EXPERIMENTALLY INDUCED DIFFERENTIATION OF SLOW TONIC AND FAST TWITCH MUSCLES IN THE CHICK

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SUMMARY

The anterior latissimus dorsi (ALD) muscle of chickens is a slow tonic muscle, while the posterior latissimus dorsi (PLD) is a fast twitch muscle. These muscles on opposite sides of a 3-week-old chick were removed, minced and replaced in the site of the other muscle and left to regenerate. The regenerating muscles were examined at various periods from 4 days onwards and their contractile properties were found to resemble those typical of the muscle they replaced and not the original muscle. The regenerating muscles from 8 days onwards displayed the morphological features of the control muscles in the contralateral site. By 14 days, differentiation was almost complete and neuromuscular junctions were seen.

It is suggested that the physiological and morphological characteristics of a muscle are determined by its position and possibly also by its innervation.

INTRODUCTION

Birds possess both slow tonic and fast twitch muscles (Hess, 1970). These physiological differences are also reflected in the fine-structural appearance of the muscle fibres. Most mammalian skeletal muscles possess only twitch fibres, but some contract slowly. If in a mammal the nerve from a slow twitch muscle is made to re-innervate a fast muscle, this muscle becomes slow contracting, and vice-versa (Buller, Eccles & Eccles, 1960). In contrast, when such an operation is performed in chickens using a nerve from a slow tonic muscle to re-innervate a fast twitch muscle, no change in contractile speed, or morphological appearance of the fibres occurs unless the operation is performed within 1 week of hatching (Hnik, Jirmanová, Vylický & Zelená, 1967; Jirmanová & Zelená, 1973).

Redifferentiation of muscle fibres in adult animals can be induced by mincing a muscle and replacing the fragments in the animal. From such a mince, myoblasts develop and fuse to form myotubes which finally differentiate into typical muscle fibres (Carlson, 1973). If in a chicken a mince from a fast twitch muscle is put in the place of a slow tonic muscle, the regenerated muscle will resemble the slow muscle and vice-versa (Gordon & Vrbová, 1975). In this study 6–12 weeks elapsed between the operation and the final physiological experiments, and so no information about the rate of regeneration and differentiation, nor of the time of re-innervation was obtained.
It was therefore important to examine the time course of these events. There is no information about the ultrastructural appearance of such regenerating slow and fast muscle minces and hence it was decided to combine the physiological analysis with an ultrastructural study of the same muscles.

METHODS

Three-week-old chickens were anaesthetized by intravenous injection of Nembutal (30 mg/kg). The anterior latissimus dorsi (ALD) and the posterior latissimus dorsi (PLD) muscles were excised under aseptic conditions and each muscle was placed in a sterile Petri dish containing 1–2 ml of sterile saline. The muscles were then cut into small pieces (1–2 mm³) with fine scissors. The small pieces of muscle obtained from PLD were put in the site of the excised ALD and vice-versa. The wound was closed and the chickens left to recover.

At different times after this operation the animals were anaesthetized with Nembutal and the regenerating and control muscles excised and mounted in a bath containing oxygenated Krebs-Henseleit solution. Contractions were elicited via bipolar silver electrodes using square wave pulses of supramaximal intensity. Isometric tension was recorded by strain gauges (Devices, Type UF1) onto a pen recorder (Devices) or photographed from an oscilloscope screen. The length of the muscle was adjusted so that maximum twitch tension was developed.

After recordings had been made on the regenerating muscles, the physiological saline was removed and replaced with glutaraldehyde fixative (see below). The muscles were maintained under slight tension for about 20 min after which they were removed and immersed in fixative. Much fibrous tissue is associated with the regenerating muscle fibres: after fixation, the fibrous tissue is white while the muscle fibres are yellow. After about 45 min fixation, the muscle fibres were carefully dissected from the fibrous tissue and cut into small pieces for further processing. They were fixed for a total time of 2–3 h.

