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FAST AND SLOW MUSCLES OF THE CHICK AFTER NERVE CROSS-UNION

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SUMMARY

1. The multiply innervated anterior latissimus dorsi (ALD) and the focally innervated posterior latissimus dorsi (PLD) muscles of the chick were investigated 2–18 months after nerve cross-union.

2. The fast PLD muscle re-innervated by the slow muscle nerve became supplied with 'en grappe' end-plates and responded to a single nerve volley with local potentials only. Control PLD muscles re-innervated by the original nerve, had 'en plaque' end-plates and responded to a single nerve volley by synchronous action potentials in the same way as normal muscles.

3. In the slow ALD muscle re-innervated with the 'mixed' PLD nerve, the type of innervation and of electromyographic response remained practically unchanged, with the exception of transplanted ALD muscles supplied with PLD nerves where, in addition to local responses, propagated action potentials were registered electromyographically in response to single nerve volleys.

4. ALD muscles of young chickens re-innervated both with an implanted purely fast muscle nerve and with the regenerated original nerve, had two types of innervation: 'en plaque' end-plates around the nerve implant, and multiple 'en grappe' end-plates in areas supplied with the ALD nerve. Accordingly, propagated action potentials were registered in response to single nerve volleys in regions near the implant, whereas local potentials were recorded in areas with original innervation.

5. Contraction velocity was not substantially altered in PLD and ALD muscles after nerve cross-union.

6. No changes were observed in the fine structure of muscle fibres in extrajunctional regions.

INTRODUCTION

In mammals, slow and fast twitch muscles undergo partial conversion towards the reverse muscle type after nerve cross-union. Their contraction velocity becomes changed (Buller, Eccles & Eccles, 1960; Eccles, 1963; Buller & Lewis, 1963, 1965; Close, 1965) and muscle metabolism is altered under the influence of the alien nerve (Drahota & Gutmann, 1963, Bücher & Pette, 1965; Romanul & van der Meulen, 1966; Guth & Watson,1967).

The question arose whether an analogous transformation of muscle type would also occur under nervous influence in those instances, when fast and slow muscles are more dissimilar in structure and function. In the chick, a pair of dorsal muscles, the slow anterior latissimus dorsi (ALD) and the fast posterior latissimus dorsi (PLD), are well suited for the study of the problem.

Both the ALD and PLD muscles consist of a practically homogeneous population of muscle fibres. The contraction velocity of ALD is 5–7 times longer than that of PLD (Ginsborg, 1960*b*; Page & Slater, 1965). Furthermore, the two muscles differ in their pattern of innervation, in their electrical response to nerve stimulation and in the fine structure of muscle fibres. While the fast PLD is a 'twitch' muscle with focal innervation, the slow ALD is composed of multiply innervated fibres with 'Felderstruktur' which have 'en grappe' end-plates (Krüger, 1950, 1952, 1958; Ginsborg, 1960*a*; Ginsborg & Mackay, 1961; Hess, 1961*a*, *b*; Silver, 1963) and a differently arranged sarcoplasmic reticulum (Page & Slater, 1965). In the ALD, only local potentials can be recorded electromyographically in response to nerve stimulation with single pulses; propagated action potentials are not evoked *in vivo* except during post-tetanic potentiation (Jirmanová & Vyklický, 1965), although under *in vitro* conditions they can be elicited even by a single volley (Ginsborg, 1960*b*). The effect of nerve cross-union has first been investigated in the PLD by Feng, Wu & Yang (1965), who found that repetitive nerve stimulation was needed to produce a maximal electrical response of the muscle, and that the speed of contraction seemed to be unaltered.

In the present study an attempt was made to learn whether the type of innervation, the electromyographic response, contraction velocity and ultrastructure of ALD and PLD muscles is altered after nerve cross-union. Preliminary accounts have already been published elsewhere (Zelená, Jirmanová & Vyklický, 1966; Zelená, Vyklický & Jirmanová, 1967).

METHODS

Surgical procedures

The operations were performed under Nembutal anaesthesia in white Leghorns of 1·5–2·5 kg body wt., with the exception of one series when young chickens were used.

Nerve cross-unions. In twelve animals, the ALD nerve was cross-united to the PLD muscle nerve. The nerves to ALD and PLD muscles which run together in a common trunk were exposed. The ALD nerve was sectioned near the muscle, while the PLD nerve was cut more centrally, so that the nerve stumps were sufficiently long to avoid traction when the nerves were reconnected by suturing the perineurium. The central stump of the PLD nerve was deviated in the opposite direction and implanted into the wing musculature to prevent mixed re-innervation. The ALD muscle was either left denervated *in situ*, or removed. Out of eleven operated animals, two PLD muscles were found to be partly re-innervated by their own nerves and were consequently discarded. As control, the original nerves to the PLD muscles were cut and re-sutured in eight animals.

In eleven animals the PLD nerve was connected to the ALD muscle in a way analogous to that described above. However, the PLD nerve is a 'mixed' nerve in the sense that it supplies not only focally innervated muscle fibres, but also a few multiply innervated fibres in the PLD muscle (Hess, 1961*b*). Furthermore, it contains an inconstant branch to the ALD muscle (Ginsborg, 1960*b*; Feng *et al.* 1965) and a small branch to its multiply innervated metapatagial part. After nerve cross-union, the ALD muscle thus became re-innervated from a nerve trunk which contained a mixed population of two types of motor nerve fibres. Although the central stump of the ALD nerve was ligated and implanted into the wing musculature, in three operated animals ALD muscles were also found to be re-innervated from the ALD nerve trunk and they were therefore not investigated further. In five animals the ALD nerves were cut and re-sutured as a control.

Transplantations of the latissimus dorsi muscles were performed in order to avoid contamination of the nerve cross-unions by the original nerve supply. The tendon of the right PLD muscle was sectioned together with the nerve and blood vessels, the origin of the muscle remaining fixed to the spine. The muscle was then dissected free, turned over across to the left side and placed above the left PLD muscle, which remained *in situ*, with its nerve supply intact. Then the left ALD nerve was cut and connected to the nerve stump of the transplanted PLD muscle. PLD muscles were transplanted in three animals. As a control, the PLD nerve of the left side was sectioned and connected to the distal nerve stump of the transplanted muscle in three animals.

In a similar way, the right ALD muscle was transplanted to the left side and connected with the left PLD nerve in four animals. As control, the transplanted ALD muscles were reconnected with ALD nerves of the left side in three animals.

