AXONAL REGENERATION IN CUTANEOUS NERVE PLEXUSES

By G. WEDDELL, *Department of Anatomy, University of Oxford*

In a recent paper (Weddell & Glees, 1941) a description was given of the early stages in the degeneration of cutaneous nerve fibres, based upon the study of large whole preparations of skin in which the nerve fibres and their endings had been stained intravitally with methylene blue. It was pointed out that the method of vital staining with weak solutions of methylene blue, followed by the examination of whole preparations, has two outstanding advantages; it permits the course of individual nerve fibres to be followed in continuity for long distances, and, since the finest nerve fibres are stained with constancy only by methylene blue, it provides a more precise picture of the more delicate histological changes which occur during the course of degeneration than can be obtained by the more commonly employed silver methods. It was also observed that complicating factors which tend to obscure the behaviour of degenerating axones and their sheaths in nerve trunks, such as the concomitant changes in vascular tissues and the presence of large numbers of macrophages, are practically absent during the degeneration of the fine nerve bundles of the cutaneous plexuses.

In the present investigation an attempt has been made to analyse the pattern of regeneration of cutaneous nerve fibres by a similar method. The mode of application of the methylene-blue technique is believed to justify the reinvestigation of certain aspects of nerve regeneration, even though a vast amount of work on this problem has already been completed (the number of publications on nerve regeneration alone collected by Rossi & Gestaldi up to 1934 amounted to 928). Further, although many excellent accounts have been given of nerve regeneration (Howell & Huber, 1892; Marinesco, 1906; Perroncito, 1907; Poscharissky, 1907; Dustin, 1910; Ranson, 1912; Kirk & Lewis, 1917; Berblinger, 1918; Cajal, 1928; Nageotte, 1932; Boeke, 1935), in the majority the investigations have been confined to the changes which occur in nerve trunks, and the observations have been based upon the examination of thin sections and not of whole preparations. There have also been relatively few observations on the regeneration of cutaneous nerve fibres. Boeke & Heringa (1924), Dijkstra (1933), and Jalowy (1935), have examined skin from specialized areas in man, birds and monkeys, but here again the observations were based upon thin sections of silver-impregnated material which do not permit of the study of the pattern of nerve regeneration over large areas of denervated skin.

MATERIAL AND METHODS

Skin from the dorsum of the ears of albino rabbits was used. It was removed at intervals ranging from 2 days to 12 months after crushing of the main dorsal ear nerve at the base of one ear in a series of twenty rabbits. In nine animals the nerves were divided and gaps of 2 mm. or more left between the ends; skin from the dorsum of the ears in these animals was removed at intervals ranging from 2 weeks to 18 months. In four rabbits, in addition to crushing the main dorsal ear nerve, an attempt was

made to denervate the back of the ear completely by crushing the nerve trunks which pass up the medial margin of the ear.

Sensory tests were applied by pin pricks and by faradic stimuli from a 'Palmer' induction coil through fine stigmatic bipolar electrodes of interpolar distance 1 mm. The tests were carried out in each case just previous to the removal of skin from the ear, and in a number of cases also during the course of regeneration. The method of carrying out such tests has already been described in detail in a previous publication (Weddell, Guttmann & Gutman, 1941). Briefly, the stimulus is applied to the skin at intervals of approximately 1–1½ sec., working from the area of sensory loss towards the innervated area until a withdrawal response is obtained. This spot is then marked on the skin with Indian ink. At the end of the test the series of spots so obtained are tattooed into the skin. Since the skin of the ear is thin and the ventral surface of the ear remains innervated throughout the experiment, a finely adjusted faradic stimulus was found to be more accurate for routine use than a pin prick; the results obtained by either method were found to be strictly comparable provided approximately threshold stimuli (determined on the opposite normal ear) were used.

A 0·02 % solution of B.D.H. standard methylene-blue stain in 0·9 % sodium chloride A.R. containing 1 % procaine hydrochloride was used, the time allowed for the staining after injection being ¾ hr. Further details of the method have been given previously (Weddell & Glees, 1941).

OBSERVATIONS

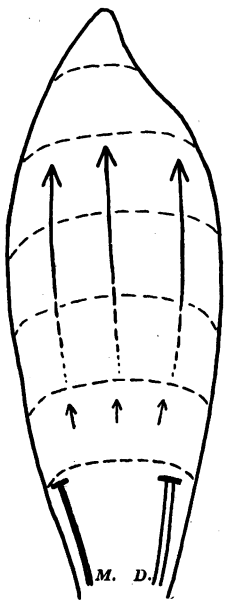
Sensory tests

(*a*) *After nerve crushing.* Where the main dorsal ear nerve only has been crushed the area of sensory loss usually involves the lateral (posterior) three-quarters of the dorsum of the ear, but the actual size and shape of the desensitized area are very variable. As early as the second day after operation there is a slight diminution in the area of sensory loss, the demarcation line having moved a small distance distally and laterally towards the denervated zone; thereafter the area of sensory loss slowly diminishes until at the end of 14 days the advancing margin of sensibility has reached some 6–7 mm. beyond the original line of demarcation. After this time the rate of advance increases considerably, reaching an average speed of 2–2·5 mm. a day. The area of sensory loss continues to diminish in a distal and lateral direction (Text-fig. 1*b*).

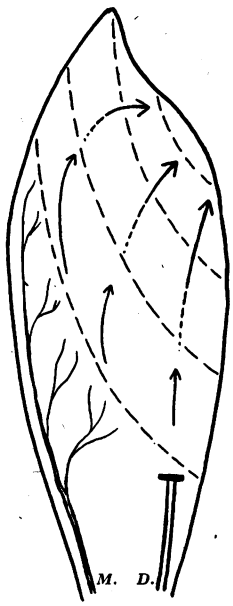
Where the dorsum of the ear has been completely denervated by nerve crushing (and only two cases proved to have been so denervated), the regeneration does not commence until 14 days have elapsed and then the advancing margin of sensibility progresses at a rate of about 1 mm. a day. The line of demarcation is difficult to define in these cases, but, ignoring certain irregularities related to precocious growth along main nerve trunks, there is a fairly straight transverse line of returning *cutaneous* sensibility, advancing up the ear, the last part of the ear to become re-innervated being the tip (Text-fig. 1*a*).

(*b*) *After nerve section.* Where only the main dorsal ear nerve has been cut and a gap of 2 mm. or more left between the ends, sensory tests carried out 2, 3 and 4 weeks following the operation show that there is a steady increase in size of the area of skin from which nociceptive responses can be elicited. The margin demarcating the sensitive from the insensitive areas advances approximately 0·5 mm. a day. As will be seen

later, this is the rate of growth of nerve fibres extending from surrounding normal nerves towards the denervated area.



Text-fig. 1*a*.



