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The formation of new motor endplates in mammalian skeletal muscle

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The ability of a denervated muscle to accept an implanted nerve or another nerve other than its own along existing pathways has frequently been reported (Gutmann & Young, 1944; Bowden & Gutmann, 1944; Aitken, 1950; Hoffman, 1951; Csillik & Savay, 1959; Guth & Zalewski, 1963).

Miledi (1962) showed that new motor endplates could be induced to develop in a part of the frog sartorius muscle which was originally free of endplates. Koenig (1963) has recently shown that new motor endplates may be formed in the sternal endplate-free zone of the pectoralis major muscle of the rat. In all these experiments a varying amount of mechanical damage was occasioned to the muscle fibres, and Miledi suggests that injury may be a significant factor in the induction of the new motor endplates. An important point still to be decided was whether new motor endplates would form in mammalian skeletal muscle which had not been mechanically injured.

Guth & Zalewski (1963) implanted the hypoglossal nerve into the devervated sternomastoid muscle in the rat and clearly demonstrated two endplates on many of the muscle fibres, separated by microdissection after cholinesterase staining. They implied that the original ending was degenerate and that the new ending was functional.

We were interested in the stages in the production and maturation of new motor endplates and have attempted to clarify a number of points arising from the above work by answering the following questions:

(1) Are new motor endplates formed in a portion of mammalian skeletal muscle which has had the original motor endplate zone removed and does this prevent atrophy of the muscle? (Series I.)

(2) Are the new motor endplates restricted to the immediate vicinity of the implanted nerve? (Series I.)

(3) Will new ectopic endings form after implantation of a muscle nerve into an otherwise intact but denervated mammalian muscle and will the original endings be re-innervated? (Series II.)

(4) Will regenerating nerves grow into a denervated but otherwise undamaged muscle from its surface and form new motor endplates? (Series III).

MATERIALS AND METHODS

The animals employed were adult albino rats weighing 200–250 g. The muscle chosen for the experiments was tibialis anterior and the common peroneal nerve was used as the implant. Serial sections of a number of normal tibialis anterior muscles

were cut in coronal and sagittal planes. These control sections were stained by a Coërs & Woolf modification (1958) of the Koelle & Friedenwald method (1949) for demonstrating cholinesterase. This enabled the normal distribution of original motor endplates in the tibialis anterior muscle to be mapped.

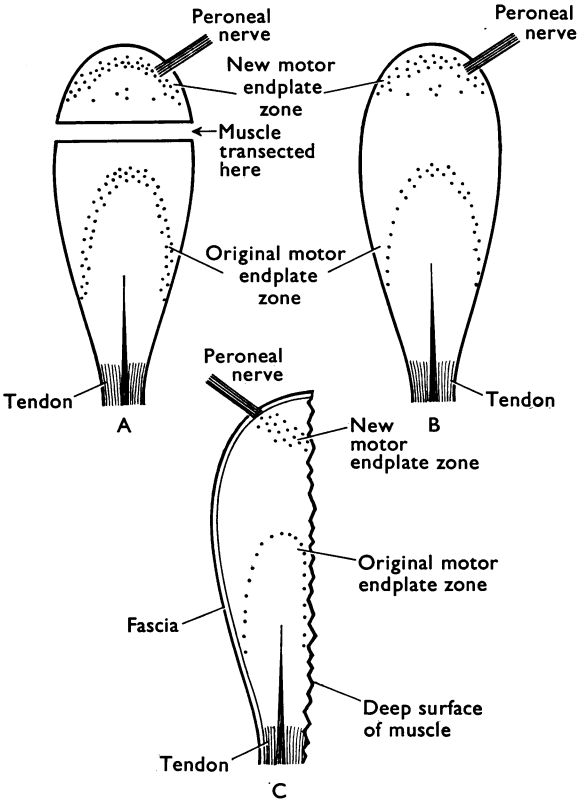


Fig. 1. A. Diagrammatic coronal section of tibialis anterior muscle showing new and original motor endplate zones and also the position in which the muscle is transected (series I). B. Diagrammatic coronal section of tibialis anterior muscle showing new and original motor endplate zones and position in which the common peroneal nerve is implanted in the muscle (series II). C. Diagrammatic sagittal section of tibialis anterior muscle showing new and original motor endplate zones and also the position in which the common peroneal nerve is placed on the surface of the proximal region of the muscle (series III).

Three series of experiments were performed. In the first series a portion of skeletal muscle, free of motor endplates was innervated. In the second series the nerve was implanted into denervated muscle retaining its original motor endplates. While in the third series the nerve was laid on the surface of the muscle with a minimum of injury to the muscle fibres.

Operative techniques

Series I. The left tibialis anterior muscle and common peroneal nerve were exposed in thirty-three animals. The nerve was carefully dissected and severed

distally as close as possible to the lateral border of extensor digitorum longus. The tibialis anterior muscle was transected about 4 mm from its proximal end and the lower three-quarters of the muscle, containing all the original motor endplates, was removed and fixed for future sectioning and staining by the cholinesterase method (see Fig. 1A). The peroneal nerve was carefully mobilized and inserted into a small incision which was made in the lateral side of the remaining upper portion of tibialis anterior. The nerve was held in place by the use of clotted human fibrin. The right tibialis anterior muscle, not operated upon, was used as a control in each case.

Owing to the difficulty experienced in retaining the implanted nerve in a small piece of muscle, the operation was altered slightly in the later forty-four animals. The peroneal nerve was first implanted into the lateral side of the denervated but otherwise intact tibialis anterior muscle and after a delay of between 60 and 100 days the animals were subjected to a second operation. The peroneal nerve was exposed and stimulated electrically. If the muscle contracted, the lower three-quarters of the muscle containing the original endplate zone was removed and placed in the fixative solution. The skin wound was then re-sutured. Biopsy was performed at times varying between 40 and 480 days after implantation of the nerve and the animals were then killed.

