NERVE REGENERATION AFTER IMMEDIATE AND DELAYED SUTURE

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INTRODUCTION

Studies of the histology of nervous regeneration have mostly been confined to the period immediately following a primary suture. But since progressive changes take place in an uninnervated peripheral stump, the conditions which new fibres will meet when growing into it will vary according to the duration of the previous degeneration. Surgeons have often emphasized the desirability of early operation on unrecovered nerve lesions: Foerster (1929) found that in cases operated more than 6 months after injury the recoveries obtained were less successful than those after earlier operation. However, little attempt has been made to discover to what extent this failure is due to changes in the nerve itself rather than to progressive changes in the denervated end-organ.

The experiments here reported were made in order to provide a thorough study of the late stages of nerve degeneration and of re-innervation after varying periods of degeneration, and thus to assist in deciding how long it is permissible to delay before operating on unrecovered lesions. Failure of recovery as a result of changes in the damaged nerve may be due to changes (*a*) in the power of the central stump to put

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out new fibres, (*b*) in the ability of the peripheral stump to receive them and to recreate a normal nerve, and (*c*) in the success of the union between the two stumps at the point of suture.

The proliferation of the Schwann cells in degenerating nerve and the increase in volume of their cytoplasm have been described by many workers (Ranvier, 1878; v. Büngner, 1891; Howell & Huber, 1892; Nageotte, 1932; Cajal, 1928; Boeke, 1935). Very many others have referred to the 'syncytial bands of Büngner', though there is some confusion as to the nature of the objects to which v. Büngner's name is attached. He himself conceived them as rods of cytoplasm in which the new axones appear during regeneration. Boeke (1935) and Bielschowsky (1935) consider that the new axones grow through the solid bands; Cajal (1928) supposed that the bands, being formed from the collapse of the tubular Schwann cell, have at least a virtual lumen, through which the axones grow. Dustin (1910) tended to the view that the axones grow along the surface of the bands, and this we shall find to be correct.

Even greater confusion exists about the fate of the walls of the tubes which contain the 'bands'. We shall see that it is these walls which maintain the pattern of the degenerated stump, make it permeable to new fibres, and also restrict the growth of the latter and determine their final diameter. Although the importance of the 'neurilemma' as a covering to the normal nerve fibre is stressed in every text-book of histology, its relations to the tube walls in degeneration are unknown.

Much of this confusion arises because of ignorance about the nature of the normal nerve sheaths and uncertainty as to their nomenclature. In an attempt to solve the problems we shall first discuss the structure of the sheaths of normal myelinated fibres, then describe the changes in the Schwann cells, neurilemma and endoneurium in degeneration, following them up to the later stages, of which existing descriptions are particularly incomplete. We shall then describe the process of re-innervation after short and long periods of degeneration, and give the results of experiments designed to examine the effect of the factors already listed on the success of regeneration after secondary suture.

MATERIAL AND EXPERIMENTAL METHODS

The experiments have mostly been with rabbits, but we have been able to make comparison with material removed by Prof. H. J. Seddon and Mr W. B. Highet at operation on cases of peripheral nerve injuries in man. We owe them our thanks not only for this material but also for much helpful discussion. It is not possible with human material to make so exact an analysis of degeneration and regeneration as with experimental animals, because after a peripheral nerve lesion the material removed at operation includes a peripheral stump which has degenerated for a known time but which has usually been to some extent re-innervated for an uncertain period. However, many conclusions drawn from the rabbit material have been verified in man, and we have also examined experimental material from dogs and cats.

In the rabbits the operations have mainly been on the tibial nerve, but the peroneal has also been used, and the latter has the advantage that recovery of its motor functions can readily be tested by looking for reflex spreading of the toes (Gutmann, 1942). The three parts of the sciatic trunk can be separated up to the head of the femur (though the sural sends a branch to the tibial in the middle of the thigh), and it is thus possible to leave the sural intact while operating on either or both of the other

two divisions. This precaution is very useful in experiments of long duration, for so long as the sensory fibres to the heel carried by the sural are intact, 'trophic' sores of the foot are much reduced. In this way animals with severed nerves have been kept in good health for over 18 months.

All operations were performed aseptically under nembutal and ether anaesthesia. The experiments fall into the following four series:

(1) *Peripheral stumps degenerated for various times.* These were obtained by resection of the tibial nerve for most of the length of the thigh, usually with the further precaution of injection of an inhibitor such as formaldehyde or gentian violet into the central stump (Guttmann & Medawar, 1942). In most cases this procedure was successful in preventing union of the stumps and re-innervation of the periphery.

(2) *Primary sutures.* These were made to study the process of re-innervation. The suture was made by the application of plasma (Young & Medawar, 1940).[1] When the tibial is cut, the ends always retract, but with practice and the use of fine watchmaker's forceps they can be brought together again and successful and uniform sutures obtained throughout a series of experiments.

(3) *Secondary sutures.* In order to obtain peripheral stumps which had degenerated for various periods of time it was necessary to follow the same procedure as in series 1. But after this the tibial central and peripheral stumps are so widely separated that suture is impossible. This difficulty was met by suturing the central stump of the freshly cut peroneal nerve into the distal stump of the degenerated tibial. Similarly, in the experiments to test changes in the power of outgrowth of the central stump after section, the neuroma on the tibial was removed and the nerve sutured on to the peripheral stump of the freshly cut peroneal.

These procedures have the disadvantage that normal functional recovery cannot be tested, but they have the advantage that uniform sutures without any tension can always be made. Further, it is the only method by which changes in the regenerative capacity of central and peripheral stumps can be separately analysed (see Kilvington, 1912).

(4) *Outgrowth from the peripheral stump after various times of degeneration.* It is well known that when a peripheral stump is left far from a central stump strands of Schwann cells grow out from it forming a 'peripheral glioma' or 'Schwannoma'. It seems very likely that this peripheral outgrowth is of great importance in ensuring a satisfactory union of the stumps when nerves are sutured, even when their apposition is optimal. So we prepared, as in series 1, a series of peripheral stumps degenerated for various times and then cut them and left them without suture to determine whether the Schwann cells progressively lose their power to grow out and thus to make a satisfactory union at suture.

HISTOLOGICAL METHODS

All material was removed at biopsy operations. The pieces of nerve were kept extended under normal tension on cardboard, and fixed at once. The selection of histological methods is not easy, for the distribution and arrangement of the cytoplasm of the Schwann and mesodermal cells in the degenerating nerve can only be studied after

[1] We are very grateful to Mr P. B. Medawar for preparing the concentrated plasma with which all the sutures were made. Without the help of this method the whole investigation would have been very much more difficult, and parts of it impossible.

the best cytological fixation, such as is given by Flemming's fluid, and in thin sections (4 μ) which can only be obtained after paraffin embedding. Bouin's fluid was much less satisfactory than Flemming's and produced serious shrinkages. Unfortunately there is no method by which fine nerve fibres can be demonstrated after Flemming or any equally good fixation, and there is no doubt that many of the conclusions reached by early workers are unreliable, being produced by too great reliance on the use of special fixatives for the silver impregnation of axones. We have made much use of the method of Bodian (1936) on material fixed in his alcohol-formol-acetic mixture no. 2 (Bodian, 1937), but the appearances have been checked by reference to better fixed material. Material impregnated by Bodian's method can be counterstained with Masson's haematoxylin, Ponceau-fuchsin, light green, and in such preparations the protoplasm of Schwann cells is often well demonstrated by a purplish brown tint produced by a combination of the red stain with an incomplete differentiation of the haematoxylin, though unfortunately it is usually much shrunk by the fixation.

We have not employed any of the existing 'specific' methods for the staining of Schwann cells. Bailey & Hermann (1938) had no success with the methods of Nageotte, and the disadvantages of frozen sections discouraged us from the use of modified Hortega methods such as that employed by Murray, Stout & Bradley (1940).

Therefore, for the demonstration of the unshrunken protoplasm of Schwann cells we have found Flemming's fluid most satisfactory. The mixture we used was: chromic acid, 1 % 15 c.c.; osmium tetroxide, 2 % 4 c.c.; glacial acetic acid, 5 drops. Flemming material was counterstained with Masson's light green trichrome and with safranin and light green. Flemming without acetic was tried, and although the fixation of cytoplasm seems equally good, staining is more difficult. Fixation in Zenker's fluid followed by staining in phosphotungstic-haematoxylin by Mallory's method shows the cytoplasm of Schwann cells quite well in degeneration, and has the advantage that myelin remains are also visible.

In our extensive use of Bodian's method on material fixed in his alcohol-acetic-formol mixture and in formol saline we have often obtained preparations in which the cytoplasm of Schwann cells is sharply impregnated. With alcohol-fixed material this impregnation is usually found when the preparations are reddish in colour rather than blue-black, though these red preparations must not be confused with those given when some of the reducing solution is carried over on the slide into the toning bath. We have made many experiments with the method, but we have not been able to trace the variable that causes the method to give sometimes a sharp black impregnation of axones against a clear background, and sometimes an impregnation of Schwann cytoplasm, with the axones more or less well demonstrated also.

Recently we have found that the surest way of obtaining Schwann cell impregnation in degeneration is as follows: Mounted sections of formol-fixed material are impregnated overnight at 37° C. in 0·5 % aqueous Protargol (no copper is added to the bath). The sections are then treated by reduction with hydroquinone, toning, and a second reduction exactly as described in a silver method for axones already published, commencing at stage 8 (Holmes, 1942). In the resulting preparations Schwann nuclei as well as other nuclei in the section are darkly stained. The cytoplasm of the Schwann cells, although shrunken by fixation and embedding, stands out as blue-black filamentous strands (see Pl. 4, figs. 24, 26 and 29).

But it must be pointed out that many of our Schwann cell preparations have been

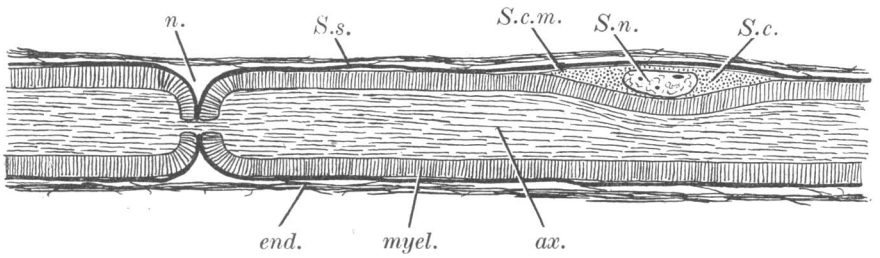
made by Bodian's original method, and we have not yet had sufficient experience with the new variant to say whether it can be used as a 'specific method for Schwann cells' under all circumstances.

Not the least of the difficulties of Bodian's method arises from variations in the composition of the Protargol, a colloidal silver preparation. We have used the Bayer brand of German manufacture and the American product of the Winthrop chemical company. Other substances marketed under the same name have not proved satisfactory.

For the demonstration of the myelin sheaths, formol-fixed material was mordanted for 14 days with 3% aqueous potassium dichromate and embedded in wax. The sections were stained overnight at 37° C. in Kulschitsky's haematoxylin, differentiated by Pal's method and counterstained with alum carmine.

Embedding has been through cedar-wood oil to wax, and usually transverse and longitudinal sections were cut from each block. We found that careful paraffin embedding need not produce appreciable distortion by differential shrinkage, even of the most delicate cytoplasm.

We owe our thanks to Mr James Armstrong for the very valuable work he has done in caring for the animals, and especially in dealing with such a large body of histological material.



Text-fig. 1. Diagram of a normal nerve fibre as it is revealed by methods here adopted. Based on tracings from the fibres shown in Pl. 1, fig. 1. The Schwann nucleus lies nearer to the node than it would usually do, but otherwise the proportions are correct. *ax.* axone; *end.* endoneurium; *myel.* myelin sheath; *n.* node; *S.c.* Schwann cytoplasm; *S.c.m.* Schwann cell membrane; *S.n.* Schwann nucleus; *S.s.* Schwann sheath (neurilemma).

THE SHEATHS OF THE NORMAL NERVE FIBRE

There is as yet no wholly satisfactory account of the relationships of the neurilemma, the Schwann cells and the endoneurium in a normal nerve fibre. In our best fixed material we see around each Schwann nucleus a small mass of cytoplasm (Pl. 1, figs. 1, 5 and 6), but we have not been able to convince ourselves that this layer extends over the whole of the myelin sheath. Nemiloff (1910), Doinikow (1911), Nageotte (1932), Cajal (1933), Rodríguez-Pérez (1934) and others have given figures showing a network of material all over the myelin, and they claim that this is the Schwann protoplasm, and some of them that it also extends within the myelin layer. But the appearances produced by their various methods are very different, and they do not demonstrate the 'protoplasmic nature' of the layer. We cannot deny the possibility that there is such a fine sheet of protoplasm, perhaps fenestrated, over the myelin, particularly as Doinikow said that it was not demonstrable in the rabbit on which most of our cytological work has been done. Sometimes, as in Pl. 1, fig. 1, there appears to be a definite edge or end to the Schwann cytoplasm, as if it does not extend

over the whole fibre. On the other hand, some of the appearances seen during degeneration can hardly be interpreted except on the assumption of a layer of Schwann protoplasm covering the myelin (Pl. 1, fig. 4 and Pl. 2, fig. 12).

If such a layer exists in rabbits' nerves it must be less than 1 μ thick, since it does not show, except near the nucleus, in transverse or longitudinal sections after Flemming fixation, which gives optimal preservation of the myelin and Schwann protoplasm (Pl. 1, figs. 5, 6). Over most of the surface of the fibre, therefore, the visible boundary adjoining the myelin is that clear membrane which Schwann first described (Pl. 1, figs. 1, 2, 5, 6; Pl. 2, fig. 7). It turns in at the nodes and is distinguished from the endoneurium outside it by being smooth and continuous rather than obviously fibrous. It often stains rather like collagen but may appear brown rather than green with Masson's light green trichrome stain after Zenker or Flemming fixation. This membrane, the sheath of Schwann, is often called the neurilemma, though this term would have puzzled Schwann. For in his time, and by some more recent authors (e.g. Cajal, 1928), the word neurilemma was used for the epineural and perineural connective tissue of the nerve trunk, and Schwann specifically stated that his sheath was distinct from the neurilemma in this sense. However, the term has been so widely used as a synonym for the sheath of Schwann that it is probably justifiable to retain it (see Young, 1942).

Outside the Schwann sheath lies the connective tissue which is definitely collagenous and does not turn in at the nodes (Pl. 1, figs. 5, 6; Pl. 2, fig. 7). This includes the sheath described by Key & Retzius (1873) and constitutes the endoneurium. To this endoneural connective tissue the term 'sheath of Henle' is often applied (see Ranvier, 1878).

There are, therefore, certainly three easily distinguishable entities outside the myelin: the Schwann cell, which may or may not cover the whole fibre, the Schwann membrane or sheath, alias neurilemma, and the sheath of Key and Retzius. Unfortunately, we still do not know enough about the chemical and physical natures or histogenesis of these layers to be able to devise an entirely satisfactory terminology. Even their appearance varies very much with the method of treatment.

Plenk (1934) holds that the situation can be described by saying that there is an inner endoneurium, which may be called the sheath of Plenk and Laidlaw, composed of a network of fine argyrophil fibres; and an outer endoneurium or sheath of Key and Retzius, of longitudinal collagen fibres. As he could not simultaneously demonstrate what is here called the Schwann sheath and the inner endoneurium Plenk believed the two to be identical. But this cannot be considered to be proved and we reluctantly conclude that it is premature to adopt the attractive solution of calling the neurilemma and the sheath of Key and Retzius the inner and outer endoneurium respectively. One danger in adopting such a terminology is that it may eventually be shown that the 'inner endoneurium' is a product of the Schwann cell, a view that has wide implications in neuropathology (Masson, 1932).

In any case it is most desirable to avoid confusion between the protoplasmic Schwann cell and the membrane here called neurilemma. It is therefore not desirable to use Schwann sheath as synonymous with Schwann cell (cf. Foot, 1940). It is the protoplasm of the Schwann cell which in the degenerated state forms the protoplasmic Schwann bands or bands of v. Büngner. The terms Schwann sheath or Schwann membrane should be used, if at all, as a synonym for 'neurilemma'. When Schwann

first described his membrane he implied that it was the outer membrane of the Schwann cell. But it may be presumed that he did not mean 'cell membrane' in the sense of modern general physiology. Though it is true that the Schwann sheath lies immediately outside the Schwann cell the preparations do not suggest that it constitutes the 'cell membrane' of the latter in the modern sense (Pl. 1, fig. 1 and Text-fig. 1).

1. *Progressive changes in the peripheral stump*

Our study of the degeneration of the peripheral stump is based mainly on nerves from forty-eight rabbits in which the stump was kept isolated, by the method described on p. 65, for periods from 6 days to 17 months. Many nerves from other rabbits have contributed details, and confirmation and comparison of many points have been made on human material, and on nerves from dogs and cats.

1·1. *Initial changes in the Schwann cells.* During the first 2 weeks after severance of a nerve, and while the axones and myelin in the peripheral stump are undergoing degeneration, the Schwann cells increase in number and amount of protoplasm. From the normal condition in which the protoplasm appears only as a small mass in the immediate neighbourhood of the nucleus, it extends and thickens till it lines a considerable part of the nerve tube, and then becomes a long strand as the myelin collapses (Pl. 1, figs. 3, 4; Pl. 2, figs. 12–14). Some stages suggest that this appearance of the Schwann protoplasm is the making manifest of a layer previously existing over the whole myelin surface (Pl. 1, fig. 4; Pl. 2, fig. 12). But even if there is such a layer it seems certain that in order to produce the voluminous strands of Schwann protoplasm seen 2 weeks after section there must be synthesis of fresh material. The nucleoli of the Schwann nuclei often become much more marked from the 4th day onwards, and this appearance may be related to the active process of protoplasmic synthesis (Pl. 1, fig. 4). However, it is also possible that this appearance is related to the division of the Schwann nuclei which is very abundant about the 9th day. Mitotic figures can still be seen after 15 days.