Text-fig. 1*b*.

Text-fig. 1*a*. Diagram illustrating the progress of sensory recovery after section of all the nerves passing up to the dorsum of the rabbit's ear. *D.*, main dorsal ear nerve; *M.*, nerves passing up the medial (anterior) margin of the ear.

Text-fig. 1*b*. Diagram illustrating the progress of sensory recovery after section of the main dorsal ear nerve only. *D.*, main dorsal ear nerve; *M.*, nerves passing up the medial (anterior) margin of the ear.

General pattern of regeneration

(*a*) *After nerve crushing.* By the exclusive use of albino rabbits, and by taking care that the methylene blue is evenly injected throughout the skin of the dorsum of the ear, it is possible to observe the progress of regeneration with a hand lens or even macroscopically. In the cleared specimens all the nerves, and to a lesser degree the blood vessels, are stained blue, while the rest of the tissue remains colourless. The blood vessels which do take up the stain can be readily distinguished from nerve trunks (Pl. 1, fig. 1). The pattern assumed by the advancing fibres is well seen 4 weeks after crushing the main dorsal ear nerve only. By this time all the main nerve trunks in the originally denervated area contain regenerated fibres and therefore take up the stain. These fibres have not yet extended into the small cutaneous nerve bundles, with the exception of a narrow zone alongside the normal intact nerve bundles passing up the medial margin of the ear. The reason for the precocious innervation of this zone is not clear and is further discussed below.

Five weeks after crushing the main dorsal ear nerve, the only portion of skin in which no small nerve bundles were seen was an area situated at the lateral margin of the ear three-quarters of the way towards the tip. This area was insensitive to nociceptive stimulation.

A study of ears at earlier stages of regeneration confirms the impression that regeneration is actually more rapid along the original main nerve trunks than in the cutaneous bundles, and that in the main nerve trunks it is also more rapid where the latter lie in closest proximity to normal intact nerves.

After complete denervation of the dorsum of the ear, the last area of skin to become re-innervated is in the midline towards the tip, nerve fibres passing first along the original main nerve trunks and only later along the smaller nerve bundles. It is interesting to note that the density of re-innervation in the latter always appears to be greater in the neighbourhood of the larger blood vessels, even when the vessels lie at some distance from the main nerve trunks (Pl. 1, fig. 1). It is possible therefore that the rapidity of regeneration of the cutaneous nerve bundles in any particular area of skin is related to its vascularity.

(*b*) *After nerve section.* After the main dorsal cutaneous nerve had been cut and a gap of 2 mm. or more left between the ends, it was found that even 4 weeks after operation practically no regeneration had taken place along the distal stump. In this case, however, definite histological evidence was obtained that new sprouts had grown out from normal intact nerve fibres at the medial margin of the ear and had extended for a short distance into the denervated zone.

Correlation of sensory tests with the pattern of regeneration

On comparing sensory tests with the general pattern of regeneration it was found that the margin of regeneration as indicated by the methylene-blue staining was always about 5 mm. in advance of the area from which nociceptive responses could be elicited. The reason for this discrepancy is discussed below. Faradic stimuli confirmed the histological evidence that nerves advance quicker along the nerve trunks than along the intervening cutaneous nerve plexuses.

In the experiment in which, after section of the main dorsal ear nerve, histological evidence was obtained that fibres had sprouted from adjoining normal nerves, there was a remarkably close correlation between the area from which nociceptive sensations can be aroused and the newly innervated area.

Histological observations

At the site of lesion. Two days after crushing the main dorsal ear nerve, regenerating nerve sprouts have penetrated into the cellular exudate between the nerve stumps; the necrotic segments of axones of the proximal stumps are still evident. Terminal swellings are seen at the end of many regenerating axones and their collaterals. Direct and proximal sprouting can be seen, and emerging from the terminal clubs are brushes of fine fibres which appear similar to those described by Cajal (1928) as direct sprouting by 'ravelling'. Net formations (Cajal, 1928) are also seen. With the exception that there is more multiple sprouting than is usually described after nerve section the picture is essentially similar to that given by Poscharissky (1907), Kirk & Lewis (1917), Cajal (1928), Boeke (1935) and other workers. There is, however, one feature of these methylene-blue preparations which deserves emphasis. A comparison of the descriptions and illustrations of other authors makes it probable that the finest fibres which can be demonstrated by intravital staining with methylene blue are often not brought to view by silver impregnation methods. Hence the picture of regenerating nerve fibres in methylene-blue preparations appears somewhat more complicated than might

be expected from previous descriptions. Even with the use of methylene blue, however, the actual terminations of the finest sprouts cannot be defined with certainty, for in some cases they are not surmounted by growth cones; on the contrary, they become progressively finer in diameter and finally pass beyond the powers of resolution of a microscope with a 1/12 in. oil immersion lens.

In the peripheral stump. The rate of regeneration of individual fibres along the peripheral stump is very variable; for some fibres 'get through' without any apparent hindrance. In one case two fibres were found to have advanced 40 mm. along the trunk 8 days after crushing the main dorsal ear nerve. The majority of such rapidly regenerating fibres are extremely fine and, unless the growing tip is expanded to some extent, their precise termination is impossible to define with certainty. Most fibres regenerate much more slowly and, as will be seen later, the more advanced fibres do not necessarily become mature or functional.

One of the most striking features in the proximal part of the distal stump, 2 weeks after crushing the main dorsal ear nerve, is the large number of fine newly regenerated fibres undergoing degenerative changes as compared with the number seen more distally. In many cases, these fine degenerating fibres can be traced back to their origin as sprouts from proximal stumps. The great majority of regenerating axones have smooth homogeneous outlines though they are not of uniform diameter. Throughout the course of the fibre there are fusiform swellings which usually occur at fairly regular intervals (Pl. 1, fig. 2).

In the smaller nerve trunks and cutaneous nerve plexus. The finest visible terminations of the nerve fibres are associated with Schwann cell pathways which retain the pattern of the cutaneous plexus in the normal ear (Pl. 1, fig. 5). On no occasion in the rabbit's ear has the tip of a regenerating nerve fibre in the cutaneous plexus been seen pursuing a course independently of a Schwann band. This is made clear by the fact that, for about 1 mm. ahead of the finest visible sprouts of nerve fibre which are detectable under the oil immersion, the nuclei and the cytoplasm of the Schwann bands are commonly stained in a characteristic fashion by intravital methylene blue (Pl. 1, fig. 4). The Schwann bands are, however, only stained when regenerating nerve fibres are growing along their course and only in relation to regenerating nerve fibres of fine diameter, for when traced proximally the bands become progressively less stained until they are no longer visible. In no case have mitotic figures been seen in Schwann band nuclei of the cutaneous nerve plexus which are stained with methylene blue. It may be noted, also, that it has not been possible with this technique to see cell boundaries between individual nuclei along the course of a single Schwann band.

