GROWTH OF NERVE IMPLANTS IN VOLUNTARY MUSCLE

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INTRODUCTION

Attempts have been made in the past to produce an excess of nerve endings in a muscle (hyperneurotization) by implanting 'foreign' nerves, but the results have been contradictory. In most reports the findings were based almost entirely on the physiological tests of transmission between nerve and muscle rather than the subsequent histological examination of the implants, but Fort (1940), working with Weiss in Chicago, found that a foreign nerve implanted into a normally innervated muscle did not make either an anatomical or a functional connexion.

Gersuny (1906) is reported by Steindler (1916) to claim that functional endings were made by a foreign implant in a normally innervated muscle of dogs, and this was confirmed by Erlacher (1914) working on monkeys and guinea-pigs. Steindler (1916), working on dogs, and Elsberg (1917) on rabbits, both state that functional connexions were not made by implanted nerves in a normally innervated muscle. In all of this work it would appear that the operation of implantation and the methods used to retain the nerve in position (stitching) would cause considerable injury to the muscle. If muscle fibres were damaged or the normal nerve supply to a muscle fibre was cut, then a new end-plate would form on the denervated muscle fibre and on stimulation a small contraction would be expected.

Implantations of nerves into denervated muscles are reported by Steindler (1916), Elsberg (1917), Weiss (1930), Fort (1940), and others. Reinnervation of the muscles in varying degrees is claimed in all cases, but in the earlier works the histological appearances were not correlated with the observations made after the stimulation of the implanted nerve.

The results of the present investigation show that in a normally innervated muscle it is very difficult to produce extra motor end-plates on the muscle fibres, but that in a denervated muscle this hyperneurotization readily occurs. The problem of multiple innervation of muscle fibres has been much discussed in the past. If muscle fibres regularly and frequently have more than one end-plate, it might perhaps be expected that new motor end-plates would develop when a 'foreign' nerve is implanted into a normal muscle. This was not found in the present series of experiments.

MATERIAL AND METHOD

In all of these experiments rabbits were used. No special precautions were taken as to size and breed of animal. In most cases the sciatic and associated nerves were exposed by an incision through the biceps muscle. In some cases the nervus gastrocnemii medialis (n.g.m.) was cut near its muscle and its end implanted either into biceps or into the lateral head of gastrocnemius. In other cases the nervus peroneus was cut and implanted into the lateral head of gastrocnemius. Many of the nerves were implanted into portions of the muscle which are usually relatively free

of end-plates. The work of Couteaux (1942) was confirmed in that normal muscles, when stained, showed regions where no end-plates could be demonstrated.

It was found impossible to implant the nerve without some damage to muscle fibres, but this was kept to a minimum. A pathway was first made between the muscle bundles with a round-bodied needle for n.g.m., or a larger instrument for n. peroneus. On a number of occasions, use was made of a guarded crochet hook to pull the nerve into the muscle. This, however, produced a great deal of unnecessary damage, and the method was abandoned.

The distal end of the cut nerve was carefully inserted into the muscle wound and held in position with a drop of human fibrinogen which was coagulated with human thrombin. The use of fibrin removes the necessity of stitching the nerve into the muscle and so lessens the possibility of muscle damage. If abundant bleeding occurred during the implantation another site for implantation was prepared near to the original site. Two implants were usually made on each rabbit, on one side into normal muscle and on the other into a muscle whose nerve or tendon was cut at the same operation. The nerves to biceps and the lateral head of gastrocnemius were identified, thoroughly crushed and a length of 1·2 cm. excised. These precautions were probably adequate to prevent reinnervation of the muscles by their own nerves during the experiment.

After 100 days the animals were anaesthetized and the response of the muscles to stimulation of the nerves was obtained, using a faradic stimulator. Platinum wire electrodes embedded in Perspex were used and placed on the nerve 2–3 cm. from the muscle. The nerve was cut proximal to the electrodes. Care was taken to remove the excess fat and organized fibrin from the nerve, especially in the region of the electrodes. After observing the results of the stimulation, the nerve was crushed as it entered the muscle and again stimulated. The crushing of the nerve blocked all indirect excitation of the muscle but did not prevent direct excitation due to surface spread or 'escape' of the stimulus.

After stimulation, the muscle with nerves attached was dissected out and placed in fixative. The type of fixation was varied according to the method of staining. Trichloracetic acid (5 %) was found to be a good fixative for a future Bodian protargol staining, but results were not consistent. After Bodian fixation (80 c.c. of 80 % alcohol, 15 c.c. of commercial formalin and 5 c.c. of glacial acetic acid), and celloidin embedding some specimens were stained with Holmes's modified Cajal technique, using buffered solutions. The most successful method was found to be fixation in 10 % formalin with 2 % pyridine in water for at least 7 days, the muscle being then washed, and frozen sections made at about 50 μ. These were stained by a modified Bielschowski-Gros technique, using pyridine as a buffer in the reducing solution of formalin. The method was as follows:

(1) Impregnation for 20 min. in the dark in 10 % aqueous solution of silver nitrate.

(2) The sections are removed and passed directly into an aqueous solution of 10 % formalin (acid) containing 2 % pyridine. This is the first of four washings in this formal-pyridine solution and the times are roughly 30 sec., 1, 4 and 8 min.

(3) After removing excess formal-pyridine the sections are placed in ammoniacal silver solution for 1 min. This solution is made by adding 880 ammonia drop by drop

to 5 c.c. of 10 % $AgNO_3$ solution until the precipitate has disappeared. Ten to fifteen further drops were then added. The importance of adding the ammonia in small quantities was shown by Silver (1942), and was confirmed in present experiments. The amount of ammonia added will vary with the pipette or dropper, but must be controlled by the staining of the section. The section should be a very faint brown colour. If too much ammonia is added, the staining is inhibited and if too little, the sections are dark and the connective tissue and vascular elements stain. The sections should be gently agitated during the time in ammoniacal silver solution.