In the early stages of degeneration the myelin first disappears from the outer surface of the sheath (Pl. 2, figs. 9, 11), and it may be that this disintegration is brought about by enzymic activity of the Schwann cells. However, we have found little evidence that the Schwann cells are actively phagocytic, and none that they become converted into special phagocytic cells. Occasionally, while the Schwann protoplasm is increasing, granules of degeneration products may become included in their cytoplasm (Pl. 2, fig. 10). We shall not deal here with the detailed changes in the myelin, which have often been described. In places the myelin may retain its original form for a surprisingly long time (25 days or more), at least over short stretches.

1·2. *Macrophages.* It is probable that the removal of myelin and axone in degeneration takes place partly by autolysis and liquefaction, but the phagocytic activity of macrophages plays a large part in the process. Histiocytes from the connective tissue of the nerve, and from the walls of the blood vessels, can be seen in all stages of conversion into macrophages, and they move among the degenerating fibres and penetrate within them (Pl. 2, fig. 8). Here they ingest myelin and axone remains and become converted into typical foam cells. In our series of rabbits' nerves they appear in small numbers about the 7th day and become very numerous by the 15th day. They appear especially early in the region of the direct trauma to the nerve; in a

human nerve which had been crushed over a length of 5 mm., macrophages were abundant on both sides of the lesion 4 days later.

In rabbits the height of the macrophage invasion is reached during the 3rd week after the injury, and thereafter their number slowly declines. Macrophages laden with debris can be seen around the blood vessels, but their exact fate cannot be determined. These cells around the blood vessels never show the maximum of vacuolation characteristic of the fully developed foam cells within the fibres, and it may be that the latter never emigrate from the tubes but are themselves removed by a later invasion.

In the later stages of degeneration removal of the remaining macrophages takes place very slowly: some were still found in a stump which had been uninnervated for 17 months, and they often seem to have shrunk into small globules which are perhaps never removed at all (Pl. 3, fig. 15).

1·3. *The Schwann tubes and the movement of Schwann cells within them.* With the disappearance of the degeneration products there is a progressive decrease in the total diameter of the tubes which remain, and whose walls are made up of the neurilemma and endoneurium. The shrinkage takes place first between the groups of macrophages, giving the characteristic varicose appearance of a degenerate fibre (Pl. 2, fig. 8; Pl. 8, fig. 62). It is important to realize that a definite tube, representing the original fibre, persists throughout the process of degeneration. These tubes may conveniently be called *Schwann tubes*. They have sometimes wrongly been called the bands of Büngner, a term which should be restricted to the protoplasmic masses contained within the Schwann tubes. There has been much uncertainty as to the fate of the outer membranes of the nerve fibre during degeneration. Cajal (1928), after some hesitation, decided that what we here call the neurilemma disappears in degeneration. On the other hand, Nageotte (1932) and Masson (1932) believed that it becomes thickened by collagenization and merged into the endoneurium. We find that it persists throughout degeneration (Pl. 2, figs. 9, 11 and 12). The endoneurium outside it becomes thickened during the later stages and may in some cases seem to merge with the neurilemma, but in other preparations the latter appears quite distinct even after long periods of degeneration (Pl. 5, fig. 35; Pl. 6, figs. 46, 47).

As the myelin and axone disappear the walls of the tube collapse, shrinking down on to the Schwann protoplasm, which thus comes to fill the tube almost completely, except for the regions still occupied by macrophages and myelin remains (Pl. 2, figs. 11, 12). This filling of the tube may depend partly on further synthesis of Schwann protoplasm, but must also result from the squeezing down of the broader masses of cytoplasm which are found in the earlier stages. Thus already by the 15th day a considerable part of the length of the smaller fibres, and the regions between the macrophages in the larger fibres, are much narrower than the original tube and are filled with Schwann cytoplasm. However, this cytoplasm is not a continuous solid mass. Even with the best cytological techniques it not only shows signs of the well-known internal fibrils (Ranvier, 1878; v. Büngner, 1891), but it also appears that each Schwann cell to some extent retains its individuality, so that separate 'Schwann fibres' are formed. It is not easy to determine how this state of affairs arises without the use of means of continuous observation, but apparently after division each Schwann nucleus collects a portion of cytoplasm in the form of an elongated structure. Thus the tube comes to contain a number of these cells, often lying alongside each other (Pl. 2, figs. 13, 14; Pl. 3, fig. 23), the separation of the cells being particularly clear

when the 'fibre' of one cell runs past the nucleus of another (Pl. 2, fig. 14). It is exceedingly difficult in such delicate protoplasmic masses to determine to what extent the Schwann protoplasm is divided up in this way into separate 'fibres'. There is no doubt that in many cases two or more nuclei lie within a single body of protoplasm, unseparated by membranes (Pl. 4, fig. 31, *S.c.*). But we have been impressed by the fact that, even after Flemming fixation, lines appear which we can only interpret as indicating a certain degree of separation of the protoplasmic masses (Pl. 3, fig. 23).

The conclusion that the collapsed Schwann tubes are filled by elongated cells with some degree of independence is confirmed by a variety of appearances suggesting that the Schwann cells move actively within the old tube, seven when the earlier stages of proliferation have been passed. At either end of a macrophage or group of macrophages there are usually collected several Schwann cell nuclei (Pl. 3, fig. 20), the regions between the macrophages having long stretches which are almost devoid of them. Moreover, the ends of nuclei that face the macrophage are often flattened (Pl. 2, fig. 13; Pl. 3, figs. 15, 22), while those nuclei which have insinuated themselves between the macrophages and the wall of the tube do not show this flattening (Pl. 2, fig. 8). The collection together of numerous nuclei and the flattening of the ends of those that lie against the macrophages might be produced as a result of active pressure by either or all of (1) the Schwann cells, (2) the macrophages and (3) the neurilemma and endoneurium. That the macrophages exert pressure on the Schwann cells is shown by the fact that in some cases Schwann nuclei have both ends flattened (Pl. 3, fig. 21; Pl. 4, fig. 26). That the Schwann cells also migrate and exert pressure on the macrophages is suggested by the reverse appearance, namely, that as the cytoplasm of the macrophages shrinks the Schwann nuclei on either side of it remain closely flattened against it. When the remains of the macrophages are reduced to a persistent small droplet a Schwann nucleus can nearly always be found pressing against either side of it (Pl. 3, fig. 22; Pl. 4, fig. 26). It cannot be entirely excluded that these appearances are due to the pressure of the contracting endoneurium, but from our knowledge of other migratory activities of Schwann cells it is reasonable to suppose that they move within the Schwann tubes.

Table 1. *Lengths of Schwann cell outgrowth observed after leaving peripheral stumps isolated for various periods of time after section of the nerve*

Period of outgrowth days	Length of outgrowth mm.	Period of outgrowth days	Length of outgrowth mm.	Period of outgrowth days	Length of outgrowth mm.
15	1·2	15	1·7	30	5·0
15	0·8	15	1·4	42	3·5
15	2·0	25	3·4	53	3·6
15	1·7	25	2·0	54	3·5
15	1·5	25	2·2	83	4·5
15	0·7	25	2·0	218	3·0
15	2·4	25	1·4	484	3·1
15	0·5				

1·4. *Outgrowth of Schwann cells from peripheral stumps.* Active movement of the Schwann cells is also responsible for the well-known phenomenon of the outgrowth of Schwann cells from the end of the peripheral stump of a divided nerve, though the progressive contraction of the lumen of the Schwann tubes may also play a part in forcing the Schwann cells out of the end of the stump. In our series we have seen

very many examples of this outgrowth (Pl. 3, figs. 16–19; Pl. 9, fig. 71). It is not always easy to distinguish Schwann cells from fibroblasts, and we cannot give any final criteria for distinguishing the nuclei of the two cell types. Those of the Schwann cells are usually regular, and those of the fibroblasts irregular in outline. In the Schwann cell nuclei there are numerous small bodies while the fibroblast nuclei contain few and large granules. A more important and reliable distinction is the mode of growth of the cells, and hence their arrangement in the outgrowth. The Schwann cells usually grow out together in groups, making a strand several cells thick in which the cells are separated by little or no collagen, while the fibroblasts, even when longitudinally orientated, do not form such intimate strands and are usually separated by more collagen. Thus the columns of Schwann cells can be clearly distinguished after Mallory's stain as multinucleate red strands running amid the blue connective tissue. By these criteria the limits of Schwann cell outgrowth from a stump can be approximately determined, though it is not easy to locate the farthest limit reached by isolated strands growing out farther than the rest. Table 1 shows the lengths of the Schwann outgrowth from peripheral stumps at different intervals after section of the nerve, the central stump having been isolated and union prevented. The values are derived from measurement on longitudinal sections, and they are conservative estimates of the maximum distance of outgrowth, for they include only Schwann bands which could be positively identified. A few pioneering cells may well extend much farther. Also no correction has been included for the factor of shrinkage during fixation and embedding, so that the actual distances of outgrowth may be greater by perhaps 50 %. But, even so, the distances of outgrowth measured by histological methods would be less than those estimated by macroscopic study (Young, Holmes & Sanders, 1940). The explanation for this discrepancy is that at the end of a peripheral stump the fibrous tissue of the perineurium and epineurium grows out with even greater activity than the Schwann tissue. Thus an apparently large and diffuse 'Schwannoma' is often found microscopically to consist largely of fibrous tissue.

From Table 1 it can be seen that Schwann outgrowth is progressive in the first few weeks after nerve section, but from study of the peripheral stumps isolated for much longer periods we can state with certainty that it does not go on indefinitely. This condition contrasts with that described by Masson (1932) when a segment of nerve is isolated by two cuts, for then a Schwann outgrowth achieving a size many times that of the original nerve arises from both ends of the stump. We have found no comparable progressive proliferation from peripheral stumps of rabbits left after a single cut. Indeed, pieces removed from the tips of stumps left for long periods of degeneration often show very few Schwann cells extending into the scar. Possibly there is therefore some atrophy of the cells of the initial outgrowth.

Although Schwann outgrowth does not continue indefinitely we have often seen quite large gaps between central and peripheral stumps bridged when the nerves were left for a considerable length of time. Such bridges, of course, include some outgrowth from the central stump, but it is possible that they owe their development to an unusually lengthy outgrowth by the Schwann cells stimulated by the existence of a convenient pathway or line of stress in the operation site. In the later stages of the proliferation the Schwann cells in the outgrowing bands become separated by thick collagenous walls, but we have not been able to decide the origin of this collagen (see Masson, 1932).

In the earlier stages of outgrowth mitotic figures can be seen in the Schwann nuclei (Pl. 3, figs. 16, 18), so that the outgrowth is due to cell division as well as to continual wandering out of cells from the peripheral stumps. But in outgrowths later than 2 months or so mitoses are very rare. The nuclei in the outgrowths (and in the peripheral stump) often show curious appearances, sometimes having transverse partial divisions (Pl. 4, fig. 28). It would not be safe, however, to consider this appearance to be evidence of amitotic division of the Schwann cells.

It is probable that there is a good deal of anastomosis between the cytoplasms of the cells of these outgrowths, but the tissue is not a uniform 'Schwann syncytium' in the sense of a single mass of protoplasm like a slime fungus. Cracks and fissures between separate cells are very obvious, and these presumably provide surfaces over which new axones can creep (Pl. 3, figs. 16, 18).

1·5. *Late changes in an uninnervated peripheral stump.* There has been some dispute as to the later stages of degeneration of peripheral nerves which have been left uninnervated for long periods. It has sometimes been suggested that the stump becomes merely a mass of fibrous tissue incapable of receiving new axones, though Spielmeyer (1922) did not accept this view. It is certainly true, as Dustin pointed out (1917), that in the region of human nerve lesions the peripheral stump becomes fibrosed and incapable of receiving new fibres; but this hardly concerns us here, since it is a special phenomenon, and the resection of such fibrosed tissue is necessary before nerve suture (but see p. 77).

1·6. *Late changes in Schwann cells.* Our observations are based on 'sterile' stumps left for various periods up to 17 months. The later changes in the Schwann cells are simply a further evolution of those we have already discussed: indeed, some of the smaller Schwann tubes reach in a few weeks the condition of the larger ones after several months. The wall of the larger tubes contracts progressively for some months, so that the tubes become narrower. This is shown clearly by the change in form of the nuclei which become extraordinarily thin and elongated and after fixation often assume a characteristic sinuous appearance (Pl. 4, fig. 25).

The Schwann cytoplasm, meanwhile, is pressed out into long strands which often have very much the appearance of nerve fibres in silver preparations (Pl. 4, figs. 26, 29 and 31). In the smaller tubes there is no room for two nuclei to lie side by side, and the result is, therefore, a strand of protoplasm with nuclei at intervals along it (Pl. 4, fig. 31). In the larger tubes, however, the condition described in the earlier stages may persist, and the tube is occupied by two or more cells pressed side by side. The apposition between them is very close, and no open spaces are found in the tubes, but in both transverse and longitudinal sections it can be seen that cracks and fissures may exist between the cells (Pl. 4, fig. 27). The longitudinal fibrils within the Schwann cytoplasm can still be seen. They are often rather clearly demonstrated by Bodian's stain after formol fixation (Pl. 4, fig. 28), and fine blue striations are seen after fixation with Zenker and staining with phosphotungstic haematoxylin. But further discussion of the nature of this longitudinal organization of the cytoplasm must await the investigation of the birefringence and other physical characteristics of the cells.

After fixation with the alcohol-formol-acetic mixture transverse sections of the tubes often show the Schwann protoplasm shrunk to a number of fine threads distributed mainly at the periphery. These threads stain with Bodian's silver method, and brown with Masson's trichrome (Pl. 5, fig. 37). Thus in such preparations it usually appears

that there are considerable spaces in the Schwann tubes. But in well-fixed Flemming material it can be seen that the protoplasm fills the whole of the tube (Pl. 5, figs. 35, 36 and 38), so that the spaces seen after alcohol fixation must result from shrinkage, and occasionally a tube with well-preserved cytoplasm can be seen after alcohol fixation, while even in Flemming material the Schwann protoplasm in the centre of the nerve is often shrunken (Pl. 5, fig. 34). Often two nuclei can be seen lying side by side or end to end within an apparently homogeneous mass of cytoplasm (Pl. 5, fig. 35), but careful inspection of the well-fixed preparations shows the same signs of division into separate cells as was seen in the earlier stages.

The appearances seen with other fixatives also yield reliable evidence that the Schwann cells are to a considerable extent separate from each other. The fine drawn-out tip of one cell can often be seen ending against the side of another (Pl. 4, fig. 27). Still more convincing is the fact that some of the cells terminate in remarkable cytoplasmic balls or clubs, often of relatively very large dimensions (Pl. 6, figs. 40–42). These formations are presumably the result of the damming up of the Schwann cytoplasm by a macrophage at an earlier stage (Pl. 2, fig. 10). After the macrophage has been removed the Schwann cytoplasm is no longer in a sufficiently fluid state to allow the ball to become pressed out into a fibre. Sometimes, also, relatively broad masses of Schwann cytoplasm which are not terminal can be seen (Pl. 6, fig. 43).

We may conclude, therefore, that in the later stages of degeneration the Schwann tubes are entirely filled with the protoplasm of very elongated cells. Often these protoplasmic masses are multinucleate, but there are also cracks between them, especially in the larger tubes. Impregnation of these cells with silver by the method described on p. 66 after formol fixation, and sometimes by Bodian's method, shows a nerve stump full of very long fibres (Pl. 3, fig. 16; Pl. 4, figs. 24, 26, 29 and 30), and as each fibre may have a smooth outline extending over several millimetres they are often extremely difficult to distinguish from nerve fibres. That they are Schwann cells may be established by the fact that the protoplasm contains a nucleus, but if this is not included in the section, it is sometimes impossible even with the highest powers of the microscope to determine whether a fibre is an axone or a Schwann cell. It is probable that the difficulty is equally acute in the study of denervated stumps by other silver methods, and as pointed out elsewhere (Young, 1942), Cajal (1928) certainly made the confusion. Pl. 4, fig. 31, shows a partly re-innervated stump in which the more lightly stained Schwann cells and darker nerve fibres can just be distinguished, but it is very easy to understand that confusion between them might arise.

1·7. *Retrograde re-innervation of peripheral stumps.* Many workers have described normal nerve fibres in peripheral stumps after every precaution had been taken to prevent their re-innervation. Lugaro (1906), Perroncito (1907) and others produced evidence that such fibres do not arise by autogenous regeneration in the isolated stump. As the difficulty of distinguishing Schwann cells from axones is so great we thought it desirable to re-examine the question of the origin of such fibres. The problem is of considerable importance, for at operation on peripheral nerve lesions a sensory response obtained from the peripheral stump is usually taken as evidence that regenerating fibres have crossed the lesion and that further recovery may take place without operative interference. If sensory fibres can be found in peripheral stumps which are without doubt isolated from their central stumps, then the value of such an operative test is doubtful.