In order to illustrate the mode of regeneration of nerve fibres through the cutaneous nerve plexus, and to demonstrate their relationship to stained Schwann bands, a series of photomicrographs was taken of a nerve fibre in its course from a small nerve trunk into the cutaneous plexus. Pl. 1, fig. 6, shows such a fibre dichotomizing where it enters the plexus. If one of the branches is followed the axis cylinder is seen to become visibly expanded at a point where the Schwann bands outlining the plexus separate. From this expanded terminal a large number of fine nerve fibrils proceed distally, apparently along the surface of the Schwann band (Pl. 2, fig. 7). Pl. 1, fig. 3, shows the appearance of such nerve fibrils somewhat further along their course, and Pl. 2, fig. 8, illustrates a point still more distally where the majority of the fibrils are so fine that they are approaching the limit of visibility. Just proximal to the expanded

terminal which gives rise to the fibrils the Schwann bands begin to stain with methylene blue and become progressively more darkly stained as the fibrils are traced distally. As already stated, they continue to take up the stain for a distance of not more than 1 mm. ahead of the finest visible nerve terminals.

With the technique employed (which involves alcohol fixation) it is difficult to say with certainty from a spread preparation whether the finest fibrils lie on the surface of the Schwann cytoplasm or whether they are situated just within it. Transverse sections of Schwann bands from selected areas at various stages of regeneration show that the larger nerve fibres are completely surrounded by Schwann cytoplasm but that the finest advancing fibrils lie towards the surface of the Schwann band and, in fact, in the most distal part of their course they appear to lie actually on the surface. Further evidence that the most distal extremities of the advancing fibres lie on the surface of the Schwann cytoplasm is furnished by some histological pictures in which one of the fine fibres may be seen to curve away from the Schwann band and rejoin it a little further on as though it had become detached during the course of preparation (Pl. 2, fig. 10).

Measurements made along twelve typical regenerating fibres showed that, although the most advanced nerve fibrils which are visible probably lie on the surface of the Schwann cytoplasm, at a distance of 3–4 mm. proximal to their tip they are completely surrounded by the cytoplasm. When, however, the distal extremity of a regenerating nerve fibre is surmounted by a growth cone (presumably indicating an obstruction to growth), the length of the part which is not surrounded by cytoplasm is considerably reduced and may even disappear (Pl. 2, figs. 9, 11).

Throughout the course of the regenerating fibres maturation is seen to be taking place. This involves the degeneration of a large number of collateral branches, and the degeneration seems primarily to affect those which had previously contained the largest number of fusiform swellings along their course, in other words, those which had presumably met with most obstruction during their outgrowth. As the number of fibres in the smaller nerve trunks and the cutaneous nerve plexus becomes reduced, the average diameter of the remaining fibres increases (Pl. 2, figs. 12–18). Concomitant with this increase in diameter is the disappearance of the fusiform swellings which form such a characteristic feature of nerve fibres in the early stages of regeneration (Pl. 2, figs. 9, 13, 21). This is well illustrated by comparing photomicrographs of two small nerve bundles taken 5 and 12 weeks respectively after crushing of the main dorsal ear nerve (Pl. 2, figs. 15, 20). On the contrary, it is also clear that the characteristic beading of the fibres of the subcutaneous and vascular nerve nets does not become apparent until maturation is completed.

In areas where regeneration is fairly advanced, fine nerve fibres can be seen approaching the skin where they begin to extend into a regenerating network distributed just beneath the epidermis. Owing to the staining of the Schwann cells at certain stages during regeneration, it is now possible to see that the Schwann cytoplasm accompanies the finest fibres through the greater part and perhaps the whole of their course (Pl. 3, figs. 22, 25). An observation of some interest is that the so-called 'Langerhans cells' are abundant in the skin of the albino rabbit's ears and these experiments show that, like the Schwann cells, they take up the blue stain when the fine regenerating nerve terminals are approaching them, i.e. when the terminals are just becoming apparent beneath the epidermis (Pl. 3, fig. 25). Boeke (1940) took the

view that these cells were in protoplasmic continuity with nerve terminals in the epidermis. Our preparations show no evidence of this; on the contrary, when they are stained with methylene blue they appear to lie in series with the Schwann cell elements which are related to the fibres in the subepidermal network. It thus appears not improbable that these 'cells of Langerhans' are nothing more than modified Schwann cells.

In tracing the course of the regenerating nerve fibres at intervals after experimental interruption, it soon becomes clear that those passing to blood vessels regenerate faster than those giving rise to nerve nets beneath the epidermis or ending around hair follicles. Fibres leave the nerve trunks and pass to the blood vessels along the course of persisting Schwann elements (Pl. 3, figs. 24, 26). During maturation, these vascular nerves, like those ending beneath the epidermis, at first appear to lie on the surface of the Schwann bands but later become surrounded by the Schwann cytoplasm. The cytoplasm related to a single Schwann nucleus extends along the individual components of the nerve nets on blood vessels for considerable distances (Pl. 3, figs. 24, 26). It is probable that these Schwann elements are identical with the 'interstitial cells' described by Lawrentjew (1926) and Boeke (1940), and thought by the latter to be nerve cells. Beading of these vascular nerves likewise does not occur until after maturation is completed (Pl. 3, fig. 23). The faster rate of regeneration of the nerve nets along blood vessels was determined by tracing the finest visible terminations along the line of the advancing cutaneous nerves, when in 15 counts along different vessels the vascular nerves were on the average 3–4 mm. ahead of the cutaneous nerves. It must be remembered, however, that nerve fibres advancing into the skin have to pass through a tortuous course in the cutaneous nerve plexus towards the skin surface, whereas vascular nerves advance more directly along the line of the vessel.

Sensory recovery

When, after nerve crushing, pin pricks first give rise to nociceptive reactions, it is found that the number of random pricks required to arouse a single such reaction is greater over a given area in which regeneration has just been completed than in a similar area in a normal ear. It is also found that a stronger faradic stimulus must be given in order to arouse nociceptive responses with each application of the electrodes. During recovery after nerve section a similar phenomenon is observed, except that nociceptive responses are still more difficult to obtain than in cases in which the nerve had simply been crushed; in addition, there is a definite latent period in the nociceptive reactions which follow the application of the stimulus. In the later stages of regeneration (3–12 months after nerve crushing) very little difference in the sensory recovery between the normal and operated side could be observed with the tests employed.

