Electron-microscopic examination shows 2 types of myofibrillar morphology in the developing, immobilized myofibres (Figs. 4–6). At all ages the small myofibres show distinct myofibrils and an extensive sarcotubular system (Fig. 5). The myofibrillar morphology of the smaller immobilized myofibres is similar to that of the non-immobilized myofibres (Fig. 4) with triads, i.e., one transverse tubule and 2 adjacent terminal cisternae of the sarcoplasmic reticulum, located at the centre of the I band between the Z disk and the A band. The larger fibres generally have ill-defined myofibrils and a poorly developed sarcotubular complex (Fig. 6) similar to the Felderstruktur morphology of the typical avian tonic muscle fibres (Shear & Goldspink, 1971). As in the tonic myofibres the triads are generally located at the Z disk in the larger immobilized fibres but are difficult to distinguish since both the transverse tubules and terminal cisternae are less well developed than in the small immobilized or control myofibres. Unlike the tonic myofibres, the M lines are clearly visible even in transverse sections (Fig. 7).

An extensive sarcotubular system is seen in large and small myofibres freed for 24 h and the myofibrils are more clearly defined (Figs. 8, 9) than in immobilized myofibres (Figs. 5, 6). A large number of the immobilized-freed, 24-h myofibrils show signs of dividing or splitting apart (Figs. 8–10). Many of the larger immobilized-freed myofibres regain a nearly normal myofibrillar and sarcotubular organization, 24 h after cast removal (Figs. 9, 10). In these fibres triads are generally found at the level of the A–I band and occasionally at the Z disk. The location of the triads is more variable, even along the length of a single myofibril, than in control myofibrils. The sarcotubular complex of the smaller immobilized-freed, 24-h myofibres is more extensive than in the larger immobilized-freed, 24-h myofibres or in the control myofibres (Fig. 8). Since both large and small immobilized-freed myofibres show notable proliferation of the sarcotubular complex within the first 24 h following removal of the cast and only a small increase in mean myofibre cross-sectional area, transverse tubular

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Fig. 8. Transverse section of a small PLD myofibre immobilized for 20 days and freed for 24 h. Note the numerous myofibrillar separations and the extensive sarcotubular complex. × 25 600.

Fig. 9. Transverse section of a large PLD myofibre immobilized for 20 days and freed for 24 h. The sarcoplasmic reticulum is less extensive than in the small immobilized, 24-h fibres and triads can be identified at the A–I bands (arrows). × 28 500.

Fig. 10. Longitudinal section of a dividing myofibril (arrows) within a large myofibre of a 27-day-old chick immobilized for 26 days and freed for 24 h. Myofilaments associated with adjacent daughter myofibrils are occasionally encountered along dividing myofibrils (triangles). × 24 700.
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and sarcoplasmic reticular development appear to precede renewed myofibrillar growth. The myofibrillar morphology and organization of the sarcotubular complex in fibres of muscles immobilized and freed for 72 h is similar to the controls.

The large number of centrally disrupted myofibrils in immobilized-freed, 24-h fibres provides an ideal model for the study of longitudinal myofibril division. Ultra-thin serial transverse sections were made through a single myofibril, from an immobilized-freed 24-h fibre of a 27-day-old chick, undergoing longitudinal division (Figs. 11–14). The myofibril split is first seen as a disruption at the centre of the Z disk (Fig. 11). Transverse serial sections through the I bands, A bands and Z disks of the 2 sarcomeres immediately preceding the disruption showed normal filament lattice arrangements with no disruptions. Serial sections through 4 successive sarcomeres adjacent to the Z disk shown in Fig. 11, show the disruption to be continuous (Figs. 12–14). The myofibrillar disruptions within the first 2 sarcomeres contain no visible elements of the sarcotubular complex (Figs. 11, 12). Elements of the sarcotubular complex, probably sarcoplasmic reticulum, have entered the disruptions of the last 2 sarcomeres and appear to be advancing through the myofibril along the previously existing disruption (Figs. 13, 14). Transverse serial sections through a large number of disrupted myofibrils show similar patterns of myofilament disruption and sarcotubular complex development. Within the small fibres, nearly 50% of the myofibrils freed for 24 h show central disruptions or splits. In the large immobilized-freed, 24-h fibres, 22% of the myofibrils show signs of splitting. The frequency of myofibril splitting in the immobilized-freed, 72-h myofibres and in control myofibres is only 10–12% at all ages studied. In both the experimental and control myofibrils, myofibrils in various stages of splitting occur at the periphery and centre of the cells with equal frequency.

