EFFECTS OF DISUSE ON GROWING AND ADULT CHICK SKELETAL MUSCLE

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SUMMARY

The effects of long-term muscle inactivity, throughout post-hatching development, have been examined. Continuous immobilization of the chicken posterior latissimus dorsi (PLD) muscle from the first hour after hatching for varying periods up to 330 days, resulted in a significantly greater decrease in myofibre size (40-64% less than control) than occurred when adult muscles were immobilized for similar periods (20-40% less than control). The myofibre atrophy resulting from long-term immobilization of adult muscle is reversible, after removal of the plaster cast. In contrast, the myofibres immobilized immediately after hatching, for similar periods of time, were unable to recover once the casts were removed. On the basis of myofibre cross-sectional area, 2 populations of cells were seen in muscles immobilized during postnatal development: small myofibres of 0.5-200 \( \mu m^2 \) and larger myofibres of 500-800 \( \mu m^2 \). The distribution of fibre cross-sectional area within immobilized adult muscles was similar to controls, suggesting a uniform response (i.e. atrophy) by all of the myofibres within the muscle. Immobilization in both newly hatched and adult PLD muscles did not appear to alter the pattern of motor endplate distribution within the muscle. Small, multiple motor endplates were observed associated with immobilized and control myofibres near their terminal ends. This finding suggests that the embryonic pattern of myofibre innervation is not entirely lost from all the fibres during postnatal development.

INTRODUCTION

Skeletal muscle size is influenced by its pattern of contractile activity. Increased demands of work result in compensatory growth, whereas disuse or inactivity induces muscle atrophy. However, the manner in which contractile activity governs muscle cell metabolism is not well understood. One procedure often used to produce a state of muscle inactivity has involved the chicken posterior latissimus dorsi (PLD) muscle from the first hour after hatching for varying periods up to 330 days, resulting in a significantly greater decrease in myofibre size (40-64% less than control) than occurred when adult muscles were immobilized for similar periods (20-40% less than control). The myofibre atrophy resulting from long-term immobilization of adult muscle is reversible, after removal of the plaster cast. In contrast, the myofibres immobilized immediately after hatching, for similar periods of time, were unable to recover once the casts were removed. On the basis of myofibre cross-sectional area, 2 populations of cells were seen in muscles immobilized during postnatal development: small myofibres of 0.5-200 \( \mu m^2 \) and larger myofibres of 500-800 \( \mu m^2 \). The distribution of fibre cross-sectional area within immobilized adult muscles was similar to controls, suggesting a uniform response (i.e. atrophy) by all of the myofibres within the muscle. Immobilization in both newly hatched and adult PLD muscles did not appear to alter the pattern of motor endplate distribution within the muscle. Small, multiple motor endplates were observed associated with immobilized and control myofibres near their terminal ends. This finding suggests that the embryonic pattern of myofibre innervation is not entirely lost from all the fibres during postnatal development.