In areas of skin which have just reached a stage in which pin pricks and faradic stimuli arouse nociceptive responses, many hair follicles are supplied only by single mature nerve fibres of small diameter, while other fine fibres may be seen still in the process of advancing towards them along the Schwann bands. Fine nerve fibres can also be seen leaving the cutaneous nerve plexus and giving rise to nerve nets situated just beneath the epithelium. These nets, however, are isolated from one another and do not form a continuous interdigitating series as in a normal ear. Finally, in such areas of skin, there are always a number of stained Schwann bands along which nerve

fibres are advancing but which have, as yet, not reached their destinations; ahead of such advancing fibrils (which are always situated in the deeper portions of the cutaneous nerve plexus) the Schwann bands are stained for as much as 1 mm.

At a slightly later date a certain number of the fibres show the presence of nodes of Ranvier. No such nodes are visible until about 4 weeks after sensation (nociceptive) has returned (Pl. 2, figs. 19–21).

In the later stages of regeneration (3–12 months after crushing the main dorsal ear nerve) the only difference which can be detected in the pattern of cutaneous innervation as compared with a normal ear is an increase in the number of collateral fibres in the nerve trunks. In addition, the average diameter of the nerve fibres throughout the ear is somewhat smaller (Pl. 2, fig. 20). On the other hand, after nerve section, even when no sensory differences can be detected between the two sides, the following histological changes are seen. In the nerve trunk there are a great many collaterals and the fibres are in general of considerably smaller diameter than in a normal trunk. The hair follicles are all innervated by fibres which are much finer than usual, but the density of the subcutaneous nerve nets is normal. In addition, a very large number of fibres of the cutaneous nerve plexus are still surrounded by blue-stained Schwann cells. It is also clear, by tracing the nerve trunk fibres towards the point of section, that far fewer fibres penetrate into the distal stump than after crushing.

On comparing the results of sensory tests with the histological pattern of regeneration in the rabbit's ear, it is apparent that the finest growing tips of the nerve fibres, and even mature nerve fibres when they are few in number, are not sufficient to mediate impulses giving rise to nociceptive responses. In fact, the advancing line of sensory change lags behind the advancing nerve sprouts by at least 5 mm. It should be emphasized, however, that the finest advancing nerve sprouts are always situated at a deeper level beneath the skin, for they are here entering the cutaneous nerve plexus through which they must pass towards the skin surface. It may well be that the discrepancy between the sensory tests and histological observations is in part due to this fact, for in striking contrast to this is the correspondence between the two advancing boundary lines when nerve fibres are extending locally through the superficial layers of the plexus and subepidermal nets towards the area of sensory loss. In these cases they correspond to within 1 mm. This close correspondence is probably also related to the slow rate of progression of these new-growing nerve fibres, which thus undergo maturation almost as quickly as they advance. Further details of this local extension of nerve fibres have been given already (Weddell *et al.* 1941).

DISCUSSION

The histological observations on axonal regeneration in the present investigation are in general agreement with those made by the majority of workers, mentioned in the introduction, who studied thin sections of nerve trunks. The use of whole preparations has, however, enabled a certain number of new observations to be made; in addition, the process of nerve regeneration can be better seen as whole. For instance, it is clear that regenerating cutaneous nerve fibres in the smaller nerve trunks and cutaneous nerve plexus follow only the course of Schwann cell frameworks all of which persist apparently unchanged in pattern after nerve degeneration. This is important, for it suggests that in human skin regenerating nerve fibres must approach the connective tissue elements of specific end-organs which also are said (Boeke, 1940) to retain their

position in the skin following nerve degeneration. Whether they approach their *correct* end-organs, however, remains uncertain.

It has been shown that typical regenerating nerve fibres in the cutaneous nerve plexuses are not surmounted by growth cones but that the termination of such fibres is so fine as to pass beyond the powers of resolution of the microscope. It has also been shown that Schwann bands are stained for 1 mm. ahead of these fine nerve terminals; it is interesting to note that staining of the Schwann bands in advance of regenerating nerve fibres has also been described in nerve trunks by Poscharissky (1907) using Stroebe's osmic acid or aniline blue staining after fixation in Flemming's fluid.

The rate of regeneration of the numerous collateral sprouts along the surface of the Schwann cells in the nerve trunks is variable and only a few of these sprouts mature and become surrounded by the Schwann cytoplasm. It is thus almost certain that in any transverse section of a regenerating nerve trunk some fibres will appear to be lying on the surface of the cytoplasm and others within it. In fact, an illustration from Cajal (1928, Fig. 81, p. 199) shows such an arrangement, although this is not specifically referred to by the author, and photomicrographs illustrating the same appearance have been given by Kirk & Lewis (1917). Whether the finest visible nerve sprouts are really external to the Schwann cytoplasm or whether they lie just within its surface layer is difficult to determine by any histological method on fixed tissues; it has been noticed, however, that the fibres do appear to be fairly easily detached from the cytoplasm without destruction of the latter, and it is thus inferred that they are external to it (Pl. 2, fig. 10). Ranson (1912), Cajal (1928) and Boeke (1935) believed that regenerating fibres lie within the cytoplasm of the Schwann cells, while Perroncito (1907) believed that they are always on the surface. Poscharissky (1907) and Kirk & Lewis (1917) showed that regenerating fibres may be either within or without the Schwann cytoplasm. These authors also described degenerative changes in the fibres located at the surface. It is evident that these conflicting statements are related to the fact that there is a change in relationship between the Schwann cytoplasm and axis cylinders during the course of maturation. The factors responsible for maturation can only be conjectured, but it seems that those fibres which become surrounded by Schwann cytoplasm continue to mature and those which for some reason do not gain the protection of the Schwann cytoplasm degenerate.

It has been shown in this investigation that all the Schwann bands outlining the position of the cutaneous nerve plexus in the later stages of regeneration are associated with fine nerve fibres even though by no means all the fibres in the proximal stump have sent out sprouts which have crossed the scar. Thus at least some of the Schwann bands must have become associated with collaterals. It also appears likely, since each Schwann band has a fibre related to it, that once maturation is completed no more fibres can cross the scar on to the surface of the bands with the possibility of survival. It follows from these observations that the extent of recovery of sensory innervation in the skin from the histological point of view does not depend mainly upon the number of fibres in any given nerve trunk supplying the skin but upon how many of the fibres are collaterals derived, more proximally, from a single source. A comparison of the average diameters of the individual fibres in normal and regenerated nerve trunks gives some histological evidence of the extent of sensory recovery, for the greater the number of collaterals the smaller is the average diameter of each nerve fibre. According to Ranson (1912) a single regenerating nerve fibre from the proximal

stump of a divided nerve may give rise to as many as fifty new sprouts. This estimate has been confirmed in the present investigation. Potentially, therefore, this greatly increases the chance of nerve fibres 'getting through', but as explained above the penetration of fibres into the distal stump does not mean that there will be a corresponding return of sensation of normal quality. It indicates only that there is a high probability of complete *histological* re-innervation of the distal stump and the skin, but the re-innervation may be effected by many fibres of reduced diameter. It has already been shown that the finest fibres do not themselves give rise to impulses which produce nociceptive responses when the usual stimuli are applied. It thus becomes clearer why the physiological quality of regeneration after crushing a nerve is so much better in the earlier stages than after nerve section. It is true that no difference in the general response to nociceptive stimulation in the later stages of regeneration after nerve section is observed, but the stimuli used are hardly adequate to detect finer changes in the quality of sensation.