Nerve implants. In five chickens of 80–140 g body wt., the nerve to the focally innervated anconeus scapularis muscle was employed to re-innervate the ALD muscle. The anconeus scapularis nerve was sectioned near the entry into the muscle, the central stump was dissected and brought to the upper surface of the ALD muscle where it was inserted into a small incision and glued to the muscle with fibrin clot. Although the ALD nerve was cut and ligated, it regenerated and re-innervated the ALD muscle in all animals of this series.

Muscles were investigated 3–18 months after the operations. Most muscles after cross-union were not studied earlier than 5 months after the operation; control muscles were tested beginning with the third post-operative month.

Electromyography

Electromyographic responses to a single volley in the peripheral stump of the nerve were recorded with a co-axial needle electrode (Adrian & Bronk, 1929). The duration of the rectangular pulse was 10 μsec and stimulus strength was adjusted to give a maximal re-

sponse. The co-axial needle electrode was found to be suitable for detecting local responses from the muscles and for distinguishing between low-voltage monophasic local potentials and propagated action potentials, which were recorded as a characteristic response to single pulses in ALD and PLD muscle respectively (Jirmanová & Vyklický, 1965). The monophasic low-voltage potentials are referred to as local potentials; however, it cannot be fully excluded that besides end-plate potentials they might also include a few propagated action potentials travelling for a short distance in a small fraction of fibres. In order to potentiate the end-plate potentials, tetanic stimulation at a frequency 200/sec was applied to the nerve for 10 sec and records were taken at 10 sec intervals after the end of tetanic stimulation.

Myography

After localizing the nerve trunks supplying both ALD and PLD muscles, the distal insertions were dissected and the muscles freed from surrounding tissues corresponding to the distal third of the muscle. The distal tendon of PLD was fixed to the myograph lever. With the ALD, a piece of the humerus was chipped off together with the muscular insertion and connected to the lever. The nerve was cut, ligated and covered with gauze soaked in paraffin, which had been shaken with physiological saline. The muscles were similarly covered up and their temperature maintained with radiant heat at 20–25° C.

Nerves were stimulated with short rectangular pulses, 0·1 msec in duration and of supramaximal strength. The muscles were stretched to optimal length, so as to give a maximal twitch response. The contraction characteristics of the muscles were recorded by an isometric myographic set-up employing an RCA 5437 valve and photographed off the screen of a double-beam oscilloscope.

Histology

Staining for cholinesterase (ChE). Animals were killed and bled. ALD and PLD muscles were removed, weighed, stretched on cork, and fixed in 4 % formaldehyde with 1·7 % Na_2SO_4 for 2 hr. After washing in distilled water, longitudinal sections 40–60 μ thick were cut and stained for ChE (Koelle & Friedenwald, 1949). Acetylthiocholine iodide was used as substrate. Sections were incubated at pH 6·2 for 45–90 min; some of them were left in the incubation medium overnight in order to obtain a general view of the distribution of end-plates in the muscle. No inhibitors were used, since Silver (1963) demonstrated that both ALD and PLD end-plates predominantly contained acetylcholinesterase, butyrylcholinesterase being undetectable in both muscles.

With some specimens, a part of the muscle was stained for cholinesterase *in toto* (Silver, 1963). The incubation times were 1–2 hr; for counting the number of end-plates, muscles were incubated overnight. After staining, the tissue was stored in 20 % glycerol. From the tissue blocks, individual muscle fibres were teased under the dissecting microscope. The size of end-plates was measured with an ocular micrometer under magnification × 400.

Staining for ChE could be safely used for detection of the newly formed end-plates, since ChE activity was found to be greatly reduced or absent at the original junctions of denervated muscles from about 6 weeks after cutting the nerve onwards.

Electron microscopy. The dorsal muscles were exposed in animals under Nembutal anaesthesia and prefixed *in situ* by 5 % glutaraldehyde, buffered at pH 7·3 with phosphate buffer. After several minutes the muscles were pinned down to a thin cork-plate, cut off at the origin and insertion and immersed in 5 % glutaraldehyde solution for 2 hr. Small blocks were then excised from the fixed tissue, washed in the phosphate buffer and post-fixed with buffered 1 % OsO_4 for 30 min. The blocks were dehydrated in alcohol, stained with 1 % alcohol solution of uranylacetate overnight, and embedded in Vestopal W (Sabatini, Bensch & Barrnett, 1963). Sections were cut with a glass knife on a Porter–Blum microtome, counterstained with lead-citrate (Reynolds, 1963) and viewed in a Tesla BS 413 microscope at 80 kV with an objective aperture of 30 μ.

RESULTS

PLD muscles re-innervated by ALD nerves

Fast PLD muscles re-innervated by slow ALD muscle nerves were found to be atrophic. The denervation atrophy, amounting to about 50 % by weight, which developed after the operation, partly receded after nerve cross-union, but wet weight of muscles supplied by the 'slow' nerve 5–6 months after nerve cross-union still remained reduced to $78{\cdot}4 \pm 8{\cdot}0$ % in comparison with contralateral normal muscles ($n = 4$). On the other hand, muscles re-innervated by their original nerves attained normal weight from the third post-operative month onwards ($98{\cdot}0 \pm 1{\cdot}5$ %; $n = 5$).

Motor end-plates. The extent of re-innervation after nerve cross-union was relatively large, but never complete. In normal PLD muscles there are no well-defined end-plate regions, but end-plates are distributed in irregular transversal bands throughout the whole muscle. In cross-innervated muscles, motor end-plates were as a rule found in the proximal two thirds of the muscle, while in the region adjacent to the spine—far off from the nerve entry—they were extremely rare or absent.

The type of end-plate in the PLD was altered after nerve cross-union. The difference in the character of innervation and in the intensity of ChE staining was already conspicuous in sections under low magnification (Pl. 1, figs. 1, 2). Inspection of single end-plates revealed differences in the configuration of the subsynaptic area (Pl. 1, figs. 3–8).

Normal PLD fibres have large 'en plaque' end-plates with intricate terminal nerve branching impressed into a network of interconnected gutters (Pl. 1, figs. 5, 7). This characteristic appearance of end-plates was gradually restored following re-innervation with the PLD nerve, although the structure of regenerated end-plates was less regular than in normal muscles (Pl. 1, fig. 6). In contrast to this, end-plates formed after nerve cross-union had a distinct 'en grappe' shape. They consisted of clusters of tiny synaptic areas characterized by weak ChE activity (Pl. 1, fig. 8). A distinct disparity was also found in the size of the two types of end-plates. Normal and self-regenerated PLD junctions occupied a muscle fibre segment about 3 times as long as that covered by the hybrid 'en grappe' end-plates, i.e. those formed in the PLD muscle by the ALD nerve, (Table 1). These hybrid junctions were even somewhat smaller than normal ALD end-plates, which were found to measure $82{\cdot}6 \pm 2{\cdot}4\ \mu$ in length on the average (measurements of 230 end-plates from three muscles).