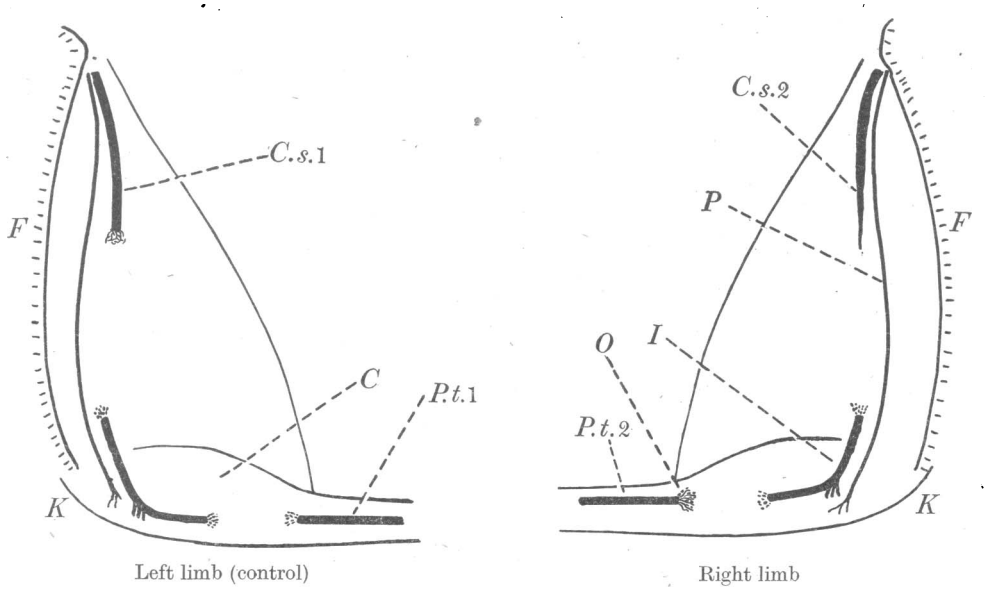
Early in our experiments we noticed that in animals in which special precautions had been taken to prevent union between the stumps, pinching or electrical stimulation of the peripheral stump after light anaesthesia always produced reflex responses. Such responses persisted even after the central end of the peripheral stump had been cut, to interrupt any fibres which might have bridged the gap in spite of all precautions. The fibres responsible for these anomalous responses must therefore enter the stump from the distribution of intact nerves, either by backward growth from the skin plexuses or by entry with the blood vessels. So far as we have been able to investigate their source systematically we find that these fibres enter from the blood vessels. Thus in rabbit 425 a length of 3·5 cm. was removed from the tibial nerve, and its central stump injected with osmium tetroxide. Sixteen months later the animal was anaesthetized and the nerve exposed. Pinching the peripheral stump was followed by reflex responses. A second pinch *central to* the first one then gave no response, but more peripheral pinches continued to do so. This shows clearly that the fibres are not derived from the central stump, but *ascend* the nerve. The peroneal nerve was then cut, but the responses were not abolished. The tibial peripheral stump was then completely exposed, but not freed from its bed, and a series of pinches made along it, beginning centrally. Reflex responses continued until the stretch of nerve just above the heel was separated from the posterior tibial artery with which it runs; after this no further responses could be obtained. Clearly the sensory fibres entered the nerve with the vessels at that point, for had they been derived from the skin plexuses the response would not have been abolished.

Similar evidence can be obtained by eliciting responses of this sort from a degenerated stretch of tibial nerve in the thigh, and then crushing the popliteal artery. No further responses can then be obtained from the nerve (see Gutmann *et al.* 1942). We conclude that few or no pain fibres run a retrograde course from the skin plexuses.

It should be noted that these retrograde fibres do not occur in a normal nerve. When a fresh nerve is exposed and cut no responses can be elicited by stimulation of the peripheral stump. After degeneration, therefore, the fibres from the blood vessels must be able to grow out into the old Schwann tubes. This has been shown to be so in an experiment in which an isolated tibial peripheral stump was prepared as above in the right hind-limb and left for 222 days (rabbit 644). After this time a second operation was performed, and the tip of the peripheral stump again cut, to interrupt any fibres from the central stump that might have found their way into it. The stump was also exposed peripherally in the shank, and a piece resected from it to interrupt any ascending fibres. The hitherto intact tibial nerve of the opposite limb was treated similarly. The animal was then left for a further 15 days to allow degeneration of any severed fibres, and outgrowth of any fibres which still remained connected with their cell bodies. Then the pieces indicated in Text-fig. 2 were removed and examined histologically. Fine nerve fibres were present in the most peripheral piece, *P.t.* 2, and they had grown out upwards from its end (Pl. 6, fig. 44). The other pieces removed contained no nerve fibres. Staining the peripheral piece with Weigert's method showed that of the fibres present a very few were medullated, but all were very small. Of course, no medullated fibres were present in the other pieces. *None of the pieces of the peripheral stump from the control side that had not been degenerated for* 222 *days contained any intact nerve fibres.*

In other similar cases we have found the same result: a few very fine medullated and unmedullated nerve fibres have grown into the long-degenerated stumps from the blood vessels, and they are sufficient to produce reflex responses on stimulation. We cannot, of course, say whether in man the sensation experienced on stimulation of such fibres would be referred to the region innervated by the normal nerve, but it seems likely that it might be deceptive in that way.

Considering that Schwann cells become structures very like nerve fibres, and that nerve fibres can enter an isolated stump from the periphery, it is not surprising that the autogenous theory of peripheral regeneration so long received the support of some experienced neurologists. They had more substantial grounds for their erroneous view than is often conceded by their opponents.



Text-fig. 2. Diagrammatic lateral views of the hind-limbs of rabbit 644 after exposure of the nerves in the thigh and shank at biopsy (see text). *C*, calf muscles; *C.s.*1, control central stump of tibial nerve 25 days after section; *C.s.*2, central stump inhibited with gentian violet 237 days previously; *F*, femur; *I*, segment of the tibial nerve at the knee, isolated for 15 days; *K*, knee; *O*, outgrowth of nerve fibres and Schwann cells from the most distal stump of the posterior tibial nerve; *P*, peroneal nerve; *P.t.*1, posterior tibial nerve on the control side; *P.t.*2, posterior tibial nerve on the long degenerated side.

1·8. *Late changes in the endoneurium and perineurium.* It would be interesting to investigate whether the decrease in diameter of the lumen of the Schwann tubes continues indefinitely. The diameter of the myelin sheath of the largest fibres in a normal rabbit tibial nerve after Flemming fixation is about 20 μ, while the largest Schwann tube in nerves similarly fixed after degeneration for from 337 to 514 days have a diameter of from 9 to 12 μ. Although we have not made careful measurements of the diameters at various times we have the impression that the tubes continually contract, but only very slowly. Thus the tubes in Pl. 10, fig. 72 are smaller than those in fig. 74. However, for the period we have studied we may conclude that the diameter of each tube is reduced by about a half. Each tube certainly remains as a definite entity. The complete process of 'degeneration' of the nerve therefore does not lead

to its conversion to a fibrous strand. Pl. 6, fig. 45, shows the pattern of Schwann tubes formed by the endoneurium after 1 month of degeneration, and although possibly some of the smallest tubes later become obliterated, the majority of them certainly persist for 18 months and probably very much longer.

As the Schwann tubes collapse their walls become considerably thicker. Both in normal and degenerated nerve the appearance of the endoneurium and neurilemma depends very much on the fixative used. In material fixed in Zenker's or Flemming's fluid the neurilemma is usually distinct as a thin sheath showing black or dark brown in haematoxylin preparations; while after Bouin or formol fixation it is not so clearly demonstrated. Similarly, in degenerated stumps the mode of fixation affects the appearance of the sheaths. After Zenker or Bouin fixation the collagen fibres of the endoneurium appear to have increased in number, while after formol or Flemming they seem to have undergone an individual thickening. But, whatever fixative is used, it is clear that there is a considerable increase in the amount of collagen between the fibres, making up to some extent the increase in the interfibrillary space produced by the collapse of the tubes. It is this collagen, no doubt, that gives the degenerate nerve its characteristic hard texture and resistance to cutting.

We have found that, close to the end of an isolated peripheral stump, collagenization of the Schwann tubes is more extensive than in the rest of the trunk, and it restricts the Schwann protoplasm to a very narrow band (Pl. 6, figs. 46, 47). It is interesting that such 'scarring' takes place even after the nerve has been cut cleanly under aseptic conditions. Its origin is obscure, but the collagen appears to lie inside the neurilemma, which is thus made visible as a very definite membrane. The Schwann protoplasm is reduced to a fine darkly staining strand at the centre of the tube, surrounded by a thick layer of substance staining green with Masson's stain though not obviously fibrous. It seems that this substance is collagen produced in the tube as a result of exposure or trauma at its cut surface.

This collagenization extends for several millimetres down stumps allowed to degenerate for a year or more. Since it restricts the lumen of the Schwann tube it presents a serious barrier to the ingrowth of new fibres (see p. 87 and Pl. 10, fig. 73). The likelihood of such scarring is a strong indication for removal of as great a length as possible from the end of long-degenerated peripheral stumps.

The perineurium, defined as the thin band of collagen which surrounds each nerve bundle in a normal trunk, shows no great thickening even after long degeneration (Pl. 4, fig. 33). The epineurium, which forms a sheath to the whole trunk and extends within it, separating the bundles with their individual perineuria, is somewhat thickened but not grossly so, and it contains many normal blood vessels.

The general appearance of a nerve that has been degenerated for a year and more is therefore not grossly dissimilar to a normal trunk. The Schwann tubes represent the original fibres, but are smaller than them. The endoneural spaces are larger, and filled with collagen, so that the whole nerve stains darkly with collagen stains. The bundles are smaller than normal, but their connective tissue sheaths are not greatly altered. The diameter of the individual bundles is reduced to about one-half, but the shrinkage of the whole nerve varies, reaching to a maximum of one-half.

2. *Re-innervation of the peripheral stump after immediate suture*

The observations described in this section were made on material from forty-nine rabbits in which the nerves were cut with scissors, sutured at once with plasma, and left for various periods from 15 to 194 days before biopsy. Some dog and human material has also been examined.

We are not concerned here with the processes by which union between the stumps is made and new axones put out by the severed fibres of the central stump, but only with what occurs in the peripheral stump when the fibres have reached it. After a good primary suture in the rabbit the first fibres appear in the peripheral Schwann tubes on about the 7th day, and the tips of the fastest axones advance along it at a rate calculated to be 3·5 mm. per day (Gutmann *et al.* 1942). We have studied the process of re-innervation mainly in longitudinal and transverse sections taken 1–2 cm. distal to the suture line on the 15th and 25th days after operation, but the later stages of innervation and medullation have also been examined. Since the rate of advance of the fibre tips is now accurately known we have been able to be certain that we were studying the relations of the axones to the Schwann cells in the very earliest stages of their association. It is very likely that many of the figures by which workers have purported to show the relations at these early stages in fact do not do so as they were not investigating conditions close to the tips of advancing axones.

2·1. *Advance of axones in the peripheral stump.* All the new fibres grow within the old tubes; those which attempt to force their way through the endoneurium or other connective sheaths are very soon blocked. Fibres running within the smaller tubes sometimes give the appearance of being in the endoneurium, but careful examination of longitudinal sections shows that every axone is within a tube containing a Schwann nucleus. It will be remembered that the Schwann tubes are almost completely filled by the Schwann cells and the extensive cytoplasm of the macrophages. It is not possible to demonstrate directly how the axones penetrate in relation to these protoplasmic masses, since the axones cannot be demonstrated in the material fixed with Flemming's fluid, which is the only method by which the cytoplasm is preserved without shrinkage and vacuolation. But, by comparing sections of well-fixed material with similar sections in which the axones are demonstrated and the Schwann protoplasm somewhat shrunken, it is possible to show with some certainty how the penetration takes place.

In nearly all cases the axones during the first few days after they have penetrated a given portion of a Schwann tube lie against the inner wall of the tube, between it and the Schwann cells and macrophages which occupy the centre (Pl. 7, figs. 48, 49, 50, 52, 53; Pl. 8, figs. 57, 58). At the level of a macrophage it is often possible to see clearly that the axones are flattened between its cytoplasm and the tube wall (Pl. 7, figs. 50, 52). In the regions between the macrophages, where the tube is occupied by the Schwann cells there is usually no doubt that at this early stage the fibres are lying between the Schwann cytoplasm and the wall (Pl. 7, figs. 48, 53). Occasionally fibres run down the centre of the tube and seem to be lying within the cytoplasm. It cannot therefore be absolutely excluded that axones do occasionally grow within the Schwann cytoplasm, but the fact that the great majority of the fibres lie at the edge of the tube strongly suggests that they grow over the surface of the Schwann cytoplasm, between the latter and the tube wall. Appearances such as those which have led

Boeke (1916) and Bielschowsky & Unger (1917) to suppose that the axones are growing down within vacuoles inside the Schwann cytoplasm may be the result of the misinterpretation of poorly fixed material, or of studying later stages when the fibres have become surrounded by Schwann protoplasm (p. 80).

On account of the possible presence of axone remains and other objects that may stain with Bodian's stain we have thought it well to include here a figure of an *uninnervated* stump, in order to prove that the objects showing in Pl. 7, figs. 48–53 and Pl. 8, figs. 57, 58, really are nerve fibres. Pl. 7, fig. 51, is from a region 100 mm. from a primary suture made 25 days previously, whereas the greatest distance at which fibres have ever been detected at this time is 80 mm. (Gutmann *et al.* 1942). The section is stained with Bodian and Masson and hence may be compared directly with Pl. 7, figs. 48–53. Pl. 7, figs. 49, 52, are indeed from sections taken 10 mm. from the same suture as Pl. 7, fig. 51. Pl. 8, fig. 56, is from the same region as Pl. 7, fig. 51, but is stained with Bodian's method alone and may therefore be directly compared with Pl. 8, figs. 57, 58. By such comparisons it is possible to say that at a point 10 mm. below a suture made 25 days previously numerous small fibres appear at the edges of the tubes and that these are not present in an unsutured stump or at this time at distances greater than at most 80 mm. from a suture.

Examination of longitudinal sections of material fixed in Bodian's alcohol fixative, stained by his method and counterstained with Masson's trichrome, often shows some quite well-fixed Schwann cells with nerve fibres running alongside them. This is most clearly shown in the re-innervation of stumps in which the internal organization of the nerve has been somewhat disturbed, as in Pl. 8, figs. 59–61, which are from a stored homograft. Here a new nerve fibre is seen running along the surface of several Schwann cells. This is especially clear at the bottom of Pl. 8, fig. 59, shown larger in Pl. 8, fig. 61, where the fibre apparently became blocked and sent out a lateral branch which left the surface of one Schwann cell (*S.c.a.*) and became attached to another (*S.c.b.*). However, the enlarged view of the upper portion of Pl. 8, fig. 59, which is shown in Pl. 8, fig. 60, shows the difficulty of making definite decisions on such points. Here it can be seen that there are various strands of Schwann protoplasm within the tube, and it cannot be decided whether the fibre is running within the somewhat shrunken cytoplasm of one cell or between that of separate cells. Pl. 8, fig. 62, shows that these conditions are not peculiar to grafted nerves, being a case from a stump re-innervated after simple suture. A single fibre can be seen running over the surface of the Schwann cells.

Pl. 7, figs. 54, 55, show in longitudinal section the numerous fibres running close to the wall of a single tube. Both are of the same tube, taken at different focal levels, in fig. 54 can be seen the fibres, and in fig. 55, taken at a lower focus, the macrophages around which they run. Fig. 55 also shows, in a neighbouring tube, two fibres running at opposite edges of a single small tube. Pl. 9, fig. 63, shows how difficult it is to be certain of the relationship of new fibres to the Schwann cells. A new fibre runs down the centre of a tube and might be held to be actually within the Schwann protoplasm. However, not only the innervated tube itself but other uninnervated tubes, shown in the figures above and below it, reveal clear fissures between separate Schwann cells, and it seems likely that the advancing fibres run in these.

In the early stages of degeneration the wall of the Schwann tube is not completely lined by Schwann cytoplasm, especially near the macrophages. Often therefore the

fibres may be without the support of Schwann protoplasm in their growth down the tube when they are passing between a macrophage and the tube wall (Pl. 7, figs. 50, 52).

It is usually supposed that the regenerating axones are provided with a bulb at their tip (see, for example, Nageotte, 1932, fig. 19), but we have rarely found these in our preparations of peripheral stumps during re-innervation, although, as has been pointed out, we have known with some exactness the point in the nerve at which the growing tips of the axones must occur. Occasionally small bulbs are formed where axones are obstructed by a macrophage, or even by a Schwann cell (Pl. 9, fig. 65), but it is probable that the growing tip is usually a tapering one, such as is seen in the earliest stages of the re-innervation of motor end-plates.

The new fibres entering a stump after primary suture are very numerous. As many as twenty have been counted around the walls of a single large tube, and ten may be present in quite a small tube. We have no evidence whether the numerous fibres in a single tube are the branches of a single axone, formed after it has entered the tube, or whether they are the processes of different nerve cells. The fibres do not run parallel to each other, and they frequently change their relative positions around the walls of the tube. It must be emphasized that these are separate fibres and not fibrils within a single axoplasm, and we agree with Bielschowsky (1935) in rejecting Boeke's interpretation that the various fibres in a single tube represent 'neurofibrils' which later become intimately associated with each other and acquire a common myelin sheath. We have seen no evidence that bifurcation and reunion of the axones, such as is found at the 'agonic' level in the cut central stump, occurs in the peripheral tubes. The individual fibres can be followed in longitudinal sections for considerable distances (Pl. 8, figs. 59, 62). However, it is worth emphasizing that they are excessively fine. The largest fibre shown on Pls. 7 and 8 is hardly above 1 μ in diameter.

Though the fibres at first lie on the surface of the Schwann cytoplasm they certainly later come to lie within it. Presumably as the axones increase in diameter (see below) they are forced into the protoplasm, which is known to have amoeboid powers, and thus flows around them. Difficulties of technique again make this process difficult to observe in fixed material. Indeed, at this stage the Schwann cytoplasm is particularly hard to demonstrate, presumably because it forms a thin sheet around the fibre. However, close to a point where a nerve had been crushed 25 days before, and at somewhat later stages after suture, it can be seen in both longitudinal and transverse sections that the axone lies within the Schwann cytoplasm, in clear distinction to its earlier position at its margin. At still later times it becomes obvious that the axones, or at least some of them, are enclosed within the Schwann protoplasm. Pl. 9, fig. 64, shows this in a preparation in which the myelin is partly preserved. When it is dissolved away, as in Pl. 10, fig. 75, the axone appears as a black dot at the centre of a 'vacuole' in the Schwann cytoplasm. This latter condition may have deceived workers who imagined that they were seeing the earliest stages of the growth of the axones within the bands.