In a previous publication (Weddell & Glees, 1941) a peculiar staining reaction was noticed in the intermediate zones (zones of overlap between normal and degenerating nerve fibres) following methylene-blue injection. In certain parts of these zones the Schwann bands become stained and the cytoplasm surrounding degenerating myelin globules takes on a fibrillary appearance. Illustrations of these processes were given and it was suggested that they most closely resembled local attempts at regeneration. It is now clear that these appearances are due to a local extension of undamaged nerve fibres both within the intermediate zone and towards the denervated area (Weddell *et al.* 1941). This process takes place in the same manner as regeneration from the proximal stump of a divided nerve, but is confined to fine nerve fibres of the cutaneous plexus.

The observation that Schwann cytoplasm accompanies the finest fibres of the subepidermal nerve net through the greater part and perhaps the whole of their course, and that the so-called 'Langerhans cells' are probably modified Schwann cells, is in accord with Trotter's interesting generalizations on the insulation of the nervous system (Trotter, 1924). Indeed, it appears that these generalizations may be extended to include what he, in common with most authors, describes as naked nerve terminals, intra-epidermal nerve fibres and nerve nets surrounding blood vessels, for these fibres seem also to be surrounded by delicate prolongations of Schwann cytoplasm as far as their terminations.

In two recent publications (Weddell, 1941 *b*, *c*) it has been suggested that since cutaneous sensory spots are each innervated by multiple nerve fibres, during the course of regeneration these will arrive at each separate spot at different times because they approach it from different directions and the ultimate course followed by the individual fibres will necessarily be of different lengths. Thus there will be a phase during regeneration in which each sensory spot is innervated by a single instead of by multiple fibres. Such a stage in regeneration has now been found to occur and hence the physiological findings of Trotter & Davies (1909) and Boring (1916) are seen to have an anatomical basis as far as the process of regeneration of cutaneous nerve fibres in the ear of the rabbit can be applied to that in man. The more rapid regeneration of nerve fibres along blood vessels is also in accord with the observations of Trotter & Davies (1909), who observed that vasomotor control returned more rapidly than skin sensation.

The general pattern of nerve regeneration throughout the skin of the whole ear has shown that regeneration of fibres from the injured nerve occurs first in the neighbourhood of adjacent normal nerves; this, it should be emphasized, is distinct from the extension of adjacent normal fibres which also occurs towards the area of sensory loss. The observation that cutaneous nerve fibres also regenerate more rapidly in the immediate neighbourhood of blood vessels suggests that both these occurrences may be in some way related to nutritional factors.

These phenomena evidently play some part in the process of diminution of areas of sensory loss which are observed clinically to take place from the periphery towards the centre of a degenerated area rather than from the centre outwards. An attempt was made to account for this phenomenon by relating it to the pattern in which the nerve fibres are distributed beneath the skin in specified areas (Weddell, 1941 *a*, *c*). In some cutaneous areas, however, the pattern of nerve distribution will not account for it entirely.

In contrast with this mode of innervation it can be seen clinically that the return of skin sensibility in regions not surrounded by areas innervated with normal nerves, such as the digits, is by an advancing wave of sensation along the line of the cutaneous nerves supplying the skin.

The more rapid regeneration of cutaneous nerve fibres in the neighbourhood of blood vessels may serve to explain the bridges of cutaneous sensibility which are commonly found, clinically, to cross an area of sensory loss during the course of regeneration from the proximal stump of the divided nerve.

It has been seen that the process of nerve regeneration throughout an area of skin involves first the rapid regeneration of nerve fibres along the main subcutaneous nerve trunks, and that it is only at a later date that fibres extend from these trunks towards the skin by way of cutaneous nerve plexuses. In other words, regenerating nerve fibres ramify in the subcutaneous tissues some time before they give extensions to the skin itself. This is an important fact from the point of view of the nociceptive sensory tests which are used to determine the progress of sensory recovery. For instance, it has been found in clinical cases that 'deep pricks' (2–3 mm. deep) will arouse pain in an area which is still anaesthetic to lighter pricks ($\frac{1}{2}$–1 mm. deep). This furnishes an explanation for the statement that one of the earliest indications of recovery of skin sensibility is a painful sensation caused by pinching the skin, for this manipulation necessarily involves the subcutaneous tissues. In any case the importance of using standard instruments, which will give approximately constant stimuli from day to day in the same patient, cannot be over-emphasized, if a true picture of the course of sensory recovery (nociceptive) is to be obtained. Cobb (1919) has already shown the importance of this from the clinical point of view.

SUMMARY

1. A descriptive account of the regeneration of cutaneous nerve fibres in the ear of the rabbit has been given.

2. The diminution of the areas of sensory loss following nerve interruption has been correlated with both the macroscopical and microscopical appearance of the regenerating nerve fibres.

3. Nerve fibres first advance along the original main nerve fasciculi beneath the skin. The first fasciculi to be re-innervated are those lying in closest proximity to

normal nerve trunks. It is also clear that cutaneous nerve bundles which are more closely related to the larger blood vessels are more rapidly re-innervated than those farther away.

4. When the nerve trunks have become re-innervated the nerve fibres pursue a tortuous course through the cutaneous plexus towards the skin surface. The finest visible terminations of the nerve fibres are associated with Schwann cell pathways which retain the pattern of the cutaneous plexus in the normal ear. On no occasion in the rabbit's ear has the tip of a regenerating nerve fibre in the cutaneous nerve plexus been seen pursuing a course independently of a Schwann band.

5. The Schwann bands become stained with intravital methylene blue during the course of regeneration when they are in relation with advancing nerve fibres of fine diameter. This staining first appears about 1 mm. in advance of the finest visible advancing nerve sprouts. No cell boundaries can be seen between the nuclei of a single Schwann band so stained, and no mitotic figures have been seen in Schwann bands of the cutaneous nerve plexus.

6. The growing tips of the finest nerve fibres cling to the surface of the Schwann cells; as they mature they become enclosed within the Schwann cytoplasm. This change in relationship is completed over a distance of 3–4 mm. in normally regenerating nerve fibres.

7. During the course of maturation the great majority of collaterals produced by a terminal growth cone degenerate and the remaining fibre or fibres increase in diameter.

8. The pattern of regeneration is discussed in relation to clinical findings and to the application of nociceptive stimuli for the determination of the position of a border of sensory loss.

