STUDIES IN NERVE REGENERATION

AND IN THE

INNERVATION OF VOLUNTARY MUSCLE

THESIS PRESENTED FOR THE DEGREE OF

M. D.

OF THE UNIVERSITY OF GLASGOW

BY

J. T. AITKEN

UNIVERSITY COLLEGE, LONDON.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

PREFACE

The work on which this thesis is based was done in the Department of Anatomy and Embryology in University College, London. The original work referred to in the text has already been published as a series of three papers in the Journal of Anatomy. The early work covered by the first paper was done in collaboration with Professor J.Z. Young and Miss M. Sharman. The microscopic preparations are in the Department of Anatomy at University College, London.

ACKNOWLEDGEMENTS

I have much pleasure in acknowledging the help which I have received from Professor J.Z. Young, M.A., F.R.S., Professor of Anatomy at University College, London. The work was begun on the suggestion of Professor Young and he has continually inspired, supervised and guided all phases of the research. Technical assistance has been given by Mr. F.J. Pittock, F.L.S., F.R.P.S. in photography and Mr. L. Middleton in histology. Many of the preparations are a credit to their skill and co-operation. In the statistical treatment of the results, I have had the help of Mr. D.A. Sholl, B.A., of the Department of Anatomy, University College, London. Many of the line drawings are the work of Miss E.R. Turlington, the Departmental Artist.

Through the kindness of the Editor, Professor C.M. West, and the Editorial Board of the Journal of Anatomy, I have been allowed to reproduce a number of figures from the papers in that Journal.

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INTRODUCTION

The processes of regeneration and repair in the tissues of the body have an obvious interest and importance and have been much investigated. The healing of bones, blood vessels, lymph vessels, skin, nerve and muscles have all been studied clinically and experimentally. Bones and skin are the tissues which have been most thoroughly investigated because of their very special importance for the well-being of the individual.

Though these processes can be watched and partially understood by a qualitative approach, full understanding and reasonable certainty of prediction can only be achieved when quantitative methods are used. Owing to the variations which are inherent in biological processes, much of the treatment of the results has to be on a statistical basis. With the help of various techniques, it should be possible to apply these statistical methods to most tissues of the body but nerve is specially susceptible to this treatment.

The injuries to the peripheral and central nervous systems encountered during a War give rise to many problems in diagnosis, treatment and prognosis. The Medical Research Council of Great Britain (1920, 1943) and Seddon (1948) have published reports on various espects of the nerve injury problem and a large amount of work was done on the experimental aspects of the problems by

Professor J.Z. Young in this country and Professor P. Weiss in America. The earliest works on regeneration of nerves known to the present writer are the papers by Cruikshank (1795) and Haighton (1795) in the Philosophical Transactions of the Royal Society, London. Cruikshank cut the vago-sympathetic trunk in dogs (the sympathetic he called intercostal) but did not allow sufficient time for regeneration. He described the neuromas at the cut ends of the nerve and the "coagulable lymph" between the ends which was "the same colour as nerve but not fibrous." He also remarked on the changes in the eyes but whether these were pupillary is not clear from the description. Haighton cut the vagus and allowed up to nineteen months for regeneration. By observing the effects produced by recutting the nerve he considered that the nerves had regenerated. He makes the statement "I am persuaded that anatomy can determine only the presence and existence of a uniting medium; but it is the province of physiology to decide whether the medium of union possesses the characters, performs the functions, of the original nerve."

The process of regeneration of nerve fibres must, as reemphasised by Young (1942), include not only the return of the nerve to the normal anatomical state but also the return to full functional activity. In this thesis the word <u>maturation</u> is used to describe the extent to which the regenerating tissue (nerve fibres) approximates to the supposed normal adult state (mature) morphologically and functionally.

The process of regeneration of the nerve fibres is dependent on the integrity of the nerve cells in the grey matter of the spinal cord or brain stem and the posterior root ganglia associated with the cranial and spinal nerves. Damage to the central cell bodies occurs in injury (fracture) and disease (anterior poliomyelitis or herpes zoster). The degree of damage to the cell body will vary considerably but our knowledge of the process of recovery in the cell body is now much more complete than it was. Studies in poliomyelitis (Bodian,Howe and others) are yielding much valuable information. Other factors have a marked effect on the process of regeneration. The effects of the size of the Schwann tubes, both in the pathway central to the lesion and in the regeneration pathway peripheral to the lesion have been discussed by Boeke (1935), Simpson and Young (1945) and by Hammond and Hinsey (1945). These authors have recorded the results obtained when a nerve with large diameter fibres is made to grow down a pathway made up of small fibres. Simpson and Young united an intercostal nerve (a mixed somatic motor and sensory nerve) to the anterior mesenteric nerve, which is chiefly composed of non-myelinated fibres. The somatic nerve regenerated but the nerve fibres were much restricted in the anterior mesenteric pathway. They were however myelinated. Hammond and Hinsey also showed that the size of the fibres above the crush or union has a marked effect

on the size of the regenerating fibres. Boeke stressed the effects of the size of the Schwann tubes on the regenerating fibres.

It is now clearly established that a most powerful influence is the contact which the regenerating fibres make with the end organs (Sanders and Young 1944; Weiss and Taylor 1944; Sanders and Young 1945; Weiss, Edds and Cavanaugh 1945; Sanders and Young 1946). Langley mentions this effect on normal nerves as early as 1922 but how this peripheral influence effects the fibres is still not known. Langley writes "I would suggest that one of the main factors in determining size (of the nerve fibres) is the nature of the tissue in which the fibre ends." The fact that in most muscle nerves there is a sensory as well as a motor component adds further complications, because the effects of the peripheral stimulus has probably a different rate and degree of action on sensory and motor fibres.

The details of the innervation of striated muscle have been considered in the last few years by Hinsey (1934), Tower (1935), Gutmann and Young (1944) and Couteaux (1947). The late E.B. Carey has published a series of papers on the structural and chemical changes which occur in the region of the motor end-plate during activity. The end-plate is now generally accepted to lie under the sarcolemma, which membrane becomes continuous with the neurilemma in the region of the end-plate. The

fine nerve branches are apparently free from any myelinated covering and they lie in grooves in the sarcoplasm. Around the fine nerve fibres, the sarcoplasm does not have any cross-striations and the inner end-plate nuclei lie in this clear sarcoplasm. The more granular outer end-plate nuclei lie on the surface of the sarcolemma covering the end-plate.

A problem which has aroused much interest and controversy concerns the number of end-plates which are present on a single muscle fibre. Garven (1926) illustrates two end-plates, one at each end, of a long fibre of the panniculus carnosus of the hedgehog. Technical problems make it very difficult to isolate mammalian muscle fibres in their total length and at the same time employ techniques of staining which show up the nerve fibres and endings. In reptiles and amphibia, the difficulties are not so great however and after staining with methylene blue or gold chloride, the muscle can be macerated and the fibres examined. The consensus of opinion, summarised by Wilkinson in 1929, is that only one ending is present on a muscle fibre. Evidence both physiological and anatomical is accumulating which indicates that Wilkinson may be wrong in his views.

Attempts have been made in the past to produce an excess of end-plates in a muscle (this condition was called "hyperneurotization" by Erlacker (1914)). The

results obtained by implanting 'foreign' nerves in a muscle with an intact 'native' nerve supply, have not been consistent. In most reports, the findings were based on physiological tests of transmission between the implanted nerve and the muscle, more than on the subsequent histological examination of the muscle.

Gersuny (1906) is reported by Steindler (1916) to claim that functional endings were made by a "foreign" nerve implant in a normal muscle of dogs. This was confirmed by Erlacker (1914) working on monkeys and guinea pigs. Steindler (1916), working on dogs, and Elsberg (1917) working on rabbits, both state that functional connexions were <u>not</u> made by implanted nerves in a normally innervated muscle. In almost all the papers which report on the histological findings drawings are given with few photographs and in some pictures it would appear that other tissues (e.g. blood vessels) were stained as well as the nerves. The techniques employed were not sufficiently discriminating or critical to allow definite statements about the morphological results, even though contraction of the muscles may have been obtained by stimulation of the implanted nerves.

In all this work it would appear that the operation of implantation and the methods used to retain the nerve in the muscle (stitching) would cause considerable injury to the muscle. If muscle fibres were damaged or the normal nerve supply to the muscle was cut, then a new end-

plate would form on the denervated muscle fibre and on stimulation of the nerve implant, a small contraction of the muscle would be recorded.

Implantations of nerves into denervated muscles were reported by Steindler (1916), Elsberg (1917), Weiss (1930) Fort (1940) and others. Reinnervation of the muscles in varying degrees is claimed in all cases. Fort, working with Weiss, has recorded the results of experiments on toads. He carried out both physiological and histological examination of the nerves and muscles and found that a "foreign" nerve implanted into a muscle with an intact nerve supply, did not make a functional connexion. The nerve fibres were seen to end blindly with no production of end-plates.

In this thesis an attempt is made to answer the following questions:-

1. Does the nature of the periphery into which a nerve fibre regenerates have an effect on the process of regeneration?
2. If so, what are the factors involved?
   a) The length of the pathway of regeneration.
   b) Different obstacles in the regeneration pathway such as smaller Schwann tubes and the scar formed when one nerve is united to another.
   c) The size of the muscle into which fibres grow.

d) Effect of making functional connexions with denervated end organs after union of a regenerating nerve to another nerve connected with its muscle.

e) Effect of forming new end organs which are functional (implants).

3. Do nerve fibres which are regenerating but are unable to reach an end organ and have formed a neuroma in fascia return up the nerve trunk?

4. It is possible to reinnervate a paralysed muscle by the implantation of a muscle nerve?

5. Is it possible to superinnervate a normal muscle?

METHODS

All the experiments were performed on rabbits. No attempt was made to use only one breed and sex but all animals were fully grown at the beginning of the experiment and at the biopsy most of them weighed about 2.5 kilograms.

In most of the experiments the nerve to the medial head of gastrocnemius (n.g.m.) in the hind limb was used. By careful dissection, it is possible to separate about 4 cm. of this nerve from the main sciatic bundle. N.g.m. passes unbranched into the belly of the muscle and there divides to supply the muscle. In all the regeneration experiments, the n.g.m. was crushed high up in the thigh with a pair of smooth fine watchmaker's forceps. Firm pressure was maintained for about 10 seconds and then the forceps were removed. It was found important to use fine smooth forceps, as toothed or dissecting forceps produced more extensive damage. The object was to interrupt the axons but to injure the neurilemmal tubes as little as possible. Weiss and his collaborators have used forceps with milled edges and in many cases the damage was very extensive, as can be shown by the time taken for the regenerating fibres to grow through the crushed region in his experiments. The site of the crush was usually marked by a drop of indian ink injected into the nearby muscle or a small piece of black ligature thread sutured near the site.

According to the purpose of the experiment the lower end of n.g.m. was:-

1. Left intact.

or 2. Cut and allowed to form a neuroma in the fascia.

or 3. Cut and united to its own peripheral stump or other muscle or sensory nerve.

or 4. Cut and implanted into an adjacent muscle.

The method of uniting the two ends of nerve was by the use of human fibrinogen which was coagulated with human thromin. The end of the nerves were brought close together and a drop of the mixed fibrinogen and the thrombin was placed over them. The amount of fibrin used should be as small as possible so as to facilate dissection at the biopsy. If too large a quantity is used, the nerves are found to be embedded in a mass of firm fibrous tissue and are difficult to isolate.

In previous work by Professor Young and his collaborators, coagulated cockerel plasma was used. The preparation of this is more difficult than human fibrin. The human thrombin and fibrinogen are obtained in sealed sterile tubes from the Lister Institute for Medical Research and by the addition of sterile water and sterile normal saline the solutions are prepared. A small quantity of the fibrinogen solution is placed in a sterile vessel and the thrombin solution is added from a sterile pipette. The solutions are mixed and as a syrupy consistency developes,

a drop of the mixture is placed where it is required. There appears to be little tissue reaction to the fibrin.

All the operations were performed under aseptic conditions and the animals were anaesthetised with Nembutal and ether and the experiment was terminated by biopsy 100 days after the operation in most cases. The sciatic nerve was exposed and the n.g.m. dissected out; the site of the crush was identified and the nerve stimulated with an induction coil and platinum electrodes. For histological examination, the nerves or muscles were removed and fixed according to the details given in the Appendix.

Owing to the great variation in the number of fibres in a regenerating nerve at different levels, the counts were made on sections cut about 1.5 c.m. distal to the crush. The sections were enlarged 750 times and projected directly onto bromide paper for photography. The number of fibres in these photographs was then counted. The fibres were grouped according to their size, for convenience in divisions of 2 microns. The size was determined by the use of dividers or a Perspex 'grid' with circles of the appropriate diameter, 1.5 mm. for the 0-2 microns group, 3 mm. for the 2. 1-4 micron group and so on. An estimate was made of the diameter of oval or crenated fibres and solid dots under 2 microns were considered as myelin debris. No attempt was made to count the non-

myelinated fibres as the staining methods were unsuitable.

The muscles used for the implantation experiments were biceps femoris and gastrocnemius lateralis. In some experiments the muscles were denervated and in others they were tenotomised. The implantation was made by carefully inserting the n.g.m. or peroneal nerve into the muscle by means of a round bodied needle. Every effort was made to do as little damage to the muscle as possible. The implant was held in position by means of coagulated human fibrin. This produced much less damage to the muscle and the nerve than stitching. If bleeding occurred during the operation of implantation, then another site was chosen. It was felt that the excessive extravasation of blood would complicate the process of nerve regeneration and prevent the new nerve fibres coming in contact with the muscle fibres.

At the biopsy the nerve was identified and by means of an induction coil, the threshold of response was determined. The nerve was then cut centrally above the crush and stimulated, crushed as it entered the muscle and again stimulated. At each stimulation the response of the muscle, if any, was noted. The nerve was then cut on the surface of the muscle and removed for histological examination; the site of the implantation was marked with indian ink and the muscle excised. The muscle was placed on a card and immersed in fixative solution. So that the

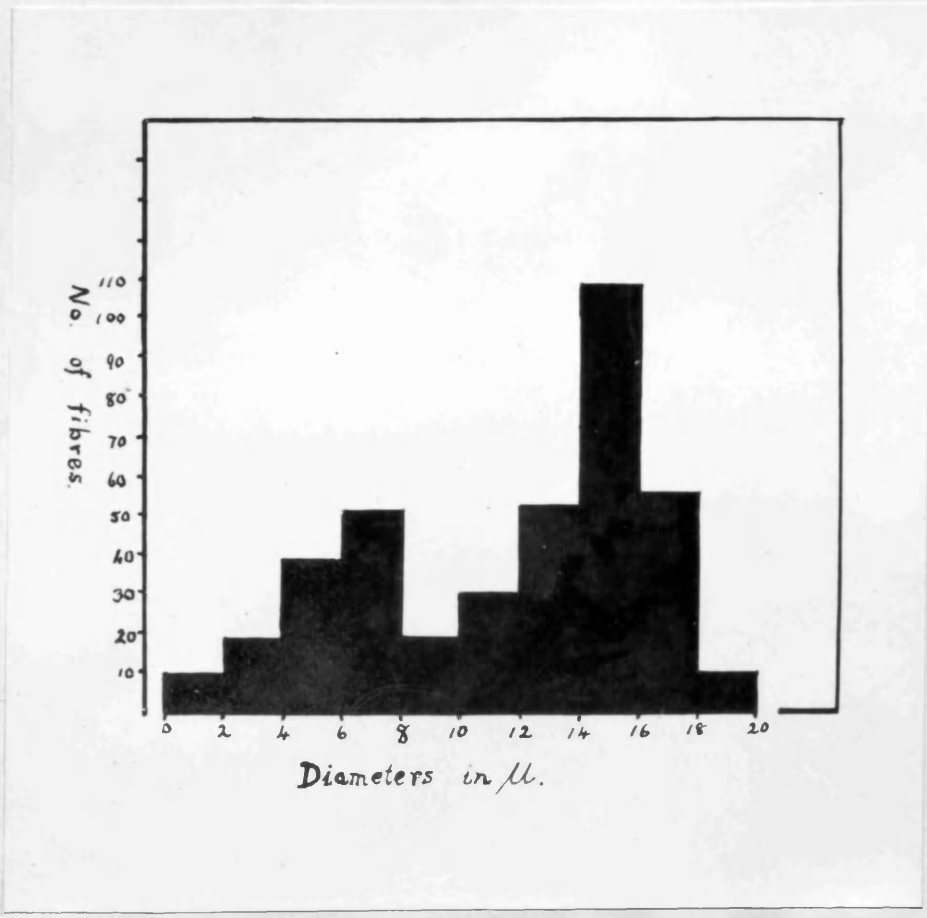
muscle could be accurately orientated later the upper end was marked with indian ink.

Some of the regenerating nerves, like most normal muscle nerves, have a fibre population which is distributed bimodally. The analysis of these distributions is statistically difficult and as an approximation the root mean square diameter (D) was calculated in some instances (Sanders & Young 1946). In some experiments it was possible to calculate a regression curve and various significance tests have also been used. This figure 'D' gives an estimate of the diameter of a 'typical' fibre of the nerve such that a number of fibres, equal to that in the nerve and each of this diameter, would have the same volume of axoplasm and myelin per unit length as is actually found distributed over the range of fibre sizes. Using 'D' diminished the effect of large numbers of small fibres, many of which may be cut twice, and magnifies the effect due to the larger fibres.

The Nerve to the Medial head of Gastrocnemius (n.g.m.)

As most of the experiments to be described were performed on n.g.m., it is necessary to describe in more detail the constitution of the nerve. It runs for about 4 cm. as a separate fasciculus in the sciatic trunk and leaves the parent nerve in the popliteal fossa to reach the gastrocnemius muscle. During its course in the thigh, it gives off no branches. Table 1(a) shows the results of differential nerve counts on seven intact nerves from animals of varying breeds, sex and weights. The left and right nerves differ very little but there is a recognisable difference between one animal and another. The extent of this last effect was not realised at the beginning of the investigations but in later experiments, care was taken to have a control experiment on the opposite side in each animal.

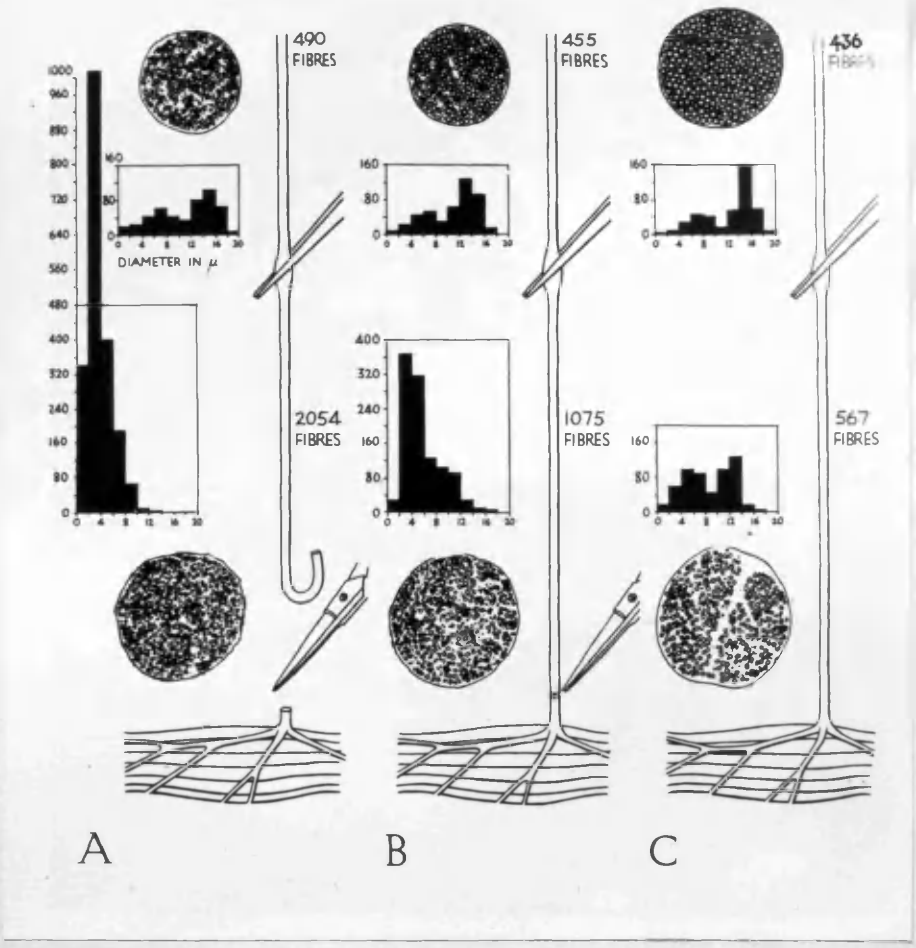
It will be noted that the distribution of fibres in the two counts made low down and high up on nerves 1619c, 1619d, and 1620c, 1620d, show that the number of fibres does not vary greatly at the two levels, nor do the fibres appear to decrease in diameter as they pass distally. Causey (1948) has confirmed these observations. As in many muscle nerves the distribution of fibre sizes is markedly bimodal with peaks at about 7 microns and 15 microns and few fibres at 10 microns. Text figure 1 is a histogram



Text Figure 1. Histogram of the fibre population of a normal n.g.m. (1619c). The total number of fibres is 392. Note the bimodal distribution of the fibre sizes with peaks at about 7 microns and at 15 microns.

of one of these nerves in Table 1a (1619c) and figure 1 is a photograph of the cross section of the nerve.

In each n.g.m. there will be both motor and sensory fibres. Eccles and Sherrington (1930), working on the cat, state that the sensory fibres make up about 30% of the total number of fibres in n. gastrocnemius lateralis. The rates of regeneration in motor and sensory fibres are probably different but little is known of the details of sensory regeneration and few, if any, muscle nerves are purely motor.



Text-fig.2. Diagrams illustrating three types of experiments and the results obtained. In all cases the nerve (n.g.m) was crushed high up. In A, the nerve was cut out of its muscle and allowed to form a neuroma in the fascia. In B, the nerve was cut and then reunited to its own peripheral stump and in C the nerve was not interfered with distally. Comparison of A & C brings out the very marked effect produced by the possibility of the regenerating fibres making a functional connexion with an end organ. Note the differences between the cross-sections of the nerves and between the histograms.