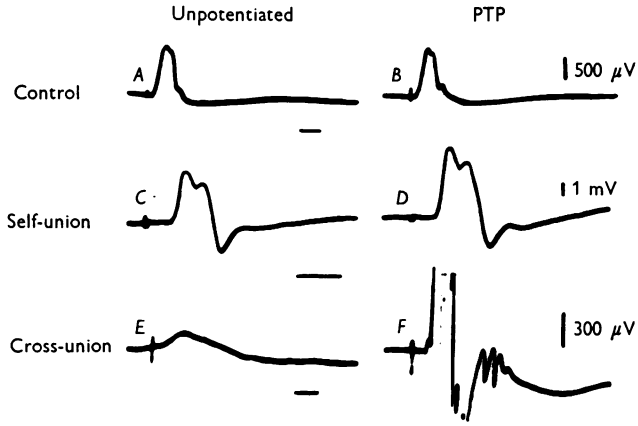
In order to learn whether the ALD nerve fibres also formed multiple endings in the alien territory of the PLD muscle, the number of end-plates was determined in samples of isolated muscle fibre segments. The results results are summarized in Table 1. After nerve cross-union most fibre

TABLE 1. Distribution and size of end-plates in isolated PLD muscle fibres

	No. of muscles	No. of fibres	Mean length of fibre segments (mm)	Distribution of fibres according to number of end-plates (%)					Mean length ± S.E. of end-plates (μ)	No. of measurements
				1	2	3	4	5		
Normal	4	124	19·2	93·5	5·7	0·8	—	—	171·0 ± 2·13	205
Self-union; 3 and 6 months after operation	2	100	16·8	64·0	28·0	6·0	2·0	—	178·8 ± 10·4	146
Cross-union; 5½–10 months after operation	4	185	8·6	69·7	20·5	4·3	3·8	1·6	79·7 ± 1·08	203

segments were supplied with one or two end-plates at a length where eight to ten junctions would occur in the ALD, and only about 10% had three or more end-plates. Genuine multiple innervation with densely distributed endings was only observed on two fibres out of the total of 185 fibres investigated. The small increment of fibres with several end-plates as well as the increase in the number of double end-plates were also found in control muscles re-innervated by the original PLD nerve.

Electromyography. In normal PLD muscles, as well as in control muscles re-innervated by the original nerve, synchronous propagated action potentials of many motor units were recorded in response to a single pulse, and no significant change of the electrical response was observed during post-tetanic potentiation (Text-fig. 1*A–D*).

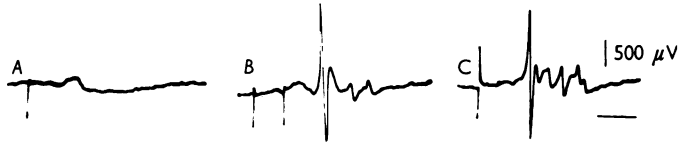


Text-fig. 1. Electromyographic records of responses in the fast PLD muscles to nerve stimulation. Control: in a muscle with normal innervation. Self-union: 4 months after re-innervation by original nerve. Cross-union: 6 months after re-innervation by the slow ALD nerve. *A*, *C* and *E* (unpotentiated): responses to single volleys before, and *B*, *D* and *F* during post-tetanic potentiation (PTP). Time scale attached to each pair of records represents 5 msec.

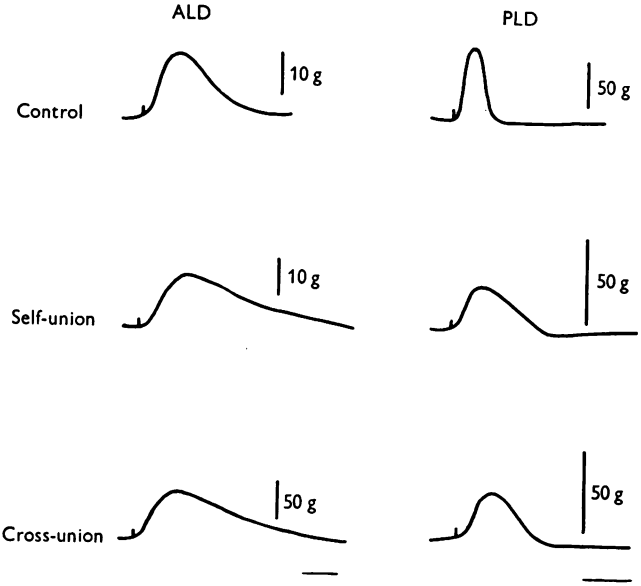
On the other hand, all PLD muscles supplied with the ALD nerve for 5–6 months ($n = 6$) invariably responded with local potentials to nerve stimulation with single pulses, whereas propagated action potentials were only recorded after repetitive stimulation and during post-tetanic potentiation, which lasted for 1–2 min (Text-fig. 1*E*, *F*).

Transplanted PLD muscles ($n = 4$) connected to the ALD nerves responded to nerve stimulation in the same way as the cross-innervated PLD muscles described above. Only local potentials were recorded in response to a single volley (Text-fig. 2*A*). As can be seen in Text-fig. 2*B*, propagated action potentials were already elicited when two impulses in a

fast sequence were used for stimulation, and they were also readily evoked following tetanic stimulation (Text-fig. 2*C*) applied for 10–15 sec. Electromyographic records from transplanted PLD controls re-innervated by the original nerve did not differ from those of normal PLD muscles.



Text-fig. 2. Electromyographic records of responses to nerve stimulation in a fast PLD muscle which had been transplanted and cross-innervated by the ALD nerve six months previously. *A*, Response to a single volley; *B*, response to two volleys at a frequency 250 c/s; *C*, response to a single volley 40 sec after 10 sec tetanic stimulation. Time scale 5 msec.



Text-fig. 3. Single twitch responses of ALD and PLD muscles in control animals and after self- and cross-union. ALD: records taken 7 months and 6½ months after self-union and cross-union of the ALD respectively. Twitch contractions to single volleys in all muscles could only be evoked during post-tetanic potentiation. Time scale for ALD 200 msec. PLD: records taken 7 and 6½ months after self-union and cross-union of the PLD respectively. In case of cross-union to PLD a twitch contraction to single volley could only be evoked during post-tetanic potentiation. Time scale for PLD 50 msec.