**DISCUSSION**

The results presented here indicate that sarcomeres of the fast-twitch myofibres of chick PLD muscle must shorten in order to grow and develop normally. Even in the very young chicks, myofibrils showed retarded growth after only 24 h immobilization. The decrease in immobilized muscle dry weight reflects a loss of insoluble contractile protein. The greater percentage decrease in cross-sectional myofibre area than in comparable muscle dry weight suggests increased development of the vascular and/or connective tissue elements in the immobilized muscles. Although the vascular system appeared normal in the immobilized muscles, the interfascicular connective tissue did appear more extensive than in the controls. Since freeing the muscles for 72 h resulted in nearly normal mean myofibre cross-sectional areas and whole muscle dry weights
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(up to 18 days post-hatching), there appears to be some regulatory interaction between the myofibre and connective tissue elements comprising the PLD muscle. In a recent study of immobilized adult cat skeletal muscles, whole muscle weight was found to decrease by a greater percentage than did fibre size (Cooper, 1972). However, in that study the percentage change in fibre size was determined from transverse diameter measurements rather than cross-sectional area measurements. When cross-sectional areas are calculated, using the reported mean myofibre diameter measurements of Cooper (1972), the changes in muscle weight and fibre size are similar to those reported in the present investigation.

In light of the uniform physiological, ultrastructural, and histochemical make-up, characteristic of newly hatched PLD myofibres (Gutmann, Hanslíková & Holečková, 1969; Shear & Goldspink, 1971; Jirmanová & Zelená, 1973; Gordon, Perry, Spurway & Vrbová, 1975), it is surprising that in the present study the myofibres are not equally affected by immobilization. The cross-innervation studies of Hník, Jirmanová, Vyklický & Zelená (1967) and Jirmanová & Zelená (1973), on young and newly hatched chick PLD muscles, show that a change in fibre type can only be brought about in newly hatched muscle fibres. These authors suggest that the ability to transform young myofibres is largely dependent upon the maturity of a particular myofibre at the time the foreign motor neuron establishes contact. Although the majority of the newly hatched cross-innervated fibres are transformed physiologically, many myofibres show only partial ultrastructural transformation (70% per muscle), i.e., as regards the sarcomembrane and myofibrillar organization. Their findings, together with those reported in the present investigation indicate that the myofibres comprising the young chick PLD muscle may not be equally differentiated on the day of hatching even though they are 100% innervated (Hirano, 1967; Gutmann et al. 1969; Grim, 1971) and show uniform histochemical properties (Gordon et al. 1975). In mammals the ultrastructural maturation of fibre type is a postnatal process related in some way to the postnatal differentiation of distinct types of motor end-plates (Walker, Schrodt & Bingham, 1968; Schiaffino & Margreth, 1969; Padykula & Gauthier, 1970). It has been demonstrated (Chinoy & Hess, 1974) that avian motor end-plates undergo similar post-hatching differentiation. Thus, depending on the state of such motor end-plate differentiation, immobilization would affect the myofibres differently. Although the immobilized myofibres do not show a measurable increase in cross-sectional area after the first 24 h following removal of the cast, they do show considerable alteration at the ultrastructural level as is evidenced by the proliferation of the sarcomembrane complex and subdivision of myofibrillar material.