(4) Transfer to water containing few drops of 880 ammonia for about 10 sec.

(5) Transfer to water containing few drops of glacial acetic acid for about 10 sec.

(6) Wash in water. The sections should be almost colourless. If they are brown then the ammoniacal silver has contained too little ammonia—probably due to evaporation. This is specially seen in hot weather.

Usually the sections were toned in 1 % gold chloride until a faint deposit could be seen on them (about 10–30 sec.). They were then fixed in a 5 % hypo solution and were then washed, dehydrated, cleared in creosote, and mounted in Canada balsam. It should be remembered that in both the toning solution and the fixing solution, bleaching occurs and loss of the finer endings will be noted if the sections are allowed to stay too long in these solutions.

To facilitate identification later, it was found advisable to mark the site of implantation and one corner of the block of muscle with indian ink. This did not interfere with the staining but was valuable as the sections could be mounted symmetrically.

The thick sections which can be stained and examined after staining by the Bielschowski-Gros methods have many advantages, though the difficulty of obtaining serial sections is a marked disadvantage.

RESULTS

Electrical stimulation of the implanted nerves gave rise to responses which were usually rather weak. When contraction of the muscle was obtained it was seen to be occurring in those fibres in the vicinity of the implant and there was never a total response of the whole muscle unless the stimulus was so strong that the effect was probably due to surface spread of the stimulus. When contraction occurred, then those fibres which responded to the stimulus did so, as far as could be detected, in their total length. Almost all the denervated muscles showed fibrillation.

In Table 1, there is listed against each animal an estimate of the extent of the muscle response to stimulation of the implanted nerve. In no experiment was the contraction vigorous enough to move the leg or the foot. Thus we have twenty cases of nerves successfully implanted into denervated muscles, and seventeen of these showed clear signs of indirect excitability through the nerve, as estimated by response at the threshold of 25 cm. or more coil distance, controlled by crushing of the nerve peripheral to the point of stimulation. In three cases the threshold was such as to raise suspicion that the response was due to 'escape' of current to the muscle fibres.

Out of seventeen cases in which implants were made into muscles with a normal nerve supply, nine showed response on stimulation of the nerve with currents weak

enough to be regarded as providing unambiguous evidence of indirect excitability. The remaining eight showed 'response' only to very strong shocks. In these cases, the response was probably due to escape of the current. The indirect responses of the muscles with the normal nerve supply were much weaker than the responses of the muscles which had previously been denervated. The results shown in Table 1 show that the implant makes a functional connexion more frequently and easily in a denervated muscle than in a normal muscle. This agrees with the previous finding (Aitken, 1949) that when a nerve regenerates into a denervated muscle its fibres mature further than do fibres growing into a muscle already innervated. The more mature nerves had many fibres whose total diameter (axon and myelin sheath) was more than 6μ and few fibres under 6μ. The less mature nerves had large numbers of small fibres (under 6μ) and few large fibres.

Table 1. *Against each animal there is given an estimate of the extent of the response to indirect excitation of the muscle by the implanted nerve*

N.g.m. = nervus gastrocnemii medialis; O = nerve pulled out; − = no response; + = slight response; + + = vigorous response.

Animal	Nerve	Response on 'normal' side to indirect excitation	Response on 'paralysed' side to indirect excitation
398	N.g.m.	−	+
404	N.g.m.	+ +	+
417	N.g.m.	+	+ +
418	N.g.m.	−	−
428	N.g.m.	+	+
443	N.g.m.	−	+
444	N.g.m.	+	+ +
446	N.g.m.	−	O
458	N.g.m.	O	+
459	N.g.m.	O	+ +
483	N.g.m.	−	+ +
484	N.g.m.	+	+ +
642	N.g.m.	+	O
643	N.g.m.	+	−
395	N. peroneus	O	+ +
446	N. peroneus	−	+
458	N. peroneus	O	−
459	N. peroneus	+	+
483	N. peroneus	+	+
540	N. peroneus	O	+
880 (50 days)	N. peroneus	−	+ +
881 (50 days)	N. peroneus	−	+ +

EXAMINATION OF THE IMPLANTS IN MUSCLE

A. *Implants in normally innervated muscle*

The regenerating nerve fibres grow out from the cut end of the nerve and form a tangled mass of fine fibres (Pl. 1, figs. 1, 2). Generally speaking the fibres are thin and lightly myelinated or non-myelinated. They pass between the muscle fibres and no very obvious cellular reaction is observed either in the connective tissue or the muscle. The fibres can often be followed for long distances and seem to end in the endomysium. The Schwann cell nuclei (Pl. 1, fig. 3) vary in their staining intensity. Some are intensely black, as though the silver were deposited on the surface, whilst in others the nucleolus and other nuclear structure can be seen. Other nuclei are mostly unstained and appear as mere shadows. Some of the nuclei probably belong to fibroblasts. In

a suitable preparation the connective tissue network can be stained and is quite different from the nerves. The undulations of the fibrous tissue are smaller and more frequent and the cell nuclei are less frequently seen (Pl. 1, fig. 4).

In those specimens in which an unmyelinated fibre lay in close proximity to a muscle fibre (sometimes for a considerable distance), no change in the constitution of the muscle was detected. Special attention was paid to the muscle nuclei, but they were neither more conspicuous nor altered in their distribution. There was no accumulation of sarcoplasm as is found in a normal motor end-plate.

If the implanted nerve grows into a region of the muscle which has motor end-plates (Pl. 1, fig. 5), then the regenerating fibres pass near to the end-plates, but there was no evidence of either attraction or repulsion between them.

These fibres, although they make no visible endings, frequently develop a myelin sheath (Pl. 1, fig. 6). This was also seen in the cross-sections of the regenerating nerves which were stained by the Flemming-Weigert method (Aitken, 1949). A few fibres reach 10–12μ in diameter (axon and myelin) but most of them are about half this size.