Contraction velocity. The contraction of the cross-innervated PLD muscles in response to a single nerve volley, 6–18 months after cross-union, could only be evoked during post-tetanic potentiation. The contraction time was

$31{\cdot}1 \pm 1{\cdot}3$ msec ($n = 4$), i.e. it was somewhat longer than in normal muscles ($22{\cdot}1 \pm 0{\cdot}43$ msec; $n = 9$) and than in muscles re-innervated by the original nerve 7–18 months after self-union ($23{\cdot}5 \pm 3{\cdot}5$ msec; $n = 3$) (Text-fig. 3, PLD). This alteration, however, appears insignificant when compared with the contraction velocity of the normal ALD muscle ($107{\cdot}7 \pm 5{\cdot}6$ msec). There was no progressive tendency to slowing down of PLD muscles after cross-union during the period between 6 and 18 months after the operation.

Ultrastructure of muscle fibres was investigated in three PLD muscles after nerve cross-union and in three normal, contralateral muscles. The typical appearance of fast muscle fibres remained preserved 6–10 months after re-innervation with the ALD nerve. Similarly as in normal PLD, muscle fibres after nerve cross-union contained well-defined myofibrils with prominent M-lines. In both normal and cross-innervated muscles, the sarcoplasmic reticulum consisted of two systems of tubules, arranged to form the characteristic triads, which were found to be regularly distributed on both sides of the Z-line (Pl. 2, figs. 1, 2).

ALD muscles re-innervated by the PLD nerves

Unlike most skeletal muscles in vertebrates, ALD muscles become hypertrophic after denervation (Feng, Jung & Wu, 1962). In our experimental series the hypertrophy caused by transient denervation receded very slowly following nerve cross-union; 5–6 months after the operation the muscles were still heavier by $43{\cdot}2 \pm 9{\cdot}4\,\%$ than the controls ($n = 6$). Even after cutting and re-suturing of the original nerve, muscle weight was increased by $15{\cdot}1 \pm 6{\cdot}6\,\%$ up to 4 months after re-innervation ($n = 4$).

In sections of both cross-innervated and self-innervated muscles a dense distribution of end-plates was found throughout the whole muscle (Pl. 3, figs. 1, 2).

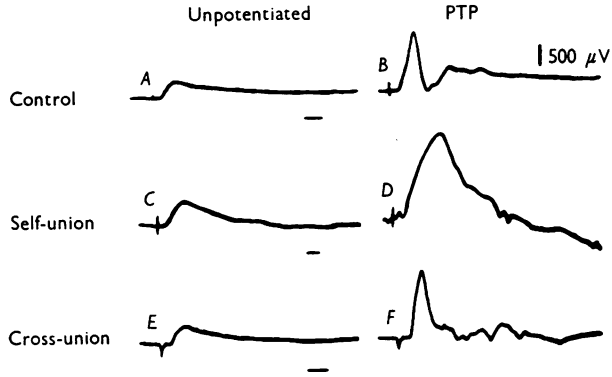
Motor end-plates. Motor end-plates found in the ALD muscle supplied with the 'mixed' PLD nerve were of two types (Pl. 3, figs. 3, 4). The overwhelming majority of the end-plates had the 'en grappe' configuration, while a few 'en plaque' end-plates were occasionally detected in some of the muscles (Pl. 3, fig. 3). In muscles re-innervated with the ALD nerves as in normal muscles, solely multiple 'en grappe' end-plates were found.

Only multiple innervation was found on a sample of 150 muscle fibres teased from three ALD muscles supplied by PLD nerves. The distances between end-plates were somewhat longer than in normal ALD muscles, but this was also the case in ALD muscles re-innervated by the original nerves.

Isolated fibre segments from transplanted muscles were too short to

allow evaluation of the innervation pattern. Extensive fibrous changes following transplantation prevented successful teasing.

Electromyography. In five out of seven cases investigated, the electrical response of cross-innervated ALD was the same as in normal muscles and those re-innervated by the original nerve (Text-fig. 4). Only local potentials were recorded from all the examined regions in response to a single volley applied to the PLD nerve and the amplitude greatly increased during post-tetanic potentiation.



Text-fig. 4. Electromyographic records of responses in slow ALD muscles to nerve stimulation. Control: in muscle with normal innervation. Self-union: 4 months after re-innervation by original nerve. Cross-union: 6 months after re-innervation with the 'mixed' PLD nerve. *A*, *C* and *E* (unpotentiated): responses to single volley before, and *B*, *D* and *F* during post-tetanic potentiation (PTP). Time scale attached to each pair of records represents 5 msec.; voltage scale, 500 μV, applies to all records.

Text-fig. 5. Electromyographic records of responses to nerve stimulation in different parts of a slow ALD muscle 3 months after transplantation and cross-union with the 'mixed' PLD nerve. *A*, Recording from muscle region near the wing; *B*, from the central part of the muscle, *C*, from the region near the spine. Time scale 5 msec.

In two cases, propagated action potentials of a short latency appeared together with local potentials in response to indirect stimulation by single pulses. However, this electrical response could only be recorded in a restricted area near the nerve entry. The major part of the muscle responded to single shocks by local potentials only.

Another picture of the electromyographic response was obtained in the transplanted muscles re-innervated by the PLD nerves. In all four cases investigated, asynchronous propagated action potentials were recorded in response to a single volley in the distal part of the muscle (Text-fig. 5*A*),

whereas only local potentials were registered near the spine (Text-fig. 5*C*). In the central region of the muscle, propagated action potentials were recorded together with local potentials (Text-fig. 5*B*). In control muscles, transplanted and re-innervated with the ALD nerves, only local potentials were found in all the investigated regions.

Contraction velocity. A twitch contraction could be obtained in the cross-innervated ALD muscle solely during post-tetanic potentiation. Contraction time of the ALD after cross-union was 136·7 msec ($n = 2$) and 169·1 msec ($n = 2$) after self-union, 10–11 months after the operations (Text-fig. 3, ALD). This means that the contraction time in both instances was somewhat prolonged, compared with the control ALD muscles ($107·7 \pm 5·68$ msec; $n = 8$).