In some experiments the normal muscle fibres from 5-week chickens were fixed in situ for 15–20 min, and then bundles of superficial fibres were removed and immersed in glutaraldehyde fixative for a further 2–3 h. The fixative was 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.2 containing 0.075 M sucrose. After fixation for 2–3 h at 4 °C, the muscles were washed in several changes of buffer-plus-sucrose solution overnight at 4 °C. The tissues were then postfixed in 1% osmium tetroxide in the buffer-plus-sucrose solution for 1 h at room temperature. They were transferred straight to 70% ethanol, dehydrated in graded ethanols, passed through propylene oxide and embedded in epoxy resin. The sections were stained with uranyl acetate and lead citrate and examined in an AEI EM801 electron microscope.

RESULTS

Contractile properties of the control and transplanted regenerating muscles

Four to eight days after the muscles were minced and transplanted small strands of muscle could be isolated from the site of the transplant. It was extremely difficult to set these up for tension recording because they were too fragile. Although some records were obtained from these early regenerating muscles, the results are considered to be unreliable.

The earliest reliable tension records were obtained from muscles 14 days after the operation. At this stage the size of the regenerating muscle is still variable. In most cases the ALD transplant at the PLD site was more organized and developed more tension than the PLD transplant at the ALD site. Fig. 1 shows an example from an experiment in which tensions from the regenerating and control muscles were
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Fig. 1. Isometric contractions in response to stimulation at 40 Hz from control ALD (A) and PLD (B) muscles, regenerating PLD-to-ALD transplant (C) and regenerating ALD-to-PLD transplant (D). The experiment was performed 14 days after the operation and the chicken was then 5 weeks old.

Table 1. Contractile characteristics of control and regenerating ALD and PLD muscles

<table>
<thead>
<tr>
<th>Time after operation</th>
<th>Muscle</th>
<th>0.5 TTP* at 40 Hz, ms</th>
<th>Maximal tetanic tension, g</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control ALD</td>
<td>344 ± 4° (5)</td>
<td>15.8 ± 2.6 (5)</td>
</tr>
<tr>
<td></td>
<td>ALD-PLD transplant</td>
<td>200 ± 5° (6)</td>
<td>2.7 ± 0.93 (5)</td>
</tr>
<tr>
<td></td>
<td>Control PLD</td>
<td>83 ± 4° (5)</td>
<td>38.6 ± 8.6 (4)</td>
</tr>
<tr>
<td></td>
<td>PLD-ALD transplant</td>
<td>938 ± 45° (4)</td>
<td>43 ± 0.18 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6-17 weeks (values taken from Gordon &amp; Vrbová, 1975)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control ALD</td>
<td>558 ± 75° (6)</td>
<td>48 ± 13 (6)</td>
</tr>
<tr>
<td></td>
<td>ALD-PLD transplant</td>
<td>158 ± 89° (6)</td>
<td>6.7 ± 1.6 (5)</td>
</tr>
<tr>
<td></td>
<td>Control PLD</td>
<td>45 ± 2° (6)</td>
<td>35 ± 8 (6)</td>
</tr>
<tr>
<td></td>
<td>PLD-ALD transplant</td>
<td>596 ± 8° (6)</td>
<td>7.9 ± 2.4 (6)</td>
</tr>
</tbody>
</table>

* TTP, time to peak tension. Values are given ± s.e. and the number of experiments in parentheses.

recorded, the transplanted ALD contracted more rapidly than the control ALD, but not as rapidly as the control PLD (see Table 1). PLD muscles in the ALD site contracted and relaxed more slowly than the control PLD and more slowly than normal ALD muscles (see Fig. 1 and Table 1). The extremely slow contraction of the PLD-to-ALD transplant may be due to the large areas of undifferentiated tissue which are still present in this muscle and these could increase the series elastic effects and reduce
the rate of tension development. At later stages of regeneration, however, the rate of tension development in the PLD-to-ALD transplant increases and is similar to that of control ALD muscles.