At each biopsy the following particulars were noted:

(*a*) The state of the implant, i.e. whether or not the nerve still remained in contact with the muscle.

(*b*) The state of the muscle, i.e. whether any marked atrophy had occurred. In most cases, the presence of fibrillation was noted.

(*c*) The response of the muscle to electrical stimulation of the nerve. The nerve was carefully dissected free of fibrous tissue. It was then stimulated approximately 1 cm from the muscle using 5 square wave pulses per second, each about 2 V and of 0·4 msec duration. The presence or absence of a muscle response was noted. Specimens in which the response was due to surface spread of the impulse were discarded from the series.

The small remaining portion of the tibialis anterior muscle was carefully dissected out, taking care not to dislodge the implanted nerve. Both the proximal edge of this piece of muscle and the implanted nerve were marked with Indian ink for reference and orientation. The specimen was then fixed in 10% formal saline and stained as indicated below.

Series II. In eleven rats the tibialis anterior muscle and the common peroneal nerve were exposed on the left side as in series I and the nerve was implanted into the upper endplate-free region of the muscle (see Fig. 1B). No portion of the muscle was removed at operation in this series. The unoperated corresponding muscle on the right side was employed as a control. At times varying between 100 and 480 days after operation, biopsy was performed and the animals were killed. At biopsy the same facts were ascertained as in series I and the whole muscle and distal portion of the nerve were removed and fixed.

Series III. In eight rats the tibialis anterior muscle and the common peroneal nerve were again exposed. Care was taken not to injure the muscle in any way. The nerve was severed distally and placed on the exposed surface of the muscle at its upper end, having first carefully incised the layer of fascia which covered the superficial

surface of the muscle. The nerve was held in place with a small amount of clotted fibrin. At times varying between 35 and 150 days the animals were biopsied as in the previous series of experiments.

Histological techniques

(1) Cholinesterase stain alone for the subneural apparatus of the motor endplates.

(2) Cholinesterase stain, counterstained by the Bielschowsky–Gros method for nerve fibres.

(3) Bielschowsky–Gros stain alone.

Following biopsy the muscle was placed in 10% formal saline for 6 h at room temperature. After washing in distilled water overnight frozen sections (50 μ thick) were cut. Most sections in series III were cut in a plane at right angles to the surface of the muscle. This enabled new motor endplates close to the anterior surface of the muscle to be seen in early regenerations and estimations to be made of the spread of the nerves through the muscle. Some of the sections were stored in 10% neutral formalin and 2% pyridine for periods up to 1 month, others were stained for cholinesterase.

(1) The Coërs & Woolf modification (1958) of the Koelle & Friedenwald stain for cholinesterase was used. The optimum incubation time was 1 h at 37 °C using acetylthiocholine iodide as the substrate. This method revealed the subneural apparatus as a localized brown deposit of copper sulphide. Some sections in each series were incubated with 10^{-3}M solution of eserine added to the substrate. The non-appearance of the brown deposit at the sites of the motor endplates indicated that specific cholinesterase was involved in the production of the copper sulphide deposits.

(2) Some sections from each muscle were stained by the above method and then placed in neutral formal pyridine in saline for periods up to 4 weeks. They were then counterstained by a modified Bielschowsky–Gros method (Gwyn & Heardman, 1965). The modifications involved were: (*a*) incubate in the cholinesterase substrate for only 15 min; (*b*) place in 10% neutral formalin and 2% pyridine in saline for at least 2 weeks.

(3) Some unstained sections from each muscle were stained by the usual Bielschowsky–Gros method.

RESULTS

Serial sections of the normal tibialis anterior muscle in both the coronal and sagittal planes showed that the original motor endplates were located unfailingly in a cup-shaped zone (Figs. 2, 20).

Tibialis anterior muscle is a bipennate muscle and has a central tendon to which most of the muscle fibres are attached. It was found that the motor endplates were situated in each case at approximately the mid-point of the individual muscle fibres. From these sections it was seen that the upper 4–5 mm of the muscle was completely free of original motor endplates.

Successful implants were made in forty-two animals out of the seventy-seven in series I, ten animals out of the eleven in series II, and seven animals out of the eight in series III.

Series I. Implants into transected muscles

In animals in which the nerve made a functional connexion with the muscle, some surface muscle fibrillation but little muscular atrophy were seen. A positive response to stimulation of the nerve was obtained after 40 days. At this time the contraction was localized to the fibres in the immediate vicinity of the implant. In animals in which re-innervation had progressed for 100 days or more, the muscular contraction following electrical stimulation of the nerve was more wide-spread.

Staining of the lower three-quarters of the muscle by the cholinesterase method revealed whether or not the whole zone of original motor endplates had been removed. These lower portions of muscle could be referred to their appropriate upper quarter after staining. By this means it was possible to state that the motor endplates in the upper quarter of the muscle were ectopic and all new.

Cholinesterase staining of the sections of tibialis anterior from series I revealed deposits of copper sulphide in the proximal quarter of the muscle in specimens which gave a muscular contraction on stimulation of the nerve. These deposits varied in position, number and configuration depending on the time between implanting the nerve and biopsy.

40 *and* 60 *days.* Localized deposits of copper sulphide were seen on the muscle fibres in the region of the implant. These did not resemble intact motor endplates in appearance (compare Figs. 3 and 30). Some deposits were diffuse and granular, while others consisted of groups of rings varying in size situated on the surface of the muscle fibres. These rings were approximately 2 μ (1·0–2·5 μ) in diameter. Some endings covered most of the width of the muscle fibre, while others were a line of rings or dots along the length of a muscle fibre (Figs. 31, 32).

Since the muscle fibres responded to indirect stimulation by contraction it was assumed at this stage that many of these deposits represented early stages in the formation of motor endplates. This was later proved to be so beyond reasonable doubt by the use of the silver counter-stain. Nerve fibres were seen entering the deposits.