ALD muscles with implanted anconeus scapularis nerves

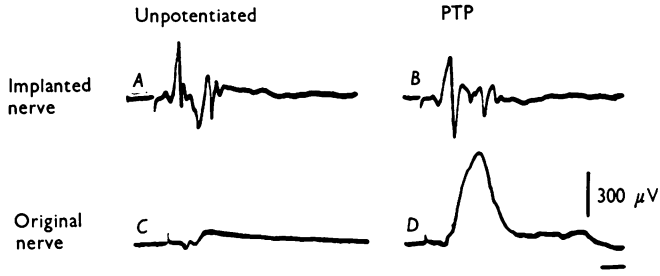
In a group of young chickens the nerve to the focally innervated anconeus scapularis muscle was implanted into the ALD near its distal insertion, where it innervated a restricted area around the site of implantation. However, the original nerve regenerated in all animals of this series. The ALD muscles investigated 3–5 months after the operation were thus supplied by two nerves.

Motor end-plates of the 'en plaque' type (Pl. 3, fig. 5) were found scattered in a narrow zone around the implanted nerve. Their distribution suggested that muscle fibres of this area had focal innervation. On the other hand, the region at the spine supplied with the original nerve was multiply innervated with junctions of the 'en grappe' configuration (Pl. 3, fig. 6). In the central zone both types of the end-plates were observed.

Electromyography. In all instances indirect stimulation of the muscle by single shocks evoked propagated action potentials in the area surrounding the nerve implant, while local potentials were registered in the region near the spine. In the zone of overlapping, local potentials with superimposed spikes appeared in response to a single volley. Text-figure 6 demonstrates the effects of separate stimulation of the anconeus scapularis nerve and of the original nerve. The electromyographic response obtained on stimulation of the implanted nerve consisted of a synchronous propagated action potentials which did not substantially change during post-tetanic potentiation. On the other hand, stimulation of the original nerve trunk elicited local potentials which greatly increased during post-tetanic potentiation.

Ultrastructure was studied in tissue blocks excised from regions next to the implanted nerve. Three ALD muscles were investigated 3–5 months after the operation. The fine structure did not show any significant alteration in comparison with that of normal, contralateral ALD muscles (Pl. 4, figs. 1, 2). In contrast to fast muscles, both normal and cross-

innervated ALD fibres had confluent myofibrils, rather thick Z-lines, indistinct M-lines and a different arrangement of the sarcoplasmic reticulum (Pl. 5), with two types of tubules about 300 and 700 Å, but with rare and irregularly distributed triads.



Text-fig. 6. Electromyographic records of responses to nerve stimulation of the slow ALD muscle of a young chick in which the 'fast' anconeus scapularis nerve was implanted 3 months previously and the original nerve re-innervated a large region of the muscle. Implanted nerve: asynchronous propagated action potentials in response to single volley stimulation of the implanted anconeus scapularis nerve. Original nerve: local potentials in response to stimulation of the original ALD nerve recorded from the region near the spine. *A* and *C* (unpotentiated): responses to single volleys before, and *B* and *D* during post-tetanic potentiation (PTP). Time scale 5 msec.

DISCUSSION

The most conspicuous change brought about in fast and slow chick muscles by the nerve cross-union was the altered character of motor end-plates. However, besides this marked local effect in junctional areas, the nerves did not appear to induce any substantial transformation of the contraction speed and of ultrastructure of muscle fibres.

Motor end-plates. All neuromuscular junctions which had differentiated in the PLD muscles after nerve cross-union invariably were of the 'slow' type, i.e. they had an 'en grappe' configuration, weak ChE activity and a reduced efficacy of impulse transmission. This seems to indicate that the ALD nerve terminals retained their properties in the alien territory of the PLD muscle and that, under their influence, the characteristics of the post-synaptic part of the new junction became altered.

Our results concerning the reduced efficacy of the 'hybrid' junctions confirm the previous observation of Feng *et al.* (1965). According to our findings, the cross-united PLD never responded by propagated action potentials and contraction to nerve stimulation at low frequencies, except during post-tetanic potentiation. Since there is no doubt that post-tetanic potentiation is a purely pre synaptic event, it seems justifiable to assume that the reduced efficacy of the hybrid junctions is due to the properties

of the ALD nerve terminals, and that it is presumably the low amount of transmitter released by the endings which prevents generation of propagated action potentials to a single volley. The degree of ChE activity at the end-plate was closely related to the type of motor endings; end-plate formed by ALD terminals had always weak activity. It is a matter of conjecture whether the presumed difference in the release of acetylcholine from 'fast' and 'slow' endings might have an effect on the diverse enzymatic equipment of the sub-synaptic membrane. In this connexion it is of interest that after vago-phrenic suture in sheep and goats, end-plates formed in the diaphragm by preganglionic fibres also had much weaker ChE activity than the normal ones (Dussardier, 1960; Couteaux, 1963). This again points to a close dependence of subneural cholinesterase on the type of nerve terminals.

As far as the 'en grappe' configuration of hybrid PLD junctions is concerned, it should be noted that end-plates of a similar appearance, though with higher ChE activity, are frequently formed in mammalian muscles by the implanted 'twitch' nerve (Koenig, 1963; Fex, Sonesson, Thesleff & Zelená, 1965; Gutmann & Hanzlíková, 1967); it seems therefore that the 'en grappe' configuration in itself, without a functional correlate, would not be a reliable criterion of the 'slow' character of the junction.

Although the average length of fibre segments from cross-innervated PLD was relatively short and not strictly comparable with those of control muscles, nevertheless it is possible to say that true multiple innervation was rare in the cross-innervated PLD. It remains a matter for further investigation to disclose which factors contributed to the resulting innervation patterns; whether the nerve fibres did not ramify sufficiently in a foreign territory, or whether the majority of muscle fibres did not accept additional end-plates.

In the experiments where the ALD muscle was supplied with fast muscle nerves, attempts to attain re-innervation solely with 'fast' nerve fibres were unsuccessful. In spite of this, one result stands out as certain: the nerves to fast muscles were capable of inducing, on the slow muscle fibres, neuromuscular junctions of the 'fast' type, i.e. those which generated propagated action potentials and had an 'en plaque' configuration with strong ChE activity. It is, however, less clear which factors favoured formation of such end-plates, and whether they differentiated in all instances, when a 'fast' nerve fibre made contact with the membrane of a slow muscle fibre.