**Ultrastructure of the muscle fibres**

*Control muscle fibres: Anterior latissimus dorsi (ALD).* This has the structural characteristics of a slow tonic muscle in a 5-week chicken. The fibres are between 10 and 33 μm in diameter, with peripheral nuclei which form bulges along their length. The myofibrils are irregular both in size and alignment; in transverse sections (Fig. 10) it is seen that they are not discrete, while their haphazard lateral alignment, emphasized by the wavy Z-lines, is apparent in longitudinal sections (Fig. 14). The sarcomeres show obvious A- and I-bands, but possibly due to the longitudinal irregularity within them, M-lines and H-zones are not seen clearly.

The sarcoplasmic reticulum forms a network between the myofibrils (Fig. 14). Associations with the transverse tubular system (T-system) occur at approximately the level of the A-I band junction and take the form of dyads and triads. These may have either a longitudinal or transverse orientation and the sarcoplasmic reticular tubule contains dense material. Quite large mitochondria are randomly distributed among the myofibrils. A few deposits of glycogen are present and lipid droplets are occasionally seen.

*Control muscle fibres: Posterior latissimus dorsi (PLD).* This is a typical twitch muscle with fibres of diameters between 12 and 18 μm. The myofibrils are discrete when seen in transverse sections (Fig. 11). In longitudinal sections (Fig. 17) the sarcomeres are in approximate alignment across the fibre. The Z-lines are straight and the A- and I-bands are clearly demarked: within the A-band, the M-line and H-zone are visible.

The sarcoplasmic reticulum consists of an irregular array of tubules around the I-bands and a few longitudinal tubules around the A-bands (Fig. 17). The triads occur in the I-bands, near to the A-I band junction and are of variable, but predominantly transverse orientation. The sarcoplasmic reticular tubules at the triad contain dense material. This arrangement of the membrane systems is slightly different in detail to that seen in fully mature PLD fibres (Page, 1969); the fibres described here are from a 5-week-old chick. Large mitochondria occur in longitudinal rows between the myofibrils. Large deposits of glycogen are present, especially around the I-bands.

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Fig. 2. PLD-to-ALD, 2-day transplant. A satellite cell (sac) with its parent muscle fibre. The basement membrane is continuous around both cells (arrows). × 27000.

Fig. 3. PLD-to-ALD, 8-day transplant. Two myoblasts (mb) in the process of fusion. Both nuclei contain prominent nucleoli. The cytoplasm contains some rough endoplasmic reticulum (er). Bundles of filaments (f) are present in the peripheral cytoplasm. × 7500.

Fig. 4. PLD-to-ALD, 8-day transplant. Filaments (f) in the peripheral cytoplasm of one of the myoblasts in Fig. 4. × 27000.
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Fig. 5. ALD-to-PLD, 4-day transplant. Degenerating muscle fibres (mf) and differentiating satellite cells ( sac) in longitudinal section. The cytoplasm of the satellite cells contains rough endoplasmic reticulum (er), small mitochondria (m) and bundles of filaments (f) aligned longitudinally. × 12000.
Structure of the transplanted regenerating muscle fibres

In the first few days after the operation, little muscle tissue is present at the transplanted sites. The muscle fibres are degenerating and are surrounded by fibrous tissue with many fibroblasts and macrophages. In many instances undifferentiated cells are seen adjacent to the muscle fibres. The degenerating fibres have a disrupted appearance with swollen cisternae of sarcoplasmic reticulum and disordered myofibrils; they were not examined in detail. In transverse section it can be clearly observed that the undifferentiated cells are within the basement membrane which surrounds a degenerating muscle fibre (Fig. 2). The same cells can be seen in longitudinal section (Fig. 5). Their cytoplasm is not highly differentiated, but it contains active rough endoplasmic reticulum and bundles of filaments. Both the rough endoplasmic reticulum and filaments have a preferred longitudinal orientation within the cell. They are identified as satellite cells.