Skeletal muscle size is influenced by its pattern of contractile activity. Increased demands of work result in compensatory growth, whereas disuse or inactivity induces muscle atrophy. However, the manner in which contractile activity governs muscle cell metabolism is not well understood. One procedure often used to produce a state of muscle inactivity has involved cutting the motor nerve supply to the tissue. [Alexsson & Thesleff, 1959; Feng, Jung & Wu, 1962; Gutmann, 1962; Miledi, 1962; Guth, 1968; Goldberg, Jablecki & Li, 1974; Jones & Vrbova, 1974]. However, it is difficult to determine whether the changes that result following denervation are due to the loss of contractile activity or to the absence of neuronal influence (i.e. the loss of nerve impulse transmission, quantal release of acetyl choline or neurotrophic-trophic substances). While denervation induces atrophy in some muscles, others undergo hypertrophy (Sola & Martin, 1953; Feng et al. 1962). Another procedure employed to create a state of muscle inactivity, without interruption of the motor nerve supply, is muscle immobilization (Crawford, 1954; Cooper, 1972; Williams & Goldspink, 1973; Shear, 1975; Tomaneck & Lund, 1974; Goldspink, 1977a, b). In adult mammalian muscle, inactivity results in a uniform decrease in myofibre girth. Freeing
the adult muscles allows the myofibres to recover their normal structure, size and metabolism. In growing muscle the role of activity is more complex and not all of the myofibres appear to be equally affected. In an earlier study (Shear, 1978) the effects of short-term disuse in growing chick muscle were examined. For that study newly hatched chick posterior latissimus dorsi (PLD) muscles were immobilized for 1–24 days. After the first day the immobilized myofibres were 25% smaller than controls and after 24 days the immobilized fibres were 70% smaller than normal. Freeing the immobilized muscles for 72 h resulted in a complete recovery, in chicks up to 21 days of age. Myofibres immobilized for longer than 21 days and subsequently freed for 72 h developed a normal myofibrillar and sarcotubular morphology, but nearly one half of the fibres remained 40% smaller in cross-sectional area than myofibres from control muscles. These findings suggest that altered patterns of muscle activity, during periods of normal myofibre growth, may influence the ability of the mature adult muscle fibre to respond subsequently in a normal manner to new functional situations.

Chick PLD muscles provide an ideal model with which to investigate the effects of activity on myofibre growth and development. It is generally accepted that the adult PLD muscle is composed of a homogeneous, fast-phasic population of muscle fibres. Each myofibre is also thought to receive a single 'en plaque', focal innervation (Ginsborg & MacKay, 1960, 1961; Hess, 1961; Silver, 1963; Gutmann & Syrovy, 1967; and Koenig, 1970). However, the cross-inervation studies carried out on the adult chick by Hnik, Jirmanova, Vylicky & Zelena (1967) and on the immature chick by Jirmanova & Zelena (1973) suggest that the myofibres of the PLD muscle are not equally differentiated on the day of hatching. The variability in maturation of newly hatched chick myofibres was also reported by Shear (1978) who showed that inactivity did not affect all of the myofibres in the PLD equally.

The disappearance of polyneuronal innervation in skeletal muscle fibres is believed to indicate the final maturation of the presynaptic elements of the neuromuscular junction. In mammalian muscle, inactivity retards and increased activity speeds up this process (Benoit & Changeux, 1975; O'Brien, Purves & Vrbova, 1977). However, this does not seem to be the case in avian muscle fibres (Renaud, LeDouarin & Khaskiye, 1978), though stimulation of the spinal motoneurons supplying the embryonic chick PLD muscle is reported to result in multiple myofibre innervation (Renaud et al. 1978). Renaud and his colleagues were unable to determine whether the multiple innervation was the result of a retarded withdrawal of multiple neuronal elements normally seen in embryonic muscle or whether newly formed axons continue to contact previously innervated muscle fibres.

In order to study the role of inactivity in a more comprehensive fashion, the effects of long-term immobilization in the PLD muscle of the chicken have been investigated. Muscle fibre size, ultrastructural morphology, and motor end-plate distribution have been examined in muscles inactivated immediately upon hatching and maintained in that state for periods up to 1 year. The muscles were freed from immobilization for varying periods in order to determine whether inactivity, during post-hatching development and growth, alters the ability of the adult myofibres to recover later.
MATERIALS AND METHODS