120 *and* 180 *days.* Many more new motor endplates had now formed and were located mainly near the proximal end of the muscle, stretching from the medial to the lateral side in a band (Fig. 6). Most were composed of small rings of deposit as in the early endplates. These endplates did not closely resemble the form of the normal motor endplates (compare Fig. 3 and Figs. 33 and 34).

240 *days.* There did not appear to be any great change in the number of motor endplates nor in their position when compared with similar sections from muscles into which the nerve had been implanted 180 days previously. However, great variation in the form which these new motor endplates presented had occurred (Figs. 7, 8).

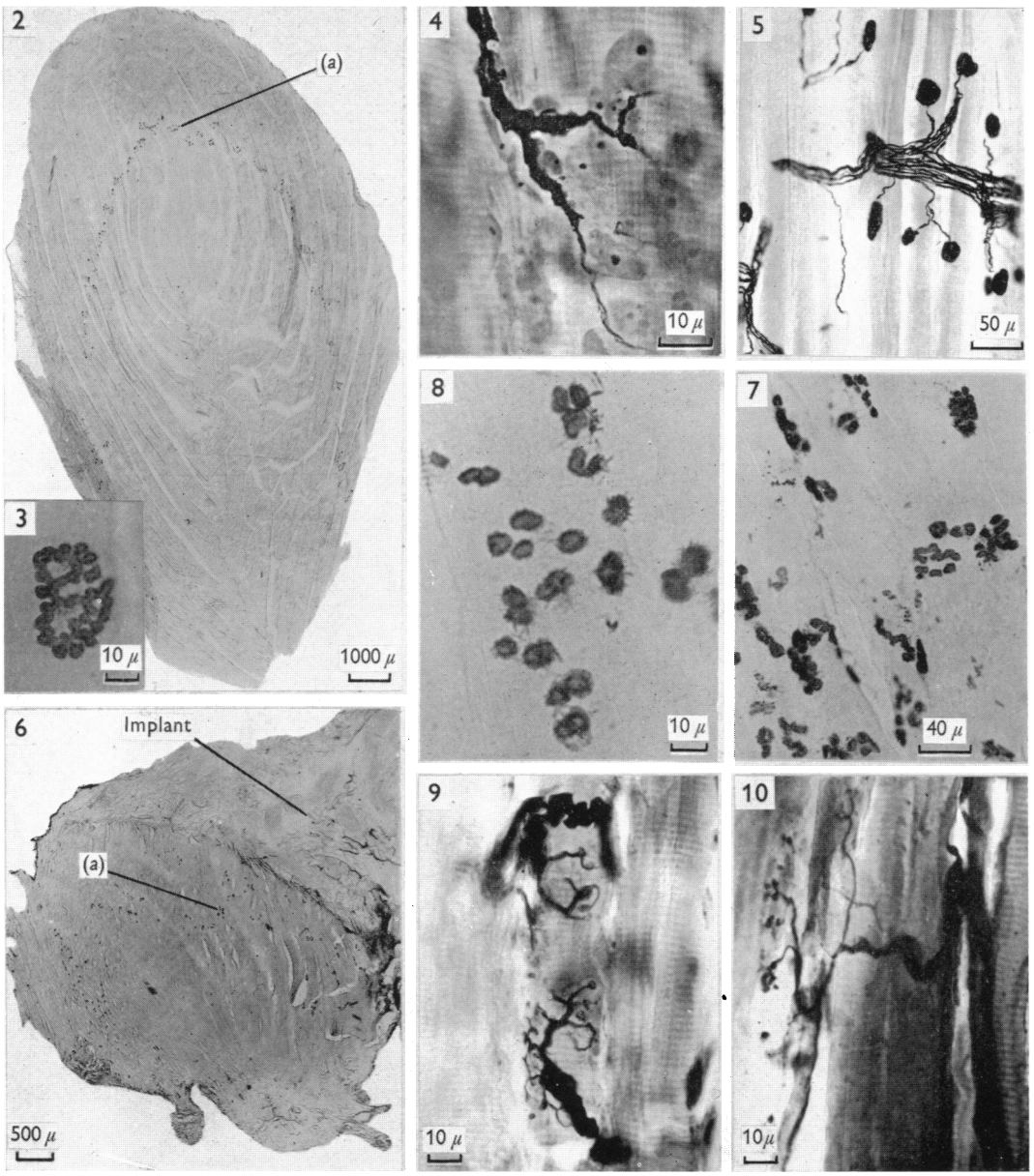
(*a*) Collections of rings stretching in a linear arrangement for up to 100 μ along the muscle fibre were seen (Fig. 35).

(*b*) In other endings the rings were gathered together and gave an overall rounded or disc-like appearance.

(*c*) Some motor endplates seemed to have a primitive gutter arrangement not unlike that found in normal motor endplates. Many of the rings making up the new endplates were incomplete.

In specimens stained by the silver method some of the nerve fibres forming the claws ended in bulbous expansions (Figs. 9, 10). A few endings consisted of a single fine nerve fibre with an accumulation of muscle nuclei alongside it.

480 *days*. The new motor endplates were more widely distributed throughout the muscle than in earlier specimens. However, the majority were still collected in a band towards the proximal margin. The individual rings of deposit making up the endings were well in evidence, but gutters were now seen, probably because some of



the rings of deposit had coalesced. Individual motor endplates had increased in size in these longer term implants but it was still difficult to say whether two adjacent collections of deposit were two endings or one ending. Owing to the thickness of the sections it was not possible to determine the ratio of the number of endplates to the number of muscle fibres. Counterstained sections showed typical new nerve fibres approaching and entering the deposits. Unfortunately the double stain often masked the fine structure of the terminal nerve branches and their associated nuclei. Silver staining alone, however, showed the terminal nerve fibres ending in claws, and accumulations of endplate nuclei in association with these fibres. A few ultraterminal nerve fibres and endings were seen.