The results of this investigation indicate that the sarcomeres of myofibrils in developing fast-twitch fibres must shorten in order to grow, split and divide longitudin-

Fig. 13. As in Figs. 11 and 12, showing the A band of the next adjacent sarcomere. Note the presence of sarcomembrane complex within the disruption (arrow). x 90000.

Fig. 14. As in Figs. 11—13, showing the A band of the next adjacent sarcomere. Note that elements of the sarcomembrane complex (arrows) are more abundant than in the previous sarcomere and appear to proceed along a single axis. x 90000.
ally. Myofibrillar division is important in that it subdivides the myofibrillar mass, allowing a well organized sarcoplasmic reticulum and transverse tubular system to develop. The myofibres of the PLD muscle require a well developed and highly ordered sarcotubular complex in order to release and sequester Ca\(^{2+}\) rapidly (Peachey & Porter, 1959; Peachey, 1968, 1970). Evidently the development of the sarcotubular complex is also dependent upon sarcomere shortening since immobilized fibres showed a decreased development of that complex in fibres of the PLD normally capable of extensive sarcotubular development (Shear & Goldspink, 1971). The retarded development of the sarcotubular complex in the large immobilized fibres is apparently reversible to the extent that muscles freed of the casts and allowed to shorten already showed extensive proliferation of that complex by 24 h, and by 72 h all of the fibres possessed a well developed highly organized sarcoplasmic reticulum and transverse tubular system and a normal myofibril morphology.

Workers in several different laboratories have reported a lateral expansion of the myofilament lattice (A and I band) during isotonic contraction (Carlsen & Knappeis, 1963; Brandt, Lopez, Reuben & Grundfest, 1967; Pringle, 1968; Goldspink, 1971; April, 1975a, b; and April & Wong, 1976). In vertebrate skeletal myofibres, Goldspink (1971, 1972) has found that during isotonic contraction the thick myosin filament lattice expands more than the Z disk lattice, thus displacing the peripheral thin filaments and causing them to pull in an oblique direction on their associated and common Z disk. According to Goldspink the resulting force acting on one Z disk by the thin filaments of 2 adjacent one-half sarcomeres might be sufficient to split the myofibrillar mass longitudinally. Such a force might be expected to increase as the total myofibrillar cross-sectional area increases, as indeed occurs when new contractile proteins are synthesized and the filaments assembled and incorporated into the growing sarcomere. The occurrence of a sarcotubular complex within the ruptured myofibrillar mass suggests that proliferation of these membranous elements further help to completely subdivide the growing myofibril.

In a recent study of myofibril growth in fish muscle, Patterson & Goldspink (1976) found that the most peripheral myofibrils incorporated new contractile filaments and divided while the more central myofibrils showed little growth or division. Also, these authors suggest that new myofilaments are added to the daughter myofibrils while they are splitting. In our observations of chick myofibril development, central and peripheral myofibrils were seen to grow and divide with nearly equal frequency. As in the fish myofibres, presumably newly assembled thick and thin filaments were observed in various stages of incorporation at the periphery of the splitting myofibrils. A possible explanation for the difference between fish and chick myofibrillar growth is that in young, newly-hatched chick myofibrils the ribosomes, upon which the contractile filaments are synthesized, are more uniformly distributed than in the young adult fish myofibrils. This, in turn would permit myofilament assembly and incorporation in a uniform fashion throughout the cross-section of the chick myofibre. We do find abundant ribosomes and polysomes throughout the cross-section of the growing myofibre. Similar observations, dealing with ribosomal distribution in developing human and avian myofibres have been reported by Larson et al. (1970, 1973). In a
recently completed study on regenerating mammalian skeletal muscle fibres (Shear & O'Steen, in preparation), splitting myofibrils were observed with equal frequency throughout the cell. Based upon our current understanding of the mechanisms of protein synthesis in eukaryotic cells, the distribution of ribosomal material would seem an important variable affecting myofibrillar growth. Further studies are required to relate changes in both ribosomal and myonuclear distribution to myofilament assembly in the growing myofibril.

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