Posterior latissimus dorsi muscles from female White Leghorn chickens were used in this study. Immediately upon hatching the PLD muscle was immobilized by fixing 1 wing against the body using a plaster-gauze cast. A description of the immobilization technique appears in a previously published paper (Shear, 1978). The newly hatched chicks adapted to single wing immobilization within 90 min. They were maintained in battery brooders separately from non-immobilized birds and were given food and water *ad libitum*. The chickens were assigned to 3 groups, according to the experimental designs described below. The birds were killed by massive intravenous injections of tubocurarine chloride (Abbott Laboratories, Chicago, Illinois), thus blocking myoneural transmission which might otherwise cause the muscle fibres to shorten. Prior to injection of tubocurarine, the chicks were anaesthetized with either nembutal (30 mg/kg IV; Abbott Laboratories, Chicago, Illinois) or ketamine (100 mg/kg IM; Parke Davis, Detroit, Michigan). The use of nembutal or ketamine prior to tubocurarine injection was done both to avoid unnecessary stress and discomfort and to prevent convulsions that might alter the appearance of the myofibrils. Ketamine was also used to anaesthetize the chicks during surgical denervation procedures.

**Immobilized adult (130-day-old) muscle**

One group of 45 chicks was maintained under control conditions for 130 days after hatching at which time the right wing was immobilized with a plaster cast. At 40-day intervals (40, 80, 120, 160, and 200 days), 3 chicks were killed and the PLD muscles from both sides prepared for electron microscopy (*n* = 3 animals for each period of constant immobilization, total = 15). The other 130-day-old animals were immobilized for identical periods (40, 80, 120, 160 and 200 days) and then freed for 10 days (*n* = 3 animals for each period, total = 15) or 40 days (*n* = 3 animals for each period, total = 15) and the PLD muscles from both sides prepared for electron microscopy. In both of the immobilized-freed groups, the chicks resumed use of the immobilized wing within 4 h following removal of the cast.

**Immobilized newly hatched muscle**

The right wing in a second group of 105 chicks was immobilized immediately upon hatching for 90, 130, 170, 210, 250, 290 and 330 days. Three chicks were killed at the end of each period and the PLD muscles from both sides were removed and prepared for electron microscopy (*n* = 3 animals for each period, total = 21). The remaining animals were immobilized from hatching for identical periods (90-330 days) and then freed of the casts for either 10, 20, 40 or 90 days (*n* = 3 animals for each period, total = 84). For example, at the end of the 90-day immobilization period, 12 chickens were freed of the casts and 3 animals each time were killed after 10, 20, 40 and 90 days. In this way we could evaluate the ability of the continuously immobilized PLD myofibres to recover.

**Muscle fibre innervation**

The histochemical technique of Kim (1972) was used to examine the postsynaptic acetylcholinesterase (AChE) activity in 3 normal 330-day-old and 3 immobilized 330-day-old chick PLD muscles. With this technique, visualization of the postsynaptic AChE activity is limited to the superficial muscle fibres. There were no apparent differences between normal and immobilized muscles (see Results). However, since both groups showed tiny reaction sites at the very ends of the muscle (origin and insertion), these regions were prepared for electron microscopy. The PLD muscles of several 330-day-old normal and continuously immobilized chicks were also denervated by transection of the entire brachial plexus. To prevent reinnervation, the proximal nerve stump was displaced medially and tied with a silk suture. These animals were autopsied 21 days postoperatively and the denervated and normal muscles from each bird prepared for electron microscopy.
Fig. 1. Changes in mean myofibre cross-sectional area of the PLD muscle immobilized 130 days after hatching for 40-130 days (●). The ability of adult immobilized myofibres to recover, following removal of the casts (---, arrows) for 10 days (■) and 40 days (▲) was also followed. The muscles were not immobilized until the animals were 130 days old. Each point represents the mean value of 500 myofibres ± the standard error of the mean, measured from 3 muscles (i.e. each point represents 1500 individual measurements) and calculated as the mean of individual differences of immobilized or freed muscles compared with an equal number of untreated contralateral control muscles.

For electron microscopy, the muscles were exposed, dissected free of connective tissue, and fixed in situ for 5-15 min by pipetting cold (4 °C) phosphate-buffered 2.5 % glutaraldehyde fixative (pH 7.2) containing 0.54 % glucose. 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 each muscle was transected into 3 parts (origin, belly, insertion), 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. 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 201 electron microscope and the magnifications calibrated using a carbon replica of a 2200 lines/mm diffraction grating.