2·2. *Increase in diameter and medullation of the fibres.* It would be most interesting to know the process by which some of the axones in the tube are selected for medullation (and, presumably, for function), while others disappear. It must in some way be connected with the rate of increase in diameter of the axones; even at quite early stages they are not all of equal size. Often there is one fibre much larger than the rest

which becomes surrounded by Schwann protoplasm and occupies the centre of the tube. But in many tubes it can be seen that more than one fibre lies within the Schwann protoplasm (Pl. 9, fig. 64; Pl. 10, fig. 75). It is not certain whether every fibre to be so included can become medullated if it acquires a sufficient diameter; certainly it is not infrequent for two or more fibres to medullate within one original tube (Pl. 9, fig. 64).

Presumably the whole course of maturation is determined by the increase in diameter of the new axones. This increase is certainly gradual and progressive along the course of the fibre. 10 mm. below a good suture made 15 days previously the largest fibres seen after shrinkage by fixation and embedding measured $1{\cdot}2\,\mu$, while the smallest were less than $0{\cdot}5\,\mu$ in diameter. The increase in diameter of the 'successful' fibres is rapid, and at 25 days the largest measure $2{\cdot}3\,\mu$; and at 100 days $7\,\mu$. Later the increase is slower. Sanders & Gutmann (1942) find that after 200 days a few of the fibres have reached the normal maximal diameter. This, measured as the total diameter of the myelin sheath in Weigert preparations, is $19{\cdot}8\,\mu$. (The equivalent maximal axone diameter of silver-stained material such as was used for the measurements given of the earlier stages would be approximately $10\,\mu$.) But even after this period most of the fibres are of less than normal diameter. Sanders & Gutmann found that the fibres in the central stump after a suture are also of reduced diameter, so it seems reasonable to suggest that some, perhaps all, of the increase of diameter of the peripheral regenerating axones is due to a downflowing of axoplasm from the central stump. Thus it may be that the axones in the peripheral stump which increase most rapidly in diameter will be those which are derived from central axones which have fewer other branches to supply. Such an arrangement would ensure that off-shoots of persistently branching fibres would be less successful than those from less branched axones, and the danger of abnormal functioning would be correspondingly reduced (see Young, 1942).

In a transverse section of a stump at 100 days there are far fewer axones in each tube than in the earlier stages (Pl. 9, fig. 66); some of those which have not increased in diameter have disappeared. In most of the tubes there is a single large fibre provided with a myelin sheath, and surrounded by a number of smaller fibres, apparently lying between its myelin and the tube wall. Two, three, or even more fibres may medullate within a single tube, and it may be that each acquires its own neurilemma and endoneurium, dividing up the original tube. But it is very difficult to make certain whether the transition from this condition to the normal regenerated nerve involves a true creation of several nerve fibres from within a single old tube, or whether some of the supernumerary fibres are later destroyed even though they have become to some extent myelinated. In the majority of the tubes there is only a single fibre left when medullation is well advanced.

We have not studied the processes by which myelin is laid down and nodes of Ranvier and other structures appear (see Gutner, 1936). Myelination seems to begin as soon as the axone is surrounded by Schwann cytoplasm: it can sometimes be seen immediately below the junction 15 days after suture. It certainly proceeds centrifugally along the fibres, but lags behind the tips of the axones along the peripheral stump (Gutmann *et al.* 1942).

3. *Regeneration after delayed suture*

This account is of three series of experiments by which we investigated separately three factors governing the success of regeneration after delayed suture. These are (1) changes in the power of the central stump to send out new axones, (2) changes in the peripheral stump which affect its power to receive these axones and to reconstitute a normal nerve, and (3) changes in the stumps which affect the quality of the junction made between them at secondary suture. The only similar experiments we have found described are those of Kilvington (1912) who went some way towards distinguishing the first two of these factors, but with whose conclusions we do not agree.

Table 2. *Test of power of outgrowth from a central stump severed again and sutured at various times after an initial cut. The distances of outgrowth were tested by pinching the peripheral stump. In the last column is shown the expected distance of regeneration, calculated from the data of Gutmann et al.* 1942

Animal	Time in days between injury and operation	Time in days between suture and biopsy	Distance of regeneration after delayed suture mm.	Distance of regeneration after control immediate suture mm.	Expected mm.
413	3	25	60	—	62
414	3	15	36	—	28
415	3	15	41	—	28
384	5	15	0	23	28
386	6	15	29	31	28
342	7	15	28	31	28
340	8	15	29	28	28
133	15	25	68	57	62
341	23	15	30	32	28
328	30	15	8	25	28
140	63	25	73	54	62
189	53	25	59	55	62
P6	103	15	30	—	28
P2	117	15	40	—	28
388	127	15	35	—	28
389	127	15	2	—	28
P4	277	25	76	69	62
P7	349	25	63	65	62

3·1. *The regenerative power of the central stump.* In eighteen animals the tibial nerve was cut high in the thigh and a gap left between the stumps so that a central neuroma was formed. Then after various periods ranging from 3 days to 11½ months a second operation was performed on each. The neuroma was removed from the central stump, and this was then joined by plasma suture to the distal stump of the peroneal nerve, which was freshly cut at a level convenient to allow suture without any tension. In most of the animals a control primary suture was performed at the same time on the other hind-limb, the tibial and peroneal nerves, hitherto intact, being cut, and the tibial central stump sutured to the peroneal distal stump. After a further period of 15 or 25 days the animal was again anaesthetized, and the distance reached by the regenerating fibres in the distal stump was tested by progressive pinching up the nerve (Young & Medawar, 1940). The value obtained for the distance of regeneration after secondary sutures of this type was compared with those found on the control sides of the animals, and also with the body of data on the rate of regeneration after primary

suture obtained from experimental work on other rabbits (Gutmann *et al.* 1942). It was thus possible within the limits imposed by this test to determine whether the outgrowth from a nerve which had been twice cut is of reduced vigour.

For convenience we may consider the results in two sets: those in which the two cuts were made within a few days of each other and those in which a longer delay was allowed.

When a central stump is cut a second time within 8 days of the original lesion outgrowth is as rapid as after a single cut and primary suture (Table 2). Indeed, in the seven animals which fall into this class all except two showed greater distances of outgrowth than were expected from the pooled results of control primary tibial-peroneal sutures. In animal 384 no innervation of the peripheral stump had taken place, but histological examination showed that the stumps had come apart. This did not occur in any of the controls, so it is an improbable event. We are not inclined to attach any significance to it as in all other animals in the series a good union was made. It is true, however, that secondary suture within a few days of the injury is made somewhat more difficult because the central stump is soft and diffuse, but the experiments leave no doubt that re-operation within a few days does not diminish the power of central outgrowth.

In the ten cases in which secondary suture was made at various times between 15 days and 11½ months there was no sign of any progressive decline in the distance reached by new fibres, and in all except two animals the distance of outgrowth was actually greater than was expected. The two exceptions, animals 328 and 389, show very low figures (Table 2). But in both cases the union was poor, and no great weight can be given to them: thus in 389 there was an unusual amount of retrograde degeneration in the central stump, presumably due to operative trauma, and there was a gap of about 2 mm. at the suture line.

But in all the other animals, even those in which the central stump had been left for nearly a year, the distance of outgrowth was normal; and we conclude that the power of outgrowth from a central stump does not diminish during the progressive formation of a neuroma.

The regenerating fibres present in the distal stump in these animals did not show any marked difference in number or diameter from those found in the control series. It would be interesting to examine the late stages of medullation after suture of an old central stump into a fresh peripheral one, but as the axones appear to increase in diameter at a normal rate during the early stages of regeneration there is no reason to believe that there is any other factor in the condition of the central stump which would interfere with recovery.

3·2. *The regenerative power of the peripheral stump.* In investigating the power of regeneration in peripheral stumps which have been allowed to degenerate for a long time we have examined not only the distances travelled by the regenerating axones within them, but also the later stages of medullation. The following account describes the experimental results. The histological observations on the re-innervation of stumps which have degenerated for a long time, forming a continuation of § 1 on the progressive changes in uninnervated nerve, are included in § 3·4 (p. 87).

Twenty-one animals have been used in the main series, the tibial peripheral stump being degenerated for times varying from 25 to 514 days (17 months), and union with the central stump being prevented by injection of the latter with some inhibiting

substance (p. 65). Then at a second operation the peroneal nerve was freshly cut, a small piece about 5 mm. long removed from the end of the tibial peripheral stump, and the peroneal central then joined with plasma to the tibial peripheral. In many cases a control primary suture of peroneal into tibial was made at the same time in the other hind-limb of the rabbit. In many of the animals the nerve was exposed after 15 or 25 days and the distance reached by the regenerating axones measured by the pinching method as before (Table 3). The table shows that of the five animals in which the nerve was degenerated for 25 days before suture four showed distances of regeneration equal or greater than those given by the control primary sutures. There is here therefore a distinct indication that either the junction is better made or fibres

Table 3. *Test of power of peripheral stump to receive new fibres after various periods of degeneration. See description of Table* 1

Animal	Days between lesion and suture	Days between suture and biopsy	Distance of regeneration after delayed suture mm.	Distance of regeneration after immediate suture mm.	Expected distance of regeneration mm.
790	25	15	40	43	28
793	25	15	38	22	28
794	25	15	39	30	28
805	25	15	31	34	28
806	25	15	31	22	28
82	33	25	66	61	62
150	38	25	65	74	62
201	45	11	13	11	11
170	53	15	23	—	28
124	54	25	49	—	62
175	91	25	47	63	62
84	126	15	33	—	28
211	150	25	15	—	62
179	236	15	26	—	28
168	241	15	32	—	28
297	309	25	25	—	62
P11	315	25	26	61	62
P8	331	25	32	59	62
P12	365	25	44	—	62
P10	365	25	67	—	62
P1	514	25	56	57	62

enter more freely into predegenerated stumps. We shall see that the former is probably the correct explanation.

Of the five animals in which the nerve was degenerated for periods between 33 and 54 days three showed slightly less than the distance of regeneration after primary suture, and three showed more than expected, but in none was the difference from normal great. After degeneration for this time, therefore, delayed is at least as effective as immediate suture.

On the other hand, of the eleven stumps degenerated more than 90 days, six showed a distance of outgrowth within expected range, and the remaining five showed only short distances of regeneration. A series of five failures is an altogether improbable number to be attributed to detached sutures or other accidents, as can be seen from the experiments listed in Table 2, and from the very many other primary sutures we have made. We must, therefore, conclude that some factor is operating in these degenerated stumps to reduce the rate of advance of regenerating fibres. But

this factor is not universal in its operation, and two of the longest degenerated stumps, P10 and P1, showed outgrowths well within the range of expectation from primary sutures. We must thus look for an influence which in some cases retards the growth of fibres after secondary suture.

3·3. *The quality of union between the stumps at secondary suture.* In searching for the reason for the poor outgrowth discussed in the last section we examined the suture regions in these animals histologically. In the animals in which the stumps had been degenerated up to 54 days excellent unions had been made, but in none of the animals degenerated for more than 90 days was the union of the same quality as that we have come to recognize after primary plasma suture. Pl. 9, fig. 67, is of a suture made into a stump that had been degenerated for 38 days: it shows a very good union. Pl. 9, fig. 68, shows that quite a good suture can be obtained after 150 days degeneration. In this animal fibres had penetrated less far into the stump than after a comparable primary suture, in spite of the closeness of the junction. Pl. 9, figs. 69, 70, show much less satisfactory sutures after degeneration of the peripheral stump for exactly one year in each case. The sutures had been made for 97 days before biopsy and these show that the union has not improved in intimacy even after a longer period. Even in the animal in which fibres had penetrated the full distance after 514 days' degeneration, examination shows that the suture, although close, has not effected an intimate union. In all these cases of suture into stumps degenerated for a long time, one obtains the impression that although the apposition between the stumps was close the union between them was not good. Instead of numerous strands running straight from one stump to the other, as in Pl. 9, fig. 67, there are at best irregular and criss-crossing fibres. In fact, the line of separation between the stumps remains sharp, instead of almost disappearing as it does after a good suture.

We conclude, therefore, that the poor union was not due to poor operative technique, except possibly in animals 179 and 297 in which we had difficulty in effecting the secondary suture, the peripheral stump being awkwardly low in the popliteal space, and embedded in fat. In the other cases the histological evidence showed that the apposition at the suture was good, but the union was bad.

3·31. *The activity of Schwann cells at secondary suture.* Since the migratory activities of Schwann cells from the cut ends of nerve stumps play a large part in effecting a union after suture, we investigated the possibility that the poor unions at secondary suture were due to a decline of the power of the Schwann cells to grow out of peripheral stumps which had degenerated for a long time. In ten animals degenerated stumps were prepared as in series 2 above by resection of a portion of the tibial nerve and inhibition of the central stump. At a second operation a piece about 5 mm. long was removed from the tip of the peripheral stump which was then left, without suture. At the same operation a piece was resected from the hitherto intact tibial nerve on the opposite side of the animal, and the peripheral stump left isolated. After a further period of from 9 to 25 days the animals were killed, and the tips of the two severed peripheral stumps removed. Longitudinal sections were cut of these pieces and of those removed at the earlier operation. It was thus possible to determine the distance and amount of Schwann outgrowth from a normal stump and from one degenerated for a long period. In Table 4 the criterion of the distance of Schwann outgrowth was the distance from the margin of the stump reached by the farthest identifiable strand of Schwann cells. Clearly this gives only a general idea of the vigour of the outgrowth,

because of the difficulty of identifying Schwann cells (p. 71), but the figures are reasonably accurate and comparable. Table 1 was obtained in a similar way and shows the distances of normal primary Schwann outgrowth from freshly cut stumps. Comparison of Tables 1 and 4 shows that after long periods of degeneration the power of Schwann cells to grow out from the stumps becomes very distinctly reduced.

But the results given in the tables also show that the most active outgrowth is given not by a freshly severed stump, but by one which has undergone a short period of predegeneration. Moreover, in the animals in which these longer distances are given the density of outgrowth was also correspondingly greater than from a freshly cut stump. Pl. 9, fig. 71, shows a very abundant growth found 15 days after severance of a nerve which had been predegenerated for 53 days.

It is somewhat surprising that there is any proliferation at all of Schwann cells when the nerve is cut a second time, for they have already gone through their degenerative change of mitosis and multiplication. The secondary outgrowth is not simply an emigration without multiplication, for mitoses can be seen in the secondary outgrowths (though they do not occur elsewhere in the twice cut stumps). Presumably the stimulus to cell division is confined to the traumatic region at the end of the nerve,

Table 4. *Lengths of Schwann cell outgrowth from peripheral stumps cut a second time after varying periods of degeneration*

Period of degeneration days	Period of outgrowth days	Length of outgrowth mm.	Period of degeneration days	Period of outgrowth days	Length of outgrowth mm.
9	30	3·5	222	15	0·5
31	25	2·5	236	15	0·5
53	15	1·4	241	15	0·3
53	25	3·3	309	25	0·2
126	15	0·8	484	9	0·4

but it is still remarkable that the Schwann cells not only retain their power of outgrowth but retain it to a greater extent than normal after a moderate amount of predegeneration.

The work of Abercrombie & Johnson (1942) provides a striking elaboration of these observations. They have investigated the migratory powers of Schwann cells in denervated nerve cultivated in vitro. They confirm Ingebrigtsen (1916) in showing that no emigration takes place from explanted normal nerves: from the stumps degenerated for 4 days and more the amount of outgrowth increases greatly, and it is at a maximum from stumps predegenerated for about 25 days. After this it falls to a plateau in stumps of about 50 days, and there is a gradual subsequent decline, though even after a year of predegeneration it never fell back to the condition of normal nerve, from which no outgrowth takes place.

These changes in the power of outgrowth of Schwann cells seem sufficient to account for the variable success of union at secondary sutures. After primary suture the Schwann cells begin to grow out after 4 days, probably as mitosis begins, and the migration during the succeeding days plays a large part in making the union. The fact that the most vigorous outgrowth is from nerve predegenerated 2–4 weeks suggests that secondary sutures made after this period would give the most successful of all junctions. As we have seen on p. 84 this is indeed the case.

This phenomenon of rapid outgrowth from peripheral stumps after short periods of predegeneration may well explain the success claimed for predegenerated nerve grafts (Ballance & Duel, 1932). Sanders & Young (1942) found that regenerating axones do not grow faster within predegenerated autografts than within fresh autografts, but that the delay at the suture line before the fibres enter the grafts is shorter for the predegenerated type. It would seem, therefore, that predegeneration does confer an advantage in grafting, by ensuring a more efficient and rapid union, though this was not the reason for which predegeneration was introduced.

The decline in Schwann outgrowth is very likely to be responsible for the formation of bad unions after very delayed secondary suture. It must be emphasized that although our investigations only tested directly the *distance* of regeneration in the stump, the poorer junctions certainly allow also a much smaller *number* of axones to reach the peripheral stump, with permanent effect on the likelihood of successful recovery. The decline of Schwann cell activity in the peripheral stump is thus a factor which indicates that it is unwise to delay suture for long periods if this can be avoided.

3·4. *Re-innervation of nerve after long periods of degeneration.* We have now to examine the process of penetration of new fibres into a stump which has degenerated for a long time, to discover whether this differs from the re-innervation of freshly cut stumps in any respect likely to impair the effectiveness of recovery. For this purpose we have had the material from all the animals listed in Table 3. Most of these were killed 25 days after suture, but to study the later stages of re-innervation some of them were left alive for up to 103 days after suture.

The chief differences between an uninnervated peripheral stump 15 days old and one of say 100 days are that in the latter the macrophages are much fewer, the diameter of the Schwann tubes has been reduced, and they are filled with Schwann protoplasm. Longitudinal and especially transverse sections (Pl. 10, figs. 73, 74) show that new fibres entering such an old stump all run within the Schwann tubes, just as they do in primary re-innervation. Pl. 10, fig. 72, shows a section similar to fig. 73 in all respects except that, as the former was 85 mm. from the suture made 25 days before, no nerve fibres are present. In transverse sections taken some 10 mm. below a secondary suture made 25 days previously, the new axones are mostly of much larger diameter than those seen after primary suture (compare Pls. 7, 8, figs. 48–58 with Pl. 10, figs. 73, 74). This may perhaps be due to the greater resistance which impedes their advance and results in the axoplasm flowing out from the central stumps, 'dilating' the axones more rapidly. So far as fixation difficulties allow, it is possible to say that there is no evidence that the axones grow out within the Schwann cytoplasm. In the early stages they usually occur at the periphery between the cytoplasm and the tube wall. However, even after 25 days, some of them give the appearance of being within the protoplasm and this is certainly the condition in later stages (Pl. 10, fig. 75).