EXPERIMENTS AND RESULTS

Section 1. Does the nature of the periphery into which a nerve fibre regenerates have an effect on the process of regeneration? (Aitken, Sharman & Young, 1947.)

To answer this question, the n.g.m. was isolated, crushed as high as possible and the length of the regeneration pathway kept standard. Table 2 shows the results obtained in three sets of experiments:-

a) Cutting the n.g.m. distally out of the muscle and allowing it to form a neuroma in the intermuscular fascial spaces (4 animals).

b) Cutting the n.g.m. distally and reuniting it to its own peripheral stump (6 animals).

c) Leaving the n.g.m. intact distally (2 animals).

The effects are summarised in Text figure 2. When the sections of the regenerating nerves are examined it will be seen that when the regenerating fibres are unable to reach an end organ and have formed a neuroma in the fascia (A)there is a large number of very small fibres and very few large fibres. On the other hand the section of the regenerating pathway in C (with the uninterrupted peripheral connexions) shows numerous large

fibres. Histograms of the spectra of the nerve fibre populations confirm these observations and show that in A the great majority of fibres are under 6 microns in diameter and very few are over 6 microns in diameter. In C the spectrum approaches that of an intact n.g.m. It is obviously bimodal with peaks about 5 microns and 13 microns whereas A is unimodal with the peak about 3 microns. Comparison of the total number of fibres shows that in A (forming the neuroma) there are 2054 fibres whereas in C there are 567. In four out of five nerves which were allowed to form neuromas, there were over 2000 fibres and in the fifth there was 1586 fibres. In Table 1, the total number of fibres in seven normal nerves is shown to be between 354 and 467 with a mean at 399.

From this it will be seen that there is a four or fivefold increase in the number of fibres between the crush and the neuroma. Above the crush, there is a slight change in the histogram and in the photograph of the cross section. There appears to be fewer large fibres and more smaller fibres but the spectrum of fibre size population is still bimodal as in an intact nerve.

The experiments represented by B give results which are between A and C. There are special obstacles in these experiments in which a nerve is reunited to its own peripheral stump because both ends of the nerve shrink and retract so that the union between them is never very close.

The regenerating fibres have to cross a gap which is bridged by coagulated fibrin and as it is impossible to ensure correct connexions many fibres go astray. Many fibres may escape into the surrounding fascia and end blindly. The importance of the fascicular arrangement within larger nerves has been shown by Sunderland (1945).

When a neuroma is formed in fascia, the regenerating nerve fibres grow to the ends of the Schwann tubes and then grow into the fascia. The neuromas contain a large number of fibres whose growing ends seem to pass between the existing fibres so that a tangled mass results. The formation of neuromas, with the resulting non-maturation of the regenerating fibres has been investigated by Sanders and Young (1944) and Weiss and Taylor (1944). These workers have stressed the fact that the increased number of regenerating fibres which occurs below a crush lesion travels down the regeneration pathway and then enters the fascia between the muscles. Most of the fibres become intertwined and form a mass of nerve fibres in the neuroma. The great majority of fibres remain small (under 4 microns in diameter) though their number diminishes as counts are made at higher levels between the neuroma and the crush.

If the nerves are left for periods longer than 100 days (Table 2 (d) and (e)) then little further change seems to occur. When sections are cut at different levels

in the regenerating nerve above a neuroma (Table 2(a) 1680 a. and h.), marked differencies in the fibre counts are found but they are mostly in the number of small fibres. Later (Section 3) it will be shown that fibres turn in the neuromas and then pass back up the central stump of the regenerating nerves as was suggested by Weiss et al (1945). The number of small fibres is greatest in the sections nearest to the neuromas.

When, in the experiments represented in Text figure 2c, the nerves were crushed only, the regenerating fibres would grow along the original Schwann tubes into the muscle. Each tube is seen to contain a number of fibres but one is usually much larger than the others. In all probability, this large fibre has come into contact with an end organ (motor end-plate) and this anatomical connexion, with the possibility of functional activity, has resulted in a marked increase in its axon diameter and in the thickness of the myelin sheath.

Examination of the nerves above the site of the crush shows that the process of regeneration has effected this proximal portion of the nerve. The changes are not very marked but in Text figure 2c, which has had the minimum of interference, the count is higher than the normal range. The effects of the regeneration are more marked in Text figure 2A in which a neuroma has formed.

In all the experiments considered in Table 2 and represented in Text figure 2, the central connexions

and the regenerating pathway are the same in all cases. Interference with the local blood supply was minimal and all nerves were treated similarly at operation. The principal blood supply to the nerves is through the longitudinal plexus in the nerve (Adams 1942, 1943; Bacsich and Wyburn 1945) supplemented by regional branches which enter the nerve at different levels. When the nerve is crushed, the longitudinal plexus will be blocked temporarily but recanalisation probably occurs rapidly. The great difference between the groups of experiments is the presence or absence of a peripheral connexion with which the regenerating fibres can make an anatomical and functional connexion.

The effect of the peripheral influence on regeneration are such that, where there is a certainty of the regenerating fibres making an anatomical and functional connexion with an end organ, there will be produced a nerve which, in cross-sectional appearance and in the histogram of the fibre population, is not unlike an intact nerve. That is, maturation of the regenerating fibres is good. Comparison of figure 2 and figure 3 shows clearly the difference between the fibre content of a nerve forming a neuroma (figure 2) and a nerve which is directly connected with end organs (figure 3).

Section 2.

Investigation of some of the factors involved in the peripheral effect.

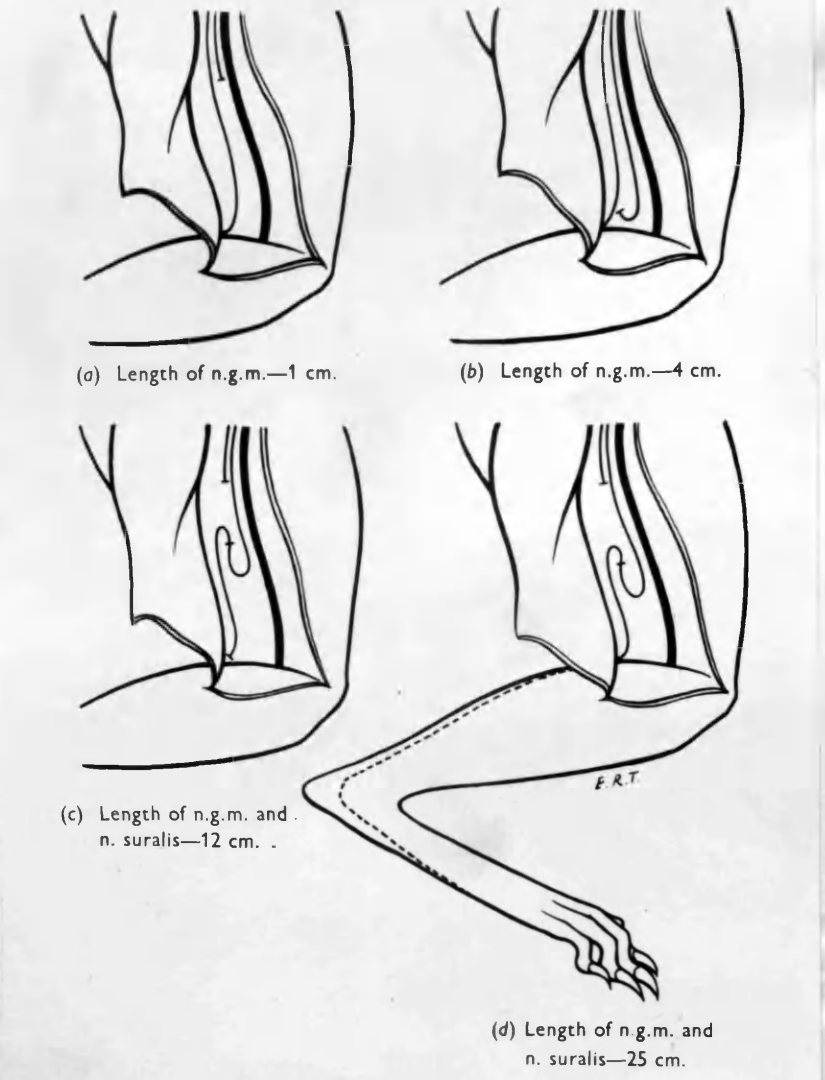
2. (a) i. The effect of the length of the pathway of regeneration (Aitken 1949).

In some animals, lengths of n.g.m. of 1 cm. and 4 cm. between the crush and the cut were taken and in other animals the n.g.m. was crushed proximally, cut distally and joined by coagulated fibrin to the distal cut end of the sural nerve. By these methods, it was possible to obtain a pathway of regeneration up to about 25 cm. in large rabbits. The method is illustrated in Text figure 3.

The experiments yielded data for the following lengths of regeneration pathway:-

a) Regeneration along 1 cm. of peripheral path-n.g.m. only.
b) Regeneration along 4 cm. of peripheral path-n.g.m. only.
c) Regeneration along 12 cm. of peripheral path-n.g.m. and n suralis cut in calf.
d) Regeneration along 25 cm. of peripheral path-n.g.m. and n. suralis connected with peripheral end organs in the skin.

The differential counts of the fibre sizes obtained from sections of the nerve about 1.5 cm. below



Text-fig. 3. Diagrams showing the four types of experiment to determine the effect of the distance travelled by regenerating fibres on the maturation of the nerves. In (a) and (b) n.g.m. only was employed but in (c) and (d) the n.g.m. was united with fibrin to the n. suralis. In all cases, n.g.m. was crushed as high up as possible.

the crush are shown in Table 3. In the shortest peripheral path (1 cm.) the counts were made on sections cut as near as possible to the neuroma. Also in Table 3 are given the total number of fibres in the sections, the number of fibres larger than 6 microns and the mean diameter of the latter fibres.

Table 3 (a) shows the figures obtained from the group of nerves which regenerated along a peripheral path of 1 cm. The total number of fibres is always large. In five specimens out of six it is over 2000 and in one of these five it is 3625. The great majority of these fibres are small in diameter. As these counts were made near to the neuroma, a large number of the smaller fibres will be counted twice as they turn and pass back up the nerve from the neuroma (see Section 3). The mean diameter of the groups of fibres more than 6 microns in diameter in all these nerves is 7.33 microns.

Table 3 (b) shows the differential counts on those nerves in which the pathway of regeneration was 4 cm. long. The fibres were fewer (never more than 1756) but the number of fibres over 6 microns in diameter was increased and the mean diameter of all the fibres over 6 microns in this group was 7.68 microns.

Table 3 (c) and (d) contain the counts made on the groups of nerves which had the longer pathways - 12 cm. and 25 cm. respectively of n.g.m. and n. suralis. These figures show a continuation of the general trend,

namely a diminution in the total number of fibres and an increase in the number over 6 microns in diameter. The mean diameter of all the fibres over 6 microns increases to 7.98 microns in the 12 cm. group and to 8.74 microns in the 25 cm. group.

On examining the figures in Table 3, and bearing in mind the results to be reported in Section 3, it was decided to consider only the larger fibre groups (i.e. those over 6 microns in diameter) and the mean fibre diameter of these fibres was taken as an index of maturation in the nerve. In this way, it was hoped to prevent the number of small fibres masking the effect of any change in the number and distribution of the larger fibres. A series of mean fibre diameters was obtained from each set of comparable experiments and considered as a sample of nerves regenerating for a given length.

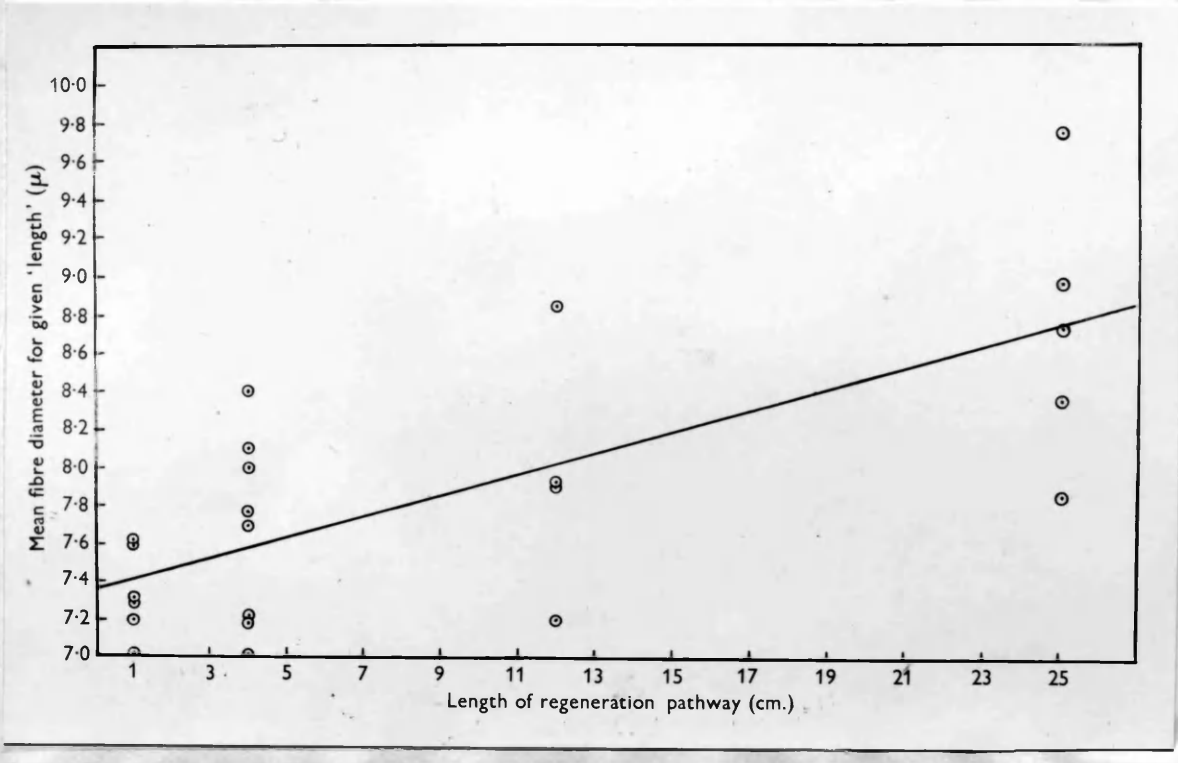
Is there any simple relationship between the degree of maturation and the length of the regeneration path? Inspection of the figures in Table 3 suggests a high degree of correlation between maturation and length regenerated, but the estimation of the correlation coefficient would only give the extent of the connexion between the two variables. It would not give any information as to the degree in which maturation is affected by the changes in the length of the path.

If, however, the mean maturation estimations for all the nerves are plotted against the length

regenerated, it appears that there is some reason to anticipate a linear relationship between the two variables. That is to say, we may reasonably expect a significant linear regression of maturation on length of regeneration pathway. This line was computed and is shown in Text figure 4.

This indicates that, over the range of the experiments, there is an increase of 0.5 microns in the mean diameter of the larger regenerating fibres in each 10 cm. travelled. That this effect is a real one, and not due to chance, is shown by the regression coefficient which is significant on the 0.001 level ($t=4.7$, $f=21$).

From these experiments, we can definitely say that regenerating fibres which grow for longer distances become larger. Nothing very definite can be said about the form of the relationship, the diameter more nearly follows the length than its logarithm, but other possibilities are not excluded. It must also be remembered that the very longest fibres make different endings from the others - in the skin rather than in a neuroma.



Text-fig.4. Linear regression of degree of maturation on the length travelled by the regenerating nerves, maturation being measured as the mean diameter of the larger ($\rangle$ 6 microns in diameter) fibres.

2. (a) ii. Time available for the process of maturation.

Though all the experiments lasted for a period of 100 days, there would be great differences in the times which would be available for the process of maturation after the regenerating fibres reached the ends of the Schwann tubes. The peripheral pathway was either 1 cm., 4 cm., 12 cm., or 25 cm. and the rate of progress of the growing fibres can be estimated from data published by Gutmann, Guttman, Medawar and Young (1942). These authors state that 5 days should be allowed for the fibres to cross the crush and 5 days to cross the union. The growth rate above the union is estimated as 5 mm. per day but only 3 mm. per day below the union. Using this data, the times, which are summarised in Table 4, are obtained.

In the longest nerves (25 cm. in length) many of the fibres would be actively growing until about 12 days before the biopsy. These specimens which formed neuromas below the knee (12 cm. in length), would have 55 days in which to mature before the biopsy. In spite of this however, as is clearly seen in Text figure 4, the mean diameter of the fibres in the former is much greater than that in the latter.

2. (b). <u>The effect of obstacles in the pathway of regeneration.</u> (Aitken, Sharman and Young 1947).

i. Smaller Schwann tubes in the regeneration pathway.

ii. The scar formed between two nerves when they are joined together.

i. The sural nerve (Figure 4) is entirely a cutaneous nerve and though it has many more medullated fibres than the normal n.g.m., the diameters of the fibres are much smaller, presumably there are none of the larger motor or proprioceptive fibres present. When the n.g.m. is united to the sural nerve and the regenerating fibres are examined in the sural nerve (Figure 5), then it is found that rarely do the fibres reach a diameter of more than 12 microns. It would appear that the Schwann tubes in the sural nerve have a constricting affect on the regenerating fibres of n.g.m.

ii. No matter how carefully the two cut ends of a nerve or nerves are placed together, there is always a measurable gap between them. This is shown in Figure 6 in which the fibres grow across a union between n.g.m. and n. suralis. The use of coagulated fibrin has tended to minimise this gap but it still persists. The union of a cut nerve to its own peripheral stump is usually very

unsatisfactory and it is more difficult to obtain a good result than in union to the cut end of another nerve, owing to the retraction which occurs in the central and peripheral sections of the nerve. Unions of n.g.m. to n.g.m. (Table 2 (b)) are not usually as good as unions of n.g.m. to n. plantaris (Table 5). Admittedly, in the case of unions with n. plantaris, there is the additional factor of the larger size of the plantaris as compared with the medial head of gastrocnemius. This factor will be discussed in the following section. Also, it is impossible to be certain that the fascicular arrangements of the two nerves are corresponding and congruous. Usually there is come twisting of the nerves and as a result motor fibres may grow down to sensory end organs in muscles or joints or even in the skin.

2. (c) The effect of the size of the muscle into which the nerves regenerate. (Aitken, Sharman and Young 1947.)

The plantaris muscle in the rabbit is large compared with the medial head of the gastrocnemius and the results of unions of n.g.m. with n. plantaris are given in Table 5. The results are better than those obtained when n.g.m. is united to itself (Table 2 (b)), and nearly as good as those obtained when n.g.m. is crushed but not cut lower down (Table 2(c)).

Though no counts of muscle fibres were made, plantaris, being the bulkier muscle and having a larger cross section, has presumably a larger number of muscle fibres and a larger number of motor end-plates. When the regenerating fibres cross the union of n.g.m. and n. plantaris they will travel down the Schwann tubes. Attetnion was drawn by Young (1942) to the multiplication of fibres which occurs below a lesion and the increased number of n.g.m. fibres (300-400 is the normal number as shown in Table 1(a)) will be able to pass down the larger number of Schwann tubes of the n. plantaris. Table 1(b) gives the figures obtained from a count of a normal n. plantaris and the total number is about 700. The very much larger number of tubes available will make it possible for a larger number regenerating fibres to make contact with motor end-

plates and so be stimulated to mature.

2. (d). The effect of making functional connexions with existing denervated end organs - degenerated motor end-plates. (Aitken, Sharman and Young. 1947).

When n.g.m. is allowed to regenerate below a crush in such a way that the new fibres travel down Schwann tubes in a nerve which is connected to a muscle, the results are very different to these obtained when a neuroma is formed in fascia, (Table 2, c and a.) Electrical stimulation of the nerves attached to the muscles gave rise to a contraction of the muscle so that a functional connexion had been made. The work of Gutmann and Young (1944) showed that the previously denervated motor end-plates had been reinnervated. The regenerating nerve fibres had grown along pathways of differing lengths into a muscle. Each original Schwann tube connected with a group of motor end-plates and as these were reached by the growing tips, the fibres ceased to grow in length but greatly increased in width and acquired a well developed myelin sheath. The number of small fibres is greatly reduced, probably by absorption above or by degeneration. The number of fibres over 12 microns is considerably larger than in a nerve ending in a neuroma (in which they are rare).

Except in those animals in which the nerves were crushed without any severance from the muscle, (Table 2. (c)), the maturation was very far from that found in a completely

intact nerve (compare Tables 1, 2(b), and 5, and Figures 1, 2 and 3). The main factors mediating against the full recovery of the nerves are the mal-union of the nerves and the fibrosis which occurs along the regeneration pathway and in the muscle itself around the end-plates if there is any delay in the process of regeneration.

2. (e). Effect of forming new motor end-plates.