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EXPLANATION OF PLATES 1–3

All photomicrographs reproduced on Plates 1–3 are from methylene-blue stained preparations of skin from the dorsum of the albino rabbit's ear.

PLATE 1

Fig. 1 (×5·5). Shows the pattern assumed by regenerating nerve fibres 4½ weeks after crushing the main dorsal ear nerve. The blood vessels can be readily distinguished from the re-innervated nerve trunks for the latter have a striated appearance. Nerve fibres can be seen entering the cutaneous nerve plexus in the neighbourhood of the nerve trunks and blood vessels. Owing to the low magnification extension into the cutaneous nerve plexus appears as a blurring around the nerve trunks and blood vessels, for the individual components of the plexus are too small to be recognized at this magnification. The areas between the nerve trunks in the upper part of the photograph are not yet re-innervated.

Fig. 2 (×550). Shows two regenerating nerve fibres in the peripheral stump of the main dorsal ear nerve which had been crushed 2 weeks previously. The fibre to the left gets progressively finer as it is traced distally until it passes beyond the powers of resolution of the microscope. The fibre to the right ends in a growth cone. A few granular remains of a degenerating collateral fibre can be seen towards the top at the right of the picture.

Fig. 3 (×900). Shows a Schwann band covered by a number of advancing nerve fibrils containing numerous swellings along their course. As the fibrils are traced distally, i.e. towards the lower end of the photomicrograph, they become progressively finer in diameter and the swellings become less obvious. The cytoplasm of Schwann bands very occasionally bifurcates forming two separate strands which soon reunite. Such a condition is shown in the upper part of this photomicrograph which was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 4 (×900). Shows two nuclei in an apparently uninnervated Schwann band. The cytoplasm between the nuclei appears to be continuous. This Schwann band was in series with that shown in fig. 8, but is situated 1 mm. ahead of the finest visible advancing nerve fibrils. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 5 (×350). Shows branches from a regenerating nerve fibre passing along persisting Schwann elements of the cutaneous nerve plexus. As the fibres are traced distally the nuclei and cytoplasm of the Schwann bands become more darkly stained. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 6 (×825). Shows a medium-sized nerve fibre (equivalent in diameter to a finely myelinated fibre in a normal rabbit's ear) about to enter the cutaneous nerve plexus where it undergoes repeated dichotomization. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

PLATE 2

Fig. 7 (×675). Shows a stained Schwann band covered by a large number of nerve fibrils sprouting from the swollen end of an axis cylinder which can be seen in the top left-hand corner of the picture. The nerve trunk giving rise to these fibrils was a continuation of one of the branches of the nerve fibre shown in fig. 6. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 8 (×1000). Shows a stained Schwann band covered by a large number of very fine nerve fibrils. Just below the upper Schwann nucleus there is a small end-bulb formed by a fine advancing fibre which can be seen in the upper part of the picture. Advancing from the tip of this end-bulb are a number of fibrils. When this band was traced distally the nerve fibrils became less obvious until the condition shown in fig. 4 was reached. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 9 (×575). Shows a regenerating nerve fibre which has become arrested. The cause of the arrest was not clear but the advancing tip was surrounded by dense fibrous tissue. The fibre is of much greater diameter than surrounding fibres which have not become arrested. No stained Schwann cells surround the fibre and no collaterals are seen in relation to it. The photomicrograph was taken 4 weeks after crushing of the main dorsal ear nerve.

Fig. 10 (× 800). Shows a fine advancing nerve fibril curving away from a Schwann band and rejoining it a short distance further on. The photomicrograph was taken 5 weeks after the main dorsal ear nerve was crushed.

Fig. 11 (× 1075). Shows an extremely fine nerve fibre surmounted by a growth cone. This fibre has probably become ensheathed within Schwann cytoplasm, for the Schwann elements are no longer stained. The fine fibrils which leave the cone become associated with stained Schwann elements in the more distal part of their course. This photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 12 (× 600). Shows a stage in the process of maturation. The collateral which contains the large fusiform swellings was found to be disintegrating when traced more distally. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 13 (× 800). Shows a disintegrating collateral lying beside a normal fibre which contains a number of swellings along its course. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 14 (× 550). Shows the point of bifurcation of a nerve fibre. One branch is degenerating and was found to have disintegrated completely when traced more distally, while the other appears to be increasing in diameter. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 15 (× 600). Shows a small nerve trunk in which the fibres are still of small diameter and contain a number of fusiform swellings along their course. There are very few regenerating collaterals, however. Compare this trunk with the nerve trunk shown in fig. 20 taken from a preparation 12 weeks after crushing of the dorsal ear nerve. This photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 16 (× 475). Shows a maturing nerve fibre which had presumably met with obstructions at two places along its course. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 17 (× 1000). Shows also a maturing nerve fibre which had probably met with obstruction during the course of regeneration. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 18 (× 900). Shows a maturing nerve fibre in which one of the sprouts has swollen considerably. A collateral of the same fibre joins the swelling which then gives rise to three further fibres. Compare with fig. 17. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 19 (× 900). Shows the outline of a myelin sheath surrounding a regenerated nerve fibre. The irregular outline of the myelin sheath indicates that the fibre has commenced to degenerate. The reason for the staining of the Schwann nucleus is not clear. Such appearances are very rare. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 20 (× 600). Shows myelinated fibres in a small nerve trunk taken from a preparation 12 weeks after crushing of the main dorsal ear nerve. Compare with fig. 15.

Fig. 21 (× 950). Shows a regenerated fibre in the cutaneous nerve plexus surrounded by a faintly outlined myelin sheath. The axis cylinder still contains swellings along its course. A node of Ranvier is faintly indicated at the top of the picture, which was taken 5 weeks after crushing of the main dorsal ear nerve.