In the case of the ALD muscles in young chickens, end-plates of the 'fast type' were always induced by the 'fast' anconeus scapularis nerve. However, following cross-innervation by the 'mixed' PLD nerve the overwhelming majority of the end-plates were of the 'slow' type. Accord-

ing to the experiments of Feng *et al.* (1965), selective re-innervation by the appropriate motor supply occurs when both ALD and PLD nerve trunks are given an equal chance to re-innervate the muscles. By analogy, our finding could be interpreted as a result of prevalence in re-innervation of the 'slow' component of the PLD nerve. It may be enquired, however, whether the relatively small number of 'slow' nerve fibres in the PLD trunk could account for the extensive re-innervation of the whole muscle after nerve cross-union. The same 'mixed' PLD nerve regularly formed junctions of the 'fast' type in transplanted ALD muscles, probably in regions which became transiently ischaemic after the operation and presumably underwent degenerative changes and subsequent regeneration. This speaks in favour of the assumption that 'fast' nerve fibres formed end-plates of the 'fast' type, if the membrane of the ALD muscles was plastic enough to respond to their induction, e.g. following injury or in young age, while in adult chick the induced end-plate differentiation might have been limited by the inherent properties of the slow muscle fibre membrane.

Ultrastructure and contraction velocity. Evidently, no alterations occur in the fine structure of extrajunctional regions of muscle fibres supplied by hybrid nerve terminals. The characteristic features of the two types of muscle fibres (Page & Slater, 1965) remained unaffected by alien innervation. This finding is in accord with the observation of Miledi & Orkand (1966), who have reported that ultrastructure of slow muscle fibres remained unaltered in a transplanted frog muscle connected to a 'twitch' nerve for more than 1 yr; however, no direct evidence was given about innervation of the fibres investigated. In our experiments, no data have been obtained, as yet, on the ultrastructure of hybrid end-plates.

Like the ultrastructure, the speed of contraction of the ALD and PLD remained practically unaltered after nerve cross-union. The slowing observed in the PLD muscle represented only about 10% of the difference between PLD and ALD mean contraction times, which is negligible compared with the effects reported in mammals (Buller *et al.* 1960; Buller & Lewis, 1965; Close, 1965). Moreover, part of the slowing could be due to the dispersion of action potentials resulting from the uneven conduction velocity in regenerated nerve fibres.

In the case of the ALD, no conclusions can be drawn from the fact that the contraction velocity was not speeded up after nerve cross-union, since the muscles investigated were supplied with a mixed population of motor nerve fibres and had mainly multiple innervation.

It appears that the possibilities of transforming fast muscles into slow by changing their innervation and vice versa are very limited in the adult chick. It is a matter for future experiments to disclose whether the muscles

are more liable to submit to nervous influences at an early age, and to what extent innervation contributes to the differentiation of the two muscles types during development.

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EXPLANATION OF PLATES

PLATE 1

Fig. 1. Regenerated end-plates in the PLD muscle re-innervated with the original nerve, 3 months after the operation.

Fig. 2. Hybrid end-plates in the PLD muscle re-innervated with the ALD nerve, 5 months after nerve cross-union.

Fig. 3. 'En plaque' end-plates in normal PLD muscle.

Fig. 4. 'En grappe' end-plates in the PLD muscle re-innervated for 10 months with the ALD nerve.

Figs. 5 and 7. Normal PLD end-plates.

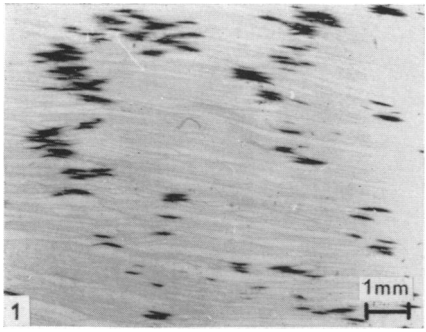
Fig. 6. A regenerated PLD end-plate 8 months following re-innervation with the PLD nerve.

Fig. 8. A hybrid 'en grappe' end-plate in the PLD muscle re-innervated with the ALD nerve, 10 months after nerve cross-union.

Longitudinal frozen sections about 50 μ thick, stained for cholinesterase. Figs. 1 and 2: incubation overnight. Figs. 3–8: incubation time about 1 hr. Scale applies to each pair of figures.