Occasionally, one or two atypical motor end-plates are found near to the neuroma. These are probably formed on a portion of a muscle fibre which has been damaged during the implantation and severed from the part of the fibre that carries a normal motor end-plate. It is interesting to speculate on the conditions in which a detached portion would call forth a response from the nerves. Pl. 1, fig. 7 shows one of these atypical motor end-plates. Though this muscle was normally innervated, the tendon had been cut at the operation. Native endings were found in abundance, and in addition a few of these new motor end-plates. The new end-plates can be identified by their position (near the neuroma) and by the thin fibre with little or no myelin which leads to them. These damaged muscle fibres, which had received a new motor ending, would contract on stimulation and would be the explanation of the contractions found in some specimens. These contractions were restricted to the vicinity of the implant and did not spread through the muscle.

Contractions of muscle were found in some specimens in which no motor end-plates at all could be demonstrated. Though serial sections were not made of the muscles, care was taken that very little tissue was lost and all sections were examined. If these cases have been correctly interpreted it must be supposed that the fine fibres in close proximity to the muscle fibres were able to stimulate the muscle fibre.

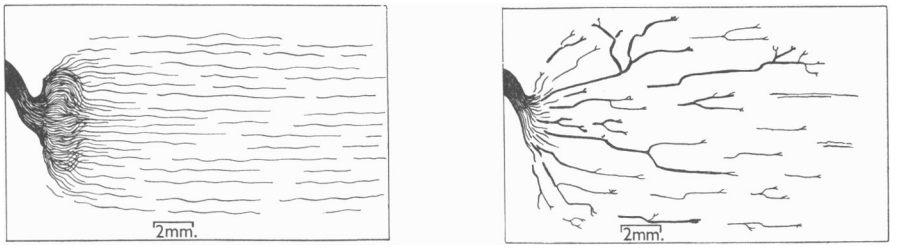
B. *Implants in denervated muscle*

(1) In a denervated muscle, the histological picture of the neuroma and regenerating fibres is very different. Examination with the low power of the microscope (Pl. 1, fig. 8) shows the regenerating fibres branching in the muscle. The fibres branch in an irregular manner and end-plates are formed soon after the division. The pattern of innervation is very like that found in an intact muscle where the large nerve fibres are seen to run across the muscle fibres and only the smaller fibres run parallel with the muscle fibres. The pattern of the nerves is quite different from that made by nerves which have been implanted into a normal muscle (Text-fig. 1).

Most of the nerves are myelinated and as seen in Pl. 1, fig. 9, the thick myelin sheath is retained to within a few microns of the end-plates. In Pl. 2, fig. 10, one

of these myelinated nerves is seen dividing into three branches. Occasionally a large myelinated fibre is seen to travel for a considerable distance. In Pl. 2, fig. 11 the fibre travels about 0·8 mm. before it divides into the smaller branches which give rise to the motor end-plates. Beyond the region where motor end-plates have formed, but in the same plane of section, the nerve fibres travel for long distances between the muscle fibres. These muscle fibres presumably have already been reinnervated and the surplus nerve fibres from the growing end of the implant pass between them. The picture in Pl. 2, fig. 12, which is an adjacent field to that in Pl. 1, fig. 8, is here very like that obtained when an implant is made into a normal muscle.

(2) Motor end-plate production. The great majority of the newly formed 'foreign' motor end-plates differ greatly in size and shape from the normal end-plate which is found in a normal muscle, but some are very like normal endings. Pl. 2, fig. 13 shows one nearly normal ending. The entering fibre divides and the two branches appear to enter opposite poles (ends) of the plate. The nucleus of a Schwann cell is seen on one branch and the end-plate nuclei are clearly seen.



Drawing of nerve implant in normal muscle Drawing of nerve implant in denervated muscle

Text-fig. 1. Drawings of two nerve implants. The nerves in the normal muscle travel for long distances with little sign of branching. The nerves in the denervated muscle branch very frequently and irregularly.

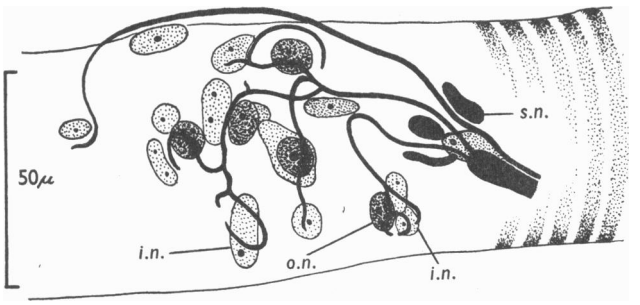
Some of the end-plates are supplied from two nerve fibres as can be seen in Pl. 2, fig. 15. Is this an example of the reinnervation of an empty normal end-plate? In this series of experiments, no evidence was seen of these denervated end-plates which, according to Gutmann & Young (1944), usually show as more darkly staining areas.

The more atypical plates varied from large claw-like endings on a muscle fibre to groups of small endings on a muscle fibre. In Pl. 2, fig. 16, one of the large claw-like endings is seen. Text-fig. 2 is a drawing of this ending. There are two main branches to this end-plate and at least nine terminal branches. The number of inner end-plate nuclei is eleven, and of outer end-plate nuclei, five. Three Schwann cell nuclei can be made out. The main nerve trunk is myelinated almost as far as the end-plate, whose overall size is 0·075 by 0·05 mm. All of these figures are within the range of figures given by Gutmann & Young (1944) for normal end-plates and reinnervated end-plates. The width of the muscle fibre at the region of the end-plate was 0·09 mm.