By 8 days after transplantation, regeneration has progressed and differentiating muscle fibres can be seen in both the ALD-to-PLD and PLD-to-ALD transplants; differences in the transplants, corresponding to their site, can already be distinguished. At both transplant sites, the amount of muscle tissue is small, and a large amount of fibrous tissue has been produced.

PLD-to-ALD transplants

Eight days after the operation, the muscle fibres regenerating from the minced PLD at the ALD site display much more variability in their degree of regeneration than those at the PLD site. The fibres are in very small bundles and are separated by connective tissue. Within this connective tissue, cells which appear similar to the satellite cells described previously can be seen; it is thought that these are myoblasts aligning themselves prior to fusion (Fig. 3). The most obvious feature of these cells is the presence of prominent nucleoli in the nucleus. The cytoplasm contains rough endoplasmic reticulum with dilated cisternae containing dense material. Bundles of fine filaments in longitudinal orientation occur in the peripheral cytoplasm of these cells (Figs. 3, 4).

Elsewhere muscle fibres with well developed myofibrils occur. The alignment of the filaments to form the A- and I-bands is regular and the A-filaments have an obvious M-line (Fig. 7). As can be seen in both Figs. 6 and 7, there are areas of well developed myofibrils interspersed with areas in which the myofibrils are being assembled. The cytoplasm between the myofibrils contains polyribosomes, Golgi complexes and the developing membrane systems. In some areas (Fig. 7), the typical network arrangement of the sarcoplasmic reticulum is already present, while nearby swollen and irregular tubules occur. No dyads or triads were observed.

At 14 days the fibres are in general more mature and vary in diameter between 16 and 28 μm. Areas of cytoplasm containing much rough endoplasmic reticulum and Golgi complexes, however, still occur especially in the peripheral regions and the ends of the fibres. The myofibrils are now well organized but they are not discrete, and vary in diameter (Fig. 12) and are not well aligned across the fibre. The Z-lines,
delimiting the sarcomeres are rather irregular and this is reflected in irregular A-I band junctions (Fig. 15). The I-band appears wider than in the 8-day muscle, though this is probably due to the state of contraction of the fibres at fixation. The A-bands now show only traces of M-lines in a few scattered instances. The sarcoplasmic reticulum forms a network around the myofibrils (Fig. 15) and longitudinally oriented dyads or triads occur near the A-I band junction. Mitochondria occur most commonly at the level of the I-bands. Occasionally a very immature fibre, such as those typical of the 8-day transplant, may still be seen.

At this stage the muscle fibres are re-innervated; neuromuscular junctions are present. The nerve endings may be quite large and contain many synaptic vesicles and some mitochondria (Fig. 16). They lie on the surface of the muscle fibre and the 2 membranes are separated by a common basement membrane. There is, at most, a very shallow depression in the surface of the muscle fibre, and there are no junctional folds.

**ALD-to-PLD transplants**

Bundles of muscle fibres originating from a minced ALD are present at 8 days in the PLD site. The fibres are closely packed and there is little extraneous connective tissue around them. While there is some diversity in the degree of organization of the fibres, in general the myofibrils are discrete and well differentiated (Fig. 8), and the structure of the sarcomeres resembles that of normal PLD muscle fibres (Fig. 9). The membrane systems are not yet fully organized, but longitudinal tubules of sarcoplasmic reticulum and developing triads can be seen. The mitochondria are rather sparse, but there are many glycogen granules. That growth and differentiation are still in progress is shown by the presence of spirally arranged polyribosomes.