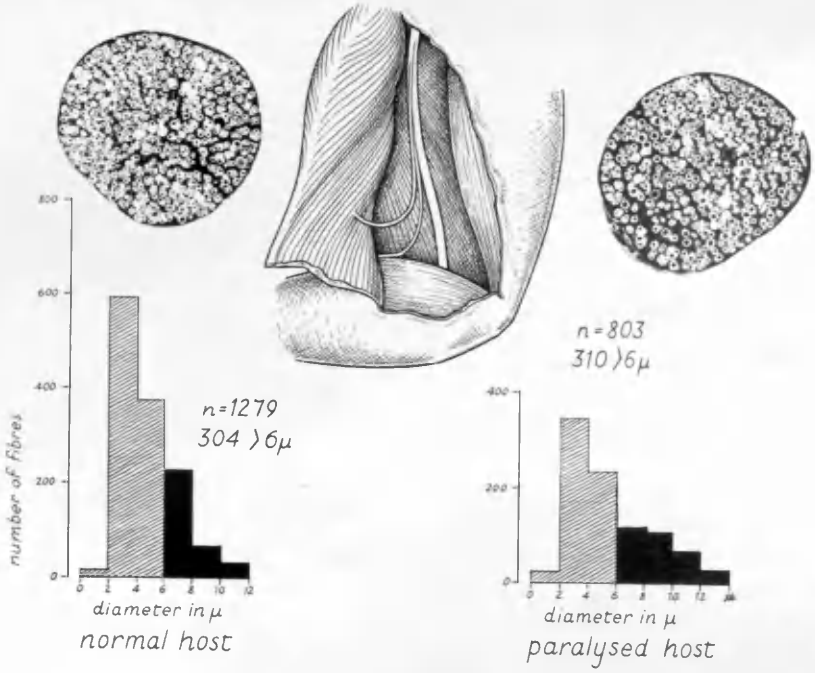
(Aitken 1950)

In these experiments, n.g.m. was isolated from the main sciatic trunk, crushed proximally, cut out of its own muscle and the cut end implanted carefully into biceps femoris or the lateral head of gastrocnemius. In a few animals, the peroneal nerve was implanted into the lateral head of the gastrocnemius without crushing. The nerve implants were held in position in the muscles by a drop of coagulated fibrin on the surface of the muscle. On one side of the animal, the implant was made into a normally innervated biceps or gastrocnemius but on the other side, these muscles were denervated. As the experiments only lasted for 100 days, no special precautions were taken to prevent reinnervation of the denervated muscles beyond severe crushings and cutting of the nerves supplying them. Attempts to formalinise the cut and crushed nerves to biceps

femoris or to the lateral head of gastrocnemius were abandones because of the danger of injury to the nerves in the sciatic trunk and especially to the n.g.m. which was regenerating. Text figure 5 shows the site of the operation on biceps and summarises the results of the histological examination of the experiments using n.g.m.

Examination of the muscles after 100 days showed that there was slight atrophy on the denervated side and almost all the denervated muscles showed some degree of fibrillation. Faradic stimulation of the implanted nerves produced a contraction in most denervated muscles and in a few of the muscles with an intact nerve supply. Platinum wire electrodes embedded in perspex were placed on the nerve 2-3 cm. from the muscle. The nerve was stimulated, then crushed near the muscle and stimulated again. This 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. When contraction occurred it was limited to the region of the implant and fibres which responded to the stimulus did so, as far as could be detected, in their total length.

In Table 6, 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 20 cases of nerves implanted into

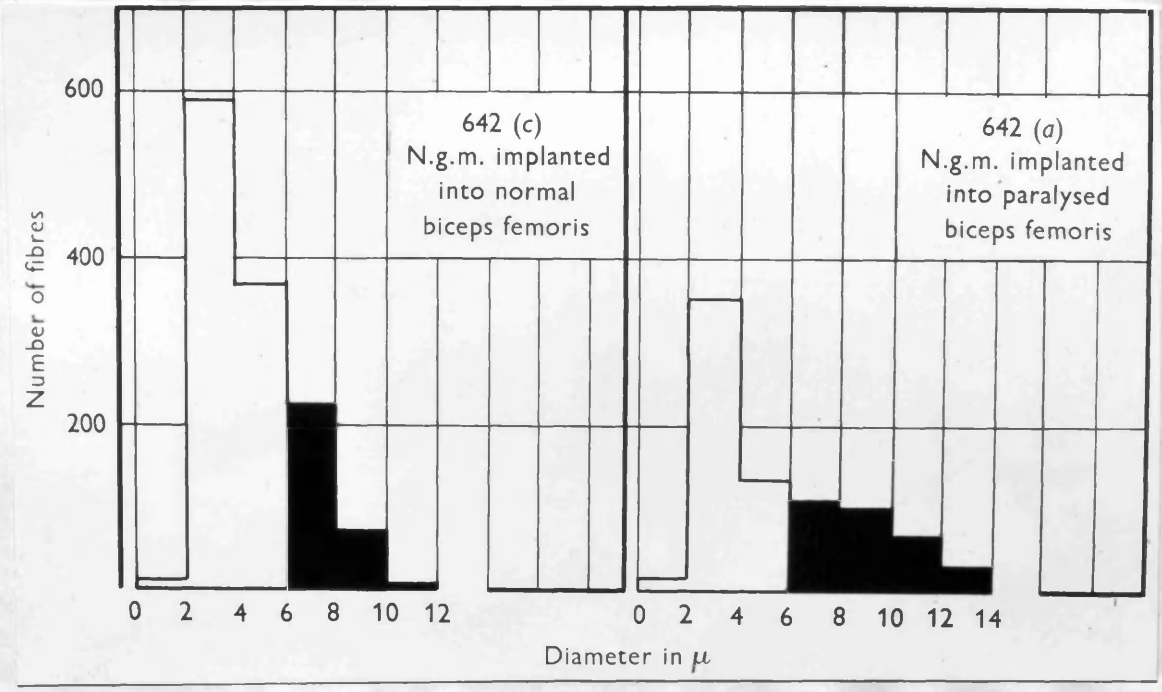


Text-fig.5. Diagram illustrating the results obtained when a regenerating nerve is allowed to grow into a normal muscle or a denervated muscle. Note the great reduction in the small diameter fibres and the increased number of large diameter fibres. The cross-sections and the histograms show this clearly.

denervated muscles (other than those whose nerves came out), and of these, all 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 only three cases was the threshold such as to raise suspicion that the response was due to 'escape' to the muscle fibres.

Out of 17 cases in which implants were made into normal muscles, nine showed a 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. The responses of the normal muscle were much weaker than the responses of the muscles which had previously been denervated. The results shown in Table 6, taken along with the results shown in Table 7 and the histograms in Text figure 6, show that the implant makes a functional connexion more frequently and easily in a denervated muscle than in a normal muscle.

When the nerves to the medial head of gastrocnemius (n.g.m.) were examined histologically, and the fibre populations compared, the figures shown in Table 7 were obtained. The larger fibres (over 6 microns) were again taken as an indication of the degree of maturation. The hypothesis that the distributions of the larger-sized fibres are the same whether the nerve



Text-fig.6. Histograms of the distributions of fibre sizes in regenerating nerves (n.g.m.) after implantation into normal or paralysed (denervated) biceps femoris. Note the reduction in the number of small fibres and the increase in large fibres (especially those over 8 microns) in the nerve implanted into the paralysed muscle.

terminates in a normal or a denervated muscle was made. Calculations on the figures in Table 7 gave the following results:-

Animal	X^2	f	$P(X)^2$
417	50.84	1	0.001
428	11.757	2	0.01
443	0.36	1	0.6
483	13.91	2	0.001
642	118.86	2	0.0001
643	21.8	2	0.001

Except in animal 443 therefore, the hypothesis is rejected in every case. When the total X^2 is considered, $X^2=217.53$, $f=10$ and $P(X)^2=0.001$. The possibility of these samples belonging to the same population is therefore exceedingly remote.

Though the root mean square diameter (D) is apt to mask particular effects, comparison of the figures for D in the two sets of experiments (Table 7) shows that except in one animal (428), the root mean square diameters are much larger on the denervated side than on the normal side. The connexion with denervated muscles has therefore had a pronounced effect on the process of regeneration, the nerves being much more mature than in those nerves connected with normal muscles.

When photographs of the two types of nerves are examined (Figures 7 and 8 taken from animal 642), it is evident that the implant into the normal muscle has many more fibres than the other. Also the Schwann tubes of the implant into the normal muscle are packed with numerous small fibres. The implants into the denervated muscle have fewer fibres and most Schwann tubes are occupied by one large diameter fibre. Histograms of these same two nerves (Text figure 6) show the differences in a very marked way. The peaks appear in each case in the 2-4 micron group but in the nerve growing into the paralysed muscle, there are less than half the number of smaller fibres found on the other side (implant into normal muscle). The total number of large fibres is not very different but whereas in the implant into the normal muscle there are 10 fibres over 10 microns in diameter, on the paralysed side there are 95.

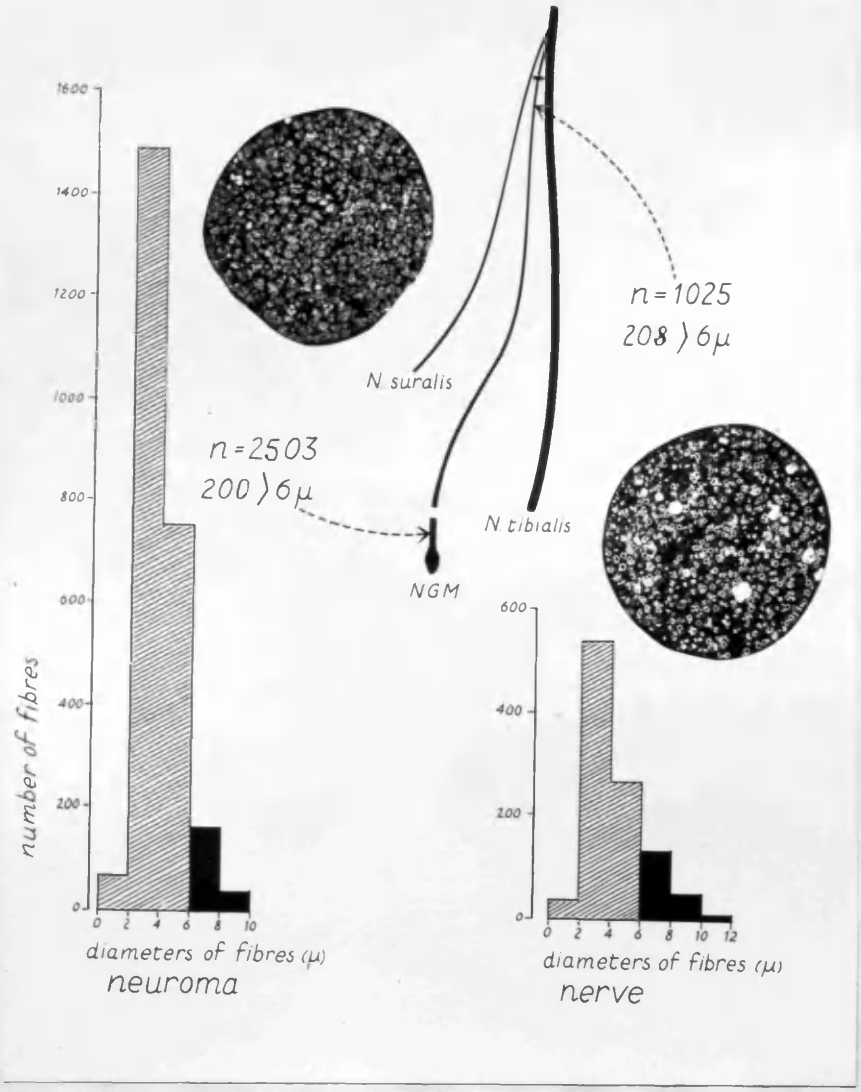
Histological examination of the muscles was done and the results are reported in Sections 4 and 5. It will be shown that many new motor end-plates formed in the paralysed muscle whereas in the normally innervated muscle, the implanted nerves formed long tortuous fibres with little or no attempt to form motor end-plates. Maturation of the nerves implanted into the paralysed muscle was much more advanced than in the implanted nerves in the normal muscles and this is correlated with the ability and freedom to form new motor end-plates.

Section 3.

Do nerve fibres which are regenerating and forming a neuroma in fascia return up the nerve trunk? (Aitken 1949)

When the regenerating nerve fibres reach the end of the Schwann tubes without making contact with an end organ, they continue to grow, become intertwined and form a neuroma. It has been suggested by some authors (Weiss et al 1945) that some of the fibres turn back and re-enter the Schwann tubes. These regenerating fibres will be travelling in a central direction (centripetally). This phenomenon was clearly demomstrated in one of the experiments where the nerve(n.g.m.) was crushed high up, and cut low down near to the muscle and allowed to regenerate and form a neuroma. The animal was re-operated on 14 days before the terminal biopsy,and the neuroma,with a short portion of the nerve, was removed. At the end of the 100 day period, the remainder of the regenerating nerve was removed. Differential counts on sections of the two specimens (185e and 185b) gave the figures which are found in. Table 8 and which are summarised in Text figure 7.

Unfortunately, owing to the nearness to the neuroma, it was impossible to obtain a section of 185e (specimen with neuroma) in which the fibres could be counted in all parts of the photograph. Many of the fibres



Text-fig.7. Diagram illustrating the results obtained when the neuroma is removed from the end of a regenerating nerve two weeks before the terminal biopsy. Note the great reduction in the number of small fibres and the relative constancy of the number of fibres over 6 microns in diameter.

were cut obliquely and the picture was confused. An estimate had to be made by dividing the photograph into 1 cm. squares and counting the fibres in as many squares as possible. In this way, the number of fibres in their size groups was counted in 426 sq. cm. The total area of the photograph was determined with a planimeter and made out to be 727 sq. cm. Estimates of the total number of fibres in the size groups was then made.

Examination of the figures in Table 8 shows that the total number of fibres in the nerve removed after 100 days is about half that found in the nerve above the neuroma removed on the 86th day. The greatest differences occur in the small fibre groups - especially those under 6 microns. In the larger fibre groups (those fibres over 6 microns), though there is a difference in the distribution of the fibres, there are 200 fibres in the portion of n.g.m. with the neuroma attached and 208 in the nerve two weeks after removal of the neuroma. The histograms in Text figure 7 illustrate the differences in the fibre distributions and larger photographs of the sections are shown in Figures 9 and 10. It will be seen that the nerve immediately above the neuroma is larger, probably due to the nearness to the neuroma. The Schwann tubes will be seen to be filled with numerous small fibres with thin myelin sheaths. The section of the nerve after removal of the neuroma shows that the Schwann tubes are almost empty. Usually there are one or two fibres and these fibres are

Section 4.

Is it possible to reinnervate a denervated muscle by the implantation of a "foreign" muscle nerve? (Aitken 1950)

The answer to this question is that reinnervation of a denervated muscle is possible to a limited degree. The methods used in these experiments to elucidate this problem are illustrated in Text figure 5. In some experiments the n.g.m. was inserted into the biceps femoris and in others the n. peroneus was implanted into the lateral head of gastrocnemius. In all cases, the host muscle, gastrocnemius or biceps, was denervated by crushing and excising the nerves which supply it. The implanted nerves were left in the muscles for 100 days after which a biopsy was performed. In two animals, the duration of the experiment was 50 days. The results of electrical stimulation of the nerves are given in Section 2(e) and are summarised in Table 6. Histological examination of the nerves is also considered in Section 2(e) and the fibre distributions are given in Table 7. A typical cross-section of the nerves is shown in Figure 8.

The histological examination of the muscles was carried out according to the modification of the Bielschowski-Gros method recorded in the histological appendix. Though serial sections of the muscle were not

obtained, great care was taken that very little tissue was lost and all the sections were examined. Other histological methods were tried. The Holmes (1942) modification of Cajal techniques, using buffered solutions, was tried on celloidin sections after fixation in Bodian's mixture of 80 cc. of 80% alcohol, 15 cc. of commercial formalin and 5 cc. of glacial acetic acid. The nerve fibres showed up well but the terminal branches were not stained (see Figure 11). 5% trichloracetic acid in normal saline was found to be a satisfactory fixative preparatory to paraffin embedding and Bodian's Protargol staining. The results of the Protargol staining were very inconsistant due partly to variations in the samples of the silver proteinate and partly to sifficulties in the cutting of the sections. Though theoretically paraffin and celloidin embedding might be preferred in that serial sections could be obtained, other factors made the choice of a modified Bielschowski-Gros technique the most satisfactory.

Using low magnification, the regenerating nerve fibres are seen branching in an irregular manner amongst the muscle fibres (Figure 12) and new motor end-plates can be seen (Figure 13). The pattern of innervation is similar to that found in a muscle with its normal nerve supply intact in which the larger nerve trunks are seen to run across the muscle fibres and only the small branches run parallel to the muscle fibres. The pattern of the

implanted nerves is quite different to that obtained when a nerve is implanted into a muscle with its nerve supply intact (Figures 22-23).

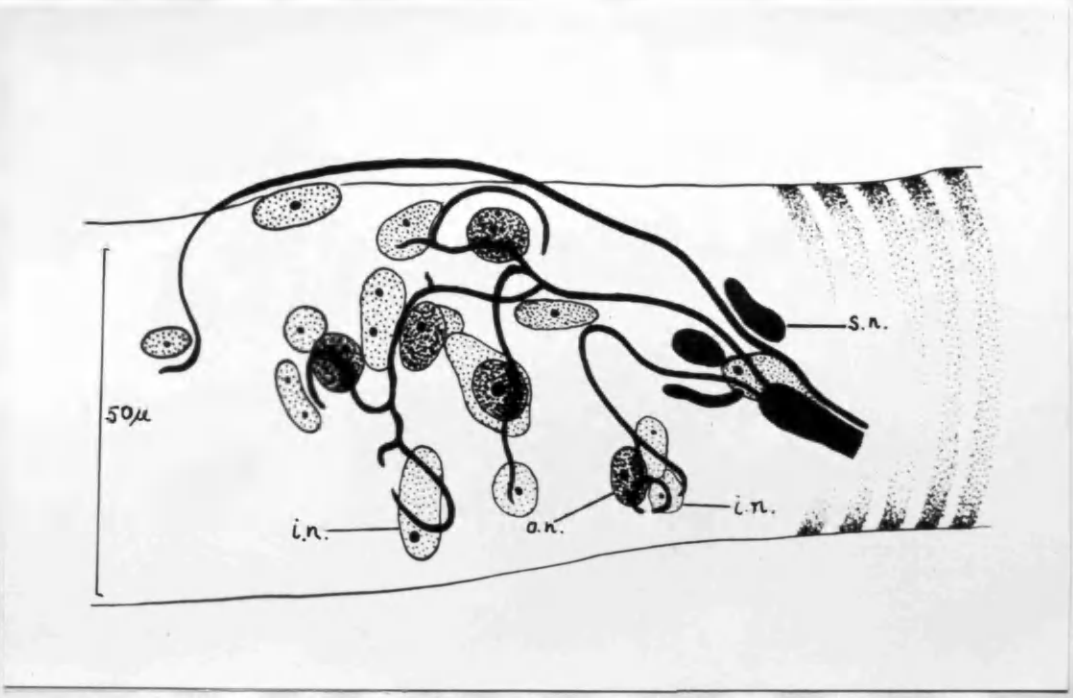
i. Examination of the Neuroma.

In the denervated muscle, most of the nerve fibres are myelinated and, as seen in Figure 13, the myelin sheath is retained to within a few microns of the end-plates. In Figure 14, one of these myelinated axons is seen to divide into three branches. Occasionally a large myelinated nerve is seen to travel for considerable distance. In Figure 15 the fibre travels about 0.8 mm. across the muscle before it divides into the smaller branches which give rise to the end-plates. Beyond the region where end-plates have formed, but in the same plane of section, the nerves travel without branching for long distances between the muscle fibres. These muscle fibres have presumably already been reinnervated and the surplus nerve fibres from the growing end of the implant pass between them. This condition is seen in Figure 16, which shows an adjacent field to that in Figure 12. The picture is very like that obtained when an implant is made into a muscle whose nerve supply is intact (Figure 23).

ii. Motor end-plate production.

The majority of the newly formed 'foreign' motor end-plates differ greatly in size and shape from the 'native' end-plates which are found in a normal muscle with its nerve supply intact, but some are very like typical normal endings. Figure 17 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. Also seen in this figure is a small nerve, showing Schwann nuclei, which finishes as a fine free ending. As the nuclei are very close together at the end of this nerve, it is possible that those which are granular and have an obvious nucleolus are end-plate nuclei or modified muscle nuclei. This may be an early stage in the developement of an end-plate.

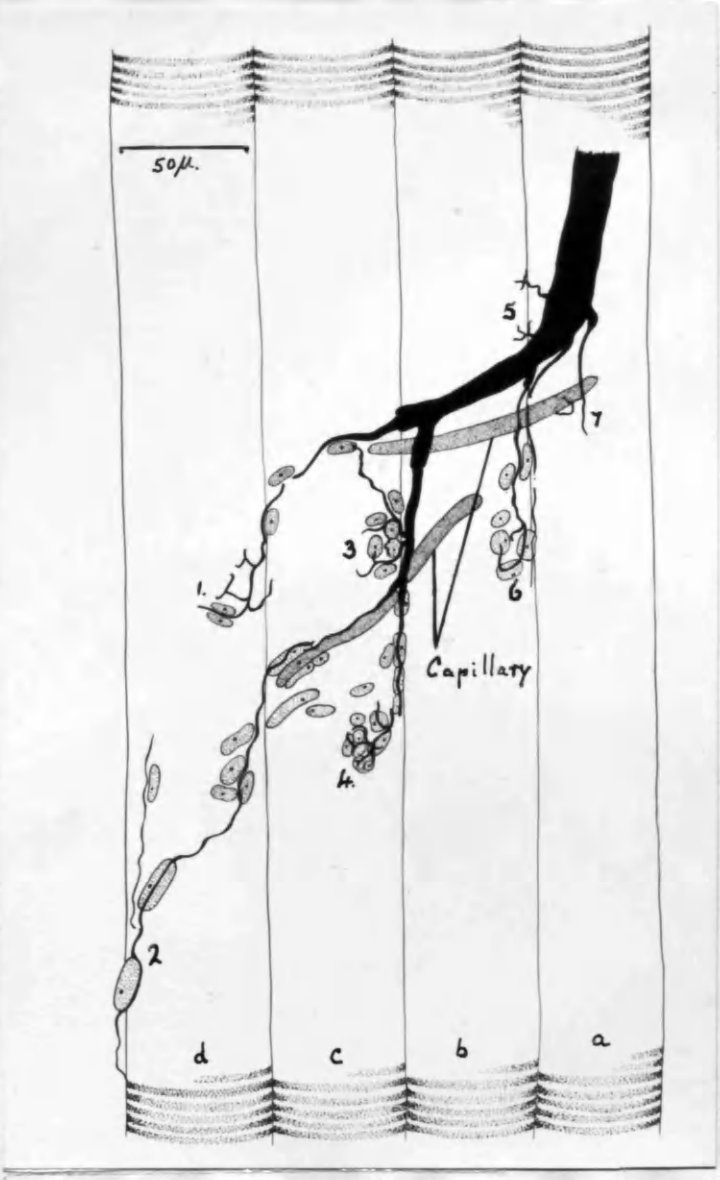
Some of the end-plates are supplied by two fibres derived from different nerves as can be seen in Figure 18. When an end-plate is supplied by two axons, it is usually impossible to say whether it is bisegmentally as well as biaxonally innervated. Is this an example of the reinnervation of an empty normal end-plate? In this series of experiments, little evidence was seen of these denervated end-plates which, according to Gutmann and Young (1944), usually show as more darkly staining areas on the muscle fibres.