On counts of fifty new motor endplates at 480 days after implantation, the number of terminal nerve branches in an endplate varied between 2 and 12 and the number of endplate nuclei varied between 3 and 26. These compared with counts on fifty normal motor endplates of between 2 and 8 branches and between 8 and 28 endplate nuclei.

A number of fine sinuous nerve fibres were seen passing between muscle fibre bundles and having no connexion with the motor endplates; these were presumably sensory or autonomic nerve fibres.

Series II. Implants into intact but denervated muscle

Specimens in this series were biopsied and examined histologically between 100 and 480 days.

The original endplate zone could still be identified in cholinesterase preparations (Fig. 11). However, there was a noticeable reduction in the number of these endplates, especially in the longer term specimens. Also some incompletely stained

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EXPLANATION OF FIGURES

Figs. 2–10 illustrate Series I.
Figs. 11–19 illustrate Series II.
Figs. 20–29 illustrate Series III.
Figs. 30–37 illustrate some stages in the maturation of motor endplates.

The sections were stained as follows:
Cholinesterase stain: Figs. 2, 3, 6, 7, 8, 11, 12, 14, 20–24, 29–37.
Bielschowsky–Gros stain: Figs. 4, 9, 10, 13, 19, 26, 27.
Cholinesterase–Bielschowsky stain: Figs. 5, 15, 16, 17, 18, 25, 28.

Fig. 2. Coronal section of normal tibialis anterior muscle showing zone of original motor endplates (a).

Fig. 3. Normal motor endplate from the same section as Fig. 2.

Fig. 4. Normal motor endplate showing terminal nerve fibres and endplate nuclei.

Fig. 5. Normal motor endplates and associated nerve fibres.

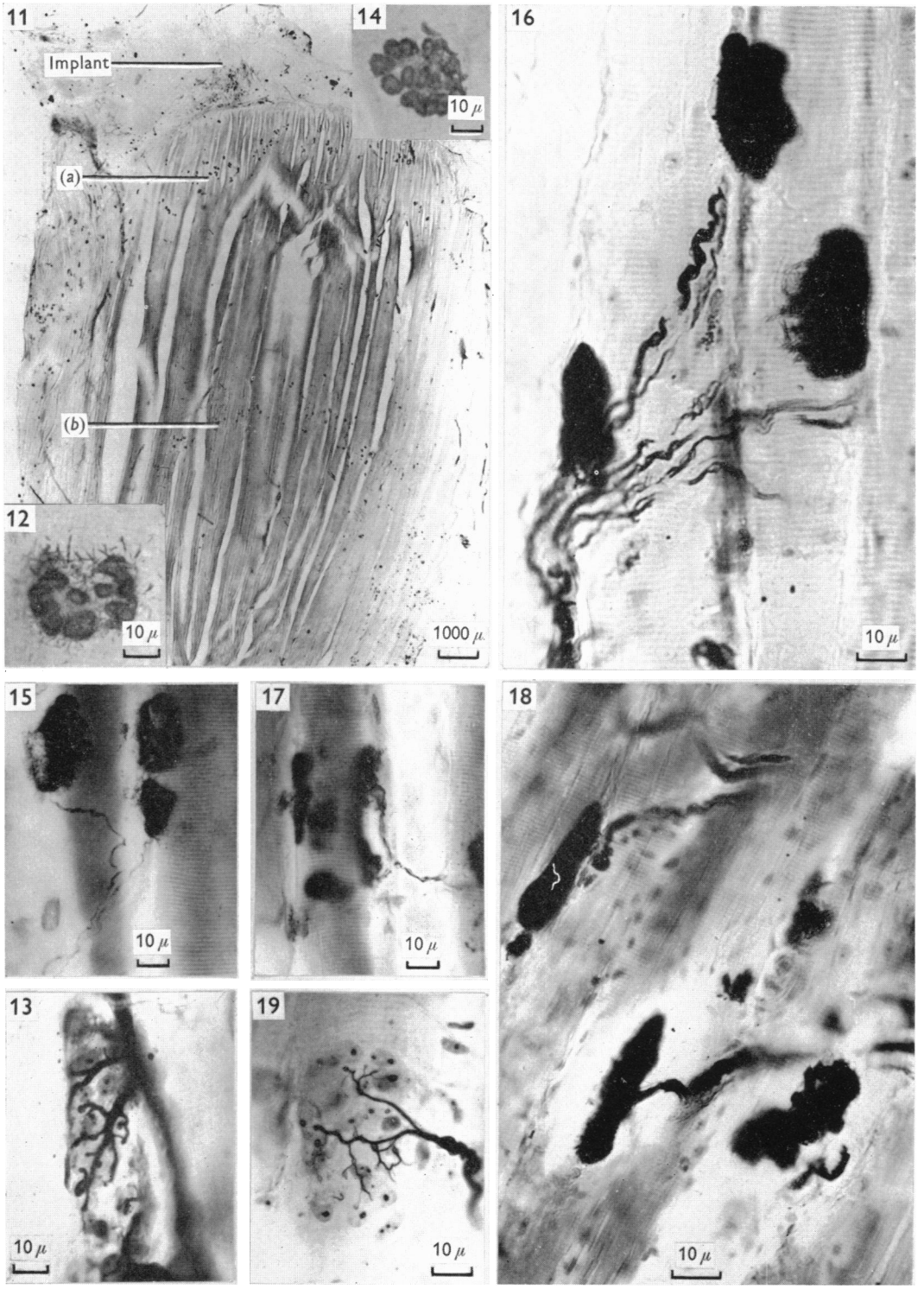
Fig. 6. Upper region of tibialis anterior muscle, normally endplate-free, showing new motor endplates (a) formed 170 days after implantation of the nerve.

Fig. 7. New motor endplates, 218 days after implantation of the nerve into the proximal endplate-free portion of the muscle.

Fig. 8. New motor endplates from the same section as Fig. 7.

Fig. 9. New motor endplates seen in proximal portion of muscle, 218 days after implantation of the nerve.