The cross-sectional areas of 500 fibres from each muscle were measured directly from low-magnification (5000 x) electron micrographs, using an electronic rolling disc planimeter (Los Angeles Scientific Instrument Co., Inc.). All of the fibres within each randomly selected fasciculus were measured and the mean myofibre cross-sectional area ± the standard error of the mean was calculated for each muscle. Individual percentage changes for the experimental muscles were calculated relative to the contralateral untreated control muscles (Shear, 1978). The coefficient of variation (CV = standard deviation/mean x 100) was calculated for each muscle in order to compare the distribution and variability of myofibre area between chicks treated by each of the methods described above (Snedecor & Cochran, 1967). Myofibril
Effects of disuse on chick skeletal muscle

39

- 40 -

- 30 -

- 20 -

- 10 -

- 500 700 900 1100 -

1300 1500 1700 -

Fig. 2. Representative histograms demonstrating myofibre cross-sectional area distributions in an adult PLD muscle, immobilized after the animal was 120 days old for a further 120 days (shaded, narrow lines) and then freed for 40 days (unshaded, heavy lines). Note the fibre cross-sectional area distribution in the 40-day-freed muscle is also typical of the distribution seen in control muscles.

cross-sectional areas were measured planimetrically from high-magnification electron micrographs (50,000–75,000 x) of 100 fibres from each muscle. Due to the variation in myofibrillar calibre at different regions of the sarcomere (i.e. the I-band and Z-discs have a smaller diameter than does the A-band), only myofibrils sectioned through the A-band were used to determine mean myofibrillar cross-sectional areas.

RESULTS

Immobilized adult (130-day-old) muscle

Immobilization of the adult chick PLD muscle for periods of 40, 80, 120, 160, and 200 days resulted in a reduction in mean muscle fibre (myofibre) cross-sectional area of 20–40% less than the mean cross-sectional area of the non-immobilized contralateral control myofibres (Fig. 1). The greatest decrease in mean myofibre cross-sectional area occurred within the first 40 days of inactivity. The mean myofibre cross-sectional area declined 20% by 40 days and was 36% less than normal after 120 days of immobilization. Between 120 and 200 days of immobilization there was only an additional 2–4% decrease in size. The variation in myofibre cross-sectional area between immobilized muscles was no greater than between contralateral control muscles (i.e. coefficient of variation for both groups < 5%). Thus, at least with regard to size, all of the myofibres within an adult PLD muscle were equally affected by immobilization (Fig. 2). Between 40 and 200 days of continuous immobilization, many fragmented and degeneration myofibrils were seen. In the earlier stages of inactivity (up to 80 days) degenerating myofibrils were observed at the periphery of the muscle fibres (Fig. 5); in later stages (120–200 days) degenerating myofibrils were observed throughout the myofibre cross-section.
C. R. Shear

Fig. 3. Changes in mean myofibre cross-sectional area of the PLD muscle immobilized immediately after hatching for 90–330 days (●). The ability of the muscle fibres immobilized during postnatal development to recover, following removal of the casts (— — —, arrows) for 10 days (■), 20 days (□), 40 days (▲), and 90 days (△) was also followed. Each point represents the mean myofibre cross-sectional area value of 500 myofibres from 3 muscles (i.e., 1500 individual measurements) and calculated as the mean percentage difference of immobilized or freed muscles compared with an equal number of unrestrained contralateral control muscles. The standard errors of the means are given for the continuously immobilized muscles and for the 90-day-freed muscles. Note that most of the recovery occurs during the first 10 days following cast removal; complete recovery does not occur even by 90 days after activity is restored.