Text-fig.8. Drawing of a large motor end-plate on a muscle fibre. Note the Schwann cell nuclei (S.n), the outer end-plate nuclei (o.n.) and the inner end-plate nuclei (i.n.) A non-myelinated fibre passes the main end-plate and ends on an adjacent part of the muscle fibre.

The more atypical plates varied from single large, claw-like endings to large groups of endings on a few muscle fibres. In Figure 19, one of the large claw-like endings is seen. Text Figure 8 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 mm. by 0.05 mm. All of these figures are within the range of figures given by Gutmann and Young (1944) for intact 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. There was no evidence of nuclear activity around this terminal branch.

Occasionally there is found a large axon which divides to supply motor end-plates to a group of muscle fibres. Text Figure 9 is a drawing of one of these large nerves. There are several muscle fibres which have each a well-formed and a very irregular ending. The muscle fibre 'a' has one end-plate, 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



Text-fig.9. Drawing of a single nerve trunk which divides into four principal and many subsidiary branches. On each of the four muscle fibres, there are two or more aggregations of muscle nuclei and clusters of fine nerve twigs suggestive of motor end plates. Some are large but others are very small. A capillary is seen to run diagonally across the muscle fibres. The terminal branches on fibre 'd' are very fine but the nuclei are orientated along the nerve. They are probably Schwann cell nuclei.

muscle nuclei. 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 Figure 20 is a photograph of this group of endings.

In the experiments where the nerves regenerate into a denervated muscle two or more motor end-plates are frequently found on one muscle fibre. Often these two end-plates are close together on the muscle fibre and the question can be raised as to whether they are not parts of a single motor end-plate. Figure 21 shows another example of this. At least four end-plates are present and appear to be on one muscle fibre. Focussing of the microscope shows that they are all about the same level in the muscle. Even if they are on the contiguous surfaces of two muscle fibres then 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.

Examination of the denervated muscles showed that the amount of interstitial fibrous tissue had increased and the fat cells were very conspicuous. The muscle fibres appeared slightly narrower than those found in the intact muscles but measurements were not made on them. It is difficult to control such factors as stretching of the muscle during the biopsy but obviously this must have a marked affect on the cross-sectional area of the fibres and will

tend to mask the affects of denervation atrophy. The differences between the intact 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.

Section 5.

Is it possible to superinnervate a normally innervated muscle? (Aitken 1950)

The results to be reported in this section were obtained from the control sides of animals in experiments on the maturation of regenerating nerves growing into paralysed (denervated) muscle and the controls for the implants in denervated muscles. The nerves were implanted in a normally innervated biceps femoris or muscularis gastrocnemius lateralis. The results of electrical stimulation of the nerves are reported in Section 2(e) and summarised in Table 6. The histological examination of the nerves is reported in Section 2(e). Figure 7 shows the arrangement

of fibres in a cross section of one of these nerves.

The histological examination of the muscles shows that the regenerating nerve fibres grow out from the cut end of the nerve and form a tangled mass of fine fibres (Figures 22 and 23.) Generally speaking the fibres are thin and finely or nonmyelinated. They pass between the muscle fibres and no very obvious cellular reaction was observed either in the connective tissue or the muscle. The fibres could often be followed for long distances and seemed to end in the fibrous tissue between the muscle fibres. The Schwann cell nuclei (Figure 24) varied in their staining properties. Some were intensely black, as though the silver was 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 these nuclei probably belong to fibroblasts. The nuclei always lie in troughs in the undulating fibre. 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 much more infrequently seen (Figure 25.)

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 (Figure 26), then the regenerating fibres pass near to the end-plates but there was no evidence of either attraction or repulsion between them.

These blind ending fibres develope a myelin sheath which is easily seen in some preparations (Figure 27.) This was also seen in the cross sections (Figure 7) of the regenerating nerves which were stained by the Flemming-Weigert method (Aitken 1949.) Though a few fibres reach 10-12 microns in diameter, most of them are about half this size and are not fully matured.

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 carried a native motor end-plate. It is interesting to speculate on the conditions in which a detached portion would call forth a response from the nerves. Figure 28 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 occasionally one 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 presumably contract on stimulation and would be the explanation of the slight 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. Some end-plates may have been missed but it must be supposed that the fine fibres in close proximity to the muscle fibres were able to transmit the impulses from the nerve to the muscle fibre.

Implants into tenotomised muscles

Another variation in the state of the normally innervated muscle into which nerves can be implanted was achieved by excising 1 cm. of the tendo calcaneum. The tenotomised muscles were examined histologically. Though partial regeneration of the tendon had occurred, the gastrocnemius, when stimulated electrically, contracted

weakly but the foot was not plantar flexed.

In these experiments, the results are not so clear as in the others. The native motor end-plates do not appear to be very different from those seen in a normal muscle (Figure 29.)

Some implants (Figure 30) give rise to many long thin fibres which coil up between the muscle fibres and around them. This is not seen in nerves which are growing into normal muscle. Sometimes one of the coiling fibres (Figure 31) is seen to form a large loop when the terminal branch turns back on the main fibre. Smaller side branches leave the same nerve. Many of the fibres, as in implants in other normally innervated muscles, are myelinated, (Figure 32) 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 (Figures 33 and 34.) They travel for long distances and then taper away. No muscle cell reaction or interstitial cell reaction was observed. Myelin sheaths with nodes of Ranvier and Schwann cell nuclei are very prominent.

Careful inspection of the material showed no regenerating nerves which had undoubtedly formed new motor end-plates except a few which were probably on damaged muscle fibres. Such plates as are present are

so typical in their characteristics that they are probably native endings.

DISCUSSION

A. Factors influencing the maturation of regenerating nerve fibres.

The following factors which are known to influence the maturation of regenerating nerve fibres will be considered:-

1. The cell body.
2. The type of lesion.
3. The pathway above and below the lesion along which the fibres have to travel.
4. The effect of making connexion with end organs.
5. The distance which has to be travelled by the regenerating fibres.
6. The time available for maturation.

7. The size of the muscle into which nerve fibres grow.
8. The functional state of the muscle into which nerves are implanted.
9. The effect of overcrowding of the Schwann tubes with regenerating fibres and their possible return up the tubes from a neuroma.

1. The cell body.

Until the recent War (1939-1945) it was recognised that the two important factors in the regenerative process of a nerve fibre were the central connexions and the nature of the regeneration pathway. Damage to the central cell bodies occurs as a result of injury (fracture) or disease (anterior poliomyelitis or herpes zoster). The degree of damage to the cell body will affect regeneration and will vary considerably and our knowledge of the process of recovery of the cell body is still not very complete. The studies of Bodian (1947), Howe and Mellors (1945) and Hyden (1943,1948) have done much to elucidate the problems, but much remains to be done. The effects of an injury on the cell body point to the fact that there is a marked degree of recovery of some cells, but the process of regeneration of the nerve fibre (maturation) is presumably much slower following injury to the cell body than when the cell body is intact.

2. The type of the lesion.

If the damage to the nerve fibres is slight (axonotmesis of Seddon (1943)), then is most cases the nerve returns almost to normal (structurally and functionally). If the damage is more extensive and severe (neurotmesis), the recovery is less complete because many fibres grow down pathways which are too small for them and motor fibres may travel to sensory end organs, or vice versa. At a union of one nerve to another, even under optimum conditions of apposition, a certain amount of scarring occurs and fibres become tortuous and even escape outside the epineurium into the fascia. These conditions all help to produce an imperfect union and hence poor maturation below the union.

3. The nerve pathway.

The diameter of the available Schwann tubes is also known to affect the process of maturation (Boeke 1935). Large motor or sensory fibres can be made to grow down small Schwann tubes in the peripheral pathway. This occurs when a muscle nerve is joined to a cutaneous nerve (n.g.m. to n. suralis) or when a somatic nerve is joined to an autonomic nerve (intercostal nerve to anterior mesenteric nerve - Simpson and Young 1945). The regenerating fibres below the union rarely reach the size of those above the union.

4. The effect of making connexions with end-organs.

The effect of the periphery on the regeneration process is now widely accepted. The idea was probably first suggested by Weiss (1936 and 1941) who held that muscles influence the activity of nerves by conditioning them to conduct messages appropriate to the function of the unit. Sanders and Young (1945), Weiss and Taylor (1945) gave the results of their experiments which showed that the peripheral connexion had a large part to play in the regenerative process.

In the present series of experiments in which the nerves were crushed only and not cut (Text figure 2), the regenerating nerves would grow back along the old Schwann tubes to the end-organs. Some of these will be motor end-plates but others will be sensory endings of different kinds. The fact that the nerves in these cases have a histological picture below the crush so near to that of an intact nerve, indicates that the motor and sensory end-organs can cause almost complete maturation of the regenerating fibres.

5. The effect of the length of the regeneration pathway.

This has been shown by varying the length of n.g.m. and uniting it with differing lengths of n. suralis. In this way it is possible to make the regenerating fibres travel up to about 25 cm. and to show that the further

they travel, the bigger they become. The groups of experiments where the pathways were 1 cm, 4 cm and 12 cm, all ended in neuromas in the fascia. The 25 cm. pathway ended in the skin. It is probable however that the nerves are all nonfunctional. Electrical stimulation of n.g.m. after cutting the central end failed to produce a response in any experiment and in the experiments with the longest pathway, nipping of the foot pads caused no visible withdrawal of the foot of the rabbit. However, where the n.g.m. has been united with n. suralis, certain other factors are introduced and must be considered. The union of the nerves, the presence of the smaller Schwann tubes in the sensory nerves and the presence of sensory end-organs in the skin are the most important factors, which might be expected to impede maturation in the longer lengths of nerves. But, in spite of this, the fibres which travelled the furthest were the most mature.

The effect of the length of the nerve regenerated was investigated by Sanders and Young (1946) who considered that the process of maturation was not more complete in the longer nerves. They employed varying lengths of the large peroneal nerve and joined it to the still larger tibial nerve. Both of these nerves have considerable mixed muscle and sensory (skin) components and the regenerating fibres would eventually make contact with end-organs, many of them on the muscle

fibres. Though the right and left sides of the animals would be partly comparable, no indication is given of the possibility of the motor end-plate connexion masking the effect of length. The difference in length was 55 mm., which, according to the present findings, would account for a shift upwards of 0.25 micron in the mean diameter of the fibres. This is a figure which could almost certainly be accounted for by the varying peripheral connexions in skin and muscle.

The rate of conduction in a nerve fibre is largely dependant on the cross-sectional area of the fibre, (Erlanger and Gasser 1937). If the longer fibres had the same cross-sectional area as the shorter fibres then, other things being equal, contraction would occur earlier in a muscle supplied by a short nerve than in a muscle supplied by a long nerve. However, recent work (unpublished) done by Dr. Fernand in the Anatomy Department, University College, London, is against this view and shows that the muscles which are more proximally placed in the limb have large nerve fibres supplying them. The functional significance of these observations is uncertain.

6. Time for maturation.

It has been suggested by Weiss et al (1945) that the nerve fibre increases in diameter after the growing end has come to rest in an end-organ. In the

In the present series of experiments, those nerves which regenerated along n. suralis into the skin of the foot had only 10-20 days in which to mature whereas those nerves which ended in neuromas in the thigh or leg had 70 or 50 days. Admittedly, in the neuromas the nerve fibres would turn wound and continue growing for some time after reaching the end of the original nerve pathway but in spite of this, the degree of maturation in the shorter lengths produced fibres of a smaller mean diameter than in the longer nerves. It has shown that when the nerves are allowed to regenerate and form neuromas for periods up to 200 days, though the number of fibres is reduced in the longer periods, there is no marked increase in the diameter of the fibres. It is therefore probable that, in considering the degree of maturation, the distance travelled is a more important factor than the time available.

7. Size of Muscle.

A regenerating nerve is able to produce a twenty to a hundredfold increase in the number of fibres below the lesion (Young 1942.) Most of these fibres are usually absorbe but if connexion is made with a nerve which originally supplie a large muscle, then many of these regenerating fibres will travel to the numerous motor end-plates. There will be few surplus fibres and so little reduction in the total number and the fibres will be well developed and mature. It may be that not only the large number of muscle fibres but also the size of the muscle fibres are factors which

help the process of maturation.

8. Effect of Muscle state.

It has long been maintained that the nerve supply of mammalian muscle is such that each muscle fibre has one motor end-plate (Wilkinson, 1929; Denny-Brown and Pennybacker, 1938), though some workers (Agduhr, 1916; Cuajunca, 1932) have reported the presence of multiple endings on a fibre. In normally innervated muscle it would appear that there is a state of equilibrium between muscle and nerve, and when a 'foreign' regenerating nerve is made to grow into a fully innervated muscle, it will lie in contact with muscle fibres which are already innervated. Yet when the nerve is examined the process of maturation is found to have proceeded farther than in a nerve of comparable length which forms a neuroma in the fascia (compare results in Tables 3(b) and 7(a)). The close proximity to muscle tissues does thus seem to have a beneficial effect on the process of maturation even though there is little or no attempt to form motor end-plates.

It is by no means certain what are the necessary anatomical relations between nerves and muscles to produce conditions which will allow of transmission of an impulse from the nerve fibre to the muscle fibre. Most normal motor end-plates are hypolemmal in position but is this a necessity? Many of the implanted nerve fibres lie between the muscle fibres and do not form

typical end-plates, but there is little proof that they are incapable of transmitting an impulse. Whether this impulse can be effective is not certain. Experiments being conducted at the present moment on devascularised muscles may throw some light on the problem. In devascularised muscles, there is a rapid regeneration of the muscle fibres (Clark and Bloomfield 1945) associated with an increase in the fibrous tissue between the muscle fibres. This fibrosis produces a considerable thickening of the sarcolemma and so may prevent the developement of new end-plates and also block the transmission of an impulse from an implanted nerve.

When a regenerating nerve is implanted into a denervated muscle the process of maturation is greatly facilitated (Table 7 (b)); not only is the number of small fibres usually reduced but the larger fibres are significantly increased. When these results are compared with those previously reported (Aitken et al 1947) for maturation in regenerating nerves following union with a muscle nerve, it is found that the maturation after implantation is poorer than after union. In the latter case, most of the motor end-plates were reinnervated and the whole muscle contracted on stimulation. Stimulation of a nerve implanted in a denervated muscle gave rise to contraction of a few bundles of muscle fibres. Histological examination of the denervated muscle revealed no innervated 'native' motor end-plate but a very different

neuroma from that which was found in a normal muscle. It would seem that the implant responds to the demand on the part of the denervated muscle and that the most potent stimulus to maturation is the opportunity to form new motor end-plates. As counts of the new motor end-plates were not made, it is impossible to correlate the degree of maturation with the number of functioning muscle units but the number of endings was never very great and they were mostly close to the implant.

The growing ends of the nerve fibres would reach the muscle in about 13 days after the operation and they would have 86 days in which to ramify amongst the muscle fibres. The results of electrical stimulation showed that the functional spread of the nerves was restricted. This was specially marked in the experiments where the implantation was made into a normal muscle.

Fort (1940), working with Weiss, has studied some of the factors involved in the establishment of neuro-muscular connexions in toads. He suggests that denervated muscle may be more 'permeable', though he admits that his evidence is inconclusive. Attempts to arrest the process of reinnervation in a denervated muscle by use of a Ringer extract of normal muscle were also not effective.

From the present work it would seem that the juxtaposition of regenerating nerve fibres and muscle fibres is sufficient to initiate the process of maturation, but that the opportunity of forming a functional motor end-plate

on muscle fibres is a very strong stimulus to continuation of the process.

9. The effect of overcrowding of the Schwann tubes and possible return of fibres up the tubes from the neuroma.

The effect of overcrowding the Schwann tubes by a large number of small fibres is most pronounced in the shorter lengths of nerve. Their numbers over the total series of nerves vary considerably (Table 3) and for no very obvious reason, even some of the implants into denervated muscle (Table 7(b)) having a total of over 1000 fibres in the groups under 6 microns in diameter. The larger sized fibres seem to form a more constant and stable series, and in the nerve which had the neuroma removed 2 weeks before biopsy there was a difference of only 8 between the number of large fibres in the specimen of nerve with the neuroma and that found in the nerve itself. It is realised that there is a difficulty in making a definite statement concerning this matter as sections cut near the neuroma are very difficult to count accurately owing to the obliquity of many of the fibres.

When Figures 9 and 10 are compared, it is seen that many of the Schwann tubes in the latter (section of n.g.m. after removal of the neuroma) contain only one fibre. During the 14 days since operation, many of the small

fibres have degenerated. Table 8 shows that the total number of fibres has been more than halved by the removal of the neuroma. This implies that in the short nerves a large proportion of the small fibres will be cut across twice in any section of the regenerating nerve. It also shows that the larger fibres (over 6 microns) are the more stable and least affected by over-crowding or turning back.

Other factors, depending on the physical and physico-chemical properties of the tissues into which the nerve fibres grow and which as yet have been little investigated, may prove more important than any which have so far been reported.

B. Growth of nerve implants in voluntary muscle.

During the course of the work on the effect of the peripheral connexions on the maturation of nerve fibres, muscle nerves were cut and the central cut ends were implanted into nearby muscles. The question was postulated "Will new motor end-plates develope in a muscle as the result of the entrance of 'foreign' nerve fibres?" The answer is "Yes" as it has been shown that in a paralysed (denervated) muscle, the 'foreign' nerve readily produces new end-plates

which transmit impulses when the nerve is stimulated. The end-plates vary considerably in size and complexity. Occasionally a nerve is seen lying close to a muscle fibre and there appear to be alterations in the muscle nuclei. Figure 36 shows this condition. There are a number of obvious Schwann cell nuclei which appear to be in greater numbers than would be expected and the sarcoplasm is more darkly stained in the region of the nerve fibre than elsewhere on the muscle fibre. Is this an early stage in the formation of a motor end-plate, or is it an atypical ending? When new motor end-plates develope, the nerve fibres must have pierced the sarcolemma. The mechanics of this process are still unknown. Presumably the growing end of the nerve has the power to produce enzymes which are responsible for the autolysis of the sarcolemma. Whether any histochemical reaction can be designed to demonstrate these early stages in the developement of a motor end-plate remains to be seen. Modifications of the Janus green B techniques (Couteaux 1946) for showing cholinesterase or the more complicated techniques using acetyl thiocholine (Koelle and Friedenwald 1949) may be possible.

In an intact 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

functional connexions with the muscle fibres. In any case, reinnervation is very much easier and more abundant in muscles which had previously been denervated.

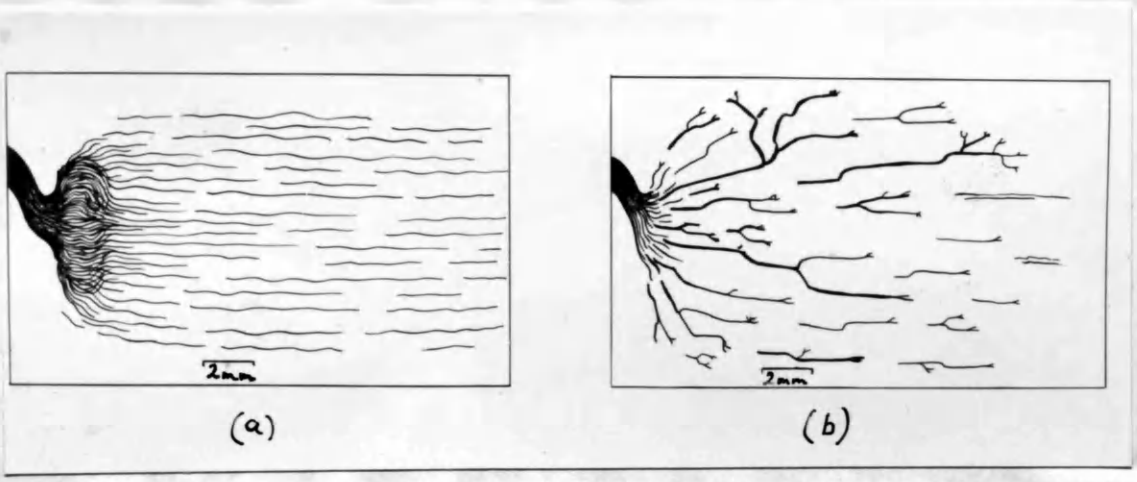
Text Figure 10 is a drawing of two sections of muscles with different implants. In (a) the implant was in a normally innervated muscle and the regenerating fibres pass for long distances in the muscle with little branching. The fibres never appear in very close proximity to the muscle fibres. In (b) the implant was in a denervated muscle. The nerve fibres are seen to divide frequently, many of them are large and have well developed myelin sheaths, and motor end-plates are found in abundance.

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 muscles?

2. To what extent does the implanted nerve spread through the denervated muscle?

1. This problem has received much attention from workers who are interested in the innervation of muscle. It would appear that there is a very great variation in the pattern of innervation between species. Multiple endings, pluriaxonal and plurisegmantal, have been reported in Amphibia and Reptiles and there is good evidence - both histological and physiological - to support this view (Kuhme 1887 and



Text-fig.10. Drawings of two sections of muscles. In (a) , the implant is in a normal muscle (nerve supply intact) and the long, thin, unbranching fibres should be noted. In (b) , the implant is in a muscle which had been denervated. The nerve fibres are thicker, branch frequently and end-plates were found in abundance.

Katz and Kuffler 1941), though most other workers (Eckhard 1849, Kulchitsky 1924 and Wilkinson 1929) find only one ending. In mammals also , the evidence is not clear. Multiple endings on the intra-fusal muscle fibres of a spiral ending have been demonstrated by Cuajunca (1932), Barker (1948) and others. On the extra-fusal fibres Agdhur (1916) illustrates two endings, one above the muscle fibre and the other on the deep surface, Garven (1925) illustrates two endings on a single fibre of the panniculus carnosus of the hedgehog. Wilkinson (1929) made a very full and careful survey of muscles from many types of animal and inspected the preparation 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. If more than one motor end-plate forms on a muscle fibre then this specificity becomes a quantitative rather than a qualitative phenomenon.