By 14 days after transplantation, differentiation has proceeded and the only sign that the fibres are not fully mature is the presence of many polyribosomes immediately beneath the sarcolemma (Fig. 19). The sarcomeres are well organized with clearly defined bands (Fig. 18). The myofibrils are discrete (Fig. 13) and are surrounded by fully developed membrane systems. Triads occur near the junction of the A- and I-bands and for the most part are transversely oriented. The sarcoplasmic reticulum consists of longitudinal tubules around the A-band and a rather more irregular network of tubules around the I-bands. The mitochondria are rather large and tend to

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Fig. 6. PLD-to-ALD, 8-day transplant. This transverse section of regenerating fibres at the ALD site illustrates the variability in the size of the myofibrils seen at this stage. The A-bands (A) have a regular lattice, the I-bands (I) are not so regular. The Z-lines (Z) are small and do not contain a regular lattice. A few rather swollen cisternae of sarcoplasmic reticulum (sr), a Golgi complex (gi) and some small mitochondria (m) are present in the cytoplasm. × 27000.

Fig. 7. PLD-to-ALD, 8-day transplant. In longitudinal section, the myofibrils at the ALD site appear banded with a regular junction between the A-bands (A) and I-bands (I). The A-band is transected by an M-line (M). The Z-lines (Z) are wide and filaments cannot be seen within them. In some areas (arrows), the sarcoplasmic reticulum (sr) forms a network, but mostly the cisternae are swollen and irregular. Spiral polyribosomes (r) are present. × 27000.
Fig. 8. ALD-to-PLD, 8-day transplant. This transverse section of part of a regenerating fibre shows well organized myofibrils with easily distinguishable A-bands (A), I-bands (I) and Z-lines (Z). The sarcoplasmic reticulum (sr) is rather dilated, but is seen around most myofibrils. Much glycogen (g) is present. × 27000.

Fig. 9. ALD-to-PLD, 8-day transplant. In longitudinal section, the sarcomeres have clearly defined A-bands (A) and I-bands (I). The Z-line (Z) is narrow and the I-filaments enter it in a regular array. The sarcoplasmic reticulum (sr) is present, particularly around the I-bands; in some places it appears very dilated (arrows). Glycogen (g) is also present. × 27000.
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be arranged in rows. A very obvious feature of these fibres is the presence of large amounts of glycogen, particularly in the region of the I-bands.

The presence of neuromuscular junctions at 14 days indicates that re-innervation has occurred. The nerve endings are quite small and contain synaptic vesicles and mitochondria (Fig. 19). They occur in depressions on the surface of the muscle fibre, but there are no distinct junctional folds.

DISCUSSION

A previous investigation in which slow tonic and fast twitch muscles were minced and transplanted to the opposite site showed that the characteristic contractile properties of the regenerated muscle fibres are determined by their innervation and not by the parent muscle (Gordon & Vrbová, 1975). These experiments were similar to those described here, but the muscles were examined a long time after the operation, so it was impossible to assess whether they differentiate immediately into fibres with the typical characteristics of their new site, or whether they first go through a transitional phase with characteristics of their original site. This present study indicates that there is no detectable transitional stage during which the muscle fibres resemble the parent muscle, and it illustrates the speed with which the regenerating muscle fibres acquire the characteristic properties of the muscle they replace.

The structural findings confirm the physiological observations and again illustrate the rapidity with which regeneration occurs. Regeneration has started 2 days after mincing and transplantation, since satellite cells are present and by 8 days these cells have produced recognizable muscle fibres, which by 14 days are fully differentiated and re-innervated.

The satellite cells were identified by their position within the basement membrane of the degenerating fibres and by their undifferentiated cytoplasm. The number of satellite cells in the normal muscles is probably very small as none was observed and hence it is probable that the satellite cells arise from the peripheral nuclei of the degenerating fibres. The formation of satellite cells by the separation of a nucleus together with a little surrounding cytoplasm by a new cell membrane appears to be the most frequent way in which satellite cells are produced (Konigsberg, Lipton & Konigsberg, 1975; Mastaglia, Dawkins & Papadimitriou, 1975; Schmalbruch, 1976; Snow, 1977a) and the presence of such nuclei in regenerating muscle fibres has been demonstrated autoradiographically (Gutmann, Mares & Stichová, 1976; Snow, 1977b, c). The presence of satellite cells within 2 days of transplantation agrees with studies of a number of denervated and minced mammalian muscles, including the extensor digitorum longus, gastrocnemius, plantaris, soleus and tibialis anterior of the rat and the rectus abdominus and diaphragm of the mouse (Bennett, Florin & Woog, 1974; Mastaglia et al. 1975; Ontell, 1975; Schmalbruch, 1976; Snow, 1977a).