Freeing the immobilized muscles for 40 days (Fig. 1) resulted in a return to a near normal mean myofibre cross-sectional area (96–98% of normal). The rate of recovery was greatest during the first 10 days following cast removal, myofibers of muscles immobilized for 40, 80, 120, 160 and 200 days having recovered approximately 80% of their total cross-sectional area. However, during this early phase of fibre recovery there was considerable variation in muscle fibre size as indicated by the large standard errors characteristic of these 10-day-freed myofibre populations (Fig. 1). In many fibres (> 50%) the myofibrils had grown 50–100% larger than myofibrils in control fibres and evidence of the various stages of myofibril division, typical of normal growing myofibrils, was common (Fig. 6). By 40 days after removal of the cast, the mean myofibre cross-sectional areas and the standard error values of all the immobilized stages had returned to normal (Fig. 1). No degenerating myofibrils were seen in any of the myofibres examined. Thus, it seems that the disuse atrophy resulting from the immobilization of adult PLD muscles was reversible.
Effects of disuse on chick skeletal muscle

Continuous immobilization of the newly hatched chick PLD muscle for periods of 90, 130, 170, 210, 250, 290 and 330 days resulted in a 40–64% reduction in mean cross-sectional muscle fibre area (Fig. 3). Inactivity during development resulted in a number of changes to the adult myofibres not previously seen in adult muscles immobilized for similar periods. On the basis of cross-sectional size 2 populations of myofibres could be distinguished. The majority of fibres (> 50%) were between 500 and 800 \( \mu \text{m}^2 \). However, a second population (20%) of very small fibres, ranging in size from 0.5 to 200 \( \mu \text{m}^2 \) was also present (Fig. 4). Identification of these exceedingly small fibres with the light microscope was not possible and positive identification and fibre counts could only be made from ultrathin sections examined with the electron microscope. It should be noted that fibres of such small calibre were not seen in juvenile, control muscles or even in developing embryonic chick PLD muscles at 4 days before hatching (Shear & Goldspink, 1971; Shear 1975, 1978). Within these small fibres, thick and thin filaments were arranged into myofibrils, generally less than 0.2 \( \mu \text{m}^2 \) in cross-sectional area. Also a rudimentary sarcotubular complex (sarcoplasmic reticulum plus transverse tubules) was present and adjacent muscle fibres were completely separated from one another by an intact basal lamina (Fig. 7). In the larger fibres, the myofibrils were not well separated from one another and the sarcotubular complex was poorly developed (Fig. 8).

Freeing the muscles for 10–90 days resulted in only a 4–16% recovery of normal mean myofibre cross-sectional area. Younger chicks (i.e. continuously immobilized up to 130 days post-hatching) showed the greatest recovery by 10 days after cast
Fig. 5. Transverse section of an adult PLD myofibre immobilized for 80 days. The myofibrils at the periphery of the fibre are fragmented and large inter-myofibrillar spaces are seen. Note many of the contractile filaments have become dissociated (arrows) from the myofibrils. × 46000.
Fig. 6. Transverse sections of an adult PLD myofibre immobilized for 80 days and then freed of the cast for 10 days. Note the large number of myofibrils in various stages of division and the well-organized thick and thin filament lattice arrays. Little inter-myofibrillar space remains, compared with Fig. 5. × 46,000.
Fig. 7. Transverse section of a small PLD myofibre from a 120-day-old chick immobilized continuously on the day of hatching. The fibre contains 3 small myofibrils and several sarcotubular elements (arrow). Note the fibre is completely surrounded by an intact basal lamina. × 103500.
Fig. 8. Transverse section of a larger PLD myofibre than shown in the preceding figure, from a 130-day-old animal, immobilized continuously from the day of hatching for 130 days. Note the large irregular myofibrils and the absence of glycogen. × 46000.
Effects of disuse on chick skeletal muscle