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. Where the

muscle was damaged during the operation, however, the implanted 'foreign' nerve produced motor end-plates. In these cases it is impossible to say whether the normal nerve to the muscle fibre had been cut or whether the muscle fibre had been divided into a part with the normal end-plate and a part without the end-plate. In either case the 'foreign' nerve would form a new end-plate. If a detached portion of the muscle fibre is reinnervated, then whatever the cause of non-receptiveness it is a reversible phenomenon and depends on a close association with the normal end-plate.

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 be induced to form on the longer portions but not on the shorter.

The results obtained 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 hyperneurotisation frequently 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 develope when a 'foreign' nerve is implanted into a normal muscle. This was not found in the present series of

experiments and the presumption is that the ordinary extra fusal muscle fibres in mammals have a limited number of end-plates and probably only one in most cases.

Most of the end-plates in a muscle are arranged in a more or less regular pattern through the muscle. When the muscle fibres are parallel, as in sartorius of most mammals, the endings are found in bands across the muscle. Some parts of the muscle, such as the upper end of sartorius, are free of endings. In gastrocnemius and biceps, 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. After 100 days denervation and using the modified Bielchowski-Gros technique, no evidence was seen of these denervated end-plates. 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 (1942). The end-plates were however found scattered through the muscle near to the implant, so no evidence was found for a neuropathic attraction of end-plates on the regenerating nerves.

If Weiss's concepts of specificity (Weiss 1947) are accepted then it would not be expected that the 'foreign' nerves would be attracted to the old end-plates though implantation of the normal nerve into its own muscle might

result in a restoration of the original pattern of innervation.

From the appearances of the regenerating nerves in the denervated muscle, it would seem that the denervated muscle fibres had a marked effect on the nerves. After the nerves came in contact with the 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 Figure 16 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 Figure 22). In other cases, the end-plates are 'in series' (Figure 21 and Text

Figure 9), and there must have been a short time between the formation of the two plates. The muscle fibre was unable, in the time available, to become non-receptive to other fibres. Figure 16 shows however, that within a short period of time, the nerves can meet with non-receptive muscle fibres and Figure 17 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.

Evidence about the development of this non-receptive state of the muscle fibres is scarce. The fact that in a denervated muscle two separate endings derived from the one axon can appear on a single muscle fibre suggests that it can hardly have been an electrical phenomenon which would presumably develope faster than nerve fibres would grow. Reorientation of the surface molecular layers of the sarcoplasm or sarcolemma might account for the non-receptive state but this again probably happens fairly rapidly. The production of some chemical which diffuses through the sarcoplasm might produce the effect. Implants into each end of a long thin muscle at different times might help to clear up this point.

Work is now proceeding to investigate further the role of the sarcolemma in this process of new end-plate production. By partially devascularising the muscle, it is possible to increase the amount of interstitial fibrous

tissue on the outer surface of the sarcolemma. This should impede and might even prevent the development of end-plates.

The implants into the tenotomised muscles show that though the muscles cannot function effectively, the end-plates do not show any gross abnomality of structure. Each muscle fibre has its end-plate or plates, and when extra fibres from a regenerating nerve implant grow amongst the muscle fibres, new endings are not formed. The nerve-muscle specificity is therefore retained in a non-functional state of the muscle.

2. In the present series of experiments the production of new end-plates was localised to the region of the implant. This may have been due to the short duration of the regenerative period (100 days). 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 denervated muscle. As itis known (Section 2(c)) that a small nerve will regenerate down a large trunk and reinnervate the muscle, it should be possible to cut a healthy nerve, split it and reinnervate its own muscle with one helf and implant the other half into a denervated muscle, but very problematical whether

a functional result would be obtained.

SUMMARY

1. In this thesis, it has been shown that the following factors in the peripheral pathway of regeneration produced a very beneficial effect on the process of maturation of nerve fibres:-

1. The possibility of making functional connexions with existing but denervated motor end-plates (nerve unions).

2. The possibility of producing new functional motor end-plates on muscle fibres which had been previously denervated (nerve implants).

3. The larger muscles produced a greater degree of maruration then the smaller muscles when nerve fibres

regenerated along existing nerve pathways into the muscles.

4. Nerve fibres which regenerated and formed neuromas in normally innervated muscle matured further than when the neuromas formed in the fascia between muscles.

5. The longer the regeneration pathway, the greater the degree of maturation.

2. When neuromas formed in fascia, the regenerating nerve fibres became very tortuous and many of them turned back up the Schwann tubes.

3. When a muscle nerve was implanted into a previously denervated muscle new motor end-plates developed especially in the region of the implant. These new endings were capable of transmitting impulses from the nerve to the muscle and producing a contraction of the muscle. This contraction was usually limited to the muscle fibres in the vicinity of the implant.

4. A normal muscle with an intact nerve supply appeared to be in a state of nerve-muscle equilibrium and when a "foreign" muscle nerve was implanted new end-plates were not formed unless the muscle had been badly injured.

during the operation.

5. From the above findings, it has been suggested that most muscle fibres have a limited number of motor end-plates and probably only one on any individual muscle fibre.

APPENDIX ON HISTOLOGICAL AND COUNTING TECHNIQUES

1. Preparations of sections of nerves.

Owing to technical difficulties, it is impossible to count living nerve fibres or to estimate their diameters. It is essential therefore to devise methods of histological preparation which will produce the minimum amount of distortion of shape and size of fibres. As nerves from different sides of the same animal or from different animals have to be compared, the methods should be capable of standardisation as far as possible. Though, of course, there are recognisable differences between the right and left sides of the same animal and between one animal and another, these factors cannot be overcome easily. In the later experiments, care was taken to avoid as far as possible the inter-animal effects. This was done by comparing experimental and control results from different sides of the same animal. By using animals of the same breed, size, age and sex, it would be possible to get statistically significant results from a smaller number of animals. The use of pure-strain littermates would have removed a number of criticisms which could be made against the techniques. All the animals were adult so that changes due to growth were eliminated.

2. Removal of nerves.

At the biopsy, the nerve (n.g.m.) was exposed and separated from the surrounding fascia and adjacent sciatic nerve and thigh muscles. Care was taken not to stretch the nerve. The adherent excess of fat and fibrous tissue was carefully dissected away before the nerve was removed from the animal. Precautions were taken to prevent drying of the specimen and artificial lights were held at a safe distance from the preparation. Normal saline was not used liberally as it was felt that this would influence subsequent fixation.

A small card with a rectangular hole cut in it was placed near the nerve which was then severed proximally about 2 mm. above the site of the crush, and distally (if necessary) and laid across the hole in the card. Slight firm pressure was sufficient to attach the nerve to the card which had previously been labelled. The card and nerve were quickly placed in the fixative solution.

In the more recent work on nerves it has been found better to fix the nerve in situ. This is done by separating the required nerve from the adherent surrounding tissue and pouring the fixative solution over and around the nerve as it lies in a trough between the muscles. In this way, there is little or no danger of stretching the nerve. Also it has been observed that

when a nerve is cut, the two cut ends are separated from each other by the retraction of the nerve above and below the cut. This retraction is presumably associated with a slight increase in the diameter of the nerve. When nerves are fixed in situ they are left in the bath of fixative solution for about 30 minutes and then cut out, placed on cards and immersed in more fixative solution. If the muscles have also to be examined histologically, then the nerves must not be fixed in situ.

3. Type of fixative, solution and duration of fixation.

If distortion has to be minimised then it is essential that the fixative solution should be capable of penetrating the tissue quickly and at the same time it should precipitate the proteins with the minimum of shrinkage. Some mixture containing osmium seemed to be the best and the following modification of Flemming's solution was used:-

1% Chromic acid in distilled water 15 cc.
2% Osmic acid in distilled water 4 cc.
Glacial acetic acid 1-2 drops (not more).

If this mixture is not freshly prepared, then the osmic acid fraction evaporates. To prevent this happening during the period of fixation of the tissue, the

specimen tube is sealed with a waxed cork. The osmic acid is responsible for the rapid penetration and fixation. So that the fixation shall be even, the nerve should not be too large in diameter and all adherent fat should be removed. If an excess of fat is present on the nerve, the fixation is not satisfactory and subsequent staining is uneven. The nerve to the medial head of gastrocnemius (n.g.m.) is about 1 mm. in diameter and the penetration is even and rapid. The peroneal and tibial nerves, however, are 2-3 mm. in diameter and the centre of these nerves is often poorly fixed. When 0.9% saline is used instead of distilled water the fixation is not so good and more fibres appear to be crenated and otherwise distorted especially towards the centre of the nerve. The amount of glacial acetic acid is also critical When it is absent from the mixture, the outside fibres only are well fixed and if it is in excess of 5 drops, the amount of shrinkage is considerable.

Using the above modification of Flemming solution the nerves were circular in outline and only occasionally fibres were seen in which the myelin sheath appeared to have ruptured. This rupturing usually occurred inwards and not outwards. Some of the fibres were crenated and others were oval in shape. Gutmann and Sanders (1943) have stated that following fixation, the shrinkage in a nerve probably amounts to about 10%. This figure however is an estimate and very difficult to check.

The nerves were maintained in the fixative solution up to 24 hours. Anytime between 12 hours and 24 hours gave good results. With less than 12 hours, the fixation was not even and with over 24 hours, the specimen becomes very brittle and difficult to cut.

4. Dehydration and Embedding.

The nerves on their cards were removed from the fixative and placed in 50% alcohol without any intermediate washing. The specimens were passed through the graded alcohols being in each solution for 15 to 30 minutes. After two baths in absolute alcohol, the specimens were cleared in cedar-wood oil. The nerves can remain a longer time in this oil than in the alcohols and in fact can be stored without any delaterious effects, though it is better to pass on through a cedar-wood oil - benzol - paraffin wax mixture (equal parts) into 55°C wax. The specimens were passed through three changes of wax and the nerve was then detached from the card and embedded. After orienting the nerve, the wax was cooled rapidly and, if the embedding wax had been filtered before use, then little or no crystallisation occurred.

When passing the nerves through the waxes and in the final embedding, care must be taken that the temperature of the wax does not rise much above 55°. If this does occur, then the specimen will not only be brittle

and difficult to cut, but it will have shrunken by an amount which is difficult to estimate.

5. Cutting and Mounting.

The blocks of wax were trimmed down and sections were cut not more than 5 microns thick. The position of the crush can often be identified by a slight swelling on the nerve but if this is not visible, then the distance of the marked site of the crush from the upper end of the nerve should have been noted. Sections of the nerve were cut at a distance of 1.5 cm. below the crush in all cases except those in which the length of nerve below the crush was 1 cm. In these latter specimens, the sections were taken as near the end of the nerve as possible - i.e. as far from the crush as can be obtained. The sections obtained were mounted and dried on to the glass slides. Again care was taken not to overheat the slides or distort the sections.

6. Staining.

After removal of the wax in xylol, the slides were stained by a modified Weigert method.

1. Pass through absolute alcohol, 90% 70% 50% to distilled water.
2. Mordant sections in 3% potassium bichromate for 12 hours at 37°C. (or 2 hours at 55°C.)

3. Rinse in distilled water.
4. Place in Kultschitzky's haematoxylin for 12-24 hours at 37°C. (or 2 hours at 55 C.)
5. Transfer direct to 3% potassium bichromate for 1 minute.
6. Wash with distilled water and differentiate with Pal's solution:- Oxalic acid.....1 gm.
   Potassium sulphite.1 gm.
   Distilled water....200 cc.
7. Wash in distilled water.....½ hour.
8. Wash in tap water.....½ hour.
9. Dehydrate and mount in Canada Balsam.

7. Photographing the Sections.

The slides were examined microscopically and a section was chosen because of its even staining, its flatness on the slide, the absence of distortion in the fibres (especially the absence of obliquely cut fibres), and the presence of the epineurium. If the epineurium is not retained then it is difficult to be sure that some of the fibres have not been lost with it. The section was projected through a microscope directly on to bromide paper. The magnification in all cases was 750x. This was checked by the use of a graticule at the beginning and end of a session of projection. The error of magnification was very small. If the section of the nerve was large, then a

composite photograph was made up by scanning across the nerve and using only the central portions of the prints. This prevented distortion due to the lens of the microscope. In the smaller nerves, the whole area could be projected on to one piece of paper.

8. Counting.

The prints were usually divided into quadrants or smaller sections by cross-ruling with ink. In this investigation, the nerve fibres were grouped according to their diameters. The groups used were 0-2 microns, 2.1-4 microns, 4.1-6 microns etc. As the photographs represented a 750x enlargement of the actual section, 2 microns would be equivalent to 1.5 mm, 4 microns to 3 mm, etc. In the early stages of the work dividers were used for estimating the diameters of the fibres and are still used for the smallest fibres. Later a perspex grid was used for the larger fibres. On this square of perspex were engraved a series of circles of differing diameters, 1.5 mm, 3 mm, 4.5 mm, up to 15 mm. The centre point of these circles was drilled so that a needle could pass through it and so pierce the photograph thus marking the fibre which had been counted.

To facilitate the counting of large numbers of fibres, the needle was mounted on a plastic non-conducting handle in such a way that when the needle was pressed on

the photograph it completed an electric circuit connected to a Post Office relay and counter (see Figure 36.) It was thus possible for attention to be concentrated on the estimation of the fibre size and the addition of the fibres was done by the counter. The small fibres were first counted and the largest fibres last.

Crenated and oval fibres were always a difficulty. The latter fibres are almost certainly distorted and an estimate was made between the longest and the shortest diameters. The crenated fibres may be shrunken slightly or the section may be taken near to a node of Ranvier. Near the nodes, the myelin sheaths seem to become naturally crenated. The largest diameter of the crenated fibres was therefore taken because it was felt that the largest diameter represented the size of the neurilemmal tube, the true diameter of the nerve.

Frequently there were seen small dots on the photograph. Unless these had definite signs of a central axon shadow, they were considered as myelin debris and not counted.

As the counting of nerve fibres is a subjective estimation of a variety of factors, it is better that one person should do all the counts so that variation may be minimised.

PREPARATION OF SECTIONS OF MUSCLES

1. Biopsy.

At the biopsy, the site of the implantation was clearly identified and marked with indian ink after the nerve had been freed from surrounding fibrous tissue and tested electrically. After removing the nerve for fixation, the portion of the muscle containing the implant was removed. The lateral head of gastrocnemius was separated from the femur and from the medial head. A portion of muscle about 3 cm. long was usually excised. Biceps was treated in a similar way - a block of tissue about 2 cm. square being removed. The specimen was placed firmly on a piece of card, labelled and the card transferred into the fixative solution.

2. Fixation.

The type of fixative solution varied according to the staining method which was being subsequently used. At the beginning, attempts were made to obtain serial sections of the muscles. For this purpose paraffin and celloidin embedding was tried. With large pieces of muscle, paraffin embedding tended to produce a very hard block and was difficult to cut. On one occasion, the block was cut on a sliding microtome, the surface of the block being coated with gum

damasc before the section was removed. Though this prevented fragmentation of the muscle, the gum was difficult to remove and interfered with the staining. For future Bodian Protargol staining, the muscle was fixed in 5% trichloracetic acid in 0.9% saline for a few days and sections cut in wax. The results however were not constant - a poor picture being obtained even of normal muscle.

After fixation in a Bodian solution (80 cc. of 80% alcohol; 15 cc. of commercial formalin and 5 cc. of glacial acetic acid) the muscle was embedded in celloidin and stained by a modification of Cajal techniques (Holmes 1942), using buffered solutions. Though many of the nerve fibres showed up well, it was not possible to identify any of the finer twigs nor motor end-plates (see Figure 11.)

The most successful and consistent results were obtained with a modified Bielschowski-Gros technique. The muscle was fixed in 10% commercial formalin amd 2% pyridin in distilled water for at least 7 days. The muscle was then washed for an hour or so and placed in a syrup of cane sugar preparatory to freezing and cutting. Sections were taken off 50-100 microns thick. To facilitate orientation of the sections before mounting, the piece of muscle was marked at one end with indian ink. Groups of five sections were placed in a series of numbered tubes containing a weak solution of formalin (a few drops in 10 ccs. of distilled water.) It was then possible to reconstruct the muscle block with reasonable accuracy. Care was taken not to lose

tissue between the sections which were all stained and mounted.

3. Staining.

1. Distilled water was always used. Each group of sections was washed in distilled water to remove debris and the remains of any sugar solution.

2. Impregnate for 20 minutes in the dark in a 10% aqueous solution of Analar Silver Nitrate.

3. The sections were removed and passed directly into 10% aqueous solution of commercial formalin containing 2% pyridine. This was the first of four washing in this formal-pyridine solution and the times were roughly 30 seconds, 1 minute, 4 minutes and 8 minutes.

4. After removing excess formal-pyridine solution, the sections were placed in a ammoniacal silver solution for 1 minute. This solution was made by adding 880 ammonia drop by drop to 5 cc. of 10% silver nitrate solution until the precipitate had just disappeared. According to the room temperature between 10 and 15 extra drops were then added to the solution. The importance of adding the ammonia in small drops rather than as a larger bulk of fluid

was shown by Silver (1942) and confirmed in the present experiments. The physical conditions of the solution are quite different when a bulk of ammonia is suddenly added. The amount of ammonia added will vary with the size of the pipette or dropper but must be controlled by the staining of the sections, which should be a faint brown colour at the end of the minute.

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 too prominently. The sections should be gently agitated during the time in the ammoniacal silver to prevent the sections resting on the bottom of the dish. Reduction of the silver then occurs rapidly with the formation of a mirror on the vessel and a deposit of silver granules on the section.

5. The sections were transferred to water containing a few drops of 880 ammonia.

6. Transfer to water containing a few drops of glacial acetic acid.

7. Wash in water. The sections should be very pale brown or almost colourless. If they are too brown then the ammoniacal silver has contained too

little ammonia - probably due to evaporation. This was specially seen in hot weather.

8. Usually the sections were toned in 1% gold chloride until a faint deposit was seen on the surface of the tissue (10-30 seconds.)

9. They were then fixed in 5% hypo solution until they were transparent.

10. Washed in water to remove all the hypo.

11. Dehydrated.

12. Cleared in creosote.

13. Mounted in Canada Balsam.

It should be remembered that, in both the toning solution and in the hypo, bleaching occurs and loss of the finer endings will occur if the sections are allowed to remain in those solutions too long. If toning is not

desired, after washing (7) transfer the sections to hypo for fixation and then carry through stages 10 to 13.

The thick sections which can be stained with Bielschowski-Gros techniques and examined at different levels of focus in the section have many advantages. A much clearer impression is obtained of the three-dimensional relationships. The difficulty of obtaining serial sections is, however, a very great disadvantage.

The thick sections, though better for visual inspection with the microscope were not so good for photographic purposes. The camera focuses on to one plane and the depth of focus is never very great. Where good photographs could not be obtained, camera lucida drawings were made.

TABLES 1 - 8.

TABLE 1.

TABLE 1. Distribution of fibres in normal n.g.m. and n. plantaris of the rabbit.

N is the total number of fibres in the nerve; D the root mean square diameter (μ).

Nerve	Breed of rabbit	Wt./lb.	Level	Diameter μ 0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16	16.1-18	18.1-20	20.1-22	N	D
				(a) Nervus gastrocnemii medialis.												
1609c	Blue Beveran	5	Low	1	14	35	49	35	20	37	62	136	27	0	416	13.68
1610a	Butterfly	4¾	Low	5	13	31	48	45	16	57	154	58	9	0	436	12.94
1611a	Butterfly	4	Low	3	10	30	53	27	35	56	121	62	5	0	402	12.84
1619c	Wild Type	3½	Low	9	18	38	41	19	30	53	118	56	10	0	392	12.74
1619d	Wild Type	3½	High	1	15	37	45	23	22	43	104	84	11	0	385	13.16
1620c	Young Flemish	4	Low	2	11	24	53	23	13	27	46	111	43	1	354	14.03
1620d	Young Flemish	4	High	2	8	35	53	27	15	35	57	125	26	0	383	13.69
1628a	Grey	8½	Middle	6	19	23	33	17	11	20	104	104	19	0	356	13.90
1629a	Wild Type	8	Middle	9	20	40	49	30	26	100	159	32	2	0	467	12.40
														Mean	399	13.26
				(b) Nervus plantaris												
1663a	Grey	8		0	15	31	48	45	56	58	36	253	111	16	669	14.80

TABLE 2

TABLE 2. Distribution of fibres regenerated: a) Below crush and above neuroma (100 days).
b) Below crush above primary union of n.g.m. with itself (100 days).
c) Below crush alone (100 days).
d) Below crush and above neuroma (150 days).
e) Below crush and above neuroma (200 days).

Diameter μ

Nerve	0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16	N	D
(a) Above neuroma.										
1554a		1282	448	244	65	3	0	0	2042	4.08
1663c	419	976	164	22	4	0	1	0	1586	3.05
1680a	125	1312	710	94	0	0	0	0	2241	3.91
1680h	342	1041	399	194	68	9	1	0	2057	4.22
1693h	408	1110	568	218	98	19	3	2	2023	4.80

Nerve	0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16	16.1-18	18.1-20	N	D
(b) Primary suture of nervus gastrocnemii medialis with itself.												
1622h	409	301	137	148	143	16	0	0	0	0	1154	4.87
1647b	262	567	238	150	128	89	11	1	0	0	1446	5.38
1653h	56	483	187	122	128	18	1	1	0	0	996	5.30
1654j	124	399	316	175	122	47	6	0	0	0	1189	5.56
1661c	30	369	318	126	107	92	30	2	1	0	1075	6.33
1693c	105	550	218	103	94	86	11	0	0	0	1167	5.51
(c) Crush of nervus gastrocnemii medialis without severance.												
1662f	15	62	101	92	49	101	128	16	3	0	567	9.41
1661f	24	157	77	92	58	122	53	10	0	0	593	9.00

Nerve	0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16	N	D
(d) Nerve left 150 days.										
1543f	893	964	349	7	1	0	0	0	2214	2.91
1543g	108	837	320	158	12	0	0	0	1435	4.12
(e) Nerve left 200 days.										
1626g	79	742	476	157	20	2	1	0	1477	4.39

TABLE 3

TABLE 3. Distribution of fibres in regenerating nerves of different lengths.