PLATE 2

Fig. 1. Longitudinal section through a normal PLD muscle fibre.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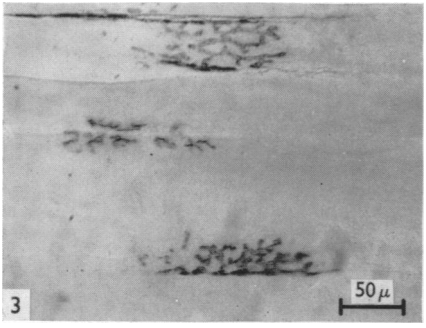
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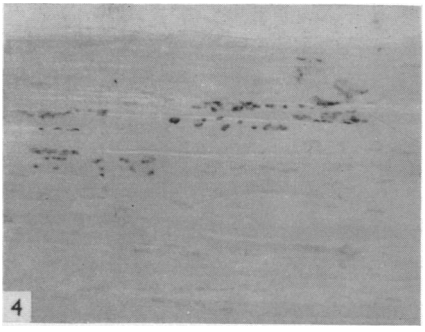
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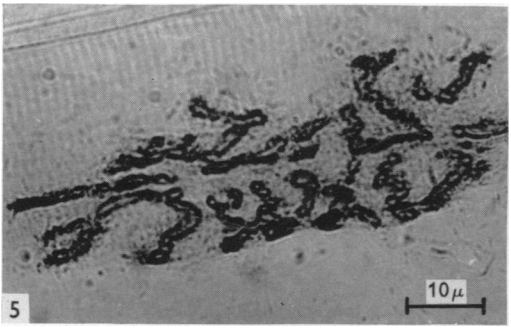
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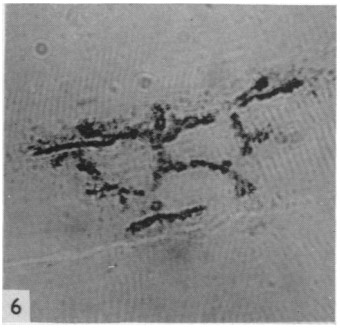
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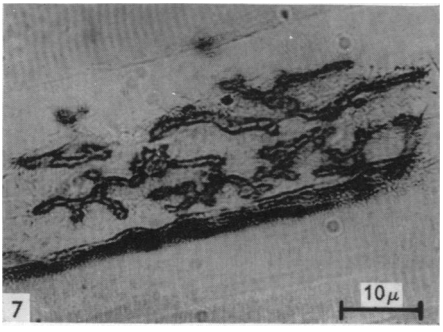
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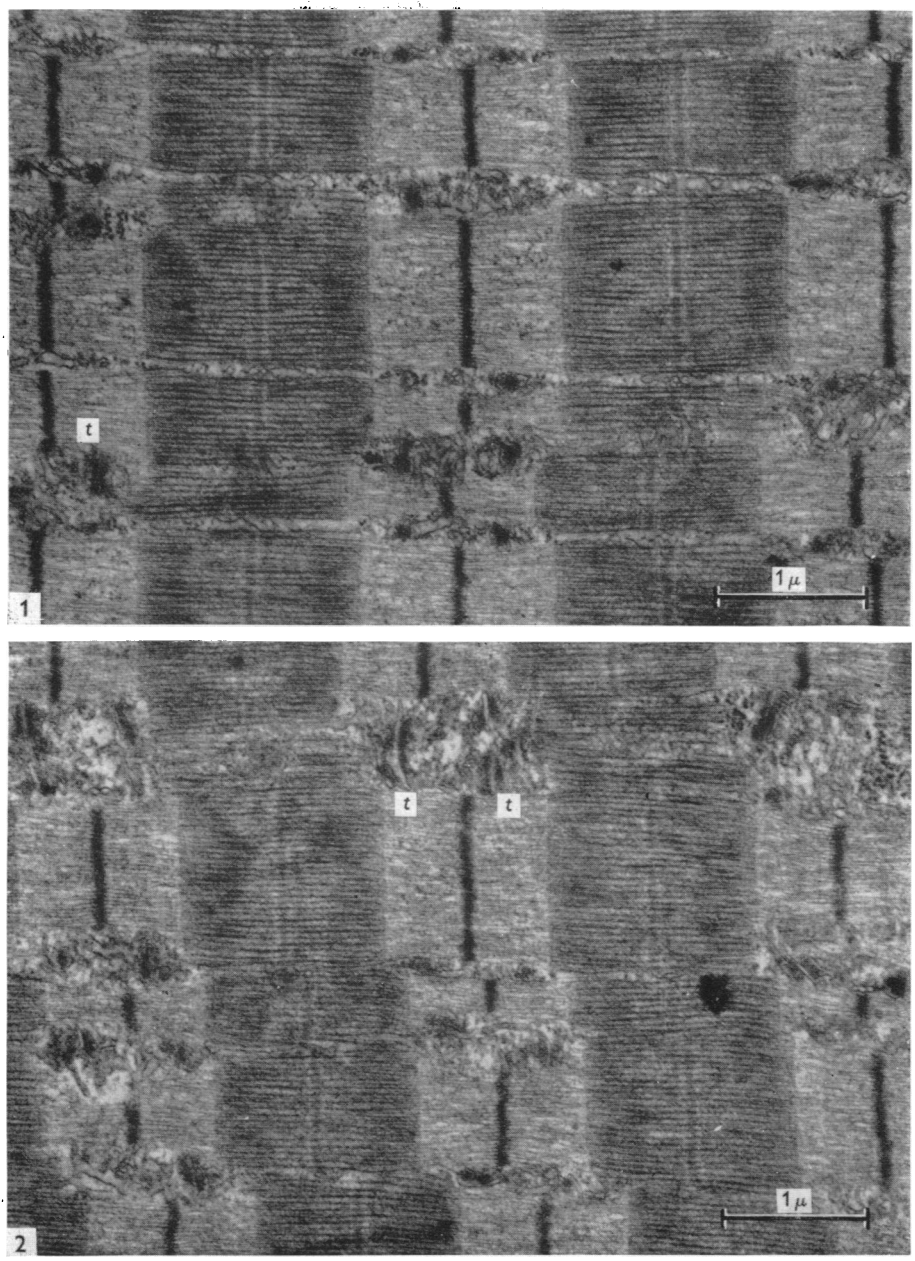
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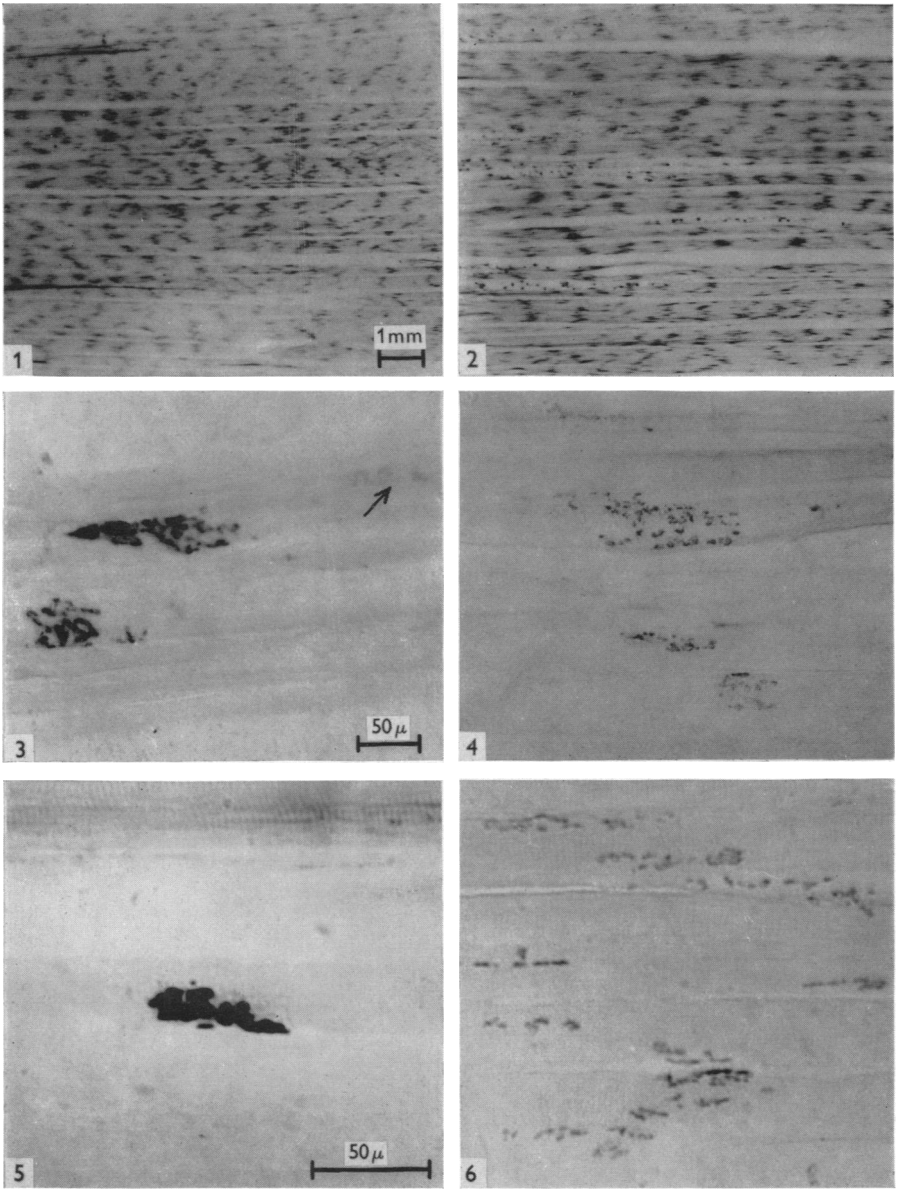
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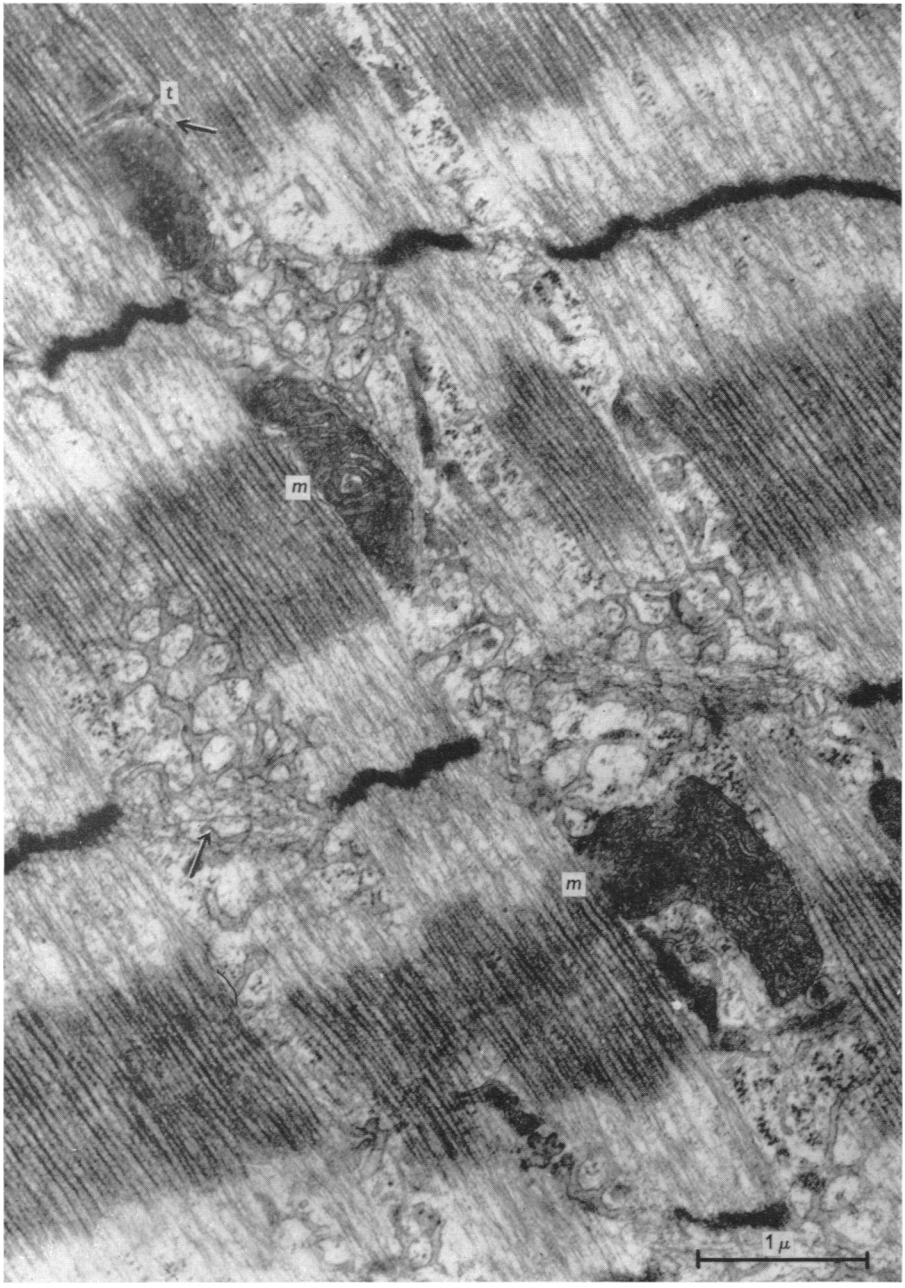