Fig. 10. New motor endplates, 218 days after implantation of the nerve, note the *en grappe* endings.


11
Implant
(a)
(b)
14
10 μ
12
10 μ
1000 μ
16
10 μ
15
10 μ
17
10 μ
18
10 μ
13
10 μ
19
10 μ

motor endplates were seen towards the edge of the sections, possibly in the final stages of disintegration.

The re-innervated original motor endplates seen following cholinesterase staining did not closely resemble intact original motor endplates even after long periods of re-innervation of up to 480 days. Most consisted of a collection of tightly packed rings of deposit (Fig. 12), and were distinguishable from the ectopic early new motor endplates in which the rings were usually less uniformly grouped together. The re-innervated original endplates retained their individual rounded or disc form. The new motor endplates showed a diversity in form from a rounded collection of rings to a linear collection of rings stretching along the muscle fibre (compare Fig. 14 with Fig. 24). The regular arrangement and position of the original endplates ensured that they were not confused with the newly formed endings. Rarely was an ending seen in the original endplate zone which closely resembled one of the new ectopic endings.

In the proximal quarter of the same muscle section, the zone usually free of endplates, many new motor endplates had formed. These stretched from the region of the implanted nerve right across the section (Fig. 11). They were mainly located towards the proximal edge of the muscle section.

This zone of ectopic new motor endplates lay parallel to the upper limit of the original motor endplate zone, although separated from it by a distance of at least 2 mm. These new motor endplates, as seen in the cholinesterase preparations, had a similar distribution to the new motor endplates in the upper end of the transected muscles in series I. They also had similar ring-like constitution and variability in size. The more mature new endplates (480 days) were little changed in form from the new motor endplates examined at 218 days in this series (Fig. 14). A silver preparation of a re-innervated original endplate is shown in Fig. 13. The thick terminal nerve branches are to be contrasted with the fine branches found in the new endplates (Fig. 19). Counterstaining the cholinesterase preparations with silver showed that the regenerated nerve fibres had grown into close relation with the copper sulphide deposits both in the original and new zones, indicating innervation of the motor endplates (Fig. 15–18).

Fig. 11. Coronal section of tibialis anterior muscle showing new (a) and original (b) motor endplate zones, 218 days after implantation of the nerve.

Fig. 12. A re-innervated original motor endplate from the same section as Fig. 11.

Fig. 13. Re-innervated original motor endplate from a section of muscle 366 days after implantation of the nerve.

Fig. 14. A new motor endplate from the same section as Fig. 11.

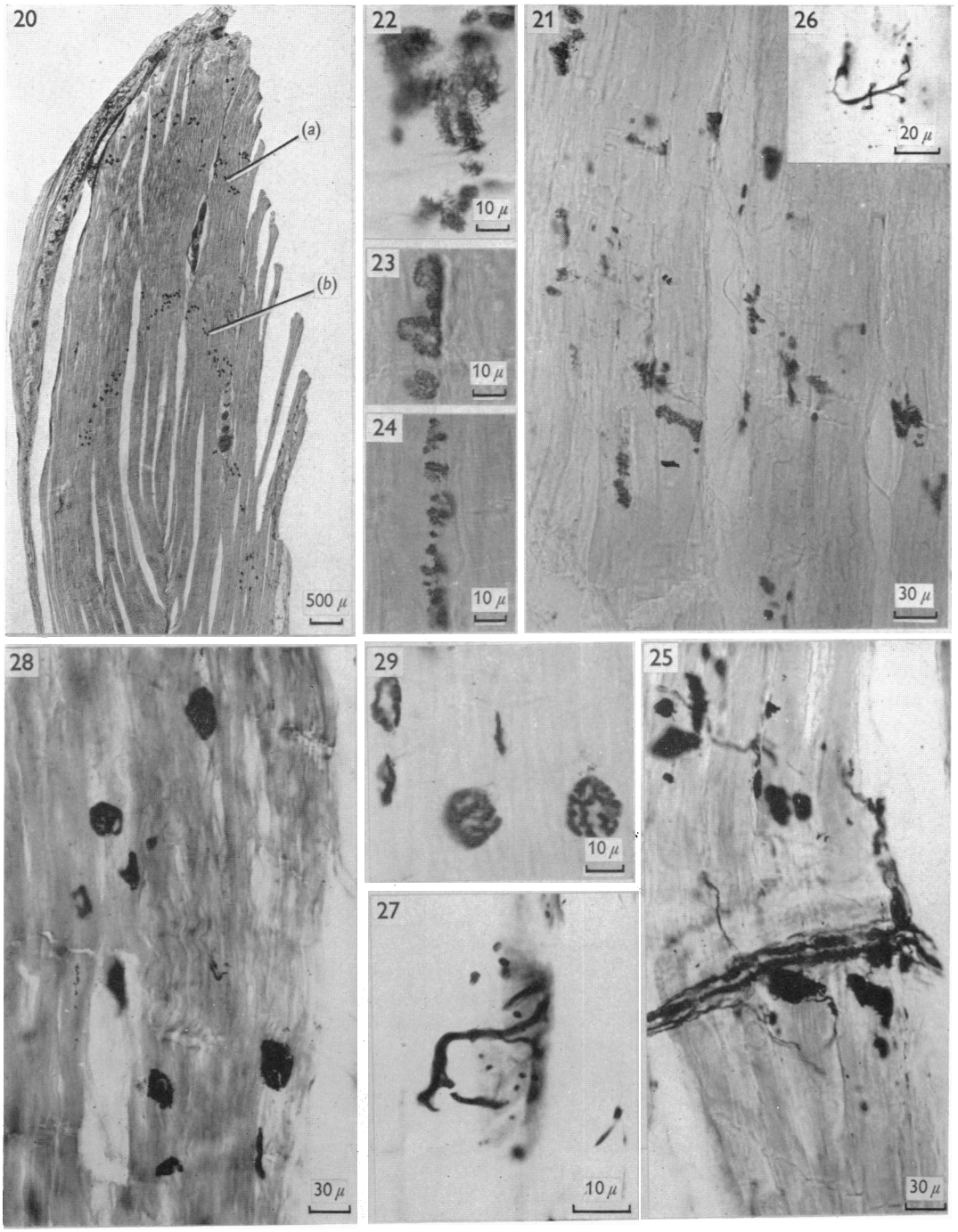
Fig. 15. Re-innervated original motor endplates and associated nerve fibres from a section of the muscle 218 days after implantation of the nerve.