removal. In older chicks the limited recovery that did occur after cast removal was
more gradual, taking up to 90 days in some muscles. However, even by 90 days
following cast removal, the mean myofibre cross-sectional area was still 40% less
than in the corresponding control muscles (Figs. 3, 4). Unlike the myofibres in muscles
immobilized after development and maturation were complete, there was a marked
decrease in the ability of myofibres immobilized throughout early post-hatching and
juvenile development to recover, once activity was resumed. Freeing the muscles
for 10–90 days did not significantly alter the frequency of large and small fibres and
both groups were seen to undergo a slight (4–16%) increase in size (Fig. 4). Myo-
fibrillar cross-sectional area was smaller (0.49 ± 0.05 μm²) in large fibres at all stages
after cast removal than in normal fibres (0.73 ± 0.03 μm²) and a well-developed sarco-
tubular complex was present. In the small fibres, there was considerable variation
in myofibril calibre within each fibre; the smallest myofibrils were generally located
at the periphery of the fibre and ranged from less than 0.1-0.5 μm² in cross-sectional
area (Fig. 9). The central myofibrils were generally larger (> 0.75 μm²) and more
irregular. A well-developed sarcotubular complex was seen throughout the small
fibres.

In a recent study by Patterson & Goldspink (1976) on growth in fish skeletal muscle
fibres, peripheral myofibrils were found to incorporate new contractile protein at a
higher rate than central myofibrils and to split (i.e. divide longitudinally) more
frequently than central myofibrils. In the present investigation, serial transverse
sections through several sarcomeres of a small fibre, 40 days after cast removal,
suggest that a similar process has occurred here. However, in previous studies dealing
with normal chick myofibril growth, all of the myofibrils within the fibre appeared to
grow and divide at an equal rate (Shear & Goldspink, 1971; Shear, 1978).

Neuromuscular junctions

The distribution of nerve terminals along the PLD muscle fibres was examined in
order to determine if immobilization during post-hatching development altered the
normal pattern of motor innervation. For this study 3 PLD muscles from 3 untreated,
330-day-old chicks and 3 muscles from animals immobilized continuously for 330
days after hatching were examined using a histochemical technique to detect and
localize acetylcholinesterase (AChE) activity. This enzyme reaction is useful for deter-
mining only the general topography of postsynaptic AChE activity, since the reaction
is limited to the superficial muscle fibres. Our gross, whole-muscle observations showed
no apparent difference in the general pattern and distribution of AChE activity in
normal and immobilized muscles. However, in both groups of muscle, small (0.5 μm)

Fig. 9. Transverse section of a small PLD myofibre from a 170-day-old animal, immo-
ibilized continuously from the day of hatching for 130 days and then freed for 40 days.
Note the large irregular central myofibrils and the smaller peripheral myofibrils. The
peripheral myofibrils appear to be dividing; however, only 10% of the postnatal
immobilized–freed myofibres showed any measurable growth. A portion of a fibroblast
cell is seen adjacent to the fibre. Satellite cells are seldom seen associated with myo-
fibres immobilized for prolonged periods of postnatal development. ×16 100.
AChE reaction sites were seen at the initial origin of the muscle within 1 mm of the spinal cord, and at the myotendinous junction of the PLD's insertion. Our attempts to tease and separate fibres showing these small terminal AChE sites were unsuccessful owing to the dense collagen network into which the very ends of the muscle fibres insert. In order to learn whether the AChE reactions located at the extreme ends of the muscle fibres represented sites of innervation, serial longitudinal ultrathin sections were made through the terminal-end sarcomeres at both the origin and insertion of the myofibres. Figure 10 shows 2 small neuromuscular junctions at the origin of the fibre. In fibres such as these, additional small neuromuscular junctions were often seen 30 or 40 μm distant from the ends (Figs. 11-14) and normal junctions were seen near the centre of the same fibre. An accurate estimate of the number of muscle fibres that have multiple neuromuscular junctions is not possible at this time. However, of all the myofibres serially sectioned at their origin, approximately 12-20% showed more than one readily identifiable junction. Multiple neuromuscular junctions were equally common among the small and large fibres of immobilized muscles. Three weeks after denervating adult PLD muscles, no identifiable neuromuscular junctions were seen, indicating that these nerve fibres originated from the brachial plexus. A study designed to measure the endplate-specific molecular form of AChE (19.5 S), using the technique as described by Vighy, DiGiamberardino, Rieger & Koenig (1976), is currently in progress. Our preliminary results indicate that by 3 weeks after denervation the endplate form of AChE (19.5 S) is equally low in all parts of the muscle (origin, insertion and belly). While further studies are needed, these early findings suggest that the axons supplying the small neuromuscular junctions are associated with the brachial plexus. It is not within the scope of the current study to determine whether the occurrence of multiple neuromuscular junctions in normal PLD myofibres is the result of polyneuronal innervation (i.e. branches of axons from several different nerve cells ending on a single myofibre) or multiple innervation (i.e. multiple branches of a single nerve cell ending on the same myofibre).