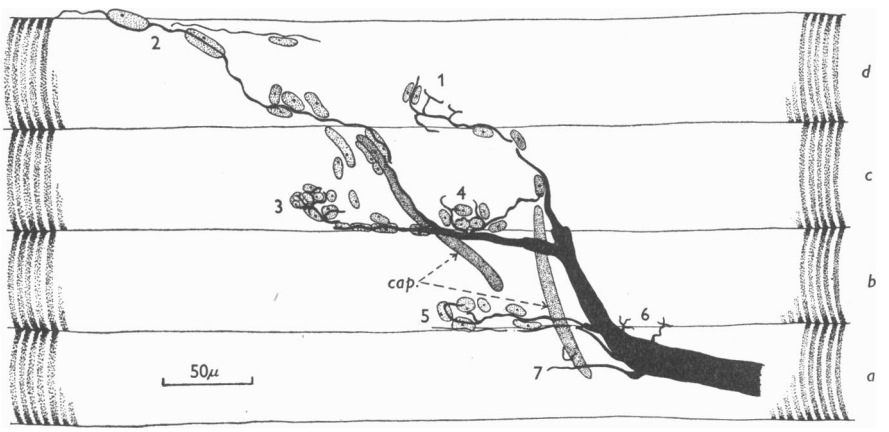
A third fine fibre can be traced past the end-plate to finish on the surface of the muscle fibre some distance beyond.

Occasionally there is found a large axon which divides to supply motor end-plates to a group of muscle fibres. In Text-fig. 3, a drawing of one of these large nerve trunks, there are several muscle fibres each with a well-formed and a very irregular

ending. Owing to slight differences in level and to the thickness of the section, it is impossible on a single photograph to show the detail, but Pl. 2, fig. 17 is a photograph of this group of endings. A muscle fibre '*a*' has one plate ending, and '*b*' has two and possibly a third, '*c*' has two and '*d*' has one end-plate and one fine fibre which comes in contact with the muscle fibre and appears to produce changes in the orientation of the muscle nuclei.



Text-fig. 2. Drawing of large new motor end-plate in muscle fibre. Note the number of Schwann cell nuclei and end-plate nuclei. A small nerve fibre which accompanies the large fibre passes by the end-plate and forms a small bare ending on the muscle surface. Abbreviations: *i.n.*, inner end-plate nucleus; *o.n.*, outer end-plate nucleus; *s.n.*, Schwann nucleus.



Text-fig. 3. Drawing of a group of large irregular motor endings which are derived from one large nerve trunk. Four muscle fibres (*a*, *b*, *c*, *d*) are each innervated by two or more end-plates (1, 2, 3...). *cap.*, capillary.

In the experiments where the nerves regenerate into a paralysed muscle two or more motor end-plates are frequently found on one muscle fibre. Pl. 3, fig. 18 shows an example of this. At least four end-plates are present and appear to be on one muscle fibre. Careful focusing shows that they are all at about the same level in the muscle. Even if they are on contiguous surfaces of two muscle fibres there is still good evidence for multiple innervation of the muscle fibres. These endings, however, all appear to arise from the same axon and so the innervation is uni-axonal and therefore unisegmental. Pl. 2, fig. 15 shows an ending which is bi-axonally innervated.

Examination of the denervated muscles showed that the amount of interstitial fibrous tissue had increased and the fat cells were very conspicuous. The differences between the normal and paralysed muscles were obvious from the beginning of the histological process. The paralysed muscle cut much more easily and the sections tended to float at the top of the collecting tubes. The blood capillary plexus always stained well in the paralysed muscle unless special care was taken to suppress it.

C. *Implants into tenotomized muscles*

The normal motor end-plates after tenotomy do not appear to be very different from those seen in a normal muscle (Pl. 3, fig. 19). Some implants (Pl. 3, fig. 20) give rise to many long thin fibres which coil up between and around the muscle fibres. This is not seen in nerves which are growing into normal muscle. Sometimes one of the coiling fibres (Pl. 3, fig. 21) is seen to form a large loop, the terminal branch turning back on the main fibre. Smaller side branches leave the same nerve. Many of the fibres, as in implants in normally innervated muscles, are myelinated (Pl. 3, fig. 22) and Schwann cell nuclei and nodes of Ranvier can be clearly seen. On some fibres the neurilemma can also be made out.

Other implants give rise to fibres which stream away between the muscle fibres (Pl. 3, figs. 23, 24). They travel for long distances and then taper away. No muscle cell or interstitial cell reaction was observed. Myelin sheaths are very prominent (Pl. 3, fig. 24) with nodes of Ranvier and Schwann cell nuclei.

Careful inspection of the material showed no regenerating nerves which had undoubtedly formed new motor end-plates. Such plates as are present are so typical in their characteristics that they are probably the normal endings.

DISCUSSION

The purpose of this investigation was to find an answer to the question: 'Will new motor end-plates develop in a muscle as the result of the entrance of 'foreign' nerve fibres?' It has been shown that in a denervated muscle the 'foreign' nerve readily produces new end-plates, which function when the nerve is stimulated. In a normal muscle, the 'foreign' nerve usually produces many long thin nerve fibres, which ramify amongst the muscle fibres and rarely form a muscle end-plate. In some experiments, however, the normal muscle contracted in response to stimulation of the implanted nerve; it is therefore probable that some of these nerve fibres make a functional connexion with the muscle fibres. Reinnervation is certainly very much easier and more abundant in muscles which had previously been denervated.

The observations made during these experiments raise two further questions. (1) What is it that prevents the regenerating fibres from forming new motor end-plates in normal muscle? (2) Would it be possible by implantation to reinnervate a muscle which had been paralysed (by denervation)?