Fig. 16. Re-innervated original motor endplates and associated nerve fibres from a section of the muscle 372 days after implantation of the nerve.

Fig. 17. New motor endplate and associated nerve fibre from a section of muscle 218 days after implantation of the nerve.

Fig. 18. New motor endplates and associated nerve fibres from a section of muscle 372 days after implantation of the nerve.

Fig. 19. New motor endplate from a section of muscle 393 days after implantation of the nerve.


20
(a)
(b)
500 μ
22
10 μ
23
10 μ
24
10 μ
21
26
20 μ
30 μ
28
30 μ
29
10 μ
27
10 μ
25
30 μ

100 *days*. Sections of the muscle stained by the cholinesterase method showed a few new motor endplates near the proximal edge of the muscle. These were particularly collected near the region of the implants and showed the early ring formation characteristic of new motor endplates. The original motor endplates were also seen but appeared somewhat degenerate, being lightly stained, fragmented and shrunken. At this stage, there was little evidence of the presence of nerve fibres in the original endplate zone on silver staining.

218 *days*. A zone of new motor endplates was seen near the upper margin of the muscle. Many of the new endplates were formed of relatively widely dispersed rings of deposit, each ring being approximately 3 μ in diameter. Other endplates in which the rings were more closely packed together (Fig. 14) showed a great variation in overall size. The fairly regular form of the re-innervated original motor endplates from the same section is illustrated in Fig. 12.

375 *and* 480 *days*. After re-innervation for these longer periods the new motor endplates were still found in a narrow zone towards the proximal end of the muscle. These new motor endplates were large and irregular in shape and many of the constituent rings of deposit appeared to have coalesced to form gutters. They were indistinguishable from the new endplates found in series I at a similar stage of re-innervation (Figs. 36, 37). Sections stained by the Bielschowsky–Gros method revealed a great diversity in the form and size of the new motor endplates. A large endplate with fine branches and numerous endplate nuclei is shown in Fig. 19.

The number of terminal nerve branches varied between 2 and 21, and the number of endplate nuclei between 5 and 22 in counts on fifty new motor endplates. A number of nerve fibre terminations had bulbous expansions. Cholinesterase staining of the re-innervated original endplates showed them to be generally more uniform in size than the new endplates.

Series III. Innervation of denervated muscle with minimal injury

The object of this series was to investigate the suggestion of Miledi (1963) that new motor endplates might form in a muscle in response to mechanical injury of the muscle fibres during re-innervation.

Following cholinesterase staining at times between 30 and 150 days, deposits of

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Fig. 20. Sagittal section of tibialis anterior muscle 63 days after re-innervation showing (a) new and (b) original motor endplate zones. The position of the new and original motor endplates has been accentuated with indian ink.

Fig. 21. New motor endplates from same section as Fig. 20.

Figs. 22–24. New motor endplates, some showing the characteristic ring formation, from the same section as Fig. 20.

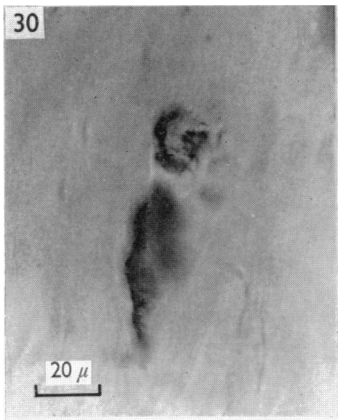
Fig. 25. New motor endplates and associated nerve fibres from upper region of muscle 100 days after re-innervation.

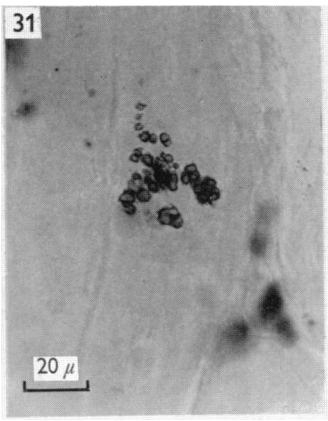
Fig. 26. New motor endplate from a section of muscle 100 days after re-innervation.

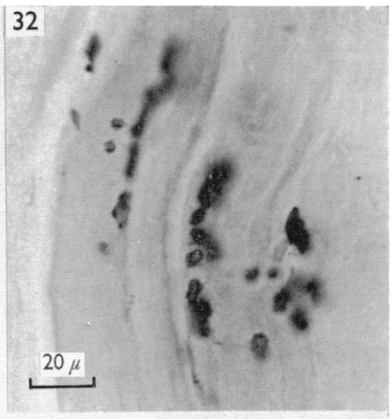
Fig. 27. New motor endplate from a muscle 100 days after re-innervation.

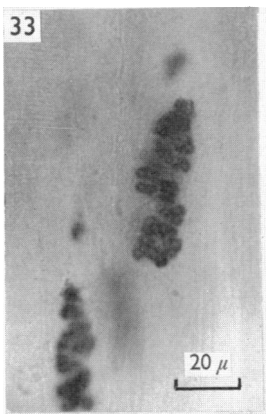
Fig. 28. Original motor endplates from a muscle 63 days after re-innervation, note the absence of nerve fibres. (Cholinesterase stain, counterstained with silver.)