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Fig. 2. Longitudinal section through a muscle fibre of a PLD muscle re-innervated with the ALD nerve for 6 months. Triads (*t*) seen on both sides of the Z-line. No alteration is seen in the fine structure in comparison with the normal PLD muscle fibre.

Glutaraldehyde and OsO_4 fixation, uranylacetate and lead stain. Scale applies to both figures.

PLATE 3

Fig. 1. Multiple end-plates in the ALD muscle re-innervated with the original nerve, $2\frac{1}{2}$ months after the operation.

Fig. 2. Multiple end-plates in the ALD muscle re-innervated with the PLD nerve 5 months after nerve cross-union.

Fig. 3. Hybrid 'en plaque' end-plates in the ALD muscle re-innervated with the PLD nerve; an 'en grappe' end-plate at the arrow. Ten months after nerve cross-union.

Fig. 4. Typical 'en grappe' end-plates in the ALD muscle 10 months after nerve cross-union. Same section as in Fig. 3.

Fig. 5. A hybrid 'en plaque' end-plate formed by the implanted anconeus scapularis nerve, 3 months after the operation.

Fig. 6. Multiple 'en grappe' end-plates in the part of the ALD muscle re-innervated with the original nerve. Same section as in Fig. 5.

Longitudinal frozen sections about 50 μ thick stained for cholinesterase. Figs. 1 and 2: incubation overnight. Figs. 3–6: incubation time 90 min. Scale applies to each pair of figures.

PLATE 4

Longitudinal section through a slow muscle fibre from the normal ALD muscle, showing the arrangement of the sarcoplasmic reticulum, with a typical lacey network of wide tubules; thin tubules at the arrow; *m*, mitochondria; *t*, triad. Glutaraldehyde and OsO_4 fixation, uranylacetate and lead stain.

PLATE 5

Longitudinal section through an ALD muscle fibre, near the implanted anconeus scapularis nerve, 3 months after the operation. Network of wide tubules; thin tubules at the arrows. No triads are to be seen in the figure. Glutaraldehyde and OsO_4 fixation, uranylacetate and lead stain.