(1) This problem has received much attention from workers who are interested in the innervation of muscle. Multiple endings, pluri-axonal and pluri-segmental, have been reported in Amphibia and Reptilia and there is good evidence—both histological and physiological—to support this view (Kühne, 1887; Katz & Kuffler, 1941), though other workers (Kulchitsky, 1924; Wilkinson, 1929) found only one ending. In mammals, however, the evidence is not so clear. Multiple motor endings on the

intrafusal muscle fibres of muscle spindles have been demonstrated by Cuajunca (1932), Barker (1948), and others. On the extrafusal muscle fibres, Agdhur (1916) illustrates two endings, one above the muscle fibre and the other on the deep surface. Wilkinson (1929) made a very full and careful survey of many types of animal and inspected the preparations of Agdhur and others. He was of the opinion that as a rule one ending is found on a muscle fibre and that if more than one occurs it must be a great rarity. Harrison (1910) and Tello (1917) have suggested the analogy of the ovum and sperm as a fair comparison with muscle fibre and end-plate. It may well be, however, that there is a relationship between the length of the muscle fibre and the number of end-plates on it. In the experiments which have been described it would appear that the normal muscle was already in a state of nerve-muscle equilibrium and that no further end-plates would form on the muscle fibres. Occasionally, however, the implanted ('foreign') nerve produced motor end-plates. In these cases it may be that the normal nerve to the muscle fibre had been cut or, more probably, the muscle fibre had been divided into a part with the normal end-plate and a part without an end-plate. In either case the 'foreign' nerve would presumably be able to form a new end-plate. If the detached portion of the muscle fibre is reinnervated, then whatever the cause of the non-receptiveness, it is a reversible phenomenon and depends on the close association with the end-plates.

If there is a quantitative relationship between the length of the fibre and the number of end-plates it should be possible to devise experiments in which the muscle fibres are broken into varying lengths. End-plates might perhaps be induced to form more readily on the longer portions than on the shorter.

Most of the end-plates in a muscle are arranged in a more or less regular pattern. When the muscle fibres are parallel, as in sartorius, the endings are found in a band across the muscle. Some parts of the muscle, such as the upper end of sartorius, are free of endings. In gastrocnemius and biceps femoris it is possible to make the implantation into a region which is devoid of normal motor end-plates. When this was done, new end-plates readily developed in a denervated muscle. If the implants were in the region of the normal end-plates, then some of these may have been reinnervated. If reinnervation had occurred regularly then the new end-plates formed by the 'foreign' nerve should follow the pattern of the normal end-plates shown by Couteaux. The end-plates were, however, found scattered through the muscle, so no evidence was found for a neuropathic attraction of end-plates on the regenerating nerves.

From the appearances of the regenerating nerves in the denervated muscle, it is evident that the muscle fibres had a marked effect on the nerves. After the nerves came into contact with the denervated muscle fibres the nerves divided up in a complicated manner, and it appears that as soon as a small branch came into very close contact with the muscle fibre, the nerve divided into numerous fine branches forming an end-plate. This is well seen in Pl. 2, fig. 11, which shows a nerve fibre crossing over numerous muscle fibres without any sign of division, and then suddenly giving rise to short branches which finish as motor end-plates on muscle fibres.

In the denervated muscles, an implanted nerve produced motor end-plates on the muscle fibres at all positions along the muscle. There did not appear to be any area which was more easily innervated than another. It would therefore appear that all

points of a denervated muscle fibre are equally receptive and capable of forming an end-plate. What is more, in these reinnervation experiments, more than one end-plate is frequently found on the muscle fibre. Sometimes the two end-plates are 'in parallel', the two fibres which supply them being about equal length and size. These end-plates probably developed on the muscle fibre about the same time (see Pl. 3, fig. 18). In other cases, the end-plates are 'in series' (Pl. 3, fig. 17 and Text-fig. 3), and there must have been a short interval of time between the formation of the two plates. The muscle fibre was unable, in the time available, to become non-receptive to other fibres. Pl. 2, fig. 11 shows, however, that within a short period of time the nerves can meet with non-receptive muscle fibres, and Pl. 2, fig. 12 shows the long straggling fibres which are found near to the new end-plates. These fibres are presumably travelling between muscle fibres which have already been reinnervated. The implants in normal muscle show this non-receptive state in a marked degree.

(2) These experiments showed that there can be reinnervation of a denervated muscle by an implanted nerve. The new end-plates, however, were found in the region of the end of the implant and not over a wide area of the muscle. This may be due to the short duration of the regenerative period. Certainly the implantation of a number of small nerves or the fascicles of a larger nerve would have a better chance of reinnervating the whole paralysed muscle.

The results which have been obtained show that the great excess of regenerating nerve fibres which is produced in a normal muscle when a 'foreign' nerve is implanted in it does not produce hyperneurotization of the muscle.

SUMMARY

1. A method of implanting nerves in muscle with the minimum of injury to the muscle is described.

2. Nerves which regenerated into a denervated muscle formed functioning motor end-plates.

3. Nerves which regenerated into normal muscle rarely formed motor end-plates and these were probably on muscle fibres that had been damaged at operation.

4. In the implants in denervated muscles, more than one ending was frequently seen on a muscle fibre.

5. Implants into tenotomized muscles formed long thin fibres which travelled between the muscle fibres and occasionally coiled round them. No regenerated endings were seen.

6. The non-receptiveness of normal muscle fibres is discussed.

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EXPLANATION OF PLATES

All sections were stained by a modified Bielschowski-Gros technique.

Figs. 1–7. Implants in normally innervated muscles.

Figs. 8–18. Implants in denervated muscles.

Figs. 19–24. Implants in tenotomized muscles.

PLATE 1

Fig. 1. N. peroneus implanted into normal lateral head of gastrocnemius shows long, thin, unbranched fibres. (483 B 9.)

Fig. 2. Same section under higher power of microscope.

Fig. 3. Implanted nerve fibre in normal muscle showing different staining of Schwann cell nuclei. (484 D 6*a*.)

Fig. 4. Section of paralysed biceps showing muscle reticulum. Note the small number of nuclei as compared with that found on a nerve fibre. Compare with fig. 3. (881 H 1.)