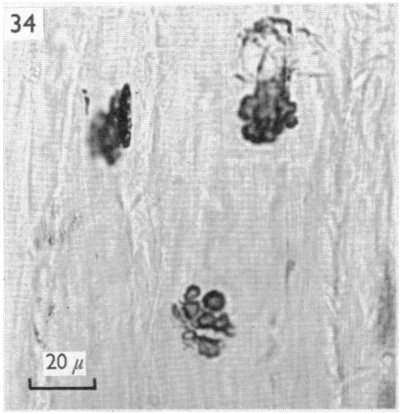
Fig. 29. Original motor endplates showing degenerative changes from a muscle 63 days after re-innervation.

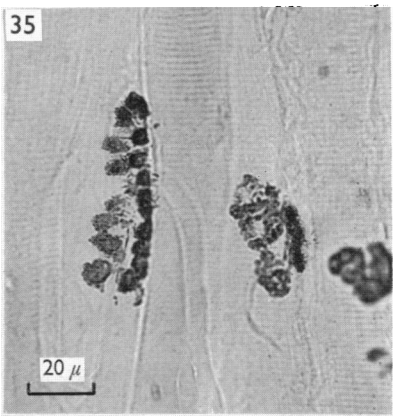

30
20 μ

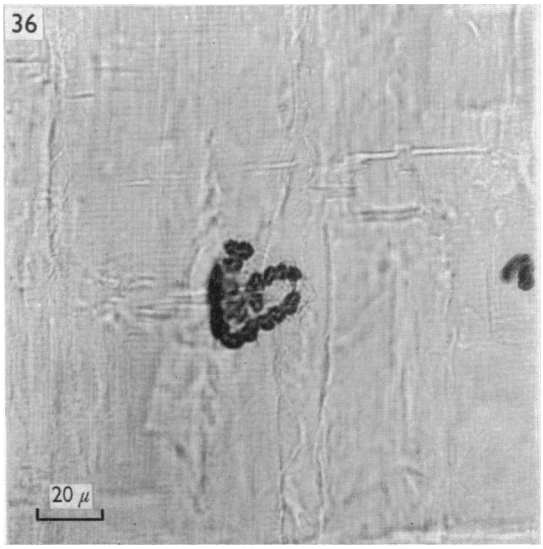

31
20 μ

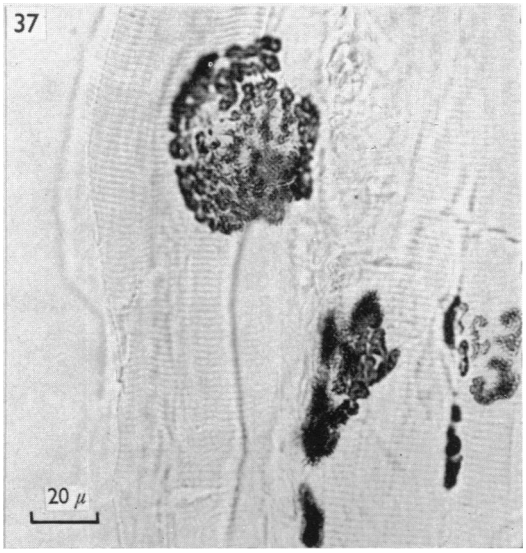

32
20 μ


33
20 μ


34
20 μ


35
20 μ


36
20 μ


37
20 μ

copper sulphide representing the site of new motor endplates were seen on muscle fibres in the upper endplate-free region of the muscles which had responded with a contraction to nerve stimulation. The deposits were not limited to the superficial region of the muscle but extended into the deeper parts (Fig. 20), particularly at 100 and 150 days following re-innervation. These deposits were similar in form to those seen in series I after an equivalent time of re-innervation but were fewer in number. They bore little resemblance in the early stages (Figs. 22–24) to the mature new motor endplates. At 30 days a localized collection of granular deposit was seen on some muscle fibres. As early as 50 days, however, rings of deposit were seen in the new motor endplates. At 60, 100, and 150 days there could be no doubt that the deposits were taking the now familiar form of new motor endplates. Counterstaining the sections with silver after cholinesterase staining revealed regenerating nerve fibres growing into close association with these new copper sulphide deposits (Fig. 25).

Bielschowsky–Gros staining of sections at the 100-day stage also revealed new motor endplates. The claws had taken on bizarre forms and a number of the nerve fibres in the endplates had a bulbous expansion at the tip (Figs. 26, 27). There were, however, fewer new motor endplates with fibres and associated nuclei at the 100-day stage than in the same stage of re-innervation in series I and II. This was probably due to the time taken for the regenerating nerve fibres to penetrate the fibrous tissue on the surface of the muscle before they came into contact with denervated muscle fibres.

Examination of the original motor endplates from the same sections (100 days) revealed that no regenerating nerve fibres had yet grown into their vicinity (Fig. 28). These original motor endplates on cholinesterase staining were shrunken, fragmented and the guttering had disappeared (compare Figs. 3 and 29).

DISCUSSION

(1) *The formation of new motor endplates*

The presence of ectopic motor endplates in the normally endplate-free proximal quarter of the muscle makes it quite clear that these are in fact new endings. It has been shown that they are innervated by staining the nerve fibres entering them. Contraction of the muscle fibres following stimulation of the implanted nerve indicates that functional connexions have been made. Silver staining of the implants

Fig. 30. Immature new motor endplate from a section of muscle 41 days after re-innervation.

Figs. 31, 32. New motor endplates from a section of muscle 63 days after re-innervation showing linear and disc-like arrangements.

Fig. 33. New motor endplates from a section of muscle 118 days after re-innervation. Note constituent rings of deposit.

Fig. 34. New motor endplate from a section of muscle 183 days after re-innervation.

Fig. 35. Linear type of new motor endplate from a section of muscle 245 days after re-innervation.

Fig. 36. New motor endplate from a section of muscle 393 days after re-innervation. Note gutter formation.

Fig. 37. Large new motor endplates from same section as Fig. 36. Note the differences in size.

into the upper endplate-free portion of the muscle showed many stages in the development of the new motor endplates, from a simple single ending of a fine nerve fibre to the more complicated claw reminiscent of the mature intact endplate.