The present results indicate that muscle fibres regenerating at the PLD site from minced ALD fibres develop more quickly than those from PLD at the ALD site. At 8 days the regenerating fibres bear a clear resemblance to the fibres normally present at their respective sites, but those at the twitch PLD site are more uniformly
and highly differentiated than those at the tonic ALD site. The myofibrils of the transplant at the PLD site display the regular banding pattern typical of PLD fibres. The Z-lines are narrow and the zig-zag of the filaments can be seen. The sarcoplasmic reticulum is not organized and triads are not seen. Thus only the final modification of the membrane systems and the formation of triads is necessary to produce the normal fibres seen at 14 days.

The fibres at the ALD site are, however, much more variable. Some possess fully developed myofibrils, while others have myofibrils in varying stages of formation. A noticeable feature is the regularity of the A-I junctions and the presence of an M-line, which are not typical of ALD myofibrils, but by 14 days the myofibrils are more like those of a normal ALD fibre. It is known that in their initial stages of development slow tonic fibres possess both slow and fast-type myosin light chains and that the fast-type light chains are gradually replaced by slow-type myosin light chains (Pette, Vrbová & Whalen, 1978; Rubinstein, Pepe & Holtzer, 1977). This sequence may also occur during regeneration and the features of the myofibrils just described may be an expression of this phenomenon. Nevertheless at 8 days the Z-line is already wide and filaments within the Z-line cannot be distinguished in either longitudinal or transverse section. In some places, the typical network of the sarcoplasmic reticulum occurs at 8 days, but mostly it is not yet organized; by 14 days the normal pattern occurs throughout the fibre.

In a series of experiments with the ALD and PLD muscles of the adult pigeon, Hikida found that minced ALD placed in the PLD site regenerates into twitch fibres, while minced ALD replaced in the same ALD site regenerates into a tonic morphology within 4 weeks, but at 18 weeks some of the tonic fibres have subsequently transformed into twitch fibres (Hikida, 1974, 1976). There is no evidence for such a transformation in the chick ALD; at 90 days, the physiological properties of regenerates from either minced ALD or PLD at the ALD site are typical of a tonic muscle (Gordon & Vrbová, 1975). It should be emphasized that the present study employed young birds, whereas Hikida used adults. This could account for the presence of occasional twitch fibres in the regenerated ALD; it is known that in old

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Fig. 10. Normal ALD, 5-week-old chicken. The myofibrils are not discrete and are randomly separated by sarcoplasmic reticulum (sr). The A-band (A) and I-band (I) lattices are irregular. The Z-line (Z) appears amorphous; there is no regular square lattice. \( \times 27,000 \).

Fig. 11. Normal PLD, 5-week-old chicken. The myofibrils vary in size, but all are discrete and separated by sarcoplasmic reticulum (sr) and glycogen (g). The A-band (A) lattice is regular, the I-band (I) lattice is not so regular. The Z-lines (Z) have a square lattice. \( \times 27,000 \).

Fig. 12. PLD-to-ALD, 14-day transplant. The transverse section resembles Fig. 10; the myofibrils are not discrete. The A-band (A) and I-band (I) lattices are irregular, and no lattices are seen in the Z-line (Z). \( \times 27,000 \).

Fig. 13. ALD-to-PLD, 14-day transplant. The myofibrils are discrete as in Fig. 11. The A-band (A) lattice is regular, but the I-band (I) lattice is not so regular. The Z-line (Z) has a square lattice. \( \times 27,000 \).
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rats many slow twitch fibres come to resemble fast twitch fibres (Gutmann & Hanzlíková, 1976).