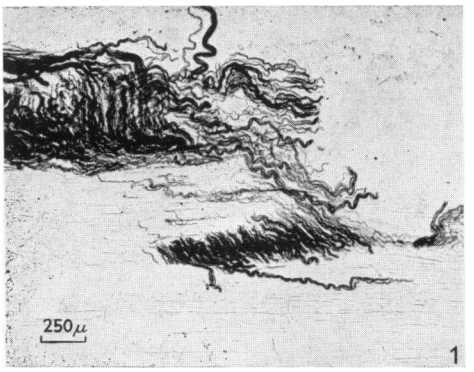
Fig. 5. N.g.m. implant near native endings. (856 G 14.)

Fig. 6. N.g.m. implant in normal muscle showing presence of myelin sheath on fibres. (484 D 14.)

Fig. 7. N. peroneus implanted into tenotomized gastrocnemius and showing large newly formed end-plates due to injury to muscle fibres during implantation. (251 B 2.)

Fig. 8. Pattern of reinnervation of paralysed gastrocnemius by implanted n. peroneus. Note the thick nerve fibres which frequently branch. (395 A 13.)

Fig. 9. Large myelinated fibres from an implant in paralysed muscle. The end-plates form at the side of the main trunk. Though only three end-plates are in view, focusing in different levels brought up three other smaller end-plates. (855 E 6.)


250μ
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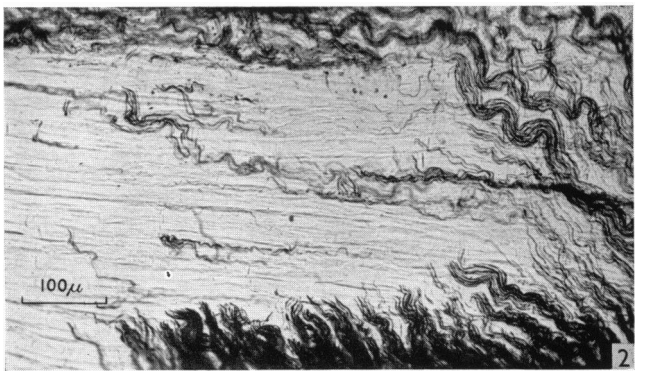
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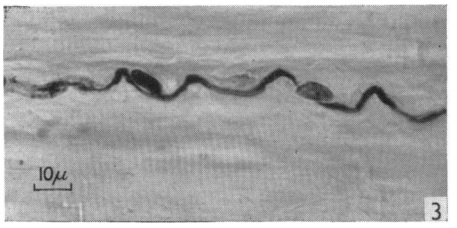
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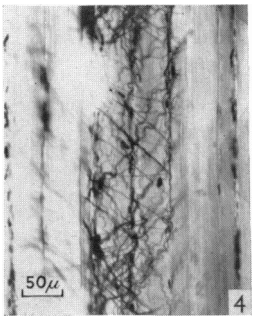
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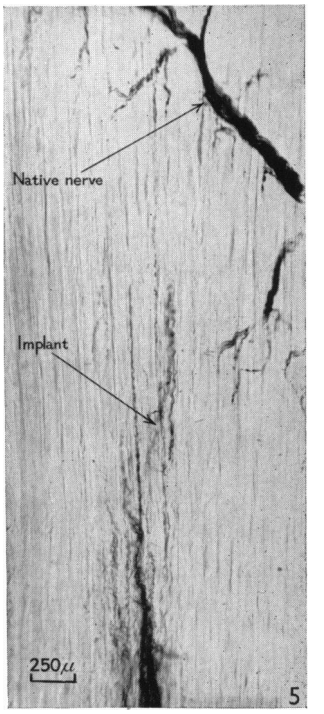
Native nerve
Implant
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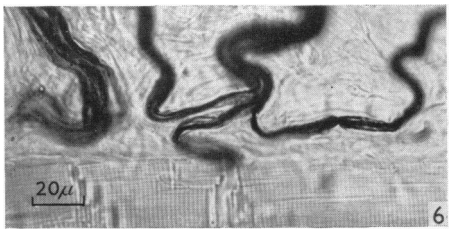
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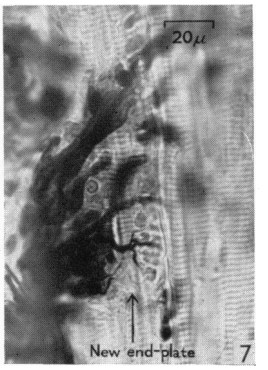
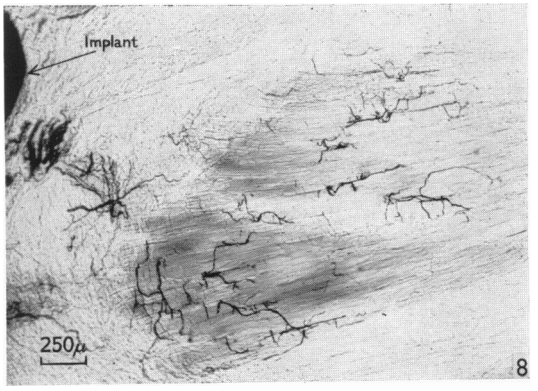
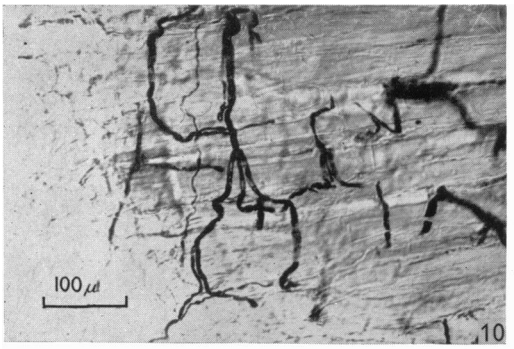