The number of fibres in each tube is definitely less than after primary suture, even at the earliest stages of re-innervation, although in these experiments the source of the new axones was always a freshly cut normal nerve. This reduction of the number of fibres in the Schwann tubes may be due either to a failure of axones to reach the peripheral stump because of the poor Schwann outgrowth, or to the reduction in the size of the lumen of the tubes, and the increase of endoneural collagen. It seems likely that this second factor is the more important, for we saw signs of a reduction

of the number of axones in the peripheral tubes after a length of degeneration which should not have caused a serious decline in the power of outgrowth of the Schwann cells (Pl. 10, fig. 74). Pl. 10, fig. 73, shows that some of the Schwann tubes have become so reduced in diameter by collagenization that it is very difficult for new fibres to enter. Probably this restriction of the tubes would have been less severe somewhat farther from the injured surface (cf. Pl. 10, fig. 72), and this case emphasizes the need for adequate resection of the peripheral stump when making a suture after long delay.

The reduction in the number of fibres entering the peripheral stump will interfere with recovery by reducing the chance that successful reconnexions will be made. It might be expected, however, that the later development of the fibres in the stump, particularly their medullation, would not be interfered with, for each axone is well in contact with Schwann cytoplasm. Actually, a stump examined at any time after a long-delayed suture shows fewer and smaller medullated fibres than a corresponding stump after a primary suture (Pl. 10, figs. 76, 77). Twenty-five days after an immediate suture there is already often a good deal of medullation in the upper part of the peripheral stump, but after a suture delayed for 514 days no medullation at all was seen after the same period.

This delay in the increase in diameter and medullation of the fibres may be due to physical conditions within the Schwann tubes, whose thick walls may resist the expansion necessary for them to contain a full-sized medullated fibre, and it may be due also to some failure of the Schwann cells in synthesizing myelin. It can hardly be directly related to inadequate vascularization of the stump, as we have already described that an apparently ample vascular supply is present. In any case there is no doubt that at 3 months after suture the regeneration of the nerve is very much less complete if the peripheral stump had previously been allowed to degenerate for a long time. Pl. 4, figs. 32, 33, show that at this time the predegenerated stump still shows an excessive collagenization and Pl. 10, figs. 76, 77, that it shows inadequate medullation. Moreover, the threshold to faradic stimulation of the peripheral stump was found to be higher 3 months after delayed than after immediate suture.

After suture to an old peripheral stump there will be at best a considerably greater delay in return of normal function than after a primary suture. It is likely, though not proved, that in such cases the recovery can never, even slowly, become complete. For as has been pointed out (Young, 1942) optimal recovery presumably requires innervation by fibres of normal diameter and medullation. We do not yet know whether the much-contracted tubes of an old peripheral stump can be gradually expanded until they are of normal diameter, but there are reasons for suspecting that they may remain permanently small. Recent experiments in which spinal nerves were sutured into post-ganglionic sympathetic nerves have shown that medullated fibres form in the latter trunks, but they remain very small (Simpson & Young, unpublished). It is clear, therefore, that the size of the tube in the peripheral stump in which a new fibre finds itself imposes a certain limitation on its growth which may have the effect of permanently specifying its diameter. Examination of later stages of medullation is now being undertaken to test whether this is really the case.

DISCUSSION

Comparison of immediate and delayed suture

The final criterion of the effectiveness of immediate and delayed suture is the degree of functional recovery that they produce. Unfortunately, there are exceptional difficulties in making such a direct comparison experimentally. The device of crossed union of nerves is essential for the making of comparable sutures, and a full analysis of functional recovery after crossed union is difficult. Gutmann (1942) has partly overcome the difficulties by comparing primary and secondary suture of tibial into peroneal nerves in the rabbit. After this anastomosis there is usually some recovery of the toe-spreading reaction which is normally effected through the peroneal nerve. He found that in rabbits in which the peroneal had been degenerated before being sutured to a tibial central stump, the recovery was always slower than the primary crossed suture and was also less complete than on the control side. However, the effects only became severe when the peripheral stump had been degenerated for more than 6 months.

There is at present no body of data comparing the results of primary and secondary suture in man. Even if the clinical results of delayed suture were known, it is most unlikely that such data would allow analysis of the factors liable to affect recovery. There are at least six factors which may affect the success of secondary suture:

(1) The power of the central stumps to send out new fibres. We have seen that this power is not affected when the axones are cut twice, whether the axones are cut within a few days or after nearly a year from the original injury.

(2) The power of the stumps to unite. From the point of view of successful functional union this depends on the outgrowth of Schwann cells from the peripheral stump. This outgrowth is at its maximum not immediately after severance of a nerve, but during the period about 2–3 weeks later. We have evidence that unions made after this interval are somewhat more successful than primary sutures. For some time longer this power to make good unions remains high, but from about 100 days onwards it declines, and long-delayed unions are less likely to be successful than primary suture.

(3) The total diameter of the peripheral stump shrinks in the later stages of degeneration, sometimes by as much as a half: it is thus more difficult to effect a good suture, and the successful re-entry of fibres into the peripheral stump is prejudiced.

(4) The shrinkage of the lumen of the Schwann tubes results in fewer fibres entering each one, and presumably reduces the chances of successful reconnexions. But the distance reached by the most advanced regenerating fibres in these old tubes is sometimes not less than after primary suture, so that they can offer favourable pathways for new growth.

(5) After delayed suture medullation is delayed in the peripheral stump, and the fibres remain of small diameter for much longer than after primary suture. It may be that this condition persists indefinitely, and, if this is so, functional recovery must remain always of inferior quality after delayed suture.

(6) The factor of re-innervation of end-organs is of great importance. It is not included in the present study, but it is probable that motor end-plates and sensory end-organs gradually disappear in denervated tissues and, of course, there is profound atrophy of the muscle fibres themselves (see Tower, 1939). Methods have been pro-

posed for avoiding muscular atrophy, fixation of joints and other progressive changes, but the destruction of end-organs cannot be prevented by any known physical treatment. Even if new and satisfactory end-organs can be formed after delayed suture the process is likely to be much slower and less satisfactory functionally than the re-innervation of old end-plates and organs in the skin which follows primary suture.

We may conclude that the processes of union of stumps and the regeneration of a new functionally efficient stretch of nerve are less satisfactory after the longer periods of degeneration. Delay in suturing for 1 or 2 months does not prejudice the chances of recovery, and may indeed even improve them; longer delays, especially those greater than 5 or 6 months, produce conditions which at least are liable to retard recovery, and may permanently prevent its completion.

SUMMARY

1. During degeneration the pattern of a nerve is maintained by the endoneurium and neurilemma, a *Schwann tube* being formed in place of each original fibre. Each tube gradually contracts, finally reaching about half the diameter of the original fibre.

2. Macrophages enter these tubes and remove the remains of axones and myelin. They are abundant from about the 8th to the 25th day and thereafter disappear gradually, some persisting for a year or more.

3. The protoplasm of the Schwann cells increases in amount and after nuclear division elongated cells are found, lying side by side in the larger tubes.

4. In early stages the Schwann cells do not entirely fill the tubes. They gradually replace the macrophages and when the tube is fully contracted its whole cross-section is filled with fibrillar Schwann protoplasm, the nuclei becoming extremely elongated. Such Schwann cells may easily be mistaken for nerve fibres, especially when stained with silver.

5. In early stages the Schwann cells probably move within the tubes. Certainly they emerge at the end of the peripheral stump and are the main agent for leading fibres back into the old tubes.

6. Growth and mitotic division take place in the Schwann tissue which has migrated from the peripheral stump. The tip of the outgrowth was found to have reached to an average distance of 1·4 mm. in 15 days, 2·2 mm. in 25 days. But the outgrowth does not continue progressively if the stump is left uninnervated.

7. Nerve fibres usually grow into isolated degenerated peripheral stumps from the blood vessels. They remain very small and are rarely medullated, but are sufficient to produce a reflex response on stimulation and an outgrowth of nerve fibres from the *peripheral* end if the stump be again cut.

8. The endoneurium and perineurium of a long-degenerated stump become moderately but not excessively collagenized. The diameter of the whole nerve may decrease by as much as one-half.

9. When new fibres grow into a freshly cut peripheral stump they run along the inner walls of the Schwann tubes. Where possible they grow along the surfaces of Schwann cells, but do not at first lie within them.

10. As many as twenty fibres may enter a single tube. One or more, rarely several, of these increase in diameter, become surrounded by Schwann cell protoplasm and medullated. The others remain for a while around the periphery of the tube and presumably finally they atrophy.

11. The power of a central stump to send out new fibres is not reduced if it be severed a second time and then sutured, either within a week or after an interval as long as a year.

12. The power of a peripheral stump to put out Schwann cells is greatest if it be cut a second time some 2–3 weeks after its first severance. Thereafter this power declines, and unions made with peripheral stumps degenerated for more than 100 days are often unsatisfactory.

13. Once within a peripheral stump which has been degenerated for a long time, however, fibres may proceed as rapidly as into a freshly cut one.

14. Fewer fibres penetrate into a stump which has been degenerated for a long time than into a freshly cut one.

15. Medullation proceeds much more slowly after delayed suture.

16. There are therefore various factors, in addition to atrophy of the end-organs, which are likely to reduce the effectiveness of recovery when suture is made after a long delay.

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EXPLANATION OF PLATES

'Masson' indicates staining with Masson's light green trichrome method. 'Mallory' indicates Mallory's aniline blue trichrome. Except where otherwise stated the material was embedded in paraffin wax through cedarwood oil. All figures, except Pl. 3, fig. 21, are of the nerves of the hind-limb of the rabbit.

Abbreviations used in the plates

ax.	axone	*mac.n.*	macrophage nucleus
ax.e.	axone end-bulb	*mit.*	mitosis
ax.r.	axone remains	*myel.*	myelin sheath
c.	collagen	*my.r.*	myelin remains
e.	epineurium	*nlm.*	neurilemma
end.	endoneurium	*no.*	node
end.n.	endoneural nucleus	*nuc.*	nucleus
f.	fibroblast	*p.*	perineurium
i.	inclusion in Schwann cytoplasm	*S.b.*	bulb of cytoplasm at the end of a Schwann cell
inc.	Schmidt-Lantermann incisure	*S.c.*	Schwann cytoplasm
mac.	macrophage	*S.n.*	Schwann nucleus
mac.c.	macrophage cytoplasm	*S.t.*	Schwann tube

PLATE 1

All photographs on this plate are to the same magnification as indicated.

Fig. 1. Normal nerve fibres in longitudinal section, showing the relations of the Schwann nucleus and the cytoplasm around it with the myelin sheath and neurilemma. A node of Ranvier and incisures of Schmidt-Lantermann are also visible. Flemming, methyl benzoate-celloidin-paraffin, Masson.

Fig. 2. Normal nerve fibre showing the relations of the neurilemma at a node. Zenker, methyl benzoate-celloidin-paraffin, Masson.

Fig. 3. The extent of the Schwann cytoplasm within the Schwann tube after 6 days' degeneration. Picric-saline, Masson.

Fig. 4. Schwann cell after 15 days' degeneration. There are two large nucleoli, and the cytoplasm of the cell appears as a network over the myelin at the upper end of the figure. Flemming, Masson.

Fig. 5. Normal nerve fibres, showing the relations of Schwann nucleus, Schwann cytoplasm, myelin sheath and neurilemma. In both fibres in which a Schwann nucleus is visible the appearances suggest that at this level at least the Schwann cytoplasm extends completely around the outer surface of the myelin. Flemming, Masson.

Fig. 6. As fig. 5. Flemming, methyl benzoate-celloidin-paraffin, Masson.

PLATE 2

Fig. 7. A normal nerve fibre showing how endoneurium and neurilemma can be recognized near a node. Zenker, Masson.

Fig. 8. Schwann tubes in a peripheral stump after 25 days' degeneration. Around the macrophages the tubes are of normal diameter, but elsewhere they are collapsing. A Schwann nucleus is insinuating itself between the cytoplasm of the larger macrophage and the tube wall. Alcohol-formol-acetic, Masson.

Fig. 9. A peripheral stump after 15 days' degeneration. The myelin sheaths are blackened with osmium tetroxide: it can be seen that the myelin is being removed first from the outer surface of the sheath. Flemming, Masson.

Fig. 10. Peripheral stump 1 cm. below a suture made 25 days before. At the top of the figure a mass of Schwann cytoplasm, with an inclusion, is dammed up against a macrophage in a tube of which the outlines are only faintly indicated. Regenerating fibres can be seen growing down the inner surface of the tube walls. Alcohol-formol-acetic, Bodian.

Fig. 11. A peripheral stump after 15 days' degeneration. The neurilemmal walls of the Schwann tubes are clearly shown. To the left a tube which has collapsed except around the macrophages is seen, and Schwann nuclei are obstructed on either side of the upper macrophage. In the right-hand tubes myelin removal is less advanced. Flemming, Masson.

Fig. 12. A peripheral stump after 15 days' degeneration, showing to the left the Schwann cytoplasm extending over the inner surface of a tube wall, and surrounding unstained myelin remains. To the right are osmiophil remains. Flemming, Masson.

Fig. 13. A Schwann tube after 16 days' degeneration. At the lower end of the figure it is of normal diameter around a macrophage, but the rest is collapsed, and contains two Schwann nuclei, one pressed against the macrophage, and several strands of Schwann cytoplasm running together inside it. Alcohol-formol-acetic, Bodian.

Fig. 14. Two Schwann cells within a single tube, of which the walls are not stained. The fibrous cytoplasm of each cell extends past the nucleus of the other. Alcohol-formol-acetic, Bodian.

PLATE 3

Fig. 15. A peripheral stump degenerated for 353 days, showing the Schwann tubes full of darkly stained Schwann cytoplasm except at a point in the middle of the figure, where it is obstructed by a small remaining macrophage island. The stump was isolated by a second operation 10 days before fixation. None of the structures shown are nerve fibres. Formol, Bodian.

Fig. 16. Columns of Schwann cells, one of them showing mitosis, grown out from the end of a peripheral stump cut 15 days before, after having previously been degenerated for 53 days. A nerve fibre threads its way along one of the columns. Formol acetic saline, Mallory.

Fig. 17. A column of Schwann cells grown out from a peripheral stump during 15 days after section. The dense bands of Schwann cells lie amongst connective tissue containing fibroblasts. Zenker, Masson.

Fig. 18. Columns of Schwann cells from same slide as fig. 16, showing by the cracks between them that they are not a homogeneous syncytium. A mitotic figure in metaphase is seen to the left. Alcohol-formol-acetic, Mallory.

Fig. 19. The outgrowth from a peripheral stump 30 days after section. The stump is at the bottom of the figure, and the darkly stained columns of Schwann cells are seen growing out from it into fibrous tissue, much of the latter apparently arising from the epineurium. Formol-acetic-saline, Mallory.

Fig. 20. A Schwann tube after 25 days degeneration. A group of Schwann nuclei is lying on either side of a macrophage within the tube; further from the macrophage there are few nuclei. Alcohol-formol-acetic, Bodian.

Fig. 21. A human nerve degenerated for 26 days, showing a Schwann nucleus lying between two macrophages and deformed by them. The outlines of the macrophages and tube walls are only very lightly stained. Formol, Bodian.

Fig. 22. Schwann nuclei after 82 days' degeneration, two of them pressing against opposite surfaces of a macrophage. Alcohol-formol-acetic, Bodian.

Fig. 23. A peripheral stump after 15 days' degeneration, showing a Schwann tube in which two clearly distinct masses of Schwann protoplasm, each with its own nucleus, are lying alongside each other. Flemming, Masson.

PLATE 4

Fig. 24. Schwann cells in a peripheral stump degenerated for 83 days and containing no nerve fibres. A single cell runs the length of the figure to the right of the middle line. The Schwann tube is very lightly stained. Formol, Protargol method (see p. 66).

Fig. 25. A Schwann cell with an extremely elongated nucleus from a stump degenerated for 335 days. Both nucleus and cytoplasm have been compressed from the condition seen in fig. 24 by the contraction of the Schwann tube. Formol, Bodian.

Fig. 26. Schwann cells from the same material as fig. 24, showing nuclei in contact with macrophages, and the elongated axone-like appearance of the Schwann cytoplasm. Formol, Protargol method.

Fig. 27. Two Schwann cells lying together in a single (unstained) Schwann tube after 85 days' degeneration. The fibrous process of one cell is alongside the nucleus of the other. Alcohol-formol-acetic, Bodian.

Fig. 28. Schwann cell after 335 days' degeneration. Its cytoplasm shows longitudinal fibrils, and its nucleus has a median transverse constriction. Formol, Bodian.

Fig. 29. Schwann cell within a Schwann tube containing a macrophage after 83 days' degeneration. Formol, Protargol method.

Fig. 30. Schwann cells and axones in a peripheral stump degenerated for 85 days. Alcohol-formol-acetic, Bodian.

Fig. 31. Same material as fig. 30 but from a region into which a few nerve fibres have penetrated. The two Schwann nuclei indicated appear to lie within the same elongated protoplasmic band. Although this is fibrous in appearance it can be distinguished from the more darkly stained axone above it. Alcohol-formol-acetic, Bodian.

Fig. 32. Transverse section of the posterior tibial nerve cut and sutured 3 months previously. Zenker, Mallory.

Fig. 33. Transverse section of the posterior tibial nerve of the opposite limb to that shown in fig. 32. On this side the nerve was first degenerated for 16 months and then re-innervated for 3 months. In spite of the re-innervation the endoneurium is still very thickened. Zenker, Mallory.