The appearance of the *en grappe* endings in some material stained by the silver nitrate method was an unusual finding in mammalian skeletal muscle. More of these types of ending were seen in the shorter term experiments. The swollen bulb at the termination of the nerve fibre branches may be the enlarged, actively growing end of the fibre.

In the cholinesterase preparations of short-term experiments, the deposits representing the new motor endplates initially have a diffuse granular form (Fig. 21). At a slightly later stage (100 days) the endplates take on the form of collections of small rings of deposit (c.f. Csillik & Savay, 1959). In longer term experiments many of these rings have enlarged in size and some of the collections of rings have taken on an elongated, linear form, i.e. the motor endplate is located along the length of the muscle fibre (Figs. 23, 35). However, many of the new motor endplates have the more rounded disc-like appearance characteristic of intact endplates in this animal. In our experimental material there seems to be some evidence for thinking that the more immature new endings are 'linear' in form as there are many more of them present in the shorter term experiments. The more mature new endings are disc-like in form. In some immature new endplates a primitive form of guttering was seen. The association of the cholinesterase deposits with the nerve endings was clearly shown in the specimens which were doubly stained. The suggestion that the rings of cholinesterase deposit surround the growing ends of the nerve branches (Couteaux, 1963) seems probable.

Signs of muscle atrophy, reduction in diameter of the fibres, centralization of the nuclei and accumulation of fat between the fibres, were not very obvious.

It is interesting to note that new motor endplates will form in the denervated muscle even in the presence of the original motor endplates. Preliminary work (Shukla & Aitken, 1963) had suggested that the ingrowing nerve fibres would enter the original endplates in preference to forming new ectopic endings. This is not so. Also, in our longest term experiments, almost 500 days, there appears to be no removal of the new ectopic endplates in favour of the original endplates that became re-innervated later, as might have been expected if the original endings exerted some preferential attraction on the growing nerve fibres. In these preparations, it is not possible to say whether the ectopic new endplate and the re-innervated original endplate are on the one muscle fibre.

Guth & Zalewski (1963) microdissected the muscles after cholinesterase staining and showed two endplates on 38 % of the fibres examined. As no silver preparations were made, it is not clear whether any of the original endplates were re-innervated. In much of the earlier work (Aitken, 1950), it was impossible to distinguish with any certainty between a newly formed endplate and a re-innervated original endplate because the muscles examined had a wide distribution of the original endplates.

The new ectopic endplates and the original re-innervated motor endplates from the same muscle, 480 days after re-innervation, cannot be easily distinguished from each other, except by position. Neither in fact resembles closely the guttered form of an original intact ending. The rings seen in the early new motor endplates are

still retained to some degree in the more mature preparations. On silver staining, however, the new ectopic motor endplates bear a close resemblance to the appearance of a normal adult motor endplate. Counts of the endplate branches and of the endplate nuclei fell within the ranges of the counts given by Gutmann & Young (1944).

Koenig (1963) implanted an adjacent nerve into the sternal end of the pectoralis major of the rat after the bulk of the muscle containing the original motor endplates had been removed, and reported the formation of new motor endplates. Few details were given of the types of ending, their stages of development or of their extent in the muscle. Hofmann, Thesleff & Zelena (1964) also found that new motor endplates could be induced to form in muscle in which the original motor endplates had been inactivated by botulinum toxin. However, in most implantations of nerves or toxins into a muscle, some injury is probably caused to the muscle fibres. It was to investigate this problem that the experiments in Series III were undertaken.

(2) *The role of mechanical injury to muscle fibres in new motor endplate production*

In most previous work the implantation of a nerve into a muscle has been performed by inserting the nerve between separated muscle fascicles, using a needle to make the separation. Also in many of these experiments the muscle was transected to remove the original motor endplate zone (Koenig, 1963; Miledi, 1962; and Series I and II above). The most careful implantation may in fact sever some of the muscle fibres, resulting in an endplate-free portion and one bearing the original motor endplate.

Miledi (1963) has suggested that damage to the muscle fibre makes it receptive to a foreign regenerating nerve even when its original innervation is intact. The present experiments amplify the preliminary findings (Gwyn & Aitken, 1964) that the nerve fibres will regenerate into a muscle from its surface in the absence of injury and form new motor endplates. It is always possible that some of the superficial muscle fibres are mechanically injured in the incision of the fascia over the muscle or that the fibrin clot itself interferes with the muscle fibres and causes damage to them. However, the presence of new ectopic endings on muscle fibres near the deep surface of the muscle as well as on the more superficially placed fibres, indicates that endplates can form in the absence of mechanical injury to the muscle fibres. In the absence of mechanical injury, it is the availability of denervated muscle fibres which would appear to be the deciding factor in the production of new motor endplates.

SUMMARY

1. In the first series of experiments, the common peroneal nerve was implanted into the proximal endplate-free region of the tibialis anterior muscle, having initially removed the lower portion of the muscle containing the original motor endplates.

New ectopic motor endplates were shown to form and to mature.

2. In the second series of experiments, the nerve was implanted into the denervated but otherwise intact tibialis anterior muscle. New ectopic motor endplates were again seen to have formed near the proximal margin of the muscle. A number

of the original motor endplates were re-innervated but there appeared to be no preferential attraction for the original endplates.

3. In the third series of experiments, the common peroneal nerve was placed on the superficial surface of the muscle with minimal injury to the muscle fibres. New, ectopic motor endplates were again seen to form throughout the proximal quarter of the muscle.

4. The various stages through which the new motor endplates pass to the mature form were shown. They were a localized granular deposit, then formation of rings into linear or disc-like collections, and the appearance of gutters probably by the coalescing of adjacent rings.

5. The relationship of the new motor endplates and the regenerating nerve fibres was followed by the use of a combined cholinesterase–Bielschowsky stain.

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