20μ
New end-plate
7


Implant
250μ
8


100μ
9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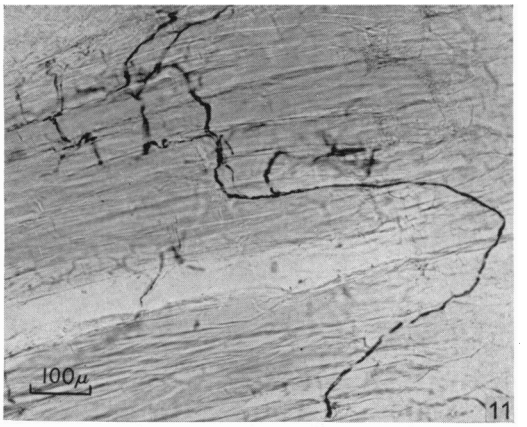
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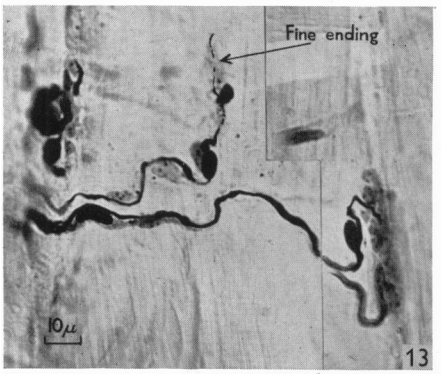
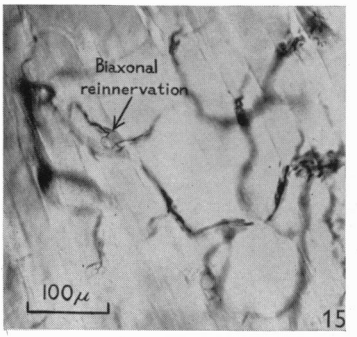
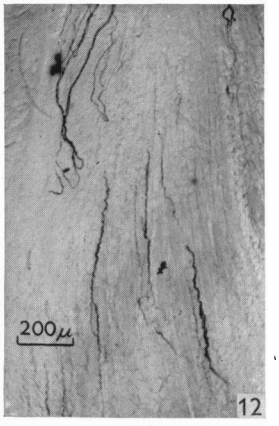

Fine ending
10μ
13


Biaxonal
reinnervation
100μ
15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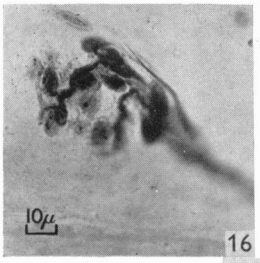
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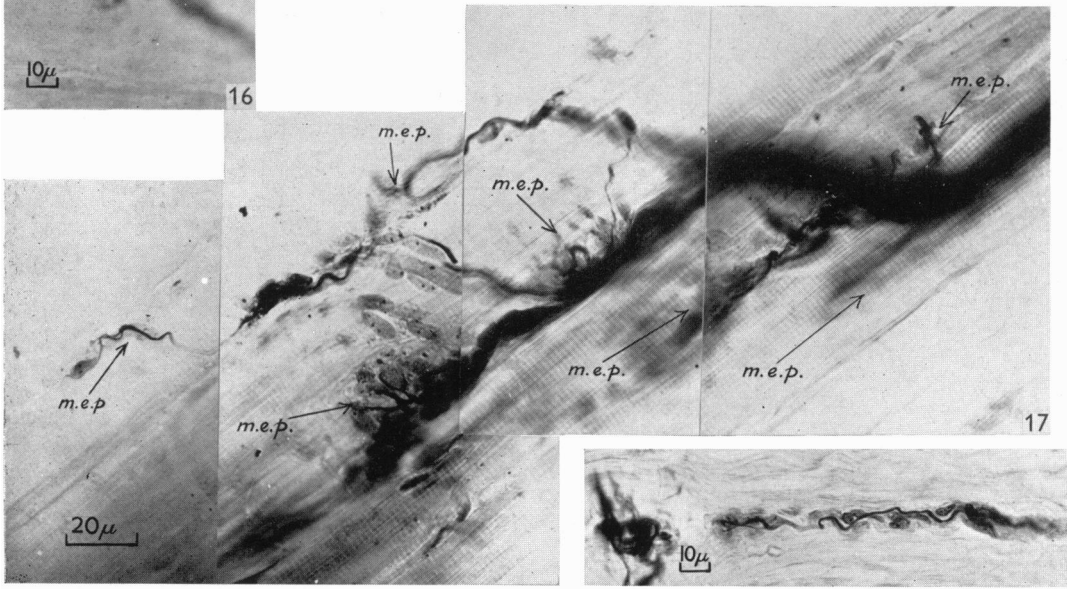
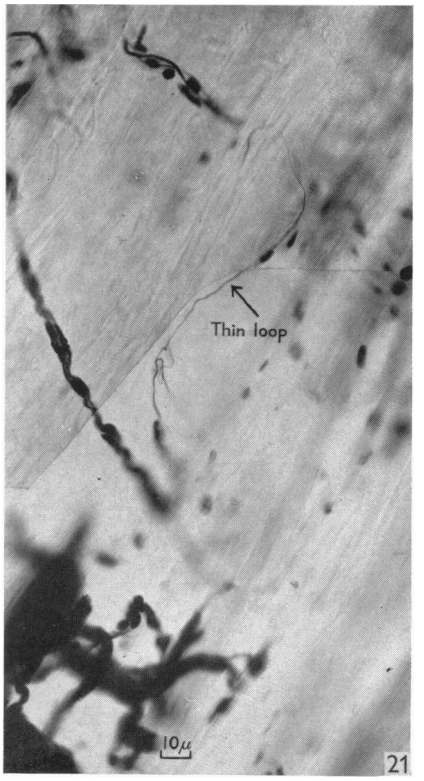
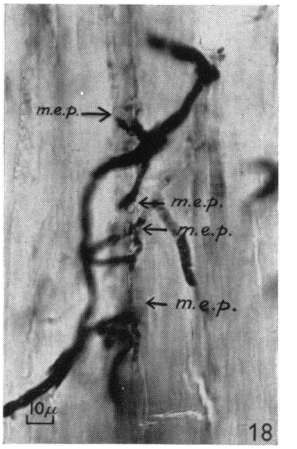