PLATE 5

All figures on this plate are to the same scale as indicated.

Figs. 34–39. Schwann tubes in long degenerated peripheral stumps, showing the varying appearances which result from shrinkage during fixation and embedding. All are stained with Masson's stain.

Figs. 34–37. From the same animal in which the nerve was degenerated for 83 days. Figs. 34–36 are of material fixed in Flemming, while that in fig. 37 was fixed in Bodian's alcohol-formol-acetic mixture. In all of them the neurilemma is distinct, as the wall of the Schwann tube. In fig. 35 a Schwann tube (*S.t.*2) containing a Schwann nucleus is filled with Schwann cytoplasm showing almost homogeneous appearance. In another tube (*S.t.*3) two Schwann nuclei with their cytoplasms lie side by side. In figs. 34 and 37 some of the tubes show the shrinkage of the Schwann cytoplasm which gives it the appearance, either of being extremely vacuolated (*S.t.*1) or of lying in strands around the inner surface of the neurilemma, leaving an apparently empty space in the middle of the tube (*S.t.*5, *S.t.*6). In fig. 36 the Schwann cytoplasm is quite well fixed, but shows irregularities as in (*S.t.*4).

Fig. 38. Stump degenerated for a longer time (241 days), showing the Schwann cytoplasm as a homogeneous mass filling all the space within the neurilemma. A suggestion of division between two separate cytoplasmic masses can be seen (*S.t.*7). Flemming, Masson.

Fig. 39. Still older stump (514 days) fixed in alcohol-formol-acetic, showing very poor fixation of the Schwann cytoplasm, which appears as a mass of separate threads. The increase in endoneural collagen is well shown. Masson.

PLATE 6

Figs. 40–42. Terminal enlargements of the cytoplasm of Schwann cells in a nerve degenerated for 335 days (formol, Bodian). In fig. 40 two strands of cytoplasm lie together in a Schwann tube, and one of them terminates in a large club-shaped structure. In fig. 41 a similar but more elongated bulb lies against a Schwann nucleus, which itself is connected with a thin strand of cytoplasm. Another similar structure is seen in fig. 42.

Fig. 43. Several strands of Schwann cytoplasm are shown in a stump degenerated for 85 days. In the middle of the figure a large mass of Schwann cytoplasm can be seen. Alcohol-formol-acetic, Bodian.

Fig. 44. The outgrowth of axones and Schwann cells from the central end of the peripheral stump from animal 644 (p. 75) degenerated for 237 days and then cut again. Alcohol-formol-acetic, Bodian.

Fig. 45. Transverse section of peripheral stump degenerated for 33 days. The Schwann cells and neurilemma are not well demonstrated, but the pattern of the Schwann tubes, maintained by the neurilemma and endoneurium, is shown. Bouin, Masson.

Figs. 46, 47. Transverse section taken a few millimetres from the central end of a peripheral stump degenerated for 310 days. The neurilemma is well preserved, but the space between it and the darkly stained bands of Schwann cytoplasm is filled with material, presumably collagen, which is stained green. Alcohol-formol-acetic, Bodian, Masson.

PLATE 7

Figs. 48–50, 52 and 53. Transverse sections of peripheral stumps taken 10 mm. below primary sutures made 25 days previously. They may be compared with fig. 51 which is of a peripheral stump degenerated for the same period but not re-innervated. All are fixed in alcohol-formol-acetic, stained by Bodian's method, counterstained Masson. In all the figures the Schwann cytoplasm is distributed around the inner surface of the walls; the lumen is occupied by macrophages and axone and myelin remains. The regenerating axones nearly all lie at the edges of the tubes and have presumably grown down between the Schwann cytoplasm (or macrophage) and the neurilemma. A few fibres (*ax.*1)

seem to lie within the Schwann protoplasm but the shrinkage makes it hard to decide the exact relations. Some axones are flattened between a macrophage and the tube wall (*ax.*2).

Figs. 54, 55. The same preparation taken at different focus to show numerous axones running in the wall of a Schwann tube, around a macrophage. In fig. 55 a small tube at the top of the figure also shows two fibres close to its wall.

PLATE 8

Fig. 56. An uninnervated peripheral stump after 25 days' degeneration, fixed in alcohol-formol-acetic and stained by Bodian's method without counterstain. Only the nuclei have taken up the silver and the outlines of the Schwann tubes are, therefore, barely visible.

Figs. 57, 58. Re-innervated Schwann tubes from sections 10 mm. below primary sutures made 25 days before. Alcohol-formol-acetic fixation, Bodian's stain, no counterstain. The section is directly comparable with the uninnervated stump in fig. 56, although the nuclei in this preparation have only slightly taken up the silver. At least thirteen regenerating axones lie around the inner surface of the walls of the large Schwann tube (*S.t.*1).

Fig. 59. Longitudinal section to show fibres growing into a homograft placed 25 days earlier. A single axone growing down a Schwann tube can be traced along the length of the figure from the top at the right of the mid-line. Peripherally, at the bottom of the figure, the axone is obstructed and sends out a smaller lateral branch shown at a higher magnification in fig. 61. Alcohol-formol-acetic, Bodian, Masson.

Fig. 60. Higher power view of a part of the field shown in fig. 59 showing the relations of a regenerating axone with the cytoplasm and nucleus of a Schwann cell.

Fig. 61. Higher power view of a part of the field shown in fig. 59, showing the blunted end of the axone, presumably caused by an obstruction, and the small lateral branch it is sending out within the Schwann tube. At this point it leaves the surface of one Schwann cell (*S.c.a.*) and becomes attached to another (*S.c.b.*), whose nucleus is somewhat abnormal.

Fig. 62. Longitudinal section of a peripheral stump 20 mm. below a primary suture made 25 days before. A single axone can be followed along a Schwann tube from the top of the figure at the right of the mid-line. It runs close to the surface of the Schwann cells. Alcohol-formol-acetic, Bodian, Masson.

PLATE 9

Fig. 63. Schwann tubes, one of them in the process of re-innervation, 10 mm. below a secondary suture made 15 days previously, the nerve having been predegenerated for 53 days. Separation between the parallel bands of Schwann cytoplasm can be seen within the tubes. Alcohol-formol-acetic, Bodian, Mallory.

Fig. 64. A stump degenerated for 150 days into which some fibres have penetrated and become medullated. In some cases there is more than one fibre within a single tube. The black points in the myelin sheaths are the result of silver impregnation after Zenker fixation. Zenker, Bodian, Masson.

Fig. 65. Peripheral stump immediately below primary suture made 82 days before. Several axones of considerable diameter are seen, and a few finer ones, one of which shows an end-bulb at a point at which it is obstructed by a nucleus. Alcohol-formol-acetic, Bodian.

Fig. 66. Transverse section 10 mm. below a primary suture made 107 days before. The Schwann tubes contain some large myelinated axones (*ax.*1) but there are also still many small axones around the inner walls of the tubes (e.g. *ax.*2). Alcohol-formol-acetic, Bodian.

Fig. 67. Longitudinal section of the suture line of a secondary suture 25 days old made after a delay of 38 days. A good union has been made between the stumps. Note that Schwann cells growing out from peripheral stumps collide in some places with fibres growing from the central stump. In figs. 67–70 the central stump is to the left. Alcohol-formol-acetic, Bodian, Mallory.

Fig. 68. Longitudinal section of a secondary suture 25 days old made after a delay of 150 days. There is a sharp demarcation between the stumps, and the union is thus less good than in fig. 67. Alcohol-formol-acetic, Bodian.

Fig. 69. Longitudinal section of a secondary suture 91 days old made after a delay of 365 days. Even though 3 months have elapsed after the suture there is still a clear distinction between the stumps, and although apposition is close, union has not been good. Alcohol-formol-acetic, Bodian, Masson.

Fig. 70. Longitudinal section of a secondary suture 97 days old made after a delay of 365 days. The union is rather better than in fig. 69, but there is much criss-crossing of axones at the suture line. Alcohol formol-acetic, Bodian, Mallory.

Fig. 71. Schwann outgrowth produced during 15 days after transection of the end of a peripheral stump that had previously been degenerated for 53 days. The stump is to the left, its end in the middle, and the whole of the right half of the picture thus shows new outgrowth. Alcohol-formol-acetic, Masson.

PLATE 10

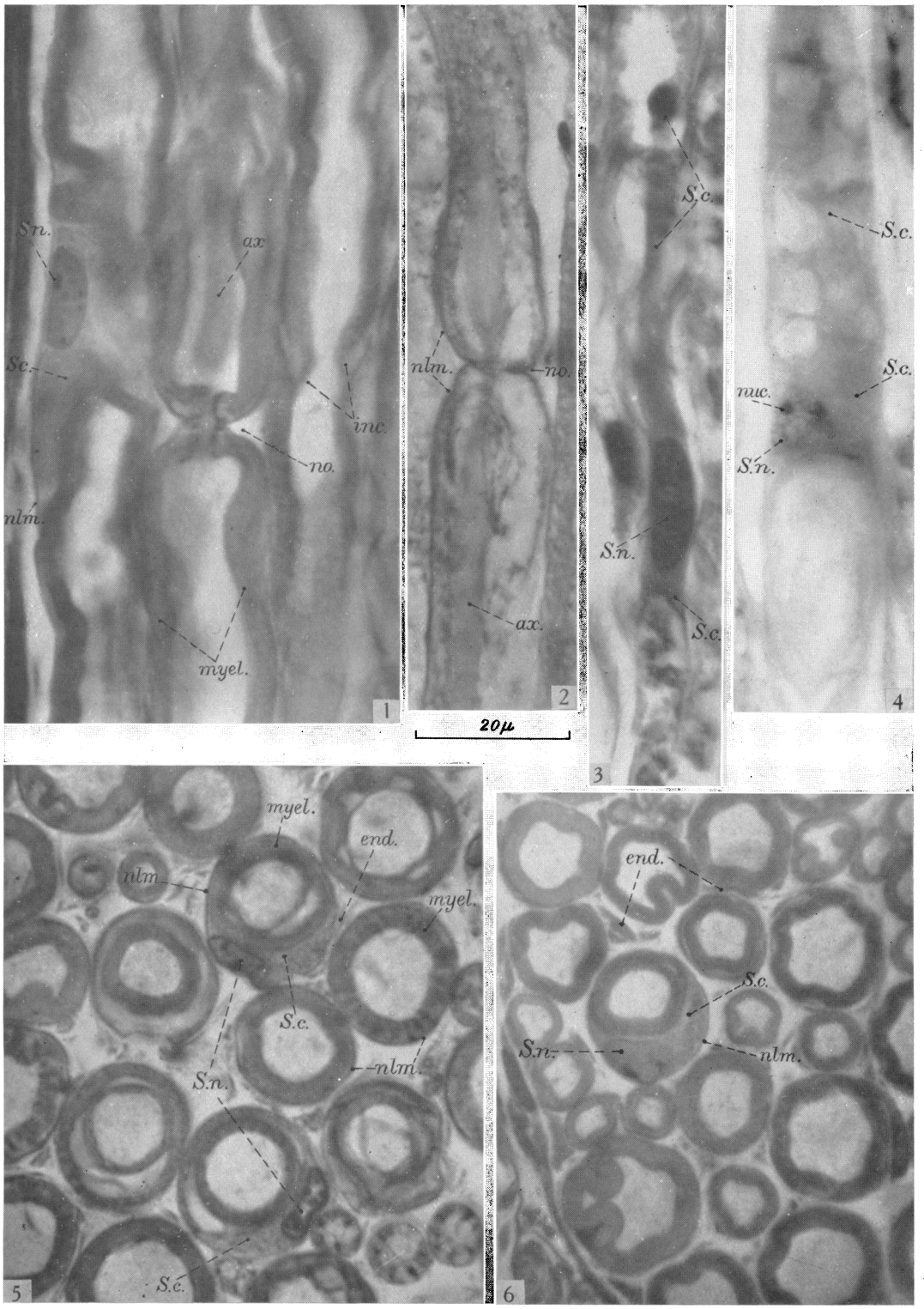
Fig. 72. A peripheral stump degenerated for 514 days. Section taken 85 mm. below secondary suture made 25 days previously. Nerve fibres have not yet reached this point. Alcohol-formol-acetic, Bodian, Masson.

Fig. 73. Same stump as fig. 72 but section taken 10 mm. below the suture. Nerve fibres are present. They are larger and fewer than after primary suture (cf. figs. 48–50, 57, 58) and the endoneurium is much thickened. Alcohol-formol-acetic, Bodian, Masson.

Fig. 74. Section 10 mm. below a secondary suture made 15 days earlier into a stump which had been allowed to degenerate for 53 days. The Schwann tubes are less shrunken than in figs. 72 and 73, but the fibres growing into them are larger than after primary suture. Alcohol-formol-acetic, Bodian, Masson.

Fig. 75. Section 10 mm. below a secondary suture made 97 days earlier after previous degeneration for 365 days. Some of the axones are now large and undoubtedly lie within the Schwann protoplasm. Alcohol-formol-acetic, Bodian, Masson.