PLATE 3

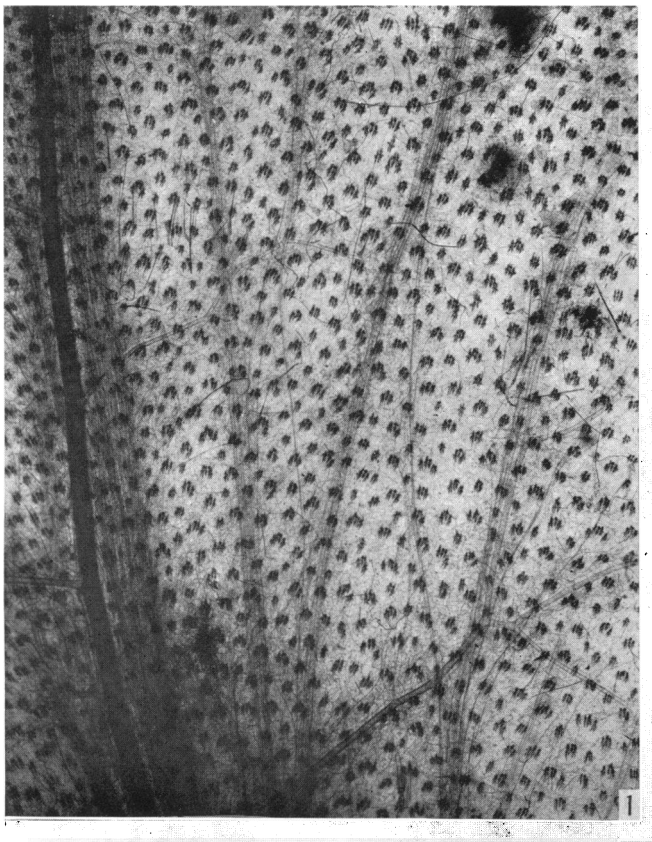
Fig. 22 (× 575). Shows regenerating nerve fibres approaching the epithelium where nerve nets are starting to form. It is clear that the fine fibres are accompanied by prolongations of the Schwann cytoplasm. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 23 (× 1000). Shows a fine regenerating nerve fibre passing along the wall of a capillary blood vessel. The fibre is not yet beaded. The faint outline of a neighbouring capillary can be seen on the right of the photomicrograph, which was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 24 (× 550). Shows a regenerating nerve net in the wall of a small artery. The Schwann nuclei can be clearly seen and the prolongations of Schwann cytoplasm can be traced for some distance along the course of the fine net fibres, the majority of which, although irregular, are not beaded in the normal manner. Compare with fig. 26. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 25 (× 350). Shows a number of so-called 'Langerhans cells' just beneath the germinal layer of the epithelium. Fine nerve fibres can be seen to approach the processes of these cells which apparently lie in series with Schwann cells. *L*, Langerhans cell; *S*, Schwann cell nucleus; *N*, fine nerve fibres passing along the processes of the 'Langerhans cells'. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.