m.e.p.
m.e.p.
m.e.p.
m.e.p.
m.e.p.
m.e.p.
m.e.p
20μ
17

10μ
14


Thin loop
10μ
21


m.e.p.
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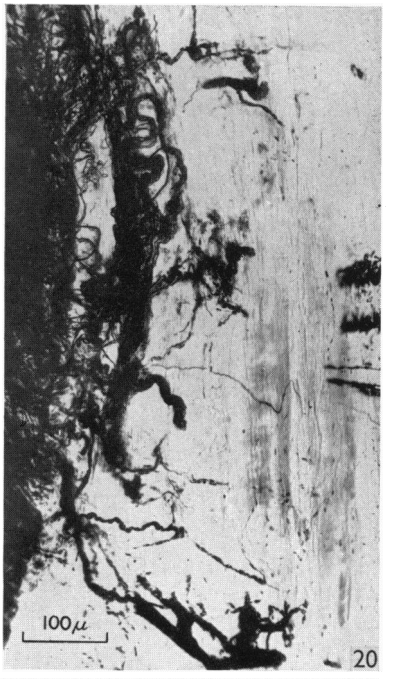
m.e.p.
m.e.p.
10μ
18


10μ
19


100μ
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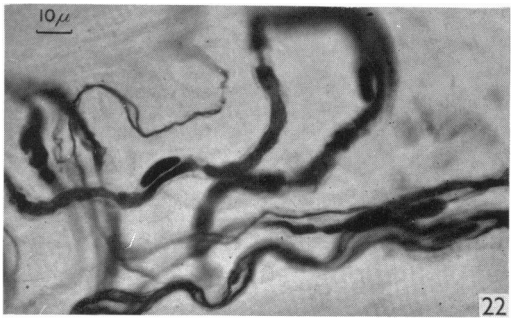
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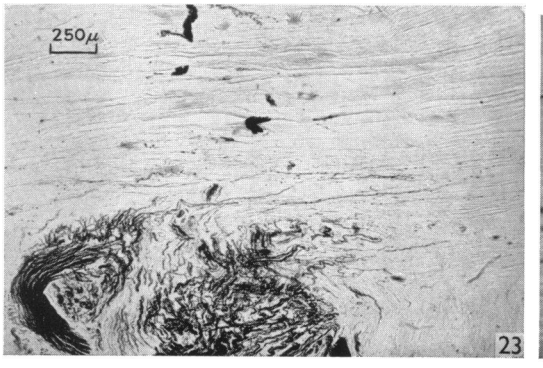
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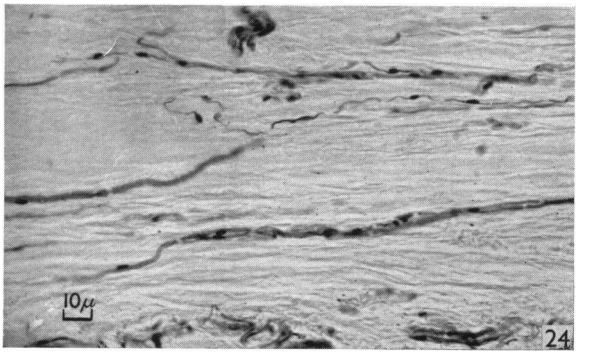
10μ
24

PLATE 2

Fig. 10. Showing the division of myelinated fibre into three branches. (395A 13.)
Fig. 11. Myelinated nerve from the implant which travels a considerable distance before dividing into smaller branches with terminal end-plates. (395A 16.)
Fig. 12. Implant into paralysed muscle. Long thin straggling fibres passing between muscle fibres which have already been reinnervated. (395A 13.)
Fig. 13. New motor end-plates formed in paralysed muscle by the implanted nerve. Note the Schwann cell nuclei, end-plate nuclei and the fine ending of the upper fibre. Retouched. (484B 6*b*.)
Fig. 14. Fine nerve fibre lying in close contact with muscle fibres. Some Schwann cell nuclei can be seen but many of the others present probably belong to the muscle. (484B 6*a*.)
Fig. 15. Bi-axonal innervation of a new end-plate formed in paralysed muscle. (855E 3.)
Fig. 16. Large new motor end-plate formed in paralysed muscle. Compare with Text-fig. 1. (855E 3.)
Fig. 17. Group of large new endings on paralysed muscle. Compare with Text-fig. 2. (855E 4.) Motor end-plates labelled *m.e.p.*

PLATE 3

Fig. 18. Group of four new endings on side of muscle fibre. All the endings are derived from the one large nerve trunk. (395A 13.)
Fig. 19. Native motor end-plates in a tenotomized gastrocnemius. (251B 39.)
Fig. 20. Implant of n. peroneus into tenotomized gastrocnemius. Note the long thin straggling fibres. A group of native endings is present at the left side of the picture. (251B 39.)
Fig. 21. Example of a long thin straggling nerve winding between muscle fibres and forming almost a complete circle. (251B 39.)
Fig. 22. Myelinated fibres of n. peroneus which was implanted into tenotomized gastrocnemius (251B 19.)
Fig. 23. N.g.m. implanted in tenotomized gastrocnemius. Note long thin nerves passing between muscle fibres with no sign of branching. (251D 36.)
Fig. 24. High-power view of these unbranching fibres. Note myelination. (251B 36.)