THE DEGENERATION AND RE-INNERVATION OF GRAFTED NERVES

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INTRODUCTION

Since the classical experiment of Phillipeaux & Vulpian (1870), many authors (e.g. Huber, 1895, 1920; Cajal, 1928; Ballance & Duel, 1932 *a*, *b*) have shown that grafted pieces of nerve may be used to conduct new fibres across the gaps caused by lesions of peripheral nerves. Various types of nerve graft have been used. Autografts (pieces of nerve from the same individual) are reported by many authors as giving successful recovery of sensation and motor function both in animals and man (see Kilvington, 1908; Eden, 1919; Huber, 1920; Cajal, 1928; Duel, 1933; Bentley & Hill, 1936; Bunnell & Boyes, 1939). The value of homografts (pieces of nerve from another individual of the same species) has been less thoroughly tested, but Kilvington (1908), Eden (1919), Huber (1920), Bentley & Hill (1940) all report successful results following homografts in animals. Heterografts (the transplantation of pieces of nerve from one species to a different one) have never been fully investigated. Huber (1895) reported success in grafting the sciatic nerves of cats into dogs, but later (Huber, 1919, 1920) suggested that heterografts are less successful than homografts or autografts. In fact, he says that in heterografts 'many of the downgrowing neuraxes pass outside the nerve transplant and thus reach the distal nerve segment'. Cajal (1928) condemns the use of heterografts, as does Eden (1919) on the basis of his own experiments and a review of previous work.

Various methods of treatment prior to the insertion of the graft have been proposed. In the case of autografts, pre-degeneration of the grafted segment has been proposed by Cajal (1928) on theoretical, and by Duel (1933) on experimental, grounds. Storage of homografts and heterografts on ice (Bethe, 1916), in saline (Tello, 1915), in a 3% aqueous solution of borax + a few drops of formalin (Bielschowsky & Unger, 1917) in vaseline or liquid petrolatum (Huber, 1920), has been tried with some success, but the possibilities of nerve storage have never been fully explored. Nageotte (1917 *a*, *c*) has advocated the use of dead grafts preserved in 50% alcohol, and claims great success in their use, which is supported by the experiments of Huber (1920), but not by the experience of clinicians (see Laduron & Christophe, 1938). Gosset & Bertrand (1938) have made use of heterografts of dog and rabbit spinal cord fixed in formol and stored in alcohol. Cajal (1928), however, states that grafts treated by boiling

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water, chloroform, formalin, or chloral hydrate, are not innervated, and the use of dead grafts still remains a controversial point.

The results of nerve grafting in clinical cases are extremely difficult to estimate, on account of the differing methods used to make the graft, and the varying standards adopted for estimating recovery. However, the extreme pessimism of British surgeons on the whole subject of nerve grafting in man (see *M.R.C.* report, 1920; Platt, 1919; Stopford, 1920; Sargent, 1920) is hardly justified. Foerster (1929), out of 21 autografts, had 5 complete recoveries, 12 ameliorations, and only 2 failures; 2 cases were not followed up. Bunnell & Boyes (1939) report 32 cases of autografting in the small nerves of the hand in which there was a high proportion of recoveries, while the thin Ballance-Duel grafts used in cases of facial paralysis show remarkable success.

The great difficulty in assessing the results of all this experimental and clinical work on the subject is that no *measure* of the efficacy of the various types of graft has been devised. Each author has claimed that some particular method of grafting gave success, but there has been very little comparison between methods on the basis of standard criteria. Moreover, there is very little information as to the nature of the histological changes which proceed in the various grafts—especially during the early stages.

The experiments described in this paper represent an attempt to compare the efficiency of the following types of nerve graft:

(*a*) Fresh autografts.
(*b*) Pre-degenerated autografts.
(*c*) Fresh homografts.
(*d*) Homografts stored in Ringer's solution at 2° C.
(*e*) Fresh heterografts.
(*f*) Dead homografts preserved in 50 % alcohol.
(*g*) Dead heterografts of alcohol-preserved spinal cord.

The comparison has been made by estimating the distance which new fibres had grown through the graft after short periods (mostly 15 and 25 days) and by careful examination of the histological conditions at these times. Such criteria are of course in many ways inferior to the test of functional recovery (which is, however, now also being applied by one of us—F. K. S.). But the use of short periods has the great advantage that it allows the investigation of numerous animals in a reasonable time, and thus avoids what we feel to be the main defect of earlier work—namely that, being neither statistical nor quantitative, it provides no basis for induction. The animals used were rabbits and the problems of comparing results so obtained with conditions in man are discussed on p. 160.

METHODS

The standard method used for assessing the value of the various types of graft is that described by Young *et al.* (1940). Grafts were 2 cm. in length, and were used to fill gaps in the resected peroneal nerve of the rabbit.

In an initial aseptic operation under anaesthesia (nembutal and ether) grafts of two different sorts were inserted in the peroneal nerve on opposite sides of the same rabbit, and fixed in position with concentrated fibrinogen solutions in cockerel plasma as described by Young & Medawar (1940). These solutions proved very convenient for the fixing of grafts, healing of the graft into the host nerve normally taking place before the clot of plasma was resorbed. By making a suitable gap of slightly less than 2 cm. in the host nerve, retraction and consequent scarring at the junctions were avoided. A standard junction was thus obtained in which there was no suture material to obstruct the downgrowth of new fibres from the central stump.

At either 15 or 25 days after operation the grafts in each animal were exposed under light anaesthesia, and the distance reached by outgrowing sensory fibres estimated. This was done by freeing the nerve from its bed, and then pinching with fine forceps every 2 mm. or so from a distal point upwards until a reflex response was obtained. Histological study of regions where the first reflex response to such pinching was obtained has shown that this simple method gives a very reliable estimate of the distance reached by (presumably) pain fibres.

After the distances of outgrowth had been measured, the grafted pieces were removed and fixed for sectioning and staining by various histological methods. The routine methods were, for axons, the Bodian silver proteinate, alone or with Masson or Mallory trichrome stains, and for myelin, Weigert-Pal and various osmium tetroxide methods.

AUTOGRAFTS

Fresh autografts. Sixty-three fresh autografts were made in rabbits, each graft consisting of a 2 cm. length of the tibial nerve inserted into a gap made in the peroneal. These two nerves lie side by side in the thigh, and only one skin incision was thus necessary both to take the graft and to insert it. The tibial nerve was used as a free graft, being entirely removed before insertion, and, since it is the thicker of the two, it offers full opportunity for the outgrowth of all the peroneal fibres. In some experiments the graft was inserted so as to maintain its original polarity; in others the polarity was reversed. There was no outstanding difference in the distances of outgrowth obtained between those grafts in which the polarity was reversed and those in which it was unchanged, nor between grafts into the peroneal of the same or the opposite side of the animal.