Figs. 76, 77. Comparable transverse sections at the same magnification taken at the same distance below primary and secondary sutures respectively, made 103 days previously in the opposite limbs of one animal. The secondary suture (fig. 77) was made after a delay of 483 days, and the myelinated fibres are smaller in diameter, fewer, and more patchy in distribution than on the control side (fig. 76). Flemming, Weigert.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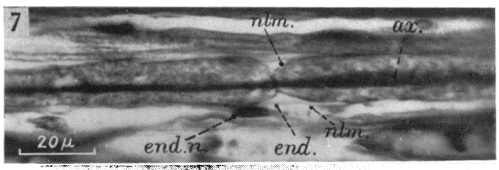
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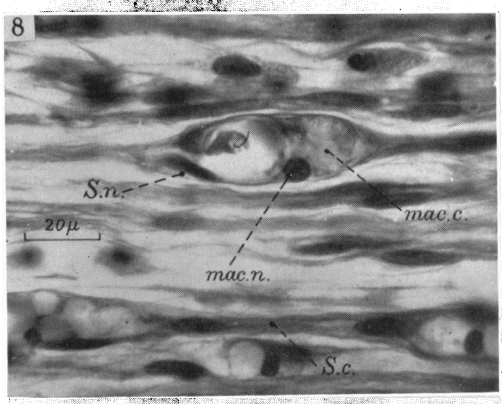
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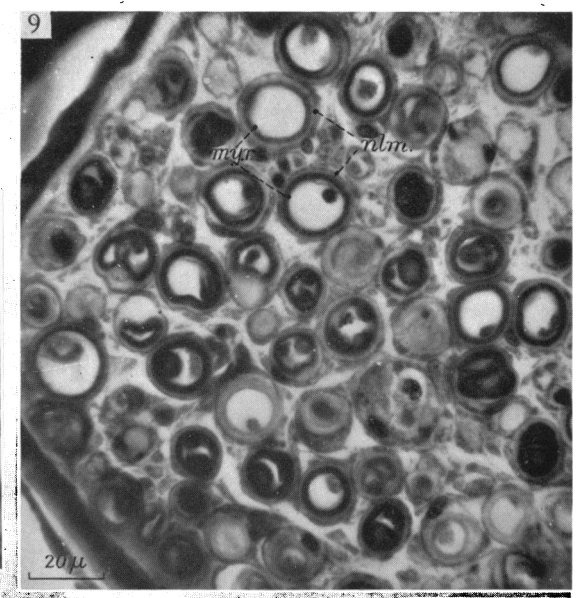
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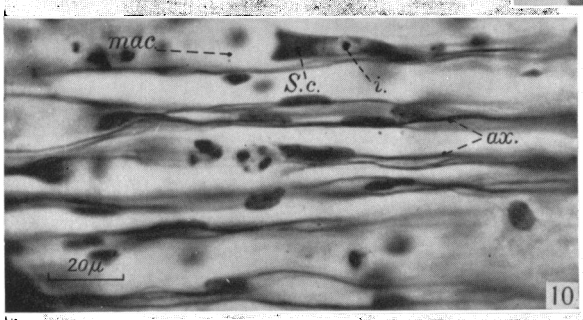
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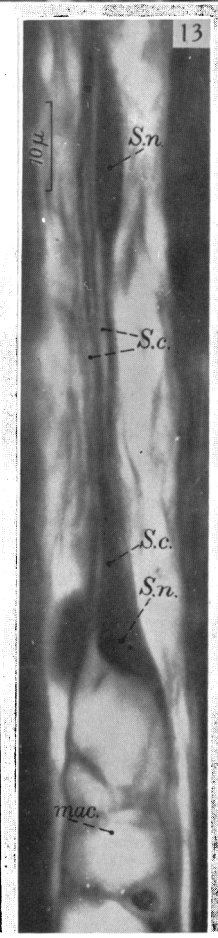
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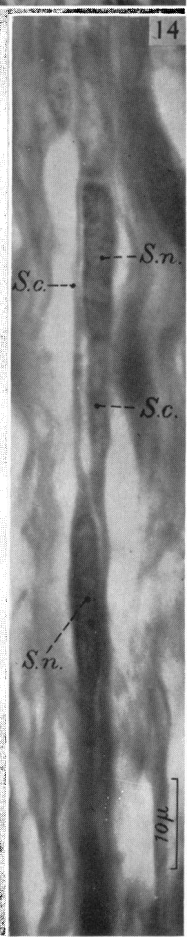
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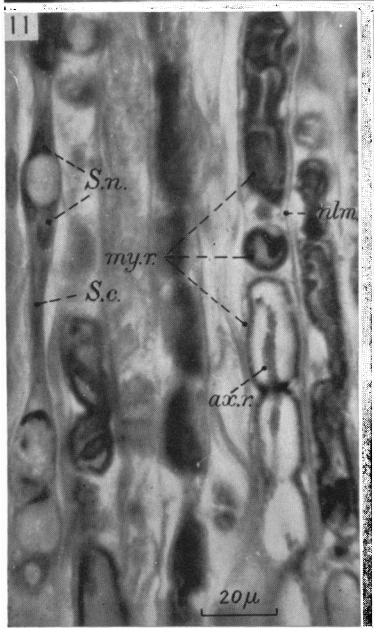
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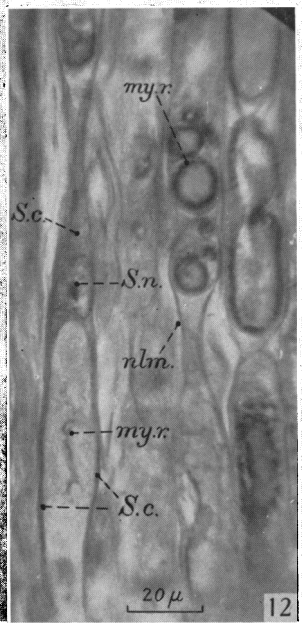
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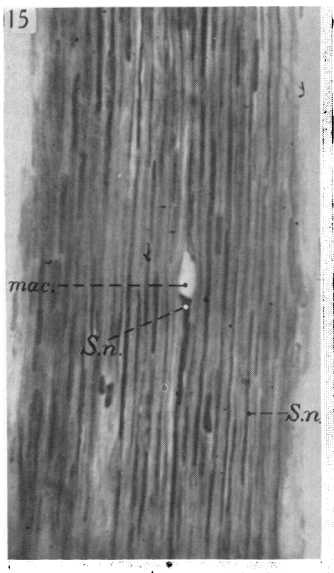
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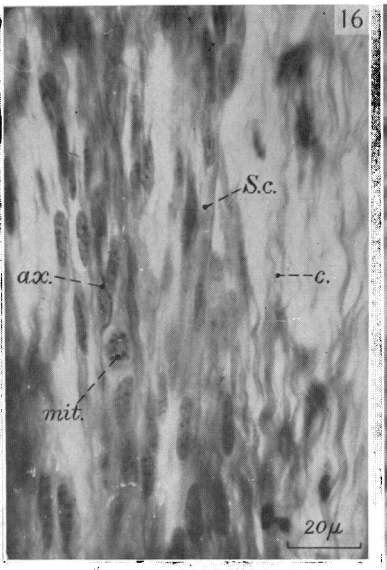
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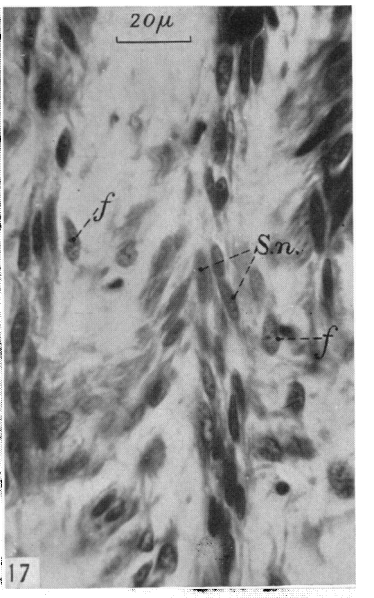
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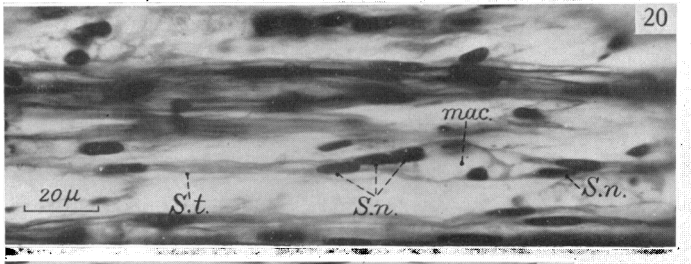
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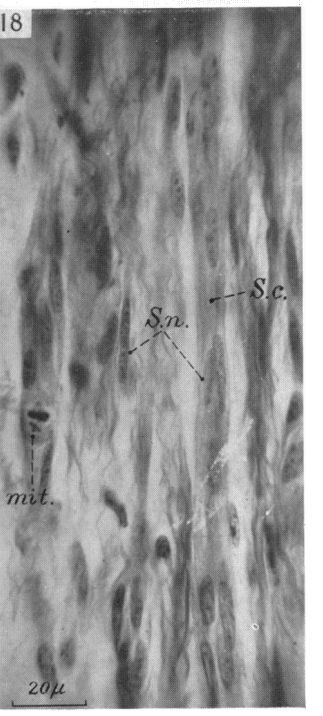
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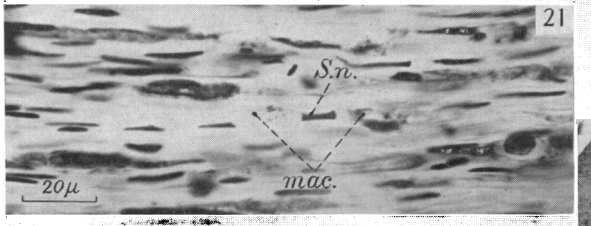
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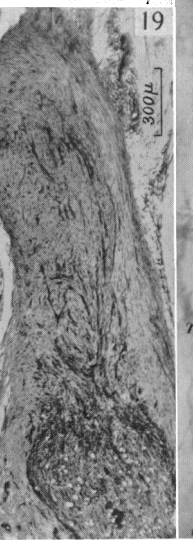
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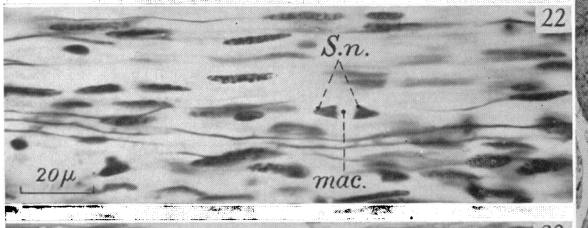
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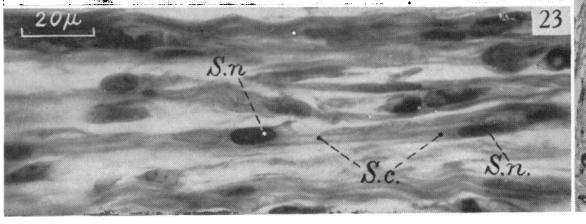
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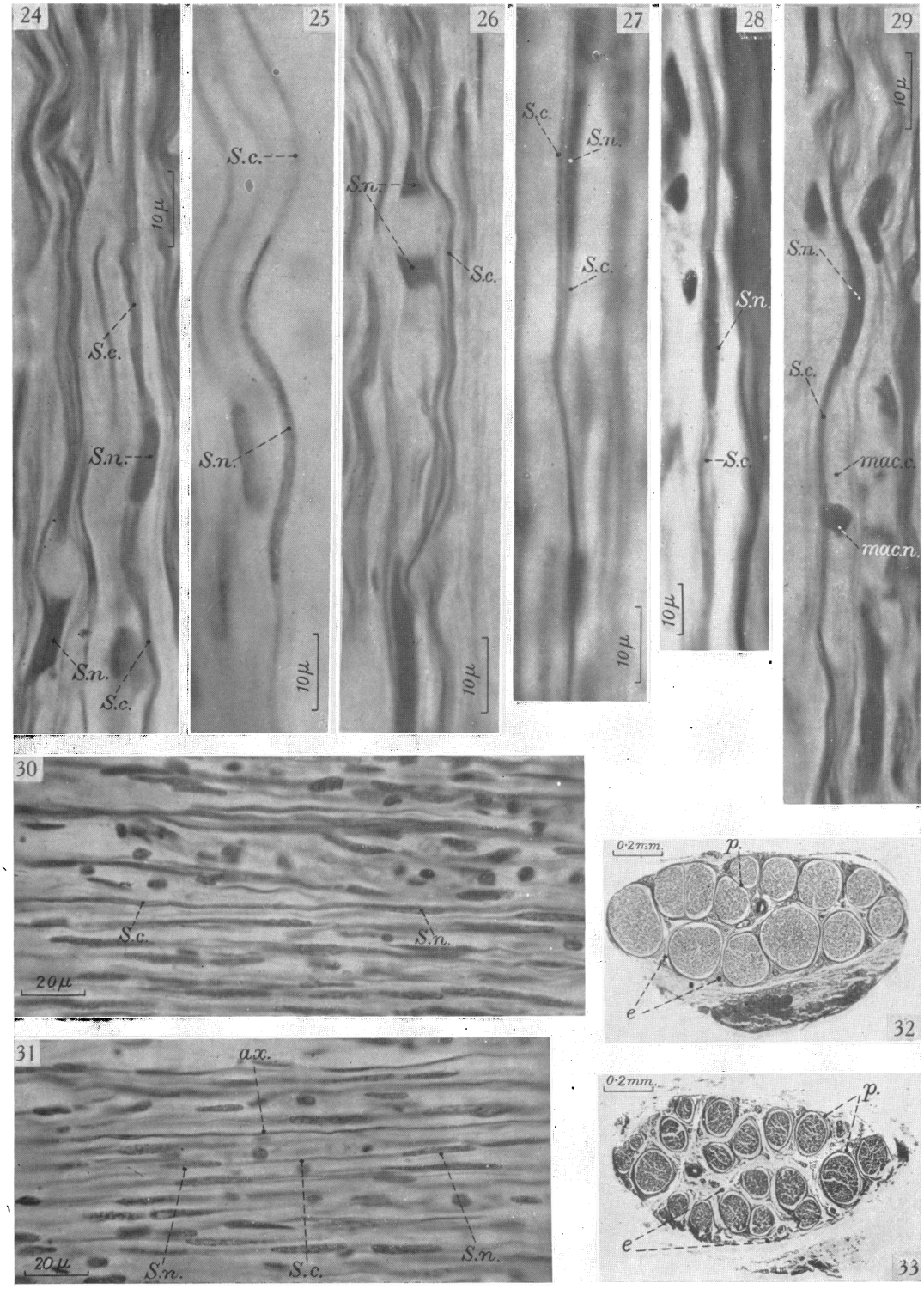
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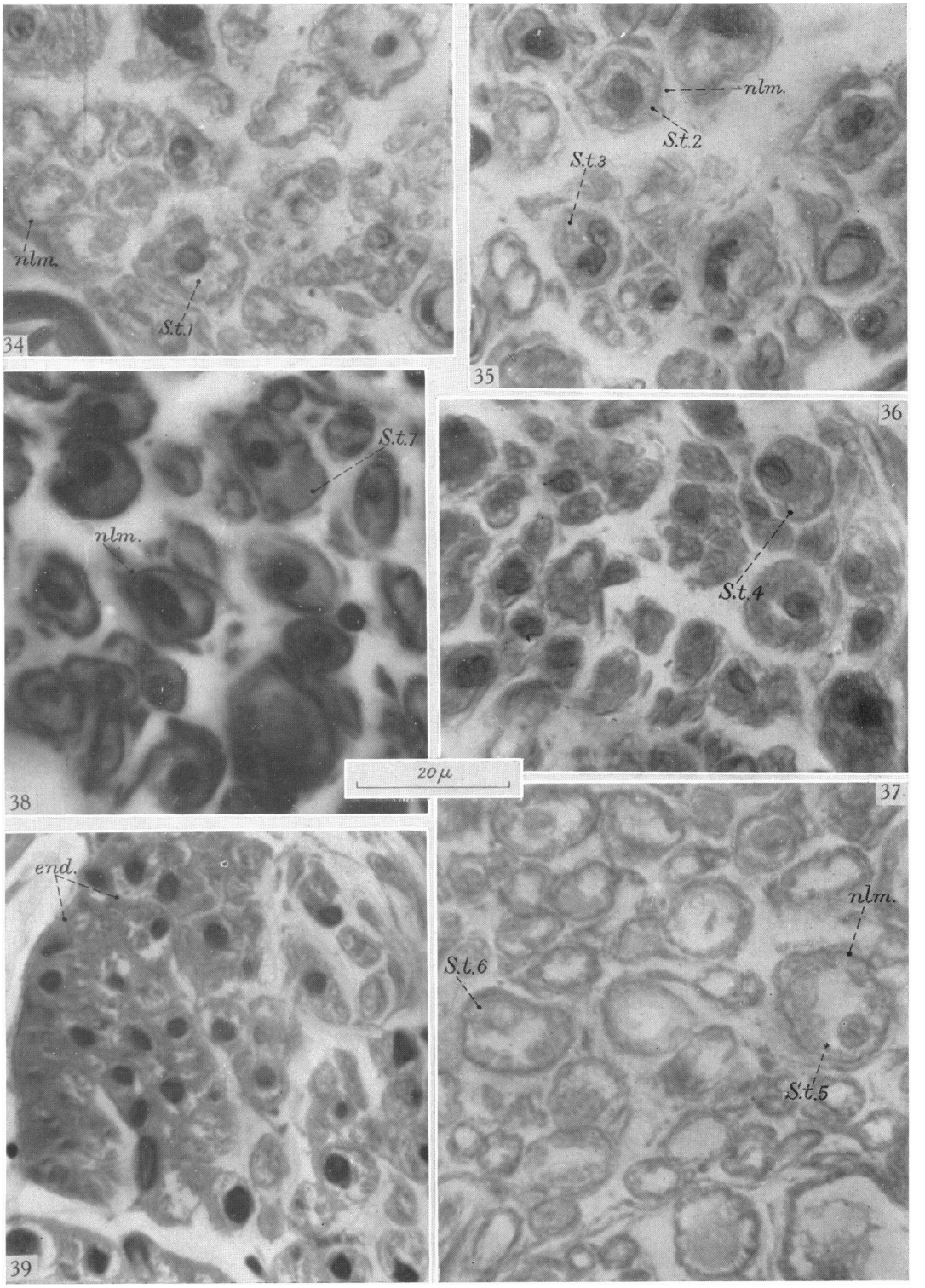
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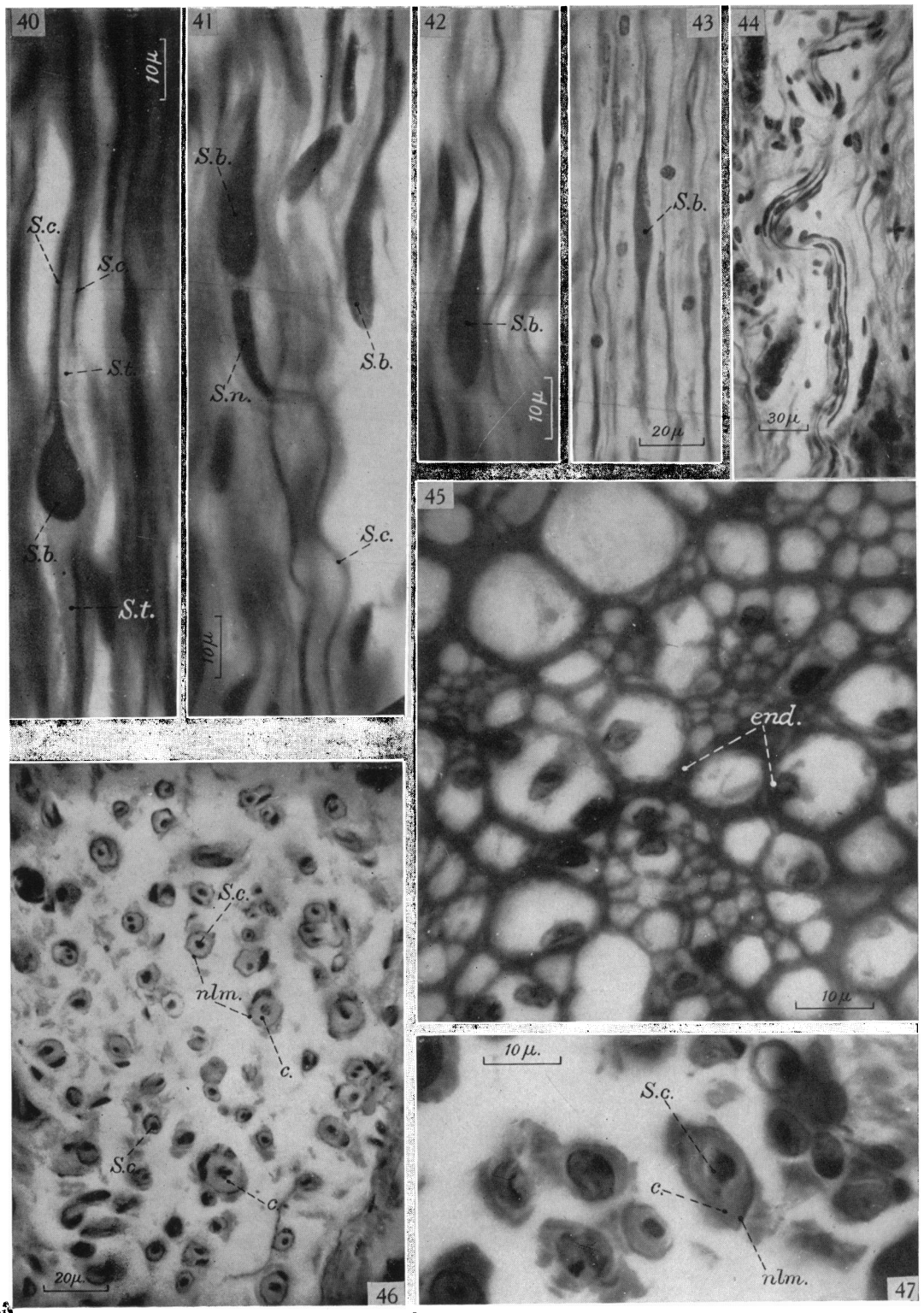
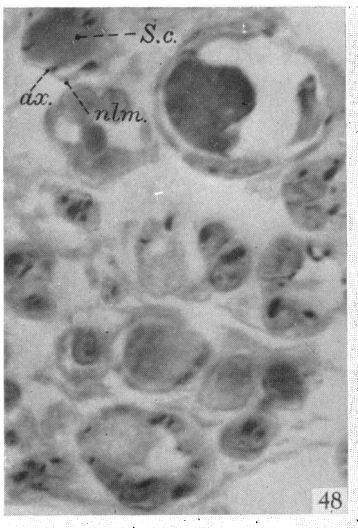
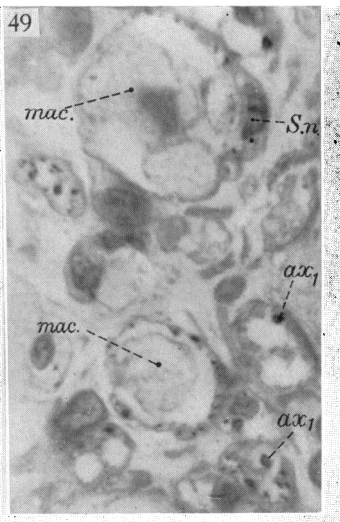
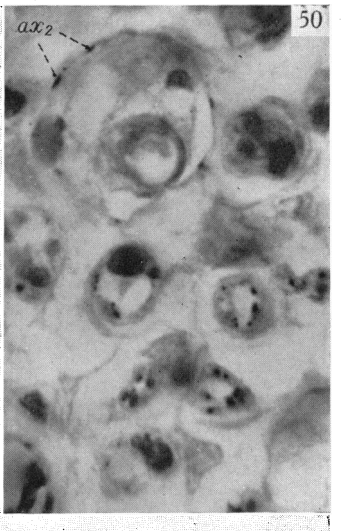
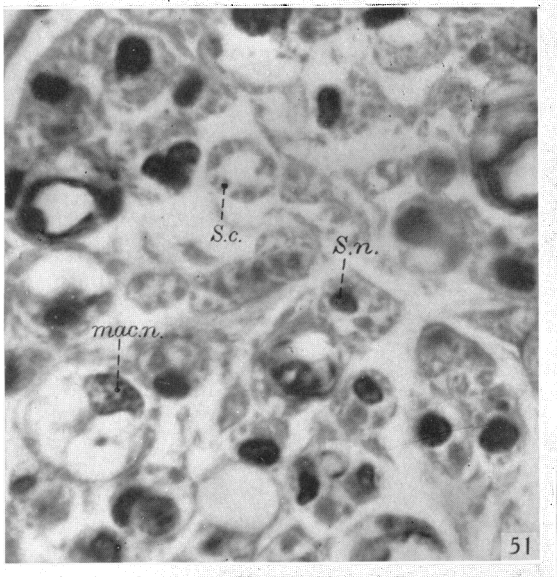