Fig. 26 (× 550). Shows a further stage in the formation of nerve nets in the wall of a small artery, the finer fibres are clearly beaded and the Schwann nuclei are no longer stained. The photomicrograph was taken 5 weeks after crushing of the main dorsal ear nerve.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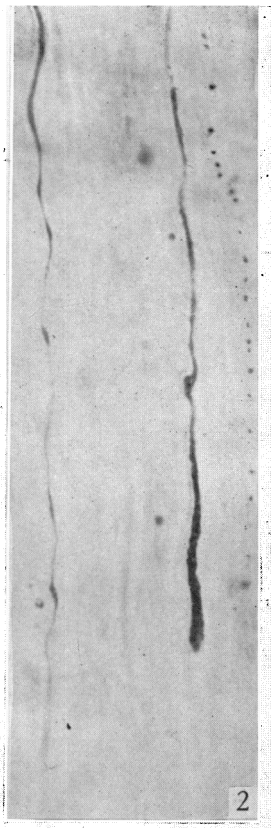
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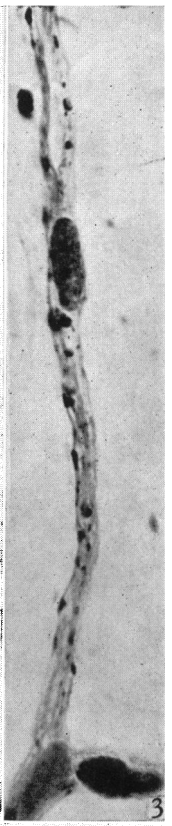
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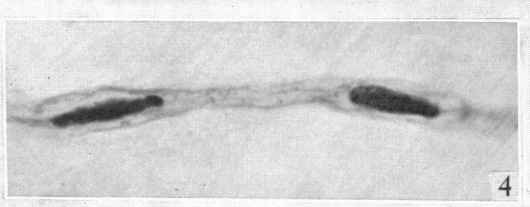
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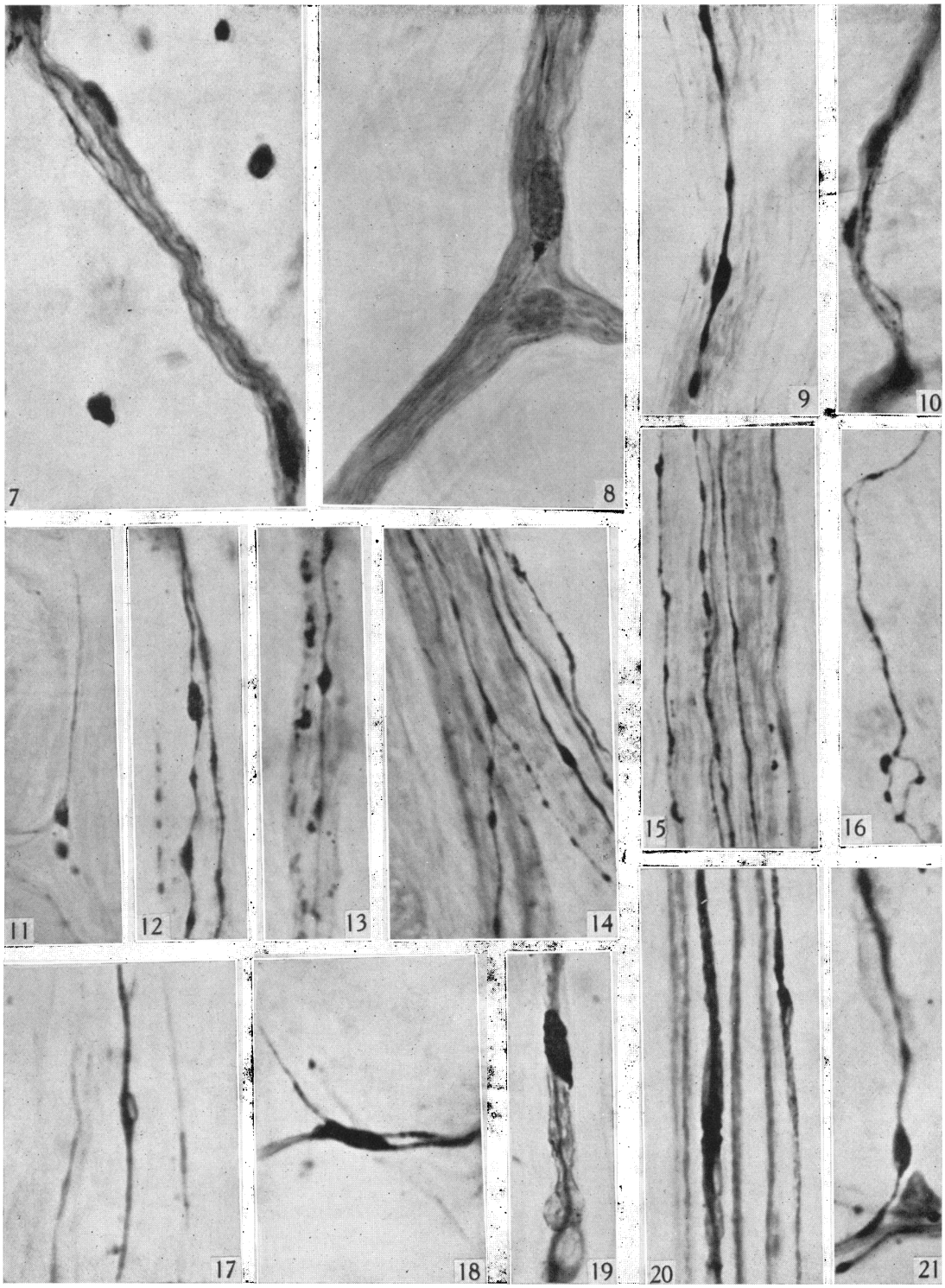
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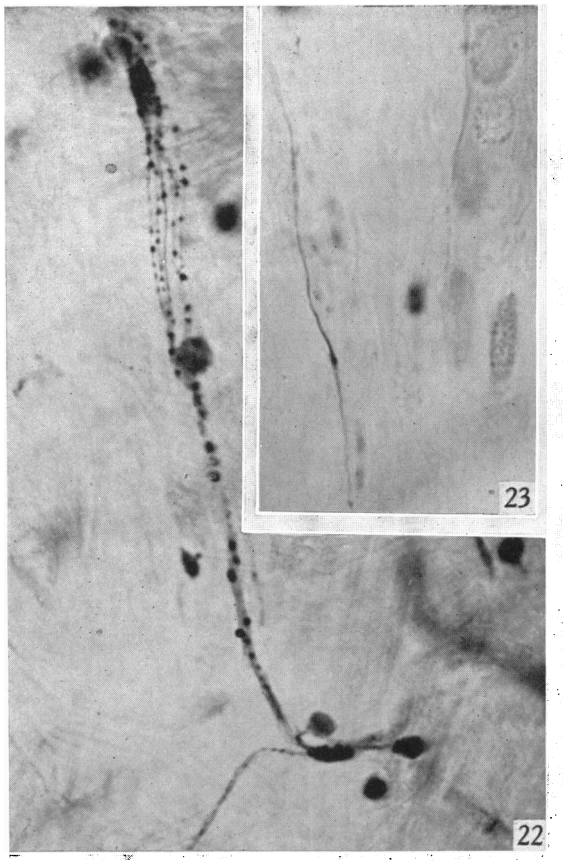
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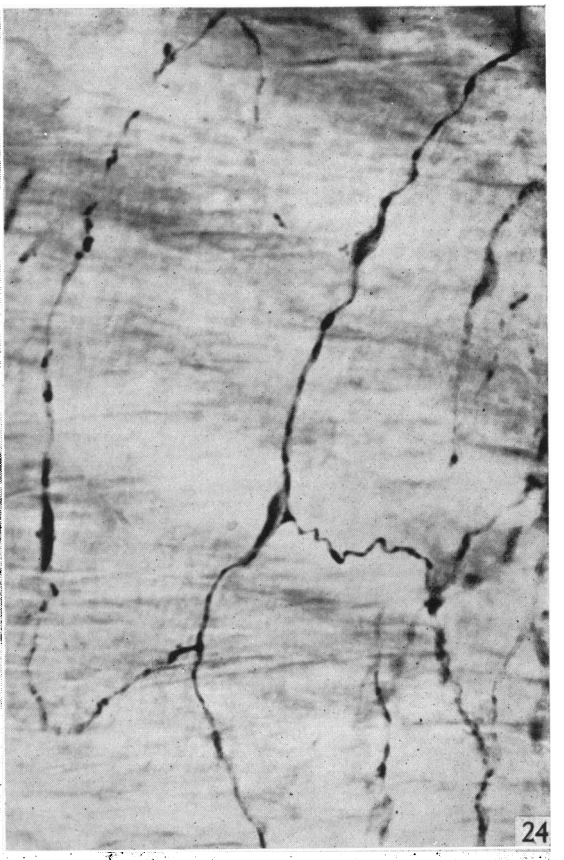
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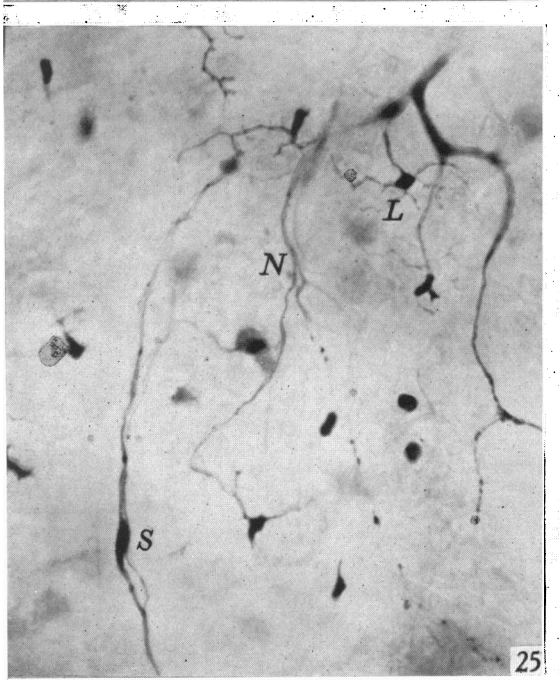
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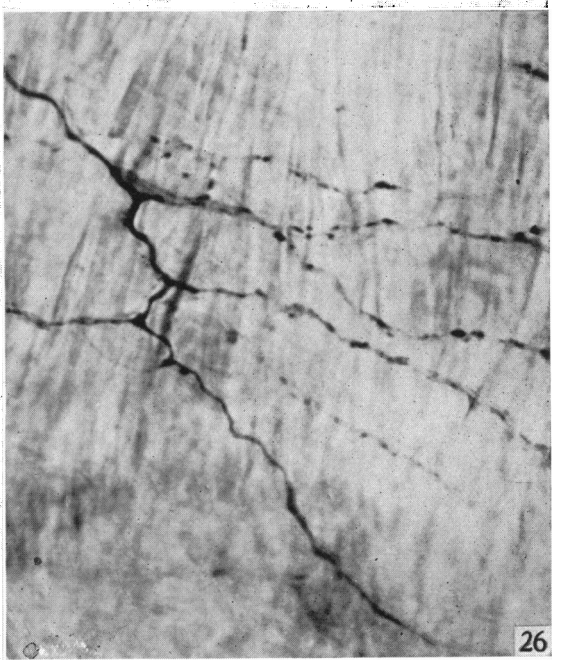
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