Specimen	Diameter (μ) 0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16	Total	No. 6μ	Mean of 6μ fibres
(a) N.g.m. with 1 cm. peripheral path.											
48 (a)	326	1254	381	91	8	-	-	-	2060	99	7.32
51 (f)	96	1528	558	207	66	8	-	-	2463	281	7.58
56 (f)	311	2513	521	198	81	2	-	-	3625	281	7.60
57 (f)	378	1576	409	199	22	-	-	-	2584	221	7.20
58 (c)	216	1115	363	150	27	-	-	-	1871	177	7.31
59 (a)	315	1750	375	23	-	-	-	-	2463	23	7.00
										Mean of group	7.33
(b) N.g.m. with 4 cm. peripheral path.											
29 (b)	132	724	318	124	41	9	3	-	1351	177	7.77
51 (a)	95	764	349	102	10	-	-	-	1320	112	7.18
55 (a)	41	540	193	125	115	41	-	-	1055	281	8.40
56 (a)	35	560	166	162	107	18	-	-	1048	287	8.00
57 (c)	85	711	311	122	3	-	-	-	1222	125	7.05
58 (a)	186	1058	368	128	16	-	-	-	1756	144	7.22
59 (b)	45	583	194	124	117	11	-	-	1074	252	8.10
185 (b)	36	518	263	138	67	3	-	-	1025	208	7.70
										Mean of group	7.68
(c) N.g.m. joined to sural nerve - total length 12 cm.											
83 (a)	35	414	172	112	160	73	6	-	972	351	8.85
89 (a)	53	589	279	119	68	11	-	-	1199	198	7.91
95 (a)	61	503	117	143	110	5	-	-	939	258	7.93
105 (b)	124	322	270	233	5	9	1	-	964	248	7.21
										Mean of group	7.98
(d) N.g.m. joined to sural nerve - total length 25 cm.											
89 (c)	20	589	277	144	114	46	-	-	1190	304	8.36
539 (d)	55	274	108	94	120	58	-	-	709	272	8.74
539 (a)	14	160	110	119	129	104	5	-	641	357	8.97
541 (a)	56	444	225	152	93	8	-	-	978	253	7.86
546 (a)	23	192	83	86	106	94	53	5	642	344	9.75
										Mean of group	8.74

TABLE 4.

TABLE 4. The estimated times allowed for maturation of the regenerating fibres of n.g.m. after travelling different distances.

Distance travelled (mm.)	Estimated time to reach end of nerve (days)	Time allowed for maturation (days)
10	7	93
40	13	87
120	45	55
250	88	12

TABLE 5.

TABLE 5. Distribution of fibres in regenerating n.g.m. below a crush and after union with nervus plantaris.

Specimen	0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16	16.1-18	18.1-20	N	D
1560b	22	169	218	153	77	104	113	33	0	0	889	8.20
1574e	19	86	100	171	160	59	7	0	0	0	602	7.40
1627j	29	113	65	145	166	69	8	0	0	0	595	7.44
1663g	15	308	156	117	89	70	5	0	0	0	760	6.15
										Mean	712	7.29

TABLE 6.

TABLE 6. Against each animal there is given an estimate of the extent of the response to indirect excitation of the muscles by the implanted nerves.

N.g.m. = nervus gastrocnemius medialis; O = nerve pulled out; - = no response; ?- = ambiguous; + = slight response; ++ = vigorous response.

Animal	Nerve	Response in "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	?-	++

TABLE 7.

TABLE 7. Distribution of fibres in regenerating n.g.m. when implanted into normal and paralysed biceps femoris muscles.

Specimen	Diameter (μ) 0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	Total	No. 6μ	Root mean sq. diameter of fibres 6μ (D)
(a) Implantation into normal biceps.										
417 (r)	52	1035	256	177	23	-	-	1790	200	7.2
428 (r)	115	716	287	165	67	8	-	1358	240	7.9
443 (c)	175	586	194	163	81	-	-	1199	244	7.7
483 (a)	316	973	419	77	216	2	-	2003	295	6.3
642 (c)	12	588	375	228	66	10	-	1279	304	7.4
643 (b)	55	426	193	189	78	1	2	944	270	7.7
(b) Implantation into paralysed biceps.										
417 (1)	6	817	394	326	160	97	11	1811	594	8.5
428 (1)	85	730	285	150	29	13	-	1292	192	7.7
443 (a)	151	612	183	134	66	9	-	1155	209	7.9
483 (d)	138	968	185	85	111	69	49	1605	314	9.7
642 (a)	13	347	133	110	105	65	30	803	310	9.3
643 (c)	44	416	216	136	98	36	5	951	275	8.5

TABLE 8

TABLE 8 (a) and (b). Distribution of regenerating fibres in the n.g.m. immediately proximal to a neuroma and in the same nerve two weeks after the neuroma had been removed.

Specimen	Diameter (μ) 0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	Total No.	6μ
	(a) Portion of n.g.m. with neuroma attached.							
185 (a)	68	1490	750	158	42	-	2508	200
	(b) N.g.m. two weeks after removal of neuroma.							
185 (b)	36	518	263	138	67	3	1025	208

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FIGURES 1 - 36.

Transverse sections of nerves were stained by a Modified Weigert technique after fixation in Flemming solution. Unless otherwise stated, all experiments lasted for 100 days.

Sections of muscle were stained by a Modified Bielschowski -Gros technique. Figure 11 is a photograph of a muscle section that was stained by Holmes' method.

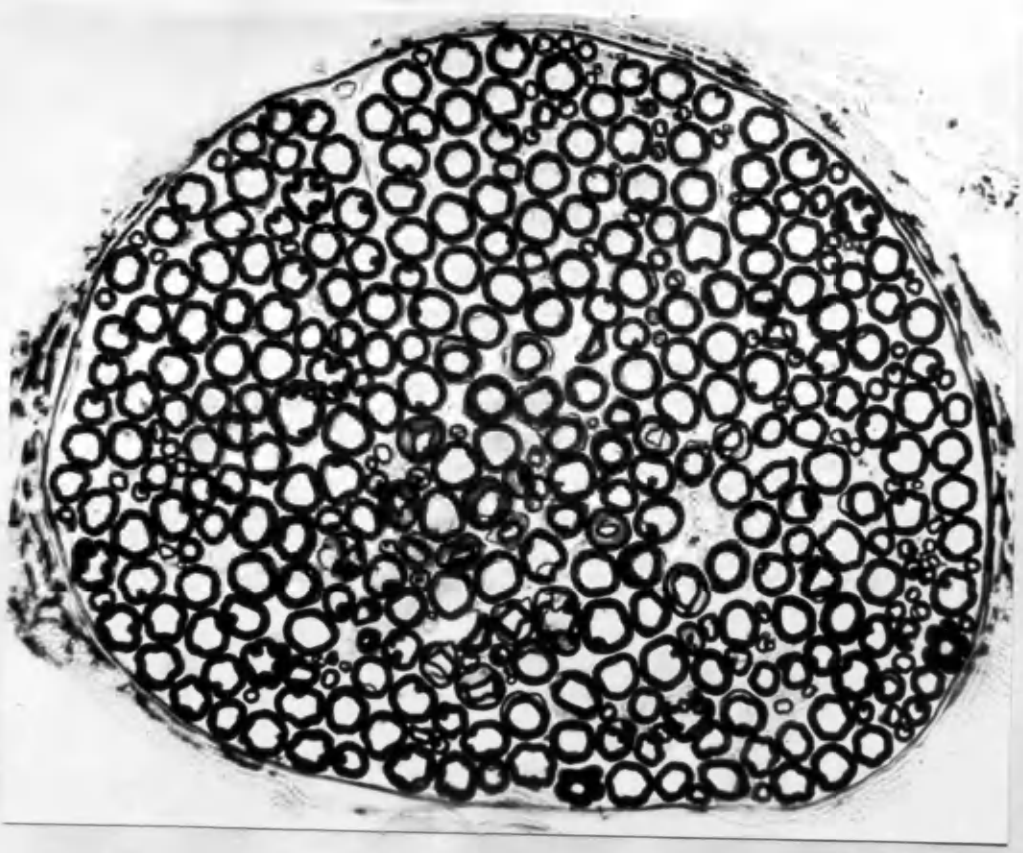

Figure 1. Transverse section of normal n.g.m.(1619c).

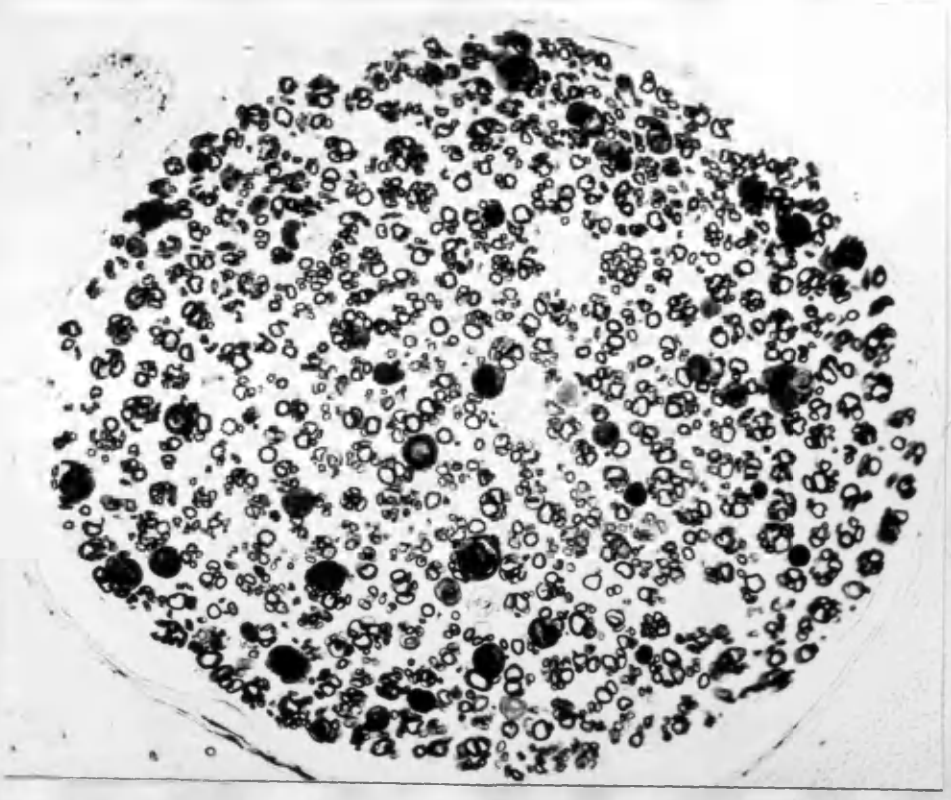

Figure 2. Transverse section of n.g.m. below the crush and above the neuroma (1554a). Note the large number of small fibres and the small number of large fibres. Compare with figure 3. The dark solid circles are stained myelin debris.

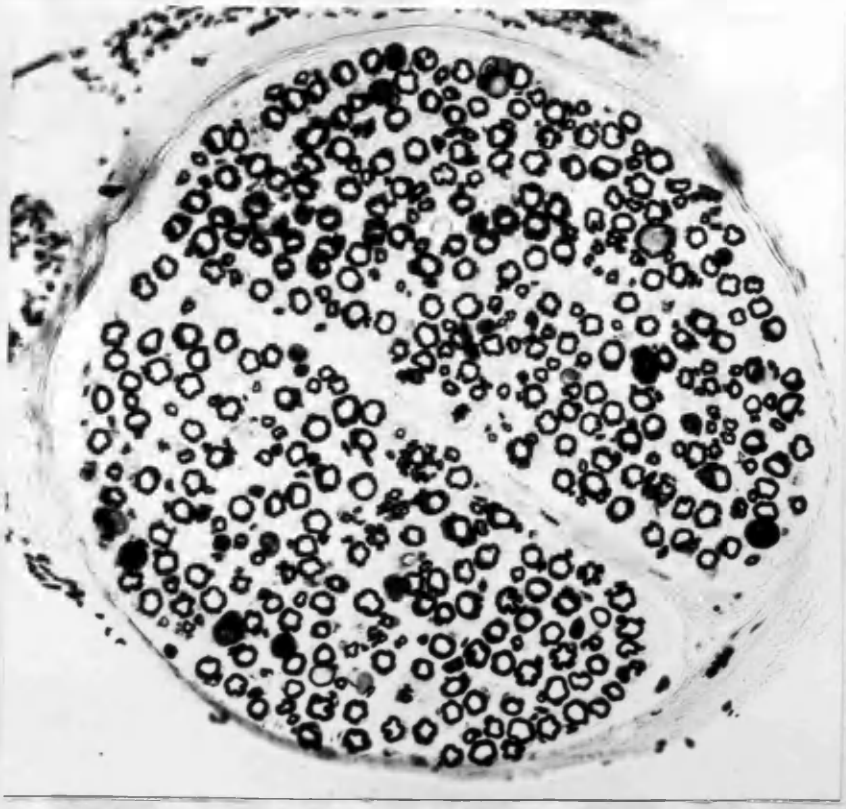

Figure 3. Transverse section below the crush in an n.g.m. in which the nerve was not severed distally. (1662f). Note the larger size of most of the fibres and the diminution in the number of smaller fibres. Compare with figure 2.

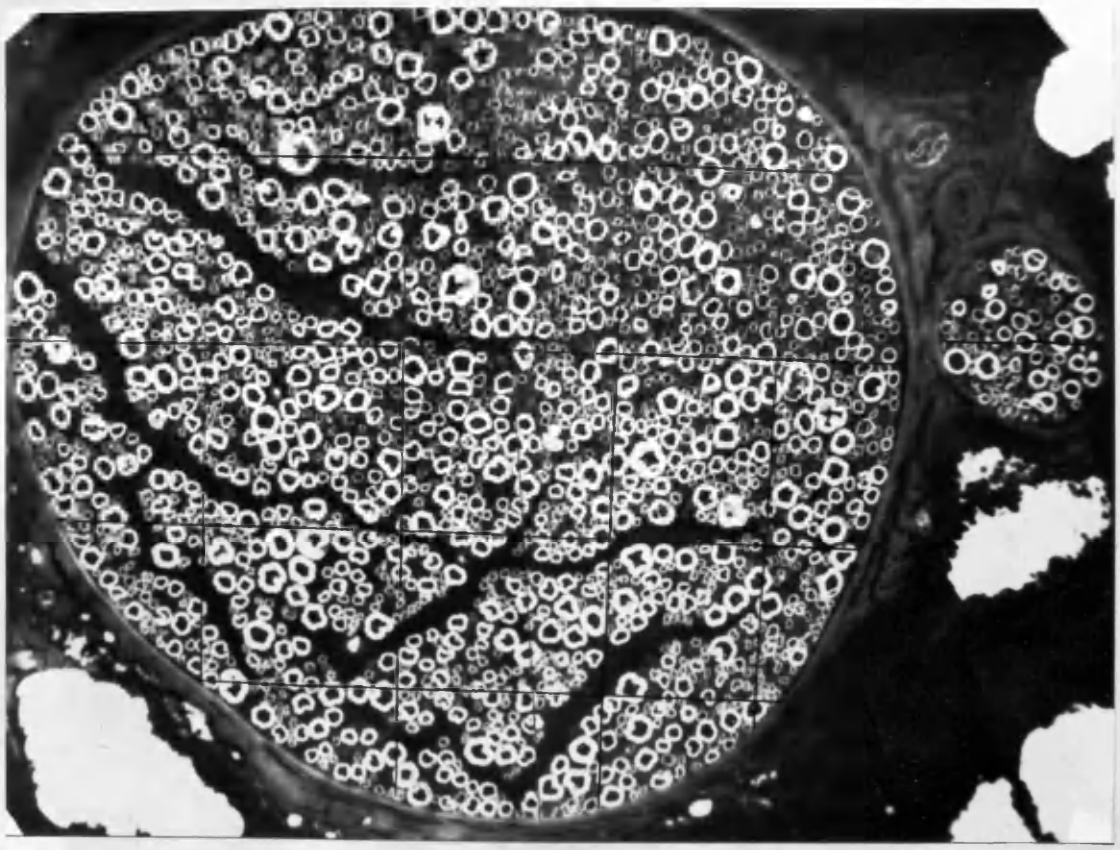

Figure 4. Transverse section of a normal n.suralis (1240C). Compare with Figure 1 and note the small number of large fibres and the large number of small fibres.

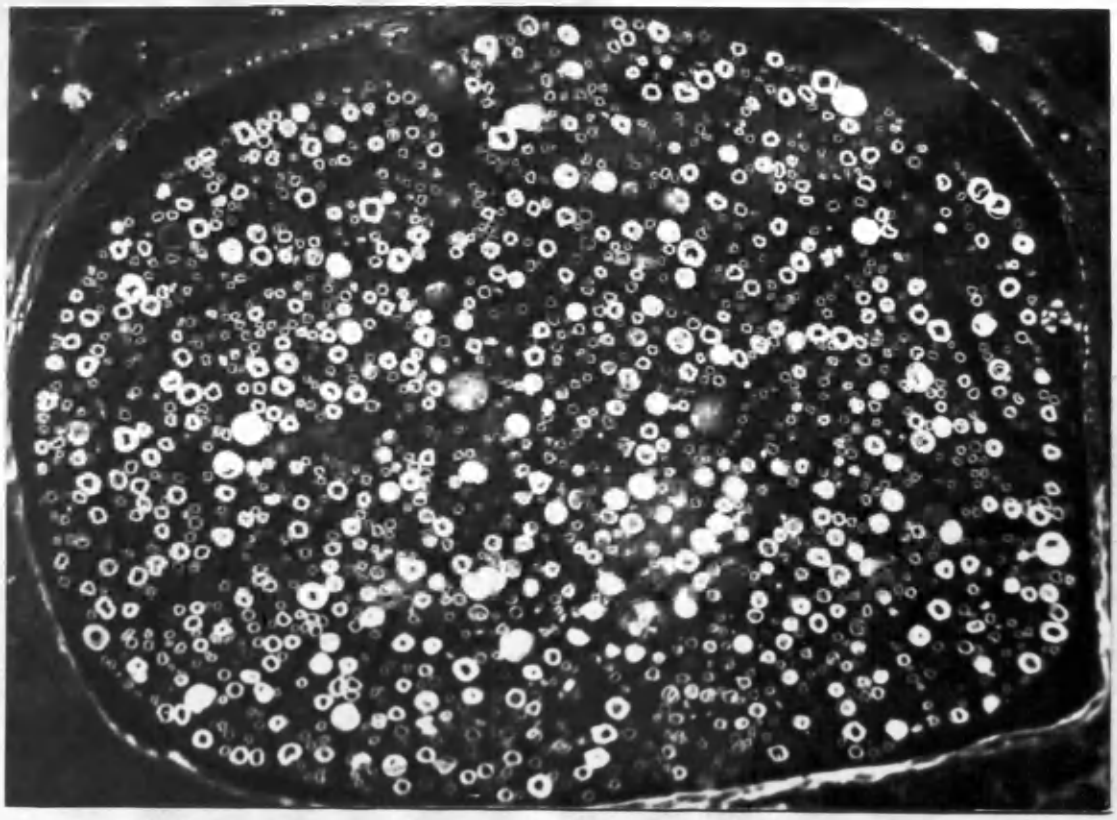

Figure 5. Transverse section of n.suralis below the union with n.g.m. (547 D). Note the regenerating n.g.m. fibres in the sural nerve.

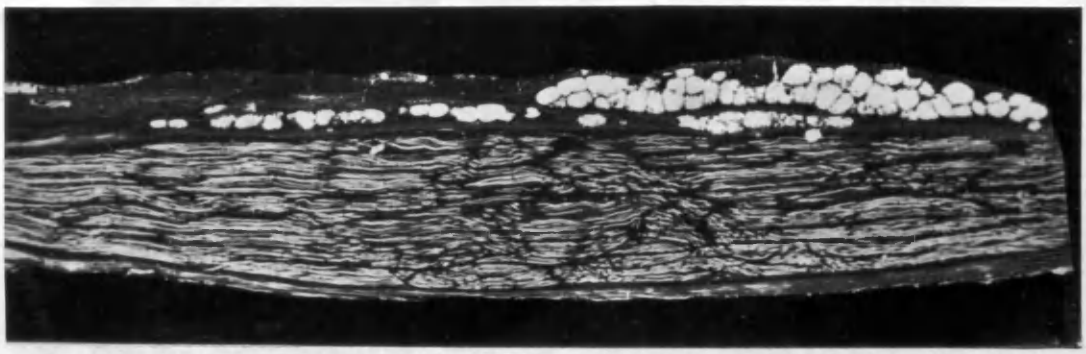

Figure 6. Longitudinal section of the union of n.g.m. to n.suralis (95d). Note the slight decussation of fibres in the centre of the picture.

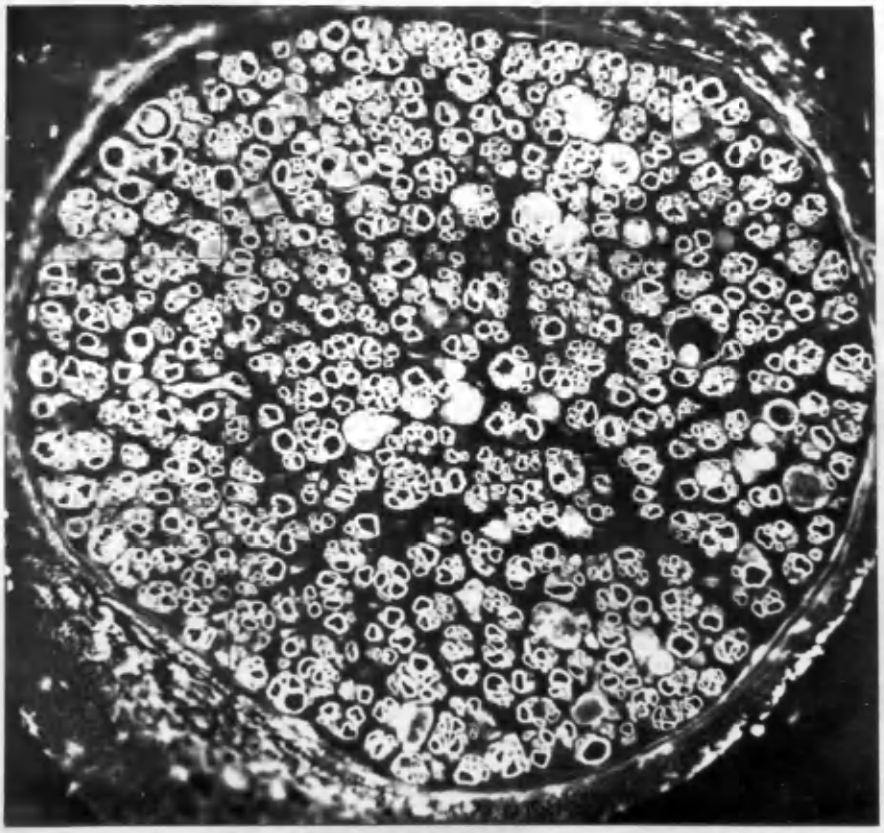

Figure 7. Transverse section of n.g.m. in which the fibres are regenerating into a muscle with intact nerve supply (642c). Compare with figure 8.