HOLMES AND YOUNG—NERVE REGENERATION

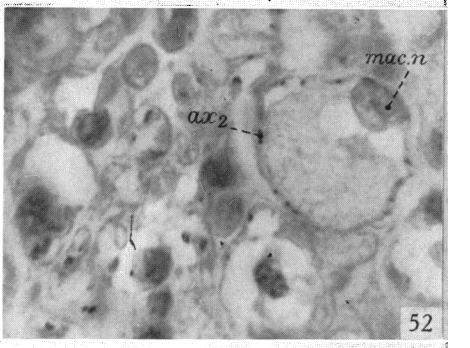

S.c.
ax.
nlm.
48

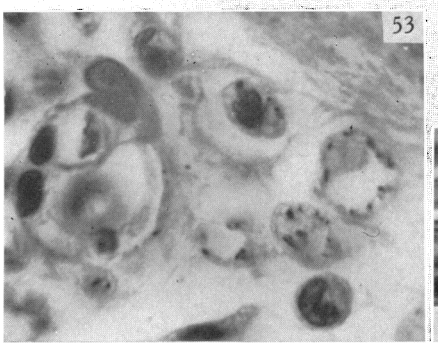

49
mac.
S.n.
ax1
mac.
ax1

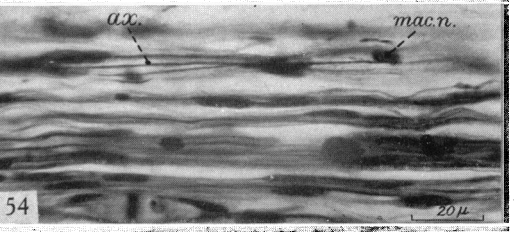

ax2
50

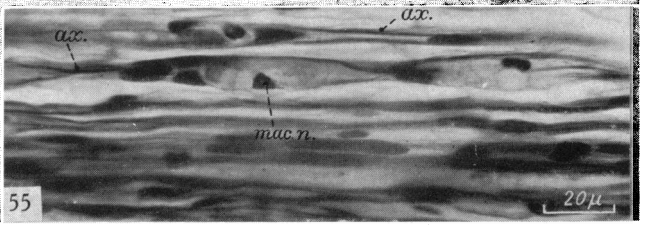

S.c.
S.n.
mac.n.
51

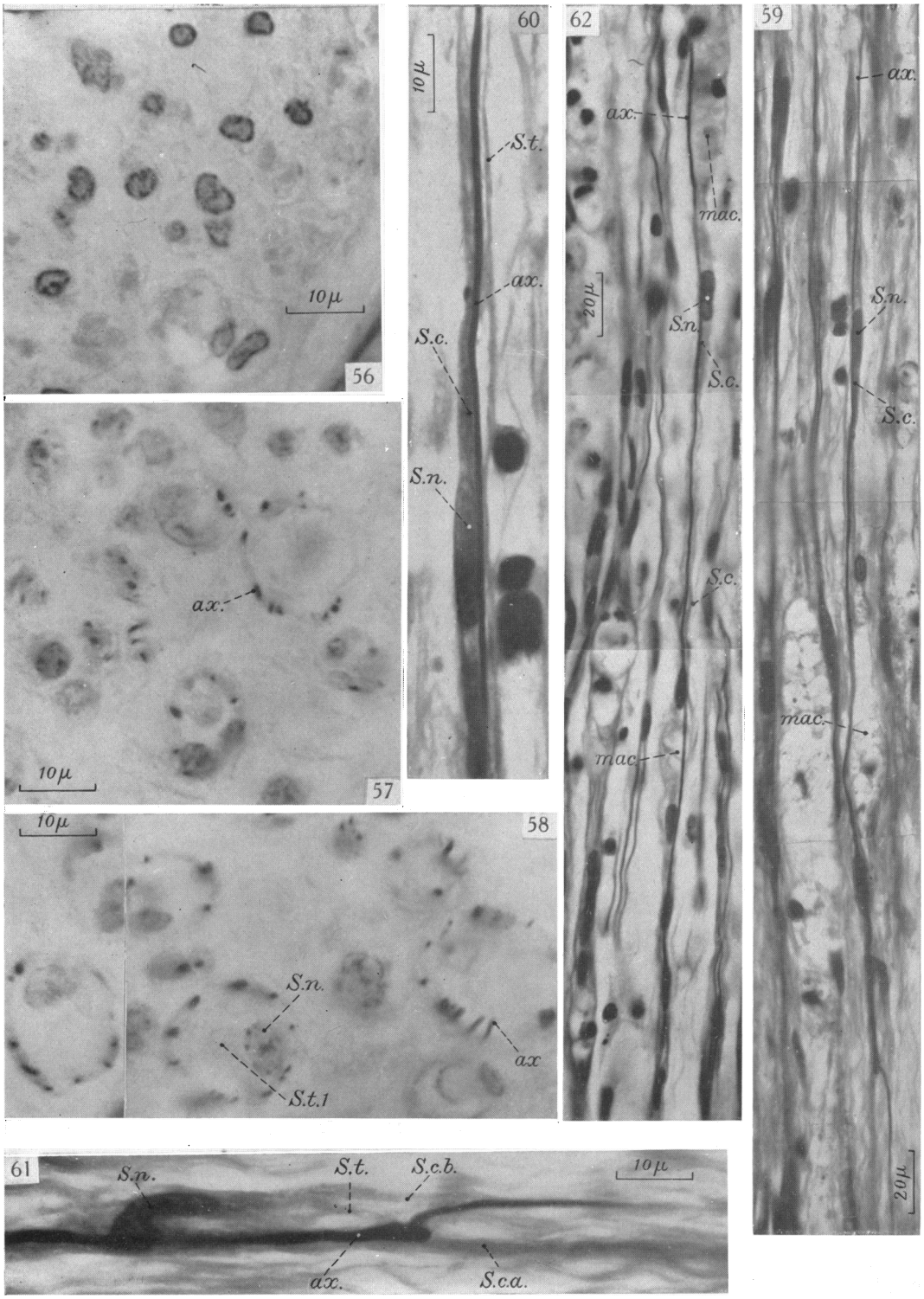
20μ

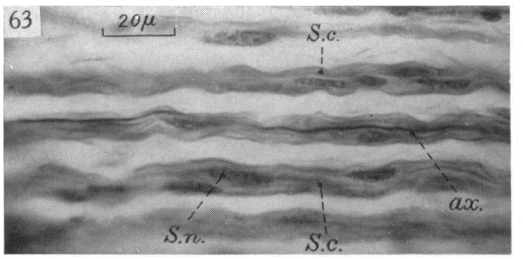

mac.n
ax2
52

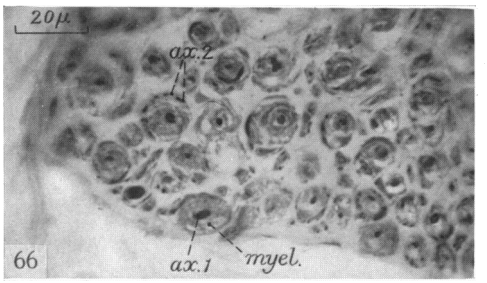

53

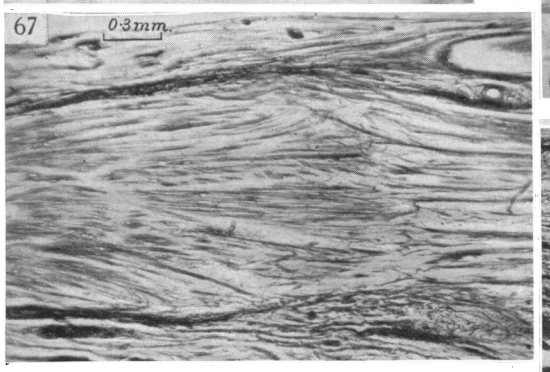

ax.
mac.n.
54
20μ


ax.
ax.
mac.n.
55
20μ

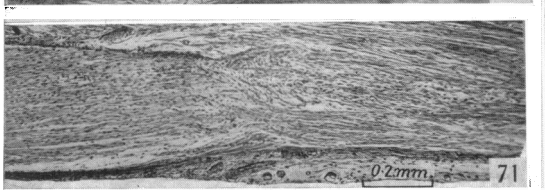

56
10μ
57
ax.
10μ
58
10μ
S.n.
ax
S.t.1
60
10μ
S.t.
ax.
S.c.
S.n.
62
ax.
mac.
20μ
S.n.
S.c.
S.c.
mac
59
ax.
S.n.
S.c.
mac.
20μ
61
S.n.
S.t.
S.c.b.
10μ
ax.
S.c.a.

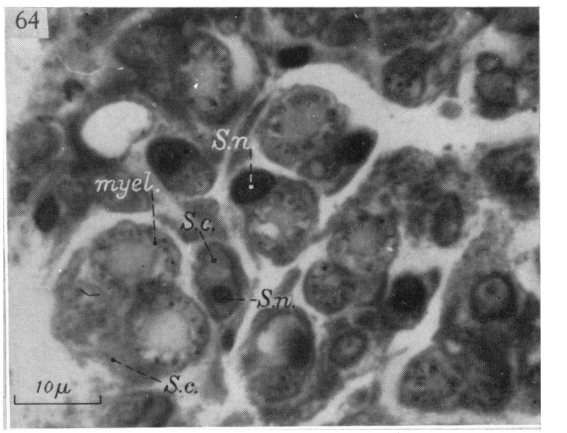

63
20μ
S.c.
ax.
S.n.
S.c.

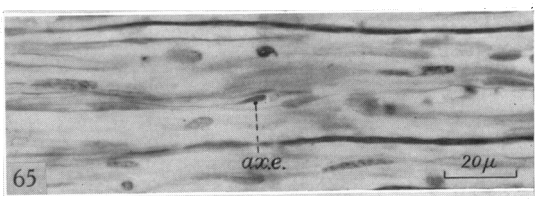

20μ
ax.2
66
ax.1
myel.

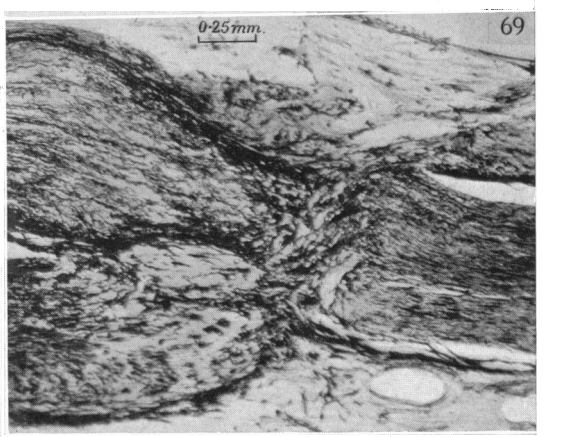

67
0·3mm.

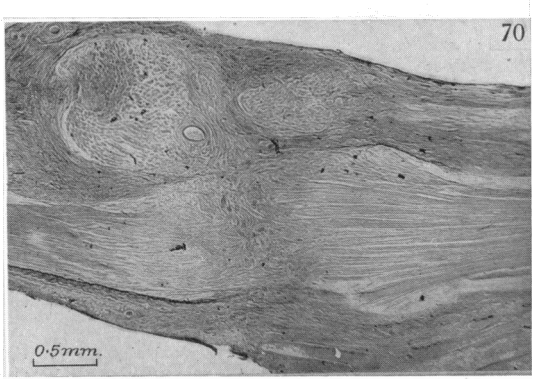

0·6mm.
68

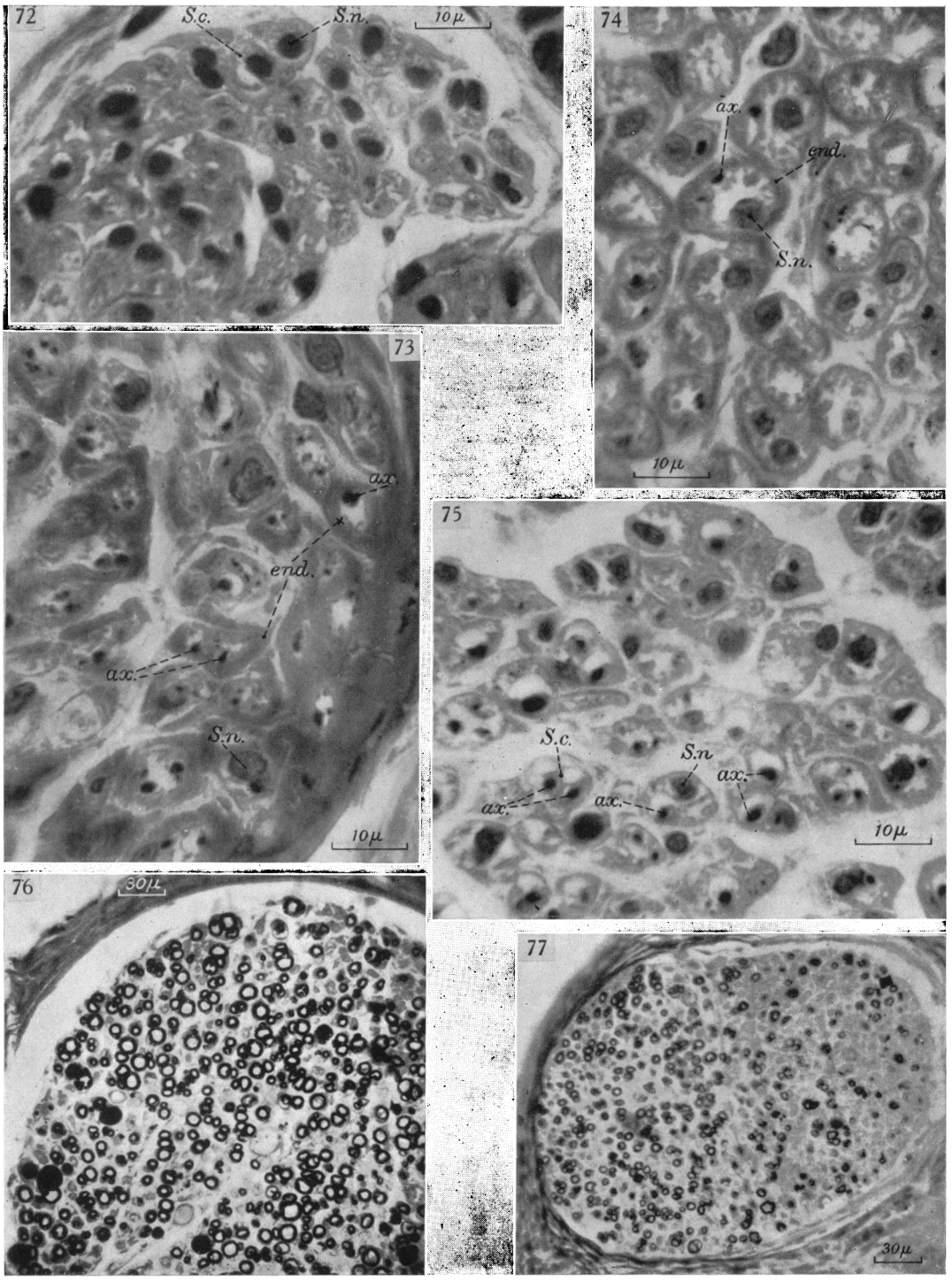

0·2mm.
71


64
S.n.
myel.
S.c.
S.n.
10μ
S.c.


ax.e.
65
20μ


0·25mm.
69


70
0·5mm.


72
S.c.
S.n.
10μ
74
ax.
end.
S.n.
10μ
73
ax.
end.
ax.
S.n.
10μ
75
S.c.
S.n
ax.
ax.
ax.
10μ
76
30μ
77
30μ