**DISCUSSION**

In the present investigation, continuous immobilization of the PLD muscle from the first hour after hatching for various periods of time up to 330 days, resulted in a significantly greater decrease in myofibre size than occurred when adult muscles were immobilized for similar periods (Figs. 1, 3). The myofibre atrophy resulting from long-term immobilization of adult muscle was completely reversed after removal of the plaster cast, in contrast to the myofibres immobilized in newly hatched chicks.

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Fig. 10. Longitudinal section of the origin end of a normal PLD myofibre. Two small motor endplates are shown (arrows). The axon terminals contain profiles of vesicles similar to those found at the primary endplate terminals. Note the relatively uncomplicated sarcolemmal infolding is typical of fast, twitch avian motor endplate specialization. Note also the extensive collagen framework at the tendinous origin, surrounding the end of the myofibre. ×14500.
Effects of disuse on chick skeletal muscle

Our previous studies suggested that immobilization during early post-hatching development might alter the ability of the myofibres to recover once normal activity was restored. In those studies, newly hatched chick PLD muscles immobilized for 21 days and subsequently freed for 72 h showed a complete recovery of normal myofibre size and structure. However, newly hatched chick PLD muscles immobilized for 27 days and freed for 72 h had a mean myofibre cross-sectional area 30% smaller than normal (Shear, 1975, 1978). The findings reported in our present study confirm and extend our earlier observations on the growth and post-hatching development in the PLD muscle of the chick: (i) the effects of muscle activity on myofibre growth are not constant during postnatal development; and (ii) the stunted or retarded myofibre growth resulting from immobilization during postnatal development is not reversible.

In the present study, disuse of the adult (130-day-old) PLD muscle results in a significant myofibre atrophy that continues for 160 days after application of the plaster cast. With regard to their size (i.e. cross-sectional myofibre area), all of the myofibres within the muscle appear to be similarly affected. The ability of the adult immobilized PLD myofibres to recover is not significantly diminished by prolonged periods of disuse. The greatest increase in mean myofibre size occurs during the first 10 days following cast removal and complete recovery occurs by 40 days. Similar rapid recovery rates have been reported in adult immobilized muscles by other workers (Thomsen & Luco, 1944; Cooper, 1972; Tomanek & Lund, 1974; Goldspink, 1977a,b).

Unlike the results seen after prolonged immobilization of adult muscle, the continuous immobilization from the moment of hatching resulted in adult muscles comprised of 2 size classes of myofibres. Within the larger myofibres, the myofibrils were less well defined than myofibrils in control fibres. The appearance of these large irregular myofibrils suggested that myofibrillar growth had occurred without myofibrillar division, as had been reported in juvenile, immobilized muscles of the chicken (Shear, 1978). The appearance of the very small (0.5-200 μm²) myofibres was unexpected. Such fibres could easily be mistaken for satellite cells, fibroblasts or even muscle spindles when examined with the light microscope. In a study on denervated developing rat and mouse skeletal muscles, small-diameter muscle fibres were seen to develop 3 weeks after nerve transection (Schultz, 1978). Schultz (1978) suggested that the small myofibres derive from satellite cells that detach from existing muscle fibres, proliferate, and then form myotubes. Evidently the small myofibres and myotubes degenerate within a few weeks due to the absence of motor innervation. In the present study the small fibres were at first thought to have a similar satellite cell origin. However, since degenerating fibres were never encountered, a more likely explanation is that the small fibres represent a population of innervated but retarded

Figs. 11–14. Longitudinal sections of the same fibre as in Fig. 10, but 1–3 mm from the terminal origin. The motor endplates seen in Figs. 11, 12 are nearer to the end of the myofibre (× 25,000 and × 9,000, respectively). The motor endplates seen in Figs. 13, 14 are much smaller and are typical of the endplates furthest from the origin of the myofibre and closest to the primary motor endplate. Figs. 13 and 14: × 20,000 and × 30,000, respectively.
or stunted myofibres. It is possible that immobilization during development reduces the number of myogenic cells that fuse with the developing myotubes and myofibres, and those myotubes and juvenile myofibres that are the least developed the first week after hatching might never acquire an adequate number of myogenic cells for normal growth, even after muscle activity is resumed. Another possibility is that the number of satellite cells associated with the developing myofibres is influenced by early muscle activity, and those myofibres that acquire the fewest satellite cells are least able to grow once activity is restored. This question requires study and is currently under investigation.

Immobilization of newly hatched and adult chick PLD muscles does not appear to alter the pattern of motor endplate distribution within the muscle. Although the histochemical localization of cholinesterase activity is limited to the superficial part of the muscle, the AChE enzyme studies indicate that the deeper myofibres have a similar distribution of motor endplates. The multiple motor endplate pattern found in both the adult immobilized and control muscles is difficult to explain. The multiple and polyneuronal innervation commonly seen in embryonic and neonatal skeletal muscle is thought to be lost during early development (Redfern, 1970; Bagust, Lewis & Westerman, 1973; Brown, Jansen & Van Essen, 1976; Korneliussen & Jansen, 1976; Riley, 1976; O'Brien et al. 1977; Rosenthal & Taraskevich, 1977; Srihari & Vrbova, 1978). Our findings indicate that in the chick PLD muscle, small multiple motor endplates remain associated with at least some of the myofibres. The endplates are found at the extreme ends of the myofibres, and more often at the origin than at the insertion of the muscle. It is not known if these endplates are functional, but they do contain vesicles and have basal laminae and sarcolemmal specializations similar to those associated with normal endplates. Furthermore, preliminary results indicate that the heavy (19.5 S) endplate form of AChE that is present at the very ends of the fibres is lost 3 weeks after transection of the brachial plexus (Shear & Oh, unpublished). In the denervated muscles small motor endplates were never observed. Therefore, the suggestion by Fisher & Goodman (1955) (see discussion by Hikida & Bock, 1971) that the origin of the PLD muscle receives motor branches from the nineteenth twentieth, and twenty-first posterior primary rami seems unlikely. In studies on polyneuronal innervation in the developing rat soleus muscle (Korneliussen & Jansen, 1976) and in the chick PLD muscle (Srihari & Vrbova, 1978) no morphological trace of degenerating axon terminals was ever found. These authors suggest that the axons retract rather than degenerate. In the light of our current findings, it seems reasonable to suggest that at least some of the redundant endplates migrate to the extreme ends of the myofibres during longitudinal growth. Experiments are currently being conducted to determine both the origin and the functional state of these small multiple nerve terminals.

The author wishes to acknowledge the help of Miss A. M. Uzdilla for skilful technical assistance and Mrs E. D. DeLong for typing the manuscript. This research was supported by grant no. AM 20131, awarded by The National Institutes of Arthritis, Metabolism and Digestive Diseases, PHS/DHEW.
Effects of disuse on chick skeletal muscle

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C. R. Shear


(Received 19 June 1980 - Revised 15 September 1980)