Figure 8. Transverse section of n.g.m. in which the fibres are regenerating into a denervated muscle (642a). Note the increase in the number of large fibres and compare with text-fig.6

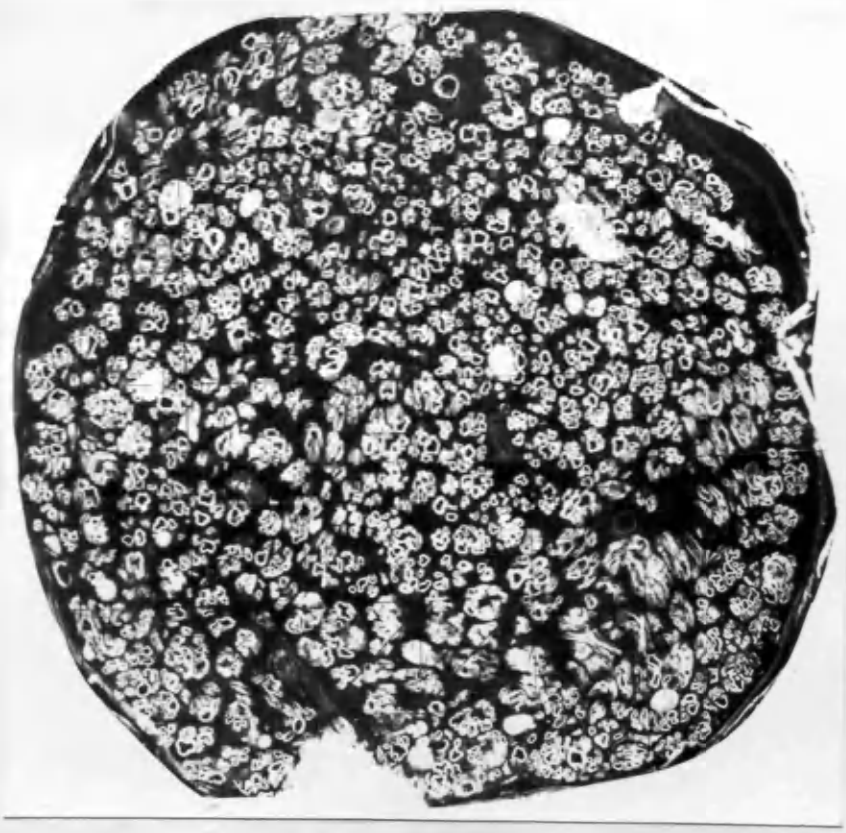

Figure 9. Transverse section of n.g.m. immediately above the neuroma in a regenerating n.g.m. (185e). The neuroma was removed two weeks before the terminal biopsy. Owing to the tortuosity of the nerve fibres, many of them have been cut obliquely. Compare with figure 10.

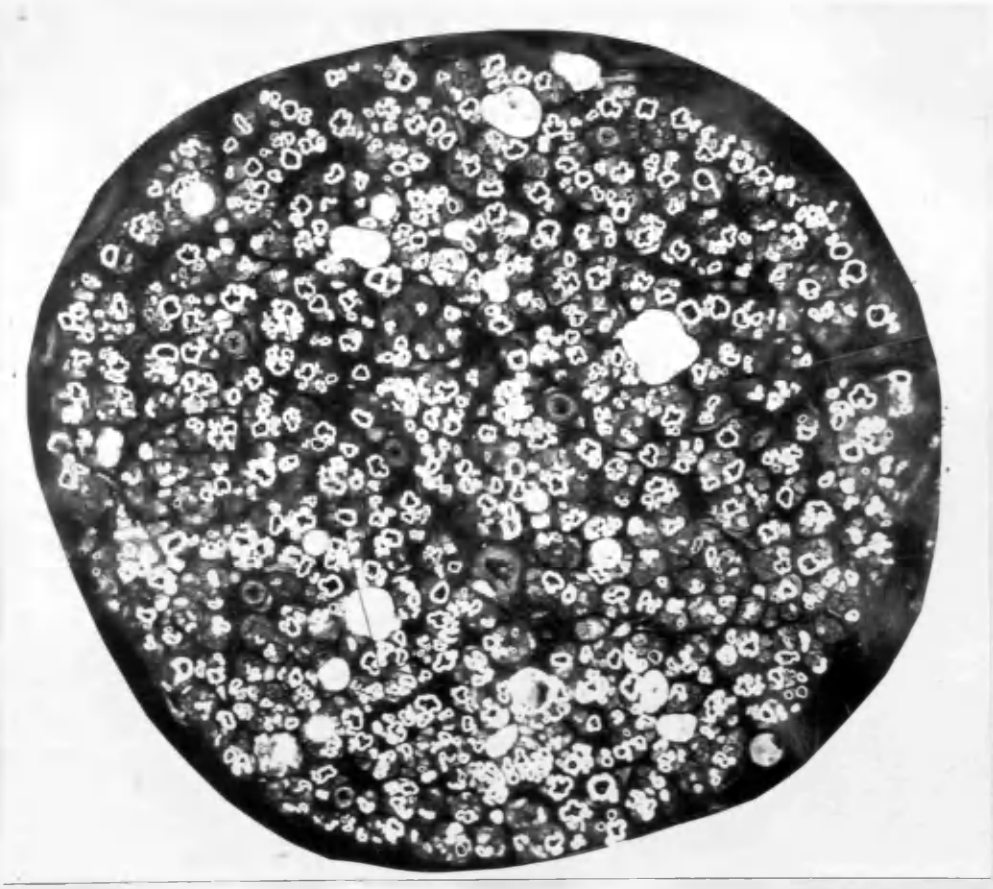

Figure 10. Transverse section of n.g.m. below the crush two weeks after removal of the neuroma.(185b) Note the great reduction in the number of fibres and compare with text figure 7.

Figure 11. Section of muscle which was stained by a Holmes' modification of the Cajal methods (47L 23). Though the fibres are clearly seen, no terminal branches or end-plates were observed.

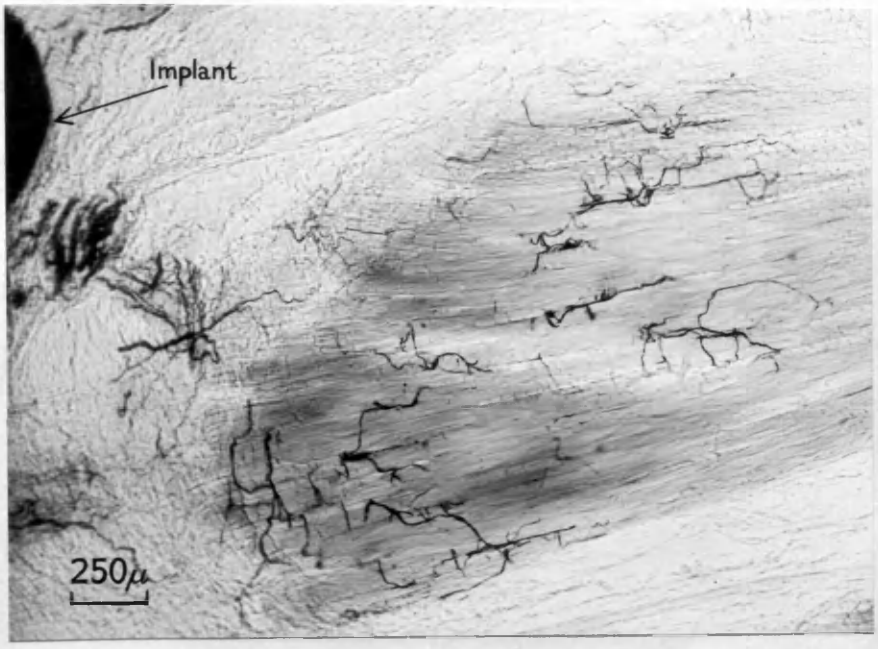

Figure 12. Sections of denervated muscle showing nerve fibres growing from an implanted nervus peroneus and reinnervating the muscle (395 A.13). Note the irregular branching pattern of the regenerating nerves.

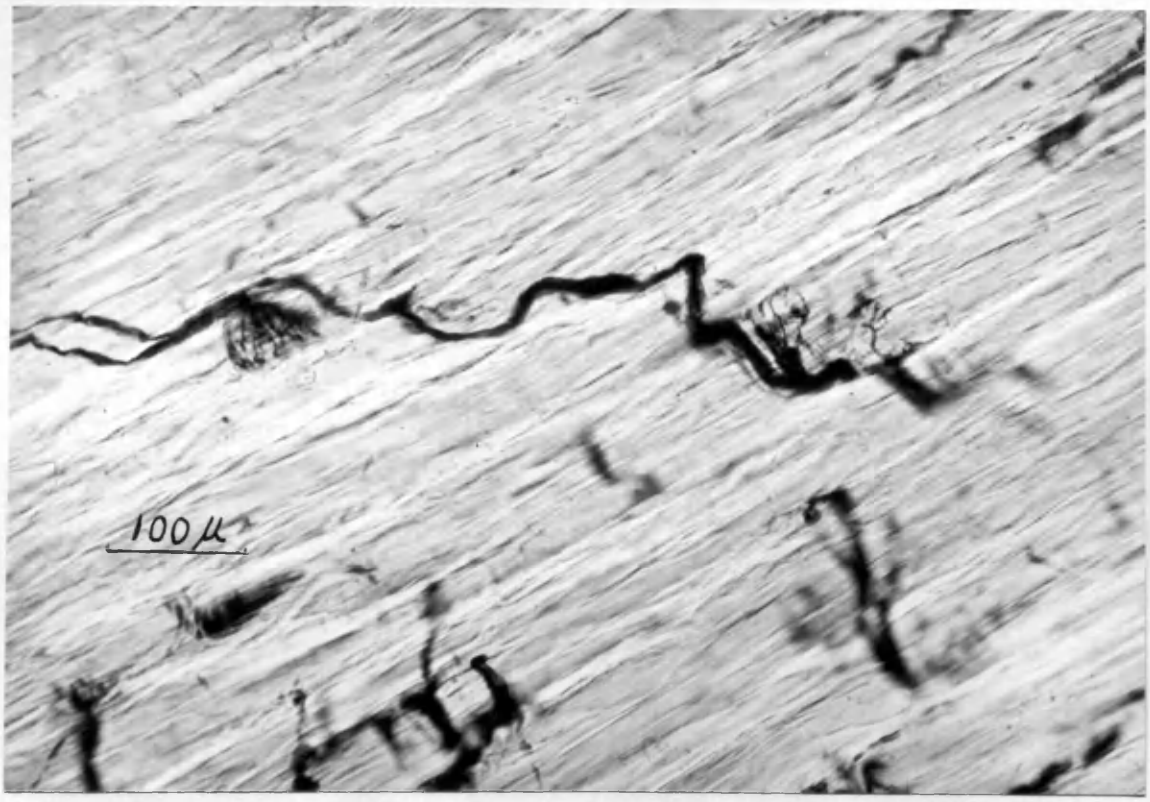

Figure 13. Showing the irregular types of ending plates which are frequently found in the nerve implants in denervated muscle (855E6). Note the nerve fibres crossing diagonally over the muscle and the larger end plates which form.

Scale 100μ

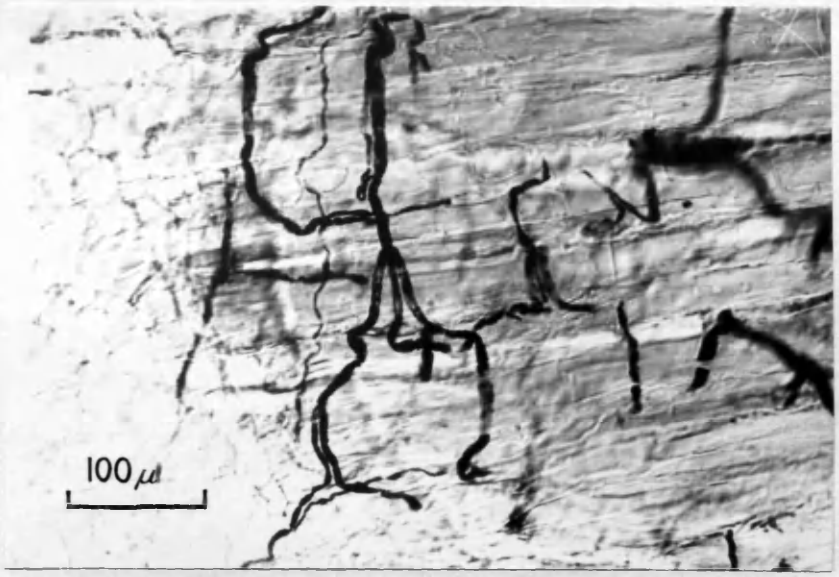

Figure 14. Section of muscle, which had previously been denervated, showing the implanted nerves. (395A 13). Note the myelinated fibre dividing into three sub-branches.

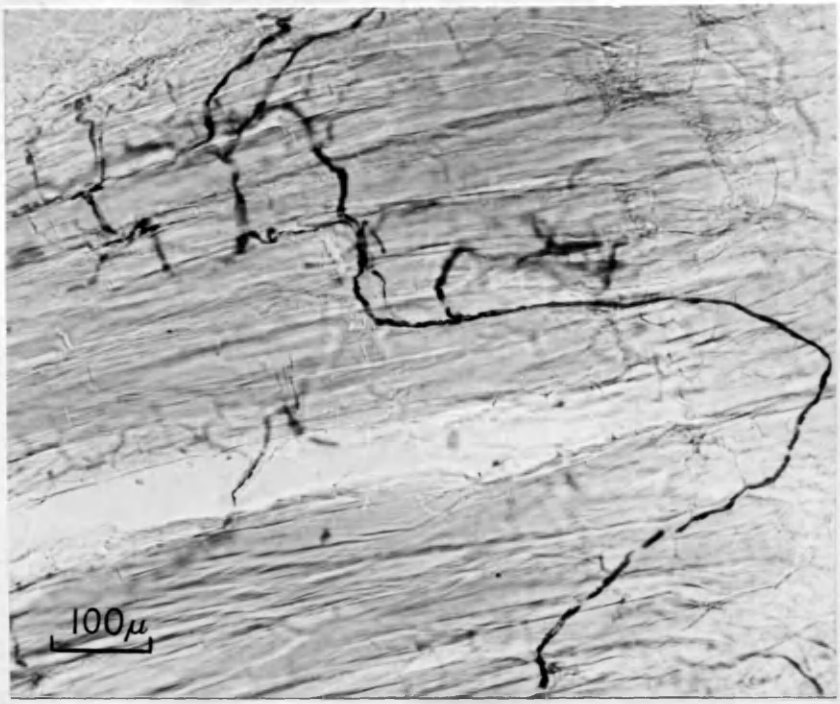

Figure 15. Section of muscle which had previously been denervated showing a nerve fibre passing across the muscle for a considerable distance (0.8 mm) before dividing up into smaller branches and forming end-plates (395 A 16)

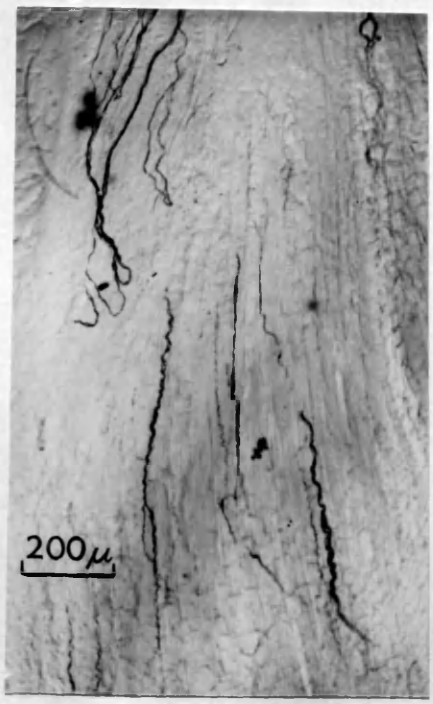

Figure 16. Section of muscle which had previously been denervated showing branches of the implanted nerve passing between muscle fibres with no sign of sub-branching. The muscle fibres had presumably been reinnervated and the end-plates would be in another microscope field or on an adjacent section (395.A13)

Figure 17. Motor end-plate formed by an implanted nerve in a previously denervated muscle. Note the nerve fibres entering opposite poles of the end-plate, the Schwann cell nuclei (black) and the inner and outer end plate nuclei. The fine nerve ending finishes on a muscle fibre and there is a certain amount of nuclear activity around the ending (484 B 6b).

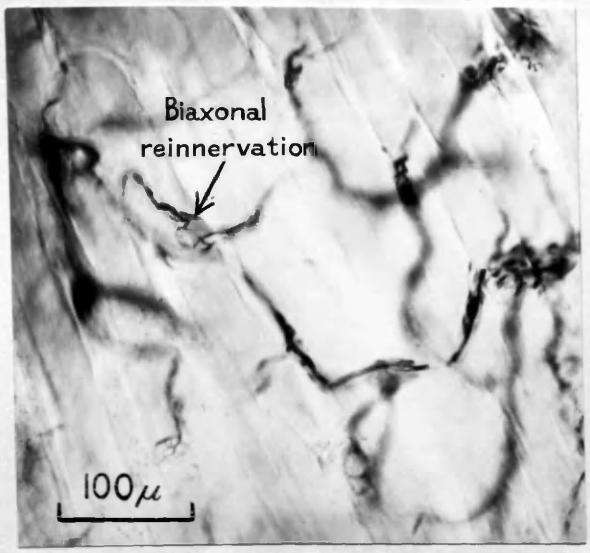

Figure 18. Section of a previously denervated muscle showing many new end plates which have been formed by an implanted nerve. Note the end-plate which appears to be reinnervated from two axons. (855 E 3 (3)).

Figure 19. Photograph of a large claw-like end plate developed on a previously denervated muscle from the regenerating fibres of an implanted nerve. (855 E. 3.) Compare with Text Figure 8.

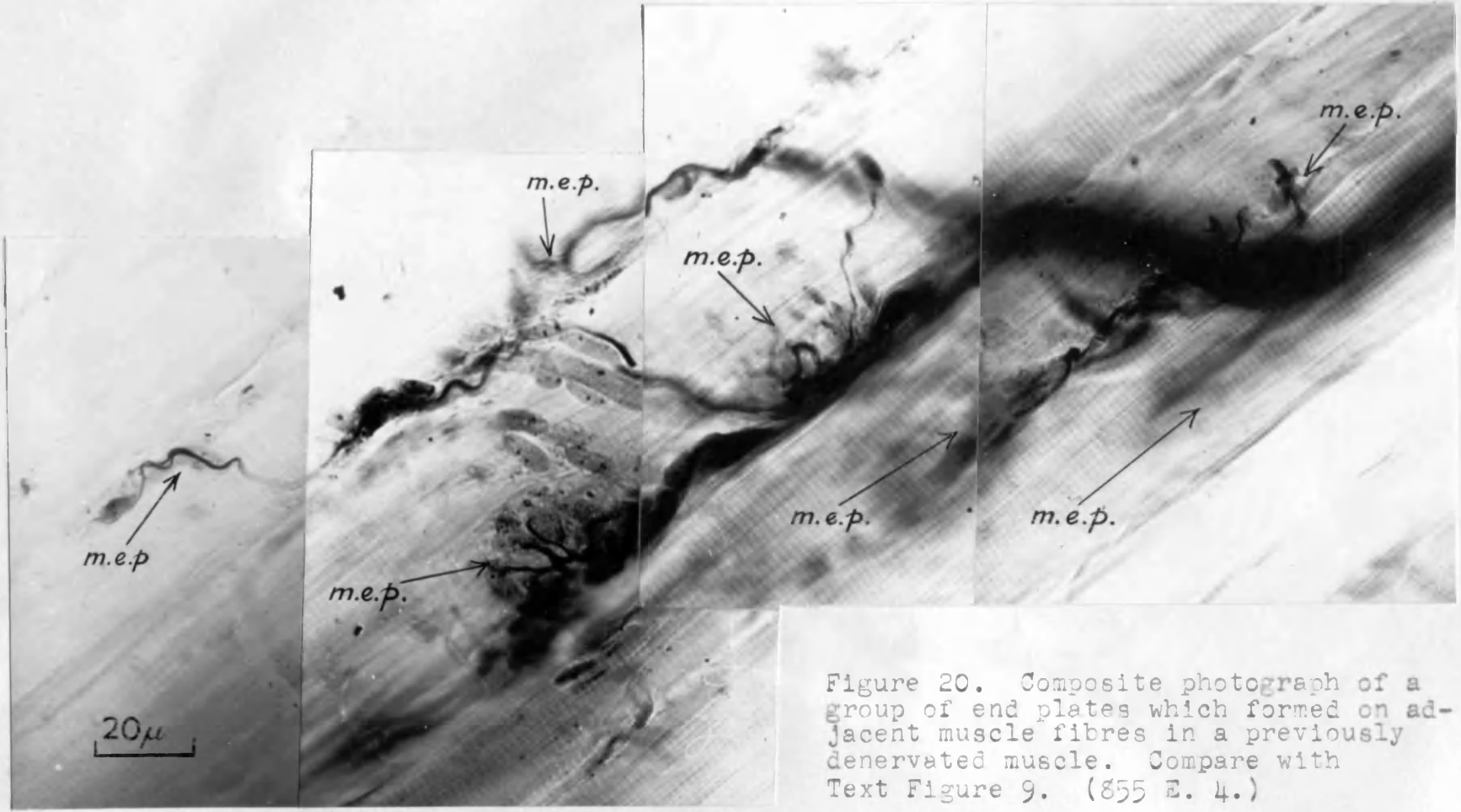

Figure 20. Composite photograph of a group of end plates which formed on adjacent muscle fibres in a previously denervated muscle. Compare with Text Figure 9. (855 E. 4.)