The control muscles in this study were the unoperated muscles from the chickens in the 14-day experiment, and thus were fixed when the birds were 5 weeks old. Their structure agrees with Page's (1969) description of the same chicken muscles. The variability in the triad orientation found in the 5-week PLD fibres was also reported by Page. We did not observe a fenestrated collar of sarcoplasmic reticulum around the A-band at this age. The ALD fibres are similar to those described by Page, except that filaments encircling the Z-line were not seen. We found no evidence to support the finding of Shear & Goldspink (1971) that normal ALD muscle fibres possess discrete myofibrils immediately after hatching.

Neuromuscular junctions were not seen in the 8-day muscles, but they are fully developed in the 14-day muscles. Innervation is said to be necessary for the differentiation of regenerating rat muscle fibres into fast and slow twitch fibres (Carlson & Gutmann, 1972; Mong, 1977); in these chicken muscles differentiation of ALD or PLD characteristics has occurred in the 8-day fibres, so it is possible that some neuromuscular contacts are already present. The neuromuscular junctions in both regenerated muscles correspond in detailed structure to those of the appropriate normal muscles (Atsumi, 1977; Hess, 1961).

These results indicate that the position and innervation of a muscle play an important role in determining its physiological and morphological properties. In addition they illustrate that satellite cell nuclei from one type of muscle can, when placed in the site of another type of muscle, form myotubes which differentiate into fibres appropriate to the new site.

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Fig. 14. Normal ALD, 5-week-old chicken. The appearance of this muscle is irregular since the Z-lines (Z) are not straight and from this it follows that the boundaries between the A-bands (A) and I-bands (I) are also irregular; M-lines are not present. The Z-lines are wide and amorphous; there is no regular array of I-filaments entering them. The sarcoplasmic reticulum (sr) forms a network around the myofibrils; dyads (d) occur irregularly. × 27 000.

Fig. 15. PLD-to-ALD, 14-day transplant. The sarcomeres are similar to those in Fig. 14, except that these sarcomeres were more relaxed or stretched on fixation, resulting in wider, more irregular I-bands (I). In places the A-band (A) is transected by an M-line (M). The Z-line (Z) is wide and amorphous and the I-filaments enter it irregularly. The sarcoplasmic reticulum (sr) is a network around the myofibrils; dyads (d) occur irregularly. × 27 000.

Fig. 16. PLD-to-ALD, 14-day transplant. A nerve ending (n) with synaptic vesicles lying on the surface of a regenerating muscle fibre (mf). × 27 000.
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Fig. 17. Normal PLD, 5-week chicken. The myofibrils are very regularly arranged with clearly delimited A-bands (A) and I-bands (I). The A-bands are transected by an M-line (M) with an H-zone on either side. The Z-lines (Z) are straight and the I-filaments enter in a regular pattern (arrow). The triads (t) are around the A-I junction and vary in orientation. The sarcoplasmic reticulum (sr) forms a random network of tubules around the I-bands and longitudinal tubules around the A-bands. Glycogen (g) is present, especially around the I-bands. x 27000.

Fig. 18. ALD-to-PLD, 14-day transplant. The sarcomeres are essentially similar to those of the normal PLD in Fig. 17. The A-bands (A), I-bands (I) and M-lines (M) appear the same, and the I-filaments enter the Z-lines (Z) in a regular pattern (arrow). The triads (t) are irregularly oriented around the I-bands. The sarcoplasmic reticulum (sr) consists of a network around the I-bands and longitudinal tubules around the A-bands. Much glycogen (g) is present. x 27000.

Fig. 19. ALD-to-PLD, 14-day transplant. A nerve ending (n) with synaptic vesicles in a depression on a regenerating muscle fibre (mf). Many spiral polyribosomes (r) are present in this area of peripheral cytoplasm. x 27000.
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