In animals opened at both 15 and 25 days autografts had always become well united with the host peroneal nerve, and had an appearance not unlike normal nerve. When pinched with forceps, such grafts did not feel hard or 'doughy', as in the case of some other types (see below). The junctions were only occasionally bulbous, and the grafted pieces retained the dimensions which they had at the initial operation. There were occasionally adhesions between the graft and its bed, although when these occurred they were

confined principally to the junctions. Any adhesions, however, were always markedly less than with fresh homografts (see below). At 15 days autografts had usually already established a vascular supply, and this came invariably from along the nerve, usually from vessels in the popliteal space. There was never an encapsulation of the graft as in the case of other types of graft (see below).

In three animals at 25 days the distal junction was found to have become detached subsequent to operation, and in each of these cases the distal end of the graft was found joined to the peripheral stump of the peroneal nerve by strands of proliferated tissue, originating partly from the graft and partly from the peripheral stump. In some cases these 'spontaneous' junctions had been penetrated by downgrowing fibres which had reached the peripheral stump by this route. Junction between a detached autograft and the peripheral stump can thus take place in a manner strictly comparable to that described by Kirk & Lewis (1917) for resected nerves which were not sutured.

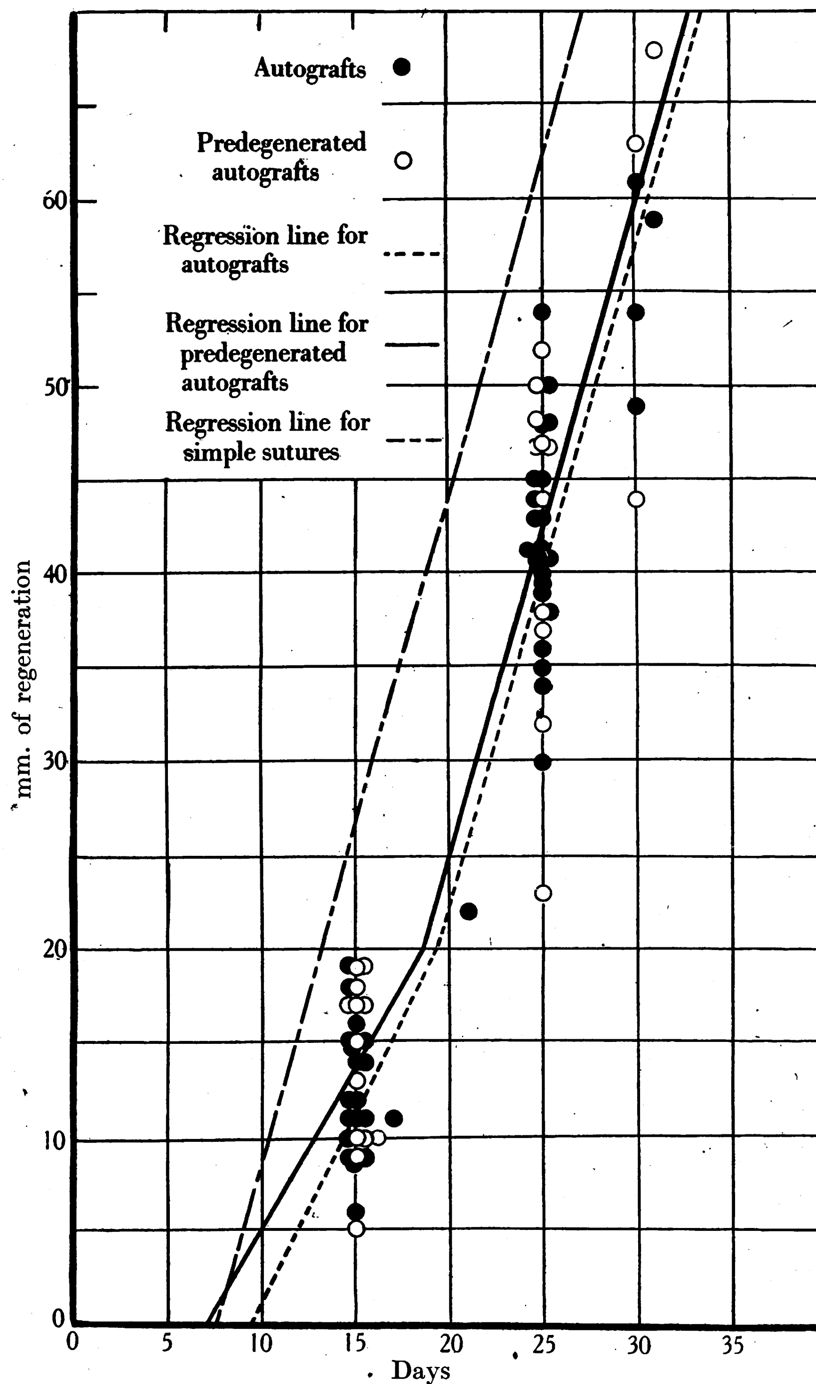
Autografts examined at 63 and 102 days still preserved the above distinctive features. There was no sign of the conversion of the graft into a connective tissue strand which has been stated to occur in man (see Stopford, 1920; Platt, 1921).[1]

Text-fig. 1 shows the distances reached by outgrowing fibres after fresh autografting. At 15 days all fibres were still in the graft, but at 25 days fibres had passed the distal junction and grown on the average 41·7 mm. from the upper junction, that is, 21·7 mm. into the peripheral stump. New fibres grow through autografts only slightly more slowly than through a normal peripheral stump. The data of Young & Medawar (1940) give the average rate of growth of pain fibres down a normal peripheral stump as 3·9 mm./day after plasma junction, with a latent period of approximately 8 days before the fibres cross the scar.[2] In the case of autografts the present data for distances reached at 15 and 25 days give an estimate for the latent period of about 9·2 days and a rate of growth of 2·0 mm./day. These rates are calculated on the assumption that growth through the peripheral stump occurs at 3·5 mm./day, i.e. that growth across the upper junction and in the graft occupies $25 - \frac{41{\cdot}7 - 20}{3{\cdot}5} = 18{\cdot}8$ days. The further assumption that there is no delay at the lower junction is discussed on p. 148.

Histology of fresh autografts. Degeneration and re-innervation proceed in fresh autografts almost exactly as in a normal peripheral stump. Proliferation of the Schwann nuclei and break-up of the myelin into ovoids proceed actively (see Pl. 1, figs. 6–8). The graft is invaded by macrophages which remove myelin remains as they do in a normal stump, although the process is rather

[1] Recently a piece of autografted nerve was removed by Prof. H. J. Seddon 5 months after insertion into a human median nerve. Histological examination showed that normal nerve structure was maintained.

[2] These figures have now been revised by additional data to 3·5 mm./day + 7·3 days.



Text-fig. 1. Distances at which reflex responses were obtained on pinching the peroneal nerve after insertion into it of pieces of tibial nerve 20 mm. long from the same animal. The lines are the regression lines, arithmetically calculated from the results at 15 and 25 days only. For comparison the line showing the rate of growth through a normal peripheral stump is added from the data of Young & Medawar (1940). The times of pre-degeneration varied from 6 to 28 days.

slower. After 25 days a section from an autograft can usually be distinguished from a normal stump by the fact that spaces in which the myelin remains lie are abnormally large (Pl. 1, fig. 7). It is not possible to say whether this condition is likely to prejudice recovery, but it may cause some of the fibres to run irregular courses.

No sign of necrosis at the centre of these grafts was seen. The tissue had been well vascularized by 25 days, but the smaller vessels were often more dilated than in a normal stump (Pl. 1, fig. 6), and occasionally there was a small lymphocytic reaction around some of the vessels (Pl. 1, fig. 7), but this was usually absent.

Junction with the central and peripheral stumps is made in a manner indistinguishable from a normal union of stumps. In particular it must be noted that the lower junction is made by the Schwann cells (presumably both those of the graft and of the peripheral stump) *before* the arrival of axons from the central stump, the union being already well made after 15 days (Pl. 2, fig. 9). This process of union is, in fact, *independent of the presence of axons*, and there should be no grounds for the fear expressed by some (e.g. Davis & Cleveland, 1934) of the delaying, and perhaps obstruction, of fibre growth on account of fibrosis at the distal junction.

The distances of outgrowth obtained at 15 and 25 days also indicate that there can be no great delay at the distal junction. The rates of growth given on p. 146 are calculated on the assumption that this delay is zero, and yet the difference in estimated rate of growth of fibres through autografts and normal peripheral stumps is only between 2·0 and 3·5 mm./day. If we assumed that the *whole* of this apparent difference is actually due to delay at the lower junction, i.e. that the actual rate of growth of fibres in both graft and peripheral stump is 3·5 mm./day, then the delay at the lower junction would be only 1·7 days. These figures support the histological findings in the conclusion that the peripheral junction is made before fibres reach it, so that when they arrive they can immediately cross.

In order to confirm this for longer grafts, in two experiments grafts of tibial nerve 5 cm. long were inserted in the peroneal nerve. The animals were reopened at the time when, by calculation from the above rate of fibre growth through autografts, fibres should have crossed the lower junction. In both cases this had indeed occurred, and fibres had travelled some way into the peripheral stump. In addition the histology of the distal junction of these long grafts, in which any 'fibrosis' should have been specially marked, showed no greater degree of fibrosis or whorling of fibres than occurs with the standard 2 cm. grafts described above (see Pl. 2, fig. 10).

There have been cases, however, in which both lower and upper junctions were not made quite closely, there being a gap of 1 or 2 mm., with considerable resultant criss-crossing. This is probably due to the slight shrinkage in length which is apt to occur in the graft, and it may be that the obstructions seen by Davis & Cleveland were produced in this way. However, it is not clear from

their paper exactly what these authors mean by the 'fibrosis' which they claim to occur. The only method of staining which they mention (Ranson's) would be suitable only for the study of axons. Their figures do not enable a comparison to be made between scars after primary and secondary suture, since those of the latter are at a much higher magnification. Thus the postulation of obstruction of fibre growth at the distal scar, which has led some to suggest a practice of severing and resuturing the lower junctions of long grafts, is still not proved by experimental evidence. This procedure might, however, become necessary if the lower (or upper) junction became detached owing to shrinkage of the graft or other causes. But figs. 9 and 10 on Pl. 2 show that under the best conditions perfectly smooth primary lower junctions can be made.

Re-innervation proceeds uniformly over all parts of these grafts. Great numbers of fibres can be seen after 15 and 25 days growing down the degenerating Schwann bands in a normal manner. There is no sign of a restriction of the innervation to the edges of the graft. Moreover, after 25 days the new fibres are becoming medullated, again in all parts of the grafts (Pl. 1, fig. 8, and Pl. 2, fig. 11). This must be an indication of the presence of live, healthy, Schwann cells. Indeed, all the evidence indicates clearly that a piece of nerve of the thickness of the rabbit's tibial is able to act as a graft in the fullest sense of the word, the grafted tissues surviving, becoming vascularized, and continuing to function in a manner approaching the normal.

Pre-degenerated autografts. A degenerated stump was prepared by cutting the tibial nerve at a preliminary operation, and leaving it for a time to degenerate. The central stump was resected for some millimetres at this operation, to avoid re-innervation of the peripheral stump during the time of degeneration. At the end of the degeneration time the animal was re-operated, and 2 cm. of the peripheral stump of the previously cut tibial was inserted into a gap in the peroneal of either the same or the opposite side of the animal. Thirty pre-degenerated autografts were made, and three sets of times of pre-degeneration were used: (1) 6–9 days, (2) 14–16 days, (3) 25–28 days.

In animals examined at both 15 and 25 days there was very little difference in superficial appearance between pre-degenerated autografts of all times of pre-degeneration and fresh autografts. Grafts were white or pink in colour, completely united with the peroneal, and well vascularized by vessels running along the nerve. When pinched, however, these grafts felt harder than fresh autografts. Bulbs did not regularly develop at either junction, but adhesions between the graft and its bed seemed to occur more frequently than in fresh autografts, and not to be confined to the junctions.

Text-fig. 1 shows the distances reached after 15 and 25 days by sensory fibres growing into pre-degenerated autografts. In some cases fibres have penetrated farther than in any fresh autograft, but the difference is not constant. In fact there is no difference in rate of fibre growth between fresh autografts and pre-degenerated grafts of any of the three times of pre-

degeneration, the calculated average rate in these grafts being 1·7 mm./day, with a latent period at the upper junction of 6·9 days, making the assumption of a rate of 3·5 mm./day in the peripheral stump and no delay at the lower junction. These figures are not significantly different from those for autografts, though the shorter latent period shown for pre-degenerated grafts is suggestive.

Duel (1933) in *Macaca* claimed a rate of regeneration through pre-degenerated grafts quicker by half than through fresh autografts. However, the numbers given are not adequate to support this contention and Bentley & Hill (1936) were unable to confirm it in cats. Since Duel's grafts were made in the facial nerve in the temporal bone, and were simply laid in position without suturing, they (Bentley & Hill) suggest that Duel's good results were due to the fact that the pre-degenerated grafts, being more rigid than fresh grafts, stayed in position better than the latter, and maintained better approximation at the junctions. Collier (1940) is also of this opinion. It is generally agreed that pre-degenerated nerve is easier to handle than fresh nerve, and for this reason may be very desirable in some situations.

Histology of pre-degenerated autografts. Histologically, pre-degenerated autografts showed no important differences from fresh autografts. The tissues of the graft survive, and the process of degeneration, begun during the period between the two operations, continues in a normal manner. After 25 days such grafts naturally show somewhat less myelin remains than do fresh autografts, and it is possible that this constitutes an advantage of pre-degeneration. With the times of pre-degeneration of the order used by surgeons and in the present experiments, this difference is not great, but it is just possible that much longer pre-degeneration times, say 2 months, might be an advantage for the grafting of thick nerves, which might survive and be re-vascularized more readily if the myelin has been removed, and when there is no active degeneration to make demands on the metabolism of the tissues. Only experiment can show whether this is so, or whether, conversely, as is possible, the active degeneration actually assists grafting by attracting new blood vessels.

The present results confirm those of Bentley & Hill (1936). Pre-degenerated autografts do not give a quicker regeneration than fresh autografts, and thus it is probably unnecessary for surgeons to go to the trouble of pre-degenerating grafts before use, except, perhaps, in the case of the facial nerve.

HOMOGRAFTS

Fresh homografts. Fresh homografts consisted of pieces of tibial nerve 2 cm. in length, taken from one rabbit, and used to bridge an appropriate gap in the peroneal nerve of a second animal. The two rabbits were opened at the same operation, and pieces of tibial nerve exchanged between them. Animals were examined at 15 and 25 days, and a total of thirty-five fresh homografts was made.

At 15 days fresh homografts showed considerable variability in appearance, although they were always completely united with the host peroneal nerve. Macroscopically, these grafts were either yellowish or reddish in appearance, and, in contrast to autografts, the graft and junctions were enclosed by a thick sheath with its own collateral blood supply. In contrast to this, vascularization of the graft itself was always along the nerve, and, although the extent of vascularization was very variable, it was always conspicuously less than in autografts. The formation of bulbs at the junctions was rare, but the grafts were often considerably swollen, the middle of the graft being blown out like a balloon. The occurrence of adhesions between the graft and its bed was also much more common than with fresh autografts.

At 25 days these general features of the appearance of fresh homografts were much accentuated: grafts were reddish or yellowish, enclosed in a thick sheath, and vascularized to a variable extent. Swelling of the graft, and adhesions, were commoner at 25 than at 15 days.

The variability of homografts shown in their appearance at biopsy appears again in the distances of outgrowth of fibres obtained at 15 and 25 days. These are shown in Text-figs. 2 and 3. At 15 days the distances at which reflex response to pinching was obtained vary from just across the proximal scar to 8 mm. past the distal scar in the peripheral stump. At 25 days they vary from only 6 mm. below the upper junction to 28 mm. below the lower one. Most of the animals at 25 days, however, gave distances between 14 and 22 mm.

When statistically treated, these results reveal a significant variability due to factors not under experimental control; a plot of error distributions reveals that the figures do not form a normally distributed population; indeed, there is a strong suggestion of two modes (Text-fig. 3). The mean distances of outgrowth have therefore little significance; they are, however, 15 mm. for 15 days, and 25·2 mm. for 25 days. It is not reasonable to obtain a value for the rate of growth through fresh homografts from these data. In favourable cases homografts give distances of outgrowth as large as those in fresh autografts (see Text-fig. 2). The main body of data, however, falls short of this. The great variability of the distances reached by fibres no doubt corresponds with the varying extent of the undesirable cell reactions described below.

Histology of fresh homografts. Degeneration and re-innervation can go on when a piece of nerve is grafted from one rabbit into another, but these processes depart in several ways from the conditions found in normal peripheral stumps or in autografts.

The break-up of myelin proceeds more slowly than usual, so that in some parts of a homograft of 25 days, especially at the centre, there may be fibres which have not yet broken up, or are in the process of doing so abnormally (Pl. 2, fig. 19). After breaking up, the myelin is found in fragments which are smaller than usual. The significance of this difference is not clear, probably it is connected with the fact that the invasion of macrophages is somewhat more marked in these grafts than in a normal peripheral stump. These

Fresh homografts

Homografts stored 7 days

Homografts stored 14 days

Homografts stored 21 days

Heterografts

Alcohol-fixed grafts (Nageotte)

Fixed spinal cord graft (Gosset)

Line for autografts

mm. of regeneration

Days

Fig. 2.

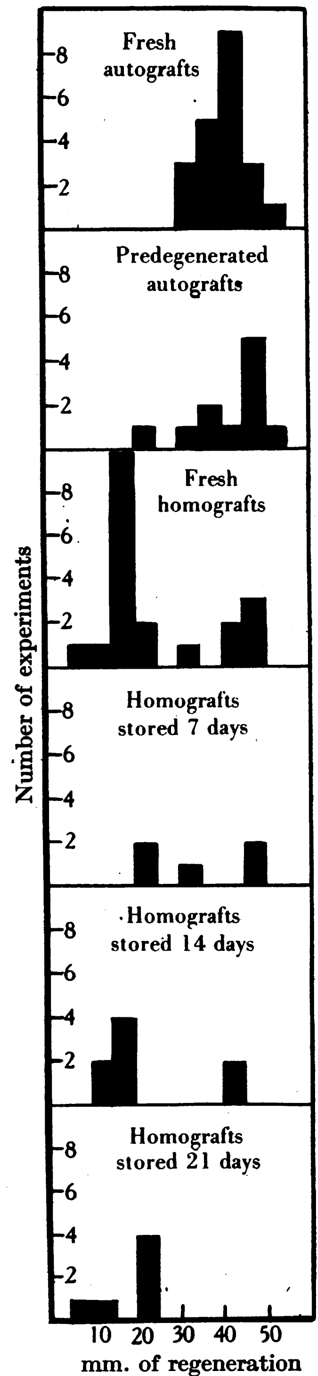


Fig. 3.

Text-fig. 2. Distances at which reflex responses were obtained after insertion into the peroneal nerve of grafts of various types. For comparison the line for fresh autografts is copied from Text-fig. 1.

Text-fig. 3. Histograms to show the frequency with which outgrowths of each distance were obtained, through various types of graft 25 days after insertion. Grouped by 5 mm. intervals.

abnormalities of degeneration result in, at best, a somewhat greater disturbance of the architecture of the nerve than is found in autografts.

In many cases the homografts produced much more extensive reactions. Large numbers of lymphocytes collected around the vessels in the graft and where host and donor tissues were in contact, especially at the junctions (Pl. 2, fig. 13). The reaction is not always equally severe. For instance, in the case shown in Pl. 2, fig. 12, it is minimal, but in other cases it may result in the filling of large parts of the graft with masses of small cells (Pl. 2, fig. 13). It must be remembered, however, that this study is limited to grafts 25 days old, and it is not possible to be certain of the fate of the tissues affected by such a reaction. Possibly the reaction may subside and leave the graft in a healthy condition. However, in some cases, after 25 days patches of necrosis were observed (Pl. 2, fig. 17) and these could hardly return to normal.

Certainly the Schwann cells of a homograft are able to multiply, even when a considerable lymphocytic reaction is present, and they produce Schwann bands which may approach normal appearance in the less disturbed parts of the graft. New nerve fibres can certainly enter in large numbers (Pl. 3, fig. 22), though in some cases with severe reactions they are noticeably fewer than in autografts. Moreover, the new fibres begin to medullate in the upper part of the graft, even after 25 days (Pl. 2, fig. 18).

There are, therefore, good reasons for supposing that the tissues of a fresh homograft in the rabbit can survive and become incorporated with those of the host nerve. In some cases this is accomplished with a small reaction, producing a condition approaching that of an autograft, though in other cases the state of affairs may be less favourable.

Homografts stored in Ringer's solution at 2° C. The practical advantages of using stored nerves from another animal (or man) would be considerable and we have made preliminary investigation of the changes occurring under various conditions of storage.

In an initial operation, pieces of tibial nerve were taken aseptically from rabbits, fastened by means of silk to small glass capillary supports without stretching the nerve, and placed in Petri dishes on pieces of Ringer-soaked gauze. These dishes were kept in a refrigerator at about 2–4° C. After periods of storage of 7, 14, or 21 days, 2 cm. pieces of stored nerve were used to fill gaps in the peroneal nerves of other rabbits. After 25 days' incubation in the host the distance of fibre outgrowth was then estimated as usual, and the grafts examined histologically. This simple technique proved moderately satisfactory, but two grafts out of twenty-four became infected.

After 25 days in the host these stored homografts showed differences in appearance from fresh homografts. Stored grafts were rarely so swollen or so strongly ensheathed as fresh homografts, and adhesions between the graft and its bed were either slight or absent. In addition they were always shrunken in length (average length of graft at 25 days = 16·1 mm.).

Between the grafts stored for 7, 14, or 21 days there were no striking

macroscopic differences after 25 days in the host. Grafts stored for all three periods varied in appearance from that of normal nerve to a pale greyish yellow, and they always possessed a thin collaterally vascularized sheath. The only consistent difference was of vascularization, the grafts stored for 7 days being as a rule better vascularized than those at 14 or 21 days.

Text-figs. 2 and 3 show the distances reached at 25 days by sensory fibres growing through stored grafts. The fibres have grown on the average farthest through grafts stored for 7 days (average distance of outgrowth = 34·5 mm.). Nevertheless, two grafts stored for 14 days gave distances of outgrowth of 46 mm., comparable not only with similar values obtained from 7-day stored grafts, but with the best results of fresh homografts and autografts. The average distances of outgrowth after 14 and 21 days' storage are respectively 24 and 18·6 mm.

In so far as averages of such scattered data have any meaning they show that fibres grow further through the grafts stored for 7 days than through fresh homografts. This is also suggested by three cases in which grafts stored for 7 or 14 days were controlled by fresh homografts on the opposite side of the same animal. In all, the fibres had grown as far or farther in the stored grafts. Of five cases in which the grafts were stored for 21 days and similarly controlled, three showed greater distances in the stored, two in the fresh homograft.

Histology of Ringer-stored homografts. Break-up of the myelin has proceeded very completely in grafts stored for all the three periods. In none of these stored grafts were there to be seen any unbroken fibres such as were found in some homografts (Pl. 3, fig. 25). Moreover, after the myelin has become broken up it is removed from these grafts by the very great invasion of macrophages which takes place, although the removal is less rapid than in a normal peripheral stump. The graft becomes filled with rows of 'foam' cells in active phagocytosis, so that the internal architecture of the nerve may become considerably upset (Pl. 3, figs. 23 and 24). Small patches of necrosis were seen in one case.

The lymphocytic reaction which is so marked a feature in fresh homografts still appears, though reduced, in those grafts which have been stored for 7 days (Pl. 2, fig. 14). But in those stored for 14 or 21 days it is at most very slight; there may be small patches of cells here and there, but there is no general reaction wherever the graft touches the host tissues. As a result, the junctions made by these stored grafts are very good, being made with a minimum of reaction on the part of the host and the graft, so that the result appears very similar to a simple suture or an autograft (Pl. 2, figs. 15, 16; Pl. 3, fig. 20).

Cells which appear to be live Schwann cells were found in the grafts after all three periods of storage and 25 days in the host (Pl. 3, fig. 21). It is not impossible that these cells have invaded the graft from the host nerve, but as they are found in all parts of the graft at 25 days, there is every likelihood

that they are cells which have survived the time of storage. They are not so numerous as the Schwann cells in a normal peripheral stump or an autograft, and, although many of them have a normal appearance, others are broader than usual, and often irregular in shape.

Thus even after storage for 21 days, and incubation in the host for 25 days, the structure of the graft is to some extent preserved. Many of the collagenous tubes remain intact and are tenanted by Schwann cells. Into these tubes new fibres can be seen to penetrate in considerable numbers, though of course they are fewer than in an autograft. A curious feature is that the growing axons are abnormally thick (compare, for instance, fig. 23 with figs. 2, 6, or 22). After 25 days the fibres begin to acquire myelin sheaths (Pl. 3, fig. 25), a sign that live Schwann cells are present in the graft.

In one case a 14-day stored graft was studied after 73 days' incubation in the host. It had the appearance of normal nerve. The junctions had been made with a minimum of criss-crossing, and, although the graft still contained some few macrophages, it was tenanted by large numbers of Schwann cells, and traversed by many new myelinated fibres.

There seems every reason to suppose, therefore, that storage of grafts in this way is possible, and may be even advantageous in that it reduces the lymphocytic reaction. Some at least of the cells of the graft can survive, and it seems likely that they would provide a basis for some functional recovery. It must not be forgotten, however, that the graft becomes filled with an excessive number of macrophages, and that the number of new fibres entering it, though considerable, is certainly less than in an autograft. Until means can be found to reduce these disadvantages, stored homografts would, *ceteris paribus*, be less likely to be successful than autografts.

FRESH HETEROGRAFTS

These grafts were 2 cm. long and of three kinds:

(1) Pieces of the whole sciatic, or the tibial only, of rats (four grafts).

(2) Pieces of the peroneal nerve of dogs (one graft).

These were used to fill gaps in the peroneal of the rabbit.

(3) In one case, 5 strands of rabbit tibial were stuck together with plasma to make a cable graft 2·4 cm. long, which was inserted into the sciatic of a dog.

At 15 and 25 days both of the first two kinds of graft had the same appearance. They were bright yellow, shrunken in length (average length of grafts at 25 days = 15·75 mm.), and never vascularized. They were enclosed in a tough fibrous sheath which was strongly adherent to the surrounding tissues. The grafts were never properly united with the peroneal nerve at the ends, and felt hard or 'doughy' when pinched.

The distances of sensory fibre outgrowth were small. At 15 days there were no fibres across the proximal scar, while at 25 days the fibres had occasionally grown a short distance (under 10 mm.) into the graft (Text-fig. 2).

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Histology of fresh heterografts. Histological examination showed that both dog and rat nerve transplanted into the rabbit peroneal set up a very marked and unfavourable reaction wherever the tissues of the two animals were in contact, that is to say, along the sides of the graft, and particularly at the ends. At these points there were vast collections of white cells, including lymphocytes, oxyphils, and some macrophages. The reaction was so great as to cause considerable swelling of the ends of the nerves, and it entirely prevented the proper 'healing' of the graft into the host nerve, so that the junction of the tissues of the two animals shows a clear line of separation even after 25 days (Pl. 3, figs. 26, 27).

Within the graft itself the reaction was much less marked. There was less invasion by histiocytes than even in a normal peripheral stump. Moreover, the Schwann cells had not undergone proliferation (Pl. 3, fig. 28), and there had been no breaking up of either the axon or the myelin (Pl. 3, fig. 29).

It may be said, therefore, that these heterografts fail to degenerate, or at best do so to a minimal extent and in an abnormal manner. This would constitute a strong contra-indication to the use of heterografts in man even apart from the cellular reactions at the ends of the graft. Under these conditions it is not surprising to find that only a very few fibres penetrate the graft, and these only for short distances.

In the one case in which rabbit nerve was grafted into a dog the results were rather different. A very extensive cellular reaction was set up, within as well as around the graft. The outlines of the latter were difficult to trace, and the tissues were penetrated by a vast number of cells, including many macrophages, polymorphs, and lymphocytes (Pl. 4, fig. 30). The first-named were especially active (in contrast to the heterografts placed in the rabbit), and had gone very far, after 25 days, towards complete removal of the graft. Such traces of structure of the latter as remained, however, showed that the Schwann cells had undergone proliferation, and along the bands so provided fibres had penetrated for a distance of 10 mm. However, they were few in number, and the whole state of the graft was not such as to provide, in itself, a good union between the stumps. It seems not impossible, however, that fibres would ultimately have reached the peripheral stump, and perhaps even have produced some functional return, though less than could be obtained with an autograft.

Blondin (1928) found functional recovery after grafts of rabbit on dog sciatics. Cajal (1928), and Eden (1919), both condemn the use of heterografts, although Huber (1895) claimed some success for them experimentally, although not so much as for fresh autografts or homografts. Both Blondin (1928) and Huber (1895) used dogs as their experimental animals. It is probable that in their experiments the grafts were largely removed, and union of fibres with their end-organs occurred by growth through the replacing tissues.

DEAD HOMOGRAFTS PRESERVED IN ALCOHOL

These grafts were prepared according to the method of Nageotte (1917*c*). Grafts were taken from an animal and fixed in 90% alcohol. At the end of 24 hr. they were transferred to 50% alcohol and kept there until required for use. 24 hr. before insertion they were taken from alcohol and washed in several changes of Ringer's solution to remove the alcohol. The grafts were always 2 cm. pieces of rabbit tibial nerve, and were inserted into the rabbit peroneal nerve in the way already described.

In contrast to fresh homografts and autografts, dead grafts examined at 15 days were never properly united with the host peroneal. They were bright yellow in appearance, felt hard when pinched, and were never vascularized. They were always enclosed in a thick, collaterally vascularized capsule, which was strongly adherent to the surrounding tissues.

At 25 days the same features were present and were even more apparent. The junctions appeared to be invaded laterally by connective tissue.

In one animal opened at 61 days the graft was found to be represented by a small nodule of bright yellow tissue not attached to either stump. It lay laterally in a thick strand of greyish tissue connecting the stumps, which had the appearance of a tissue proliferation of double origin from the central and peripheral stumps of the peroneal, similar to the spontaneous junctions first noted by Kirk & Lewis (1917).

At 15 and 25 days outgrowing fibres were only rarely found to have grown across the proximal junction. Text-fig. 2 shows that the only growth obtained was in one case where at 25 days pinching showed that the fibres had apparently grown 7 mm. into the graft; but the histology showed these fibres to be growing down outside the graft.

Histology of alcohol-preserved homografts. After 15 days the graft is beginning to be invaded by macrophages. This process goes on especially at the ends, and at this stage invasion of the graft from the sides is slight. The reaction produced is not a very violent one, but is limited to a gradual macrophage invasion, removing the grafted tissues. *The nerve fibres certainly do not penetrate the original tissues of the graft* but advance with the macrophages, and at the boundary of the digested and undigested tissue form a plexus (Pl. 4, fig. 31), many being diverted laterally and showing large end-bulbs and other irregular formations. Other strands of outgrowing fibres progress down outside the graft.

By 25 days these processes have gone further. Macrophages are now attacking the sides of the graft. Short portions at the ends have already been completely removed, leaving a mass of macrophages with some fibroblasts. Through this mass Schwann cells and nerve fibres grow out from the central stump, and Schwann cells alone from the peripheral. In this way new nerve is being built as fast as the old is removed, but of course with considerable criss-crossing at every level (Pl. 4, fig. 32).

In the graft examined at 61 days these processes had been carried much further. The original tissues of the graft had been almost completely destroyed by macrophages (Pl. 4, fig. 34), and were being actively replaced by those of the host, so that at the edges of the graft, and especially at the ends, stretches of fibroblasts and Schwann cells, running in longitudinal directions, had appeared (Pl. 4, figs. 33, 35). In this way a tissue resembling normal nerve was being rebuilt, and in places, particularly at the edges, consisted of dense bundles carrying considerable numbers of nerve fibres. The centre of the graft had been less fully replaced, and carried few fibres, though it is possible that it would have come to carry more later. There is thus no doubt that the graft has served, in a sense, to effect union of the stumps, partly by providing a bridge *outside* which fibres can grow, and partly by actual replacement of a region of the graft by new nerve. So far as these cases show, however, there is *no retention of the tissues of the original graft*, which are replaced by a mass of macrophages, within which new and longitudinally orientated tissues appear.

Thus it may be that alcohol-fixed homografts act as a guide for proliferations from both stumps, which grow out as the graft is gradually removed, and eventually meet and become innervated. Huber (1920) reported functional recovery with these grafts, but he only studied late regenerates, by which time the actual graft could easily have been removed and replaced. Nageotte (1917*b*, *c*) also studied alcohol-fixed grafts histologically, and claims to have found them innervated in the dog at 56 days. It is probable, however, that little of the graft is left at 56 days, and, as Nageotte seems only to have studied transverse sections of his grafts, he may have observed an innervated proliferation. The present results support those of Cajal (1928). The actual graft in alcohol-fixed grafts is not innervated.

If the success found with alcohol-fixed grafts in animals is due to their action as guides for proliferations growing out from the stumps of the host nerve, we have an explanation for their lack of clinical success (see Laduron & Christophe, 1938). In a wound in man involving severance of a nerve trunk, the considerable scarring of the bed in which the graft would lie would not be likely to favour the outgrowth of proliferations replacing the graft. In any case the amount of criss-crossing and loss of fibres would be such as to prevent the achievement of any satisfactory return of the complex functions of a mixed nerve such as the median (see below, p. 161).

DEAD HETEROGRAFTS OF ALCOHOL-PRESERVED SPINAL CORD

One of these grafts was prepared and inserted according to the method of Gosset & Bertrand (1938). The spinal cord of a rat was fixed in situ in a mixture of 20 parts of neutral formaldehyde with 80 parts of water. Next day it was removed from the vertebrae and placed in 50 % alcohol for a week. 24 hr. before insertion a 2 cm. length was taken and washed in several changes

of sterile Ringer's solution.[1] It was then taken and inserted into a gap in the peroneal nerve of a rabbit.

At 25 days this graft was bright yellow, shrunken, and not vascularized. It felt 'doughy' to pinching, which showed a distance of outgrowth of 5 mm. (Text-fig. 2), and was enclosed in a thick capsule, strongly adherent to the surrounding muscle.

Histology of alcohol-preserved spinal cord graft. Histological examination of the grafted piece of spinal cord recovered after 25 days showed the least favourable reaction of all the types of graft examined. The whole graft was enclosed in a fibrous capsule, within which it was being vigorously attacked by giant cells (Pl. 4, figs. 36, 38). These form a complete row all round the graft, and would presumably ultimately remove the whole of it. There has been no union of the graft with either central or peripheral stump, and there seems no likelihood that such a union would have taken place if the graft had been left for a longer period. Outgrowth had taken place from both stumps *alongside* the graft (Pl. 4, figs. 36, 37) and it is possible that in time a union would be formed in this way *outside* the graft. This union might make a basis for some functional recovery in animals, and perhaps even in man, but, as explained on p. 161, such recovery could at best only be partial on account of the small number of fibres which would return to appropriate end stations after the passage of such a long gap in irregular bundles. In any case the graft is not serving to unite the stumps by virtue of its nature as a piece of spinal cord, but merely as a support along which outgrowth from the stumps may take place. As shown above, this is part of the function of an alcohol-fixed homograft, but the reaction to the two types of graft is not similar—at least in the rabbit. The bed of macrophages left in place of a piece of alcohol-fixed nerve provides a possible basis for the formation of new nervous tissue, whereas the complete encapsulation of the piece of spinal cord would seem to prevent its penetration by nerve fibres even after removal of the grafted tissue. It would require investigation of further cases to establish the reason for this difference in behaviour of the host towards pieces of material which are both treated as foreign bodies, in the sense that no part of them is incorporated into the host.

Gosset & Bertrand (1938) claimed success for these grafts in four dogs, as judged by the recovery of a normal chronaxie in the paralysed muscles, but recovery of sensation and function was not adequately studied. From the above experiment it seems unlikely that recovery could be anything but partial in the rabbit.

This unfavourable result contra-indicates the use of 'Gosset' grafts in man. Even if they do support Schwann bands growing beside them from the cut stumps, they are likely to fail in a wound for the same reason as alcohol-fixed homografts.

[1] Gosset & Bertrand used serum for this washing. It is unlikely that the change to Ringer influenced the final result.

DISCUSSION

In the application to man of the results of these animal experiments it is necessary to consider possible differences in (1) tissue reactions, (2) size, and (3) complexity of functional requirements. As regards the first, the reactions produced by any type of graft are unlikely to be identical in man and rabbit, but it seems probable that autografts can survive, degenerate, and become re-innervated in both.[1] There is ample evidence (Foerster, 1929; Bunnell & Boyes, 1939; Ballance & Duel, 1932 *b*) to show that under some conditions functional recovery can take place after autografting in man. No full histological study has been made of the fate of human autografts, though those which have been recovered have been reported as converted into scar tissue (Stopford, 1920; Platt, 1921). It may be that this is the fate of a graft placed under unfavourable conditions. But, as well as the present work, the clinical experience of workers as varied as Foerster, Duel & Ballance, and Bunnell & Boyes shows that autografts can survive (see also footnote, p. 146).

The factor of size has never been carefully studied. The grafts in the present series were all less than 4 mm. in diameter. Those in the facial canal and the extremities were also small, while those of Foerster were cable grafts. Bielschowsky & Unger (1917) claim that in dog grafts the centre of the graft becomes necrotic and does not carry any new fibres, unless it becomes very quickly vascularized. Maccabruni (1911) found that when the graft was thick there was a necrotic central zone, and the degeneration was atypical, while this was not true of small, thin, grafts. Bunnell & Boyes (1939), in the cat, found fibres more abundant in the periphery of their grafts, while there was necrosis at the centre.

There seems no reason to suppose that long grafts would be any less successful than short. The rate of growth in autografts is little less than in a normal peripheral stump, and in the three experiments of the present series in which grafts of 5 or 6 cm. were taken, fibres had proceeded at this rate along

[1] Through the co-operation of Miss E. Bülbring of the Department of Pharmacology we were able to show that similar changes occur in the early stages after grafting in dogs. A 4 cm. length of tibial nerve was inserted with plasma into a gap in the peroneal nerve of the same dog. When examined 21 days later this autograft had the appearance of a normal peripheral stump. Sections showed that degeneration, removal of myelin, and Schwann cell proliferation had proceeded almost normally. There were rather more macrophages in the graft than in the peripheral stump, and in a few places there were collections of polymorphs and lymphocytes, but although the graft was 5 mm. thick there was no sign of necrosis at its centre. Nerve fibres were growing down the graft.

In three other dogs homografts of 35, 45 and 28 mm. were inserted into the sciatic. These grafts, when examined 12, 25 and 29 days later, appeared much swollen. Sections showed extensive collections of lymphocytes and polymorphs within and around the grafts. Many macrophages were present, but the break-up and removal of axons and myelin had proceeded much less far than in the peripheral stumps, or in the autograft described above. Moreover, there had been very little proliferation of the Schwann cells. Some nerve fibres were growing down the grafts.

We are most grateful to Miss Bülbring for her help in showing, by these experiments, that in dogs, as in rabbits, autografts degenerate almost normally, but that there is some abnormality in homografts.

the whole graft. Moreover, as shown on p. 148, there is no reason to suppose, that the lower junction is necessarily badly made in the absence of nerve fibres.

The case of homografts is less easy to discuss because the possibility of differences between man and rabbit is considerable. Only experiment can show whether the somewhat unfavourable reactions observed in the rabbit are found also in man, even between members of the same blood group. These reactions appear to be due to some substance in the graft, which is removed or destroyed during storage, and this, or some other method of treatment, may be found valuable in man.

The fact that no proper degeneration occurs in heterografts confirms the observations of Merzbacher (1905), and shows very clearly that the processes of 'degeneration' are not a mere passive breaking up, even in the case of myelin, but an active process produced by the cells of the nerve.

Factors affecting the quality of recovery. A nerve graft in man is only valuable if it conducts to the correct part of the periphery an adequate number of sensory and motor nerve fibres. Since the number of different types of fibre in a nerve is generally greater in man than in animals and the functions to be repaired correspondingly more delicate, this requirement restricts the possibilities of successful human recovery. It is therefore essential in man to use only those means of bridging gaps which give every possible opportunity to the new fibres. For instance, alcohol-fixed nerves, after being destroyed by macrophages, may be replaced by a new outgrowth of nervous tissue (p. 157). It is even possible that by this means a functional recovery can be obtained in animals (Nageotte, 1917*c*; Huber, 1920). But the criss-crossing which is bound to occur repeatedly as the new fibres advance against the barrier of unremoved graft (see Pl. 4, fig. 32) must reduce the number of fibres which return close to their original endings. In a simple nerve whose fibres are of a few types this may not matter, but it may well prevent all useful recovery of delicate functions such as those of the median nerve in man. Again, such procedures as the fixed spinal cord graft in the present series encourage, or at least allow, outgrowth from central and peripheral stumps by which union can be effected (see Young *et al.* 1940). But, for the above reason, recovery produced in this way is not likely to be complete in man.

It is important finally to consider whether the condition in autografts is likely in any similar way to prejudice the quality of recovery. Certainly a great number of fibres can penetrate an autograft. No careful counts have yet been possible, but the density does not appear to be greatly less than in a normal peripheral stump. However, a possible adverse factor in autografts is the disturbance of the structure of the nerve which is produced by the delay in the removal of myelin remains. Comparison of figs. 3 and 7 in Pl. 1 suggests that this might be an important effect. However, the disturbance is usually less extreme than that shown in fig. 7.

The interposition of an extra point of junction must also be a factor militating against the success of any graft. A good deal of criss-crossing is

inevitable, although, as has been shown (p. 148), there is no reason to suppose that fibrosis need occur at a well-made junction.

These then are factors which must tend to reduce the number of fibres returning to 'correct' endings through a graft. It would be a mistake to give them undue emphasis as contra-indications for grafts until it is known to what extent the superfluity of fibres produced during regeneration allows for loss during transit. It may be that within reasonable limits, and assuming approximately correct apposition of bundles, re-connection of paths is always a statistical accident, so that a small reduction of numbers does not greatly affect the result. The impossibility of reaching any decision on the point at present emphasizes the importance of devising in the future a method for assessing the *quality* of recovery both in man and in animal experiments.

SUMMARY

1. Various types of nerve graft were inserted into the peroneal nerve of the rabbit. After 15 or 25 days, and in some cases longer, examination was made of the distance of outgrowth of pain fibres, and the histological changes in the graft.

2. The Schwann cells of an autograft survive and multiply, and the myelin breaks up and is removed, though rather slowly. New pain fibres grow through the graft at the rate of 2·0 mm./day (cf. 3·5 mm./day in a normal peripheral stump). After 25 days fibres are already medullating in an autograft.

3. Fibres crossed the lower junction of autografts with a maximum delay of 1·7 days. Histologically it can be seen that the junction is made by Schwann cells and is not dependent upon the presence of nerve fibres. This is so even for grafts 5 cm. in length. There is, however, a tendency for grafts to shorten, which may lead to bad junctions. Apart from this there is no reason to resort to resuturing a lower junction.

4. Nerve fibres do not grow faster through pre-degenerated than through fresh autografts. Any advantage of pre-degeneration must therefore be in the consistency of the graft.

5. Homografts often set up a considerable lymphocytic reaction which may lead to partial necrosis. Proliferation of Schwann nuclei and myelin break-up proceed in the graft, though the break-up may be abnormal. The distances reached by new fibres are more variable and smaller than in autografts. However, the new fibres medullate in the graft. In the favourable cases homografts therefore provide a possible basis for recovery.

6. In homografts stored for 7–21 days in Ringer at 2° C. the lymphocytic reaction is reduced or absent. There is a great invasion by macrophages, but Schwann cells survive and proliferate, though not normally, and myelin break-up proceeds. New fibres grow into the graft to distances which are

variable, but sometimes greater than in control fresh homografts. However, the fibres are abnormally thick and rather few in number.

7. Heterografts of dog or rat in rabbit set up a vast reaction at their edges, and do not degenerate. There is no Schwann proliferation or myelin break-up, and few new fibres enter the graft. A graft of rabbit into dog produced a very great reaction by which most of the graft was removed.

8. Pieces of nerve fixed in alcohol and used as suggested by Nageotte are removed by macrophages. New nerve fibres do not enter the actual transplanted tissue, though they may grow well into the bed of macrophages and fibroblasts which replaces it, and in this way reach the peripheral stump. Reunion by such a method involves at best much criss-crossing.

9. An alcohol-preserved piece of formol-fixed rat spinal cord (Gosset) was encapsulated and attacked by giant cells, New outgrowths passed around it but not within it.

10. This study of the early fate of grafts shows that in the rabbit autografted nervous tissue can survive, with degeneration and proliferation of Schwann cells, making a good basis for recovery. Homografts can also do so, but in some cases set up reactions. These reactions are reduced by previous storage of the graft. Heterografts set up a very great reaction and do not degenerate. They and alcohol-fixed grafts only provide for reunion of stumps after destruction and replacement by host tissues and are therefore unlikely to provide for a successful recovery in man.

This work has been made possible by the assistance of the Rockefeller Foundation, and we are deeply grateful to the Foundation and its Officers for their continued support.

We wish to thank Dr P. B. Medawar for assistance with statistical work and Prof. E. S. Goodrich, F.R.S., for reading the manuscript of this paper.

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EXPLANATION OF PLATES 1–4

Except where stated the figures are of material removed 25 days after the initial suture or graft in the rabbit.

Plate 1

Fig. 1. Simple junction with plasma of peroneal into tibial nerve, made 25 days previously. The central stump (peroneal) lies to the right. Bodian's stain. (82e.4.1.)

Fig. 2. Portion of tibial nerve 1 cm. below a simple plasma suture made 25 days previously. Note numerous Schwann nuclei, ingrowing axons, macrophages, a narrow blood-vessel. Bodian's method. (185c.5.9.)

Fig. 3. Portion of peroneal nerve 6 mm. below the lower junction of a homograft made 25 days previously. More or less regular arrangement of Schwann bands, but with some interference by myelin remains. No