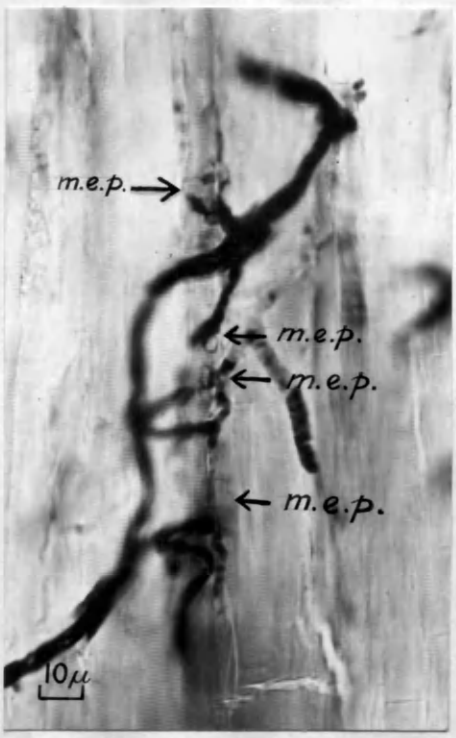

Figure 21. Group of at least four motor end plates (m.e.p.) on the adjacent sides of two muscle fibres. A blood capillary is also seen under the muscle fibre. (395A. 13).

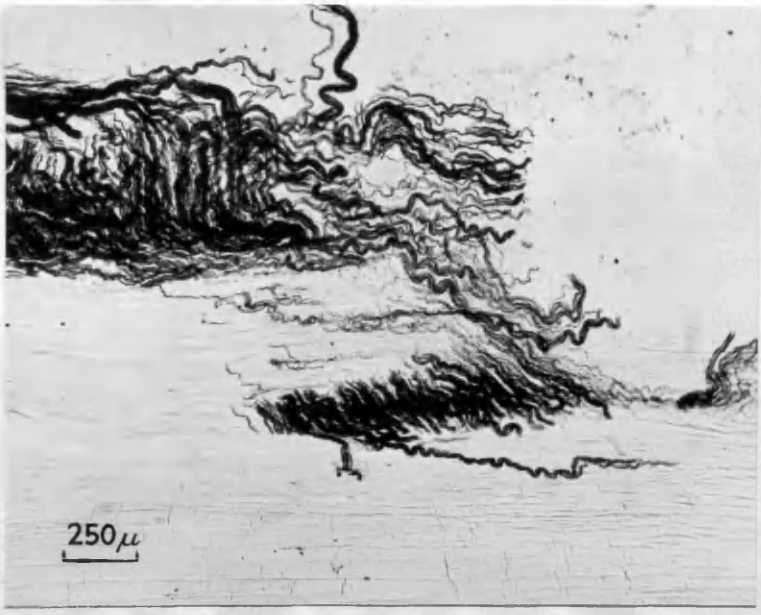

Figure 22. Section of a muscle whose nerve supply is intact showing the neuroma formed by an implanted peroneal nerve (483 B. 9.) Compare this picture with Figure 23 and Figure 12.

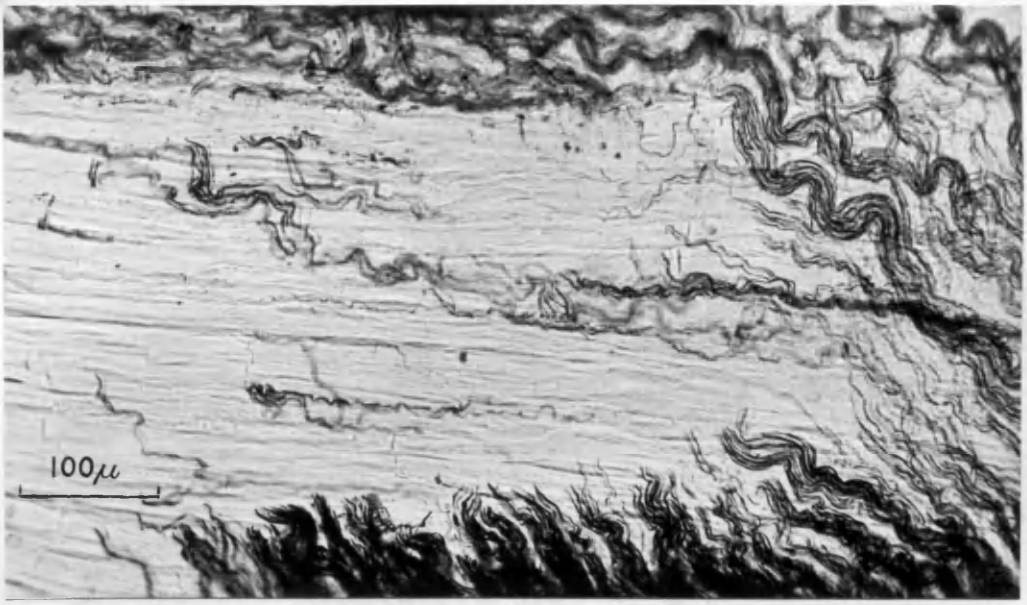

Figure 23. Section of muscle whose nerve supply is intact showing part of a neuroma formed by an implanted nerve. Note the thin fibres which travel unbranched in the fibrous tissue between the muscle fibres. (483 B. 9).

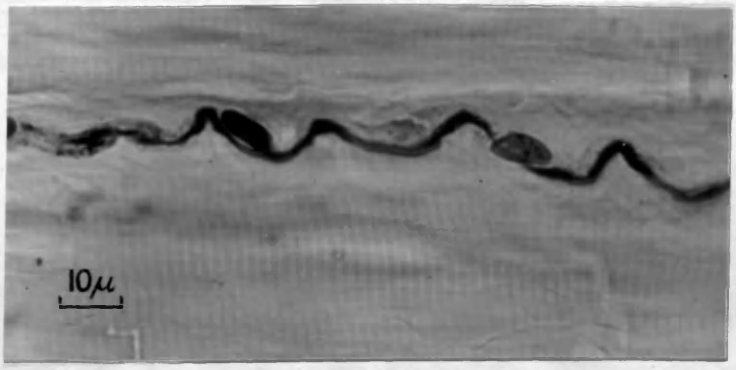

Figure 24. High power view of a nerve fibre. Note that it is travelling in the fibrous tissue between two muscle fibres. The nuclei show different degrees of staining with the silver. The black nucleus is that of a Schwann cell and the others may be in Schwann cells or fibroblasts. (484 B. 6a.)

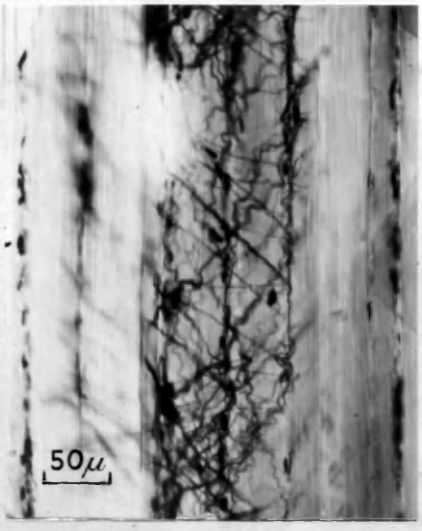

Figure 25. Section of a paralysed muscle showing the fibrous reticulum which surrounds the muscle fibres. Note that some of the fibres are very tortuous and that the nuclei are few in number. Compare this picture with that in Figure 24.

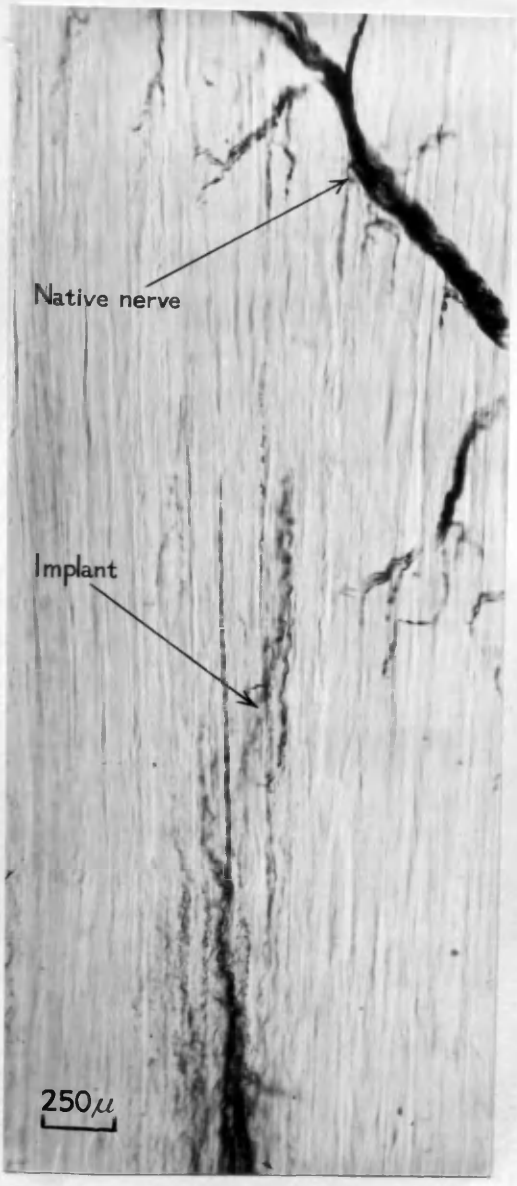

Figure 26. Fibres growing out from the implanted nerve are seen passing near to the native nerve supply. (856 G. 14.)

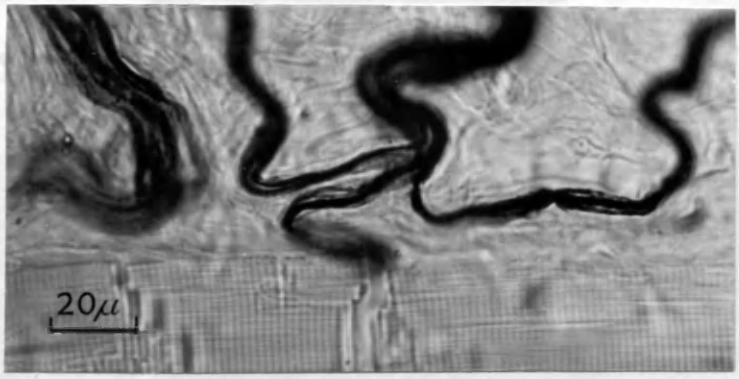

Figure 27. Implanted nerve fibres in a muscle whose nerve supply is intact. (484 D. 14.) Note the presence of the myelin sheath and the nodes of Ranvier.

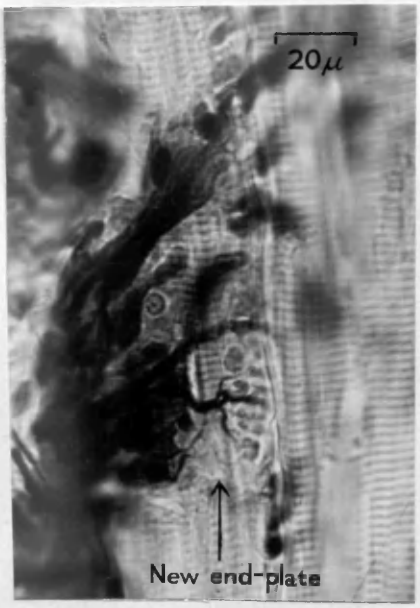

Figure 28. New end plate which has formed near to the neuroma of an implanted nerve. (251 B. 2.) Note fine, short fibre which divides up to form the motor end plate.

Figure 29. Motor end plates in a tenotomised muscle. (251 B. 39.)

Figure 30. Implanted peroneal nerve in gastrocnemius muscle which had been previously tenotomised. A group of native endings is present at the foot of the picture. Note the tortuous nerve fibres passing into the muscle. (251 B. 39.)

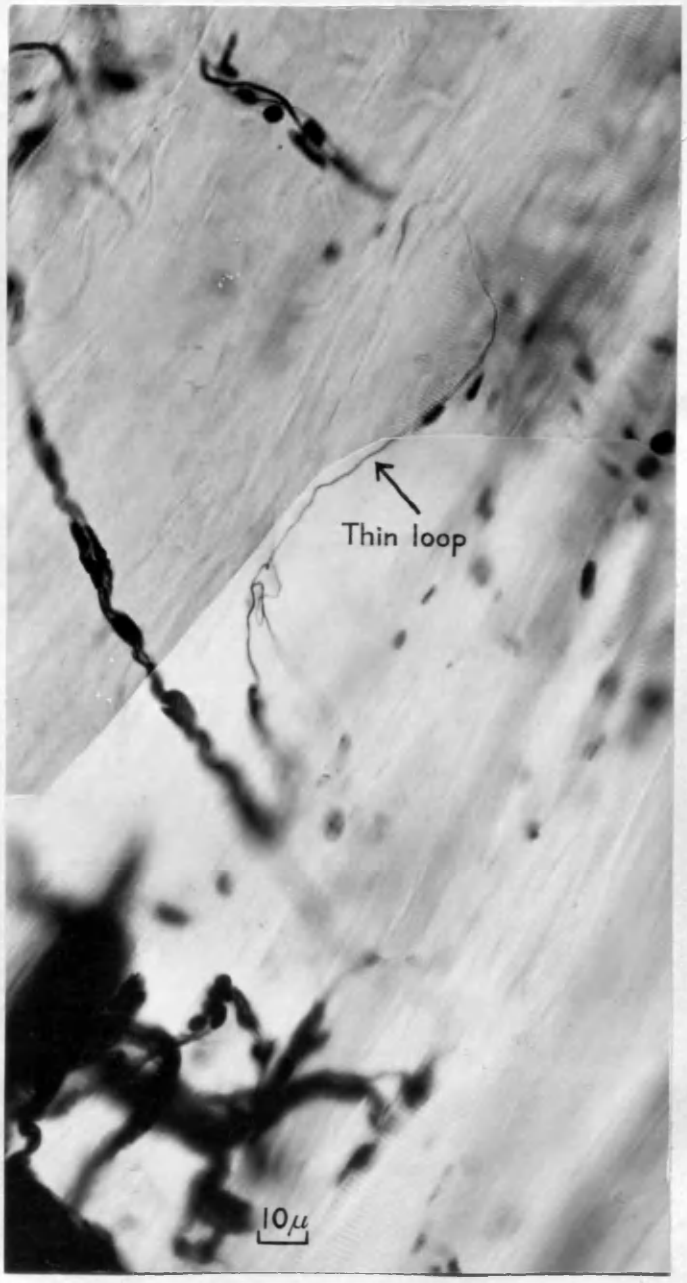

Figure 31. Example of a long, thin, straggling nerve fibre passing between muscle fibres and forming an almost complete loop. (251 B. 39.)

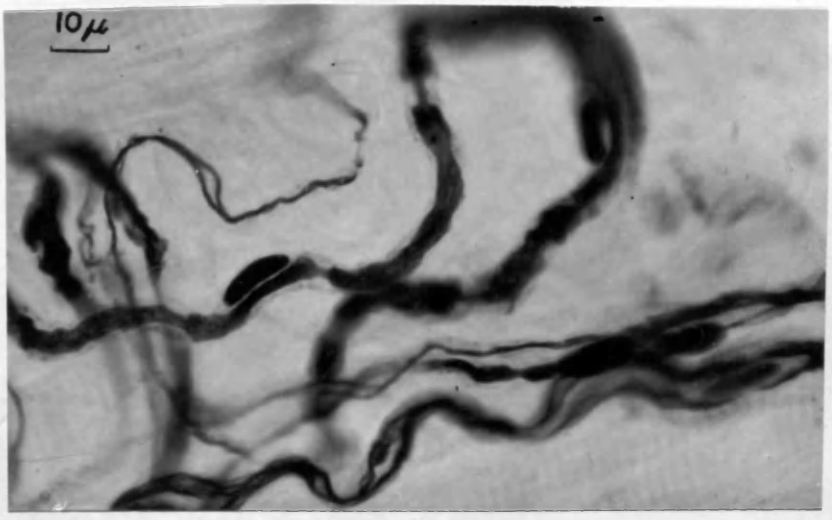

Figure 32. Myelinated nerve fibres growing from an implanted peroneal nerve into a tenotomised muscle. (251 B. 19.)

Figure 33. Nerve fibres growing out from an implanted n.g.m. into a tenotomised muscle (gastrocnemius). Note the unbranching straight fibres. (251 D. 36.)

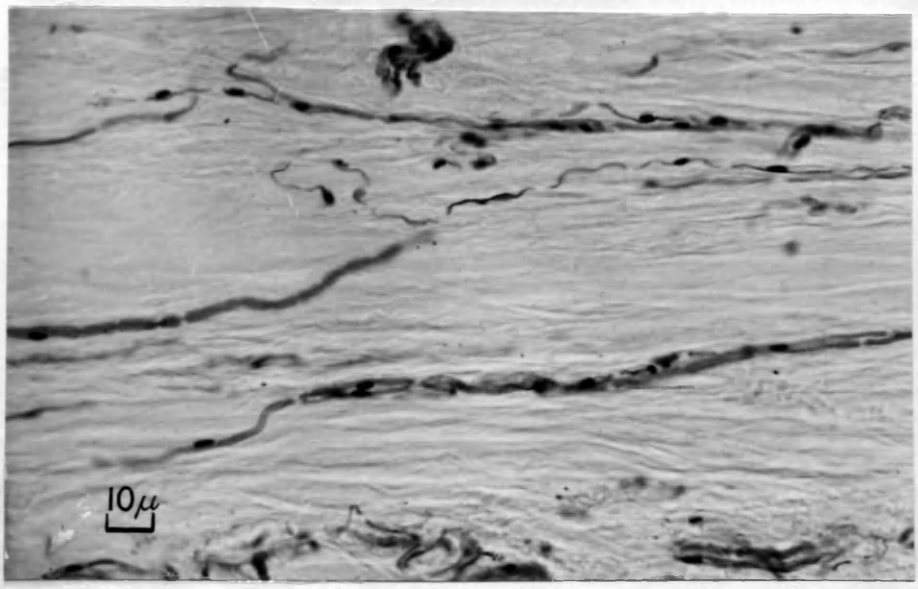

Figure 34. High power view of some of the fibres seen in Figure 33. Note the lack of branching in the presence of a myelinated sheath. (251 D. 36.)

Figure 35. Fine nerve fibre lying in close contact with muscle fibres. Some of the nuclei which are stained belong to Schwann cells (the darkest staining nuclei) but many of the others probably are muscle nuclei which have reacted to the near presence of a nerve fibre. (484 B. 6a.)

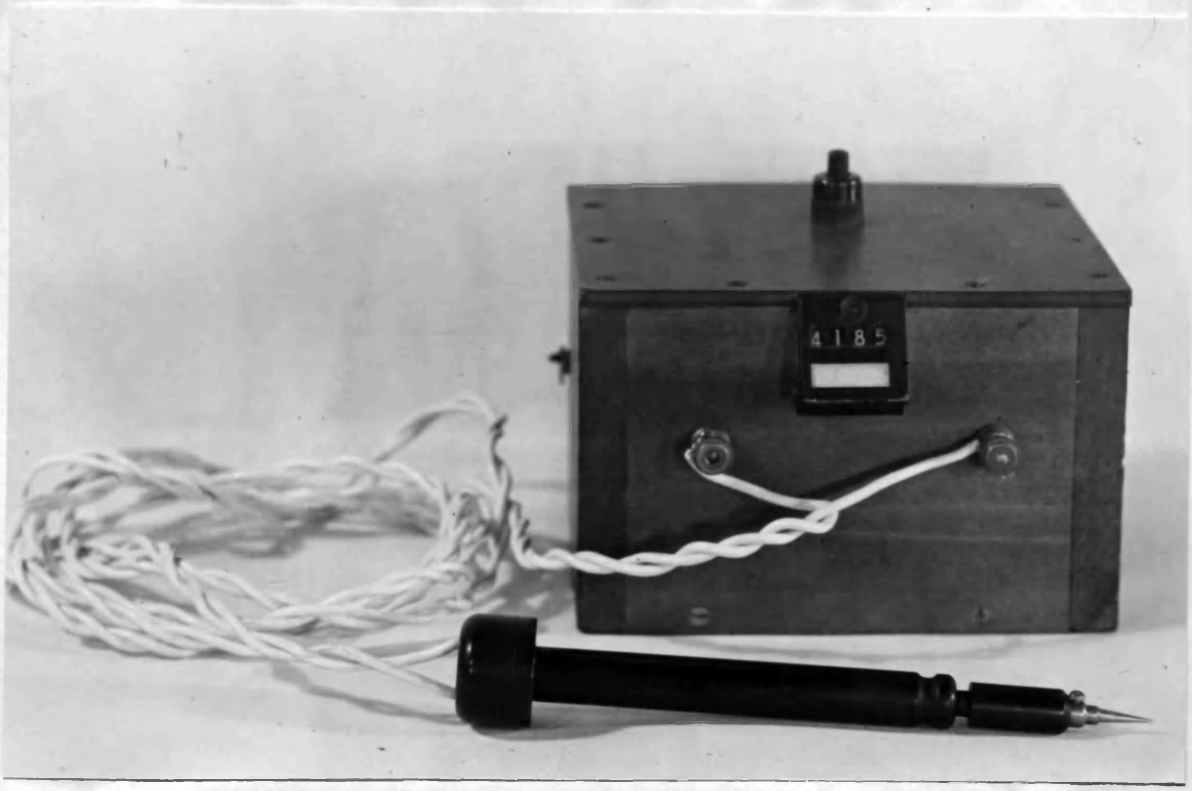

Figure 36. Photograph of the mounted needle used for marking off the nerve fibres and the Post Office Relay and counter which is activated by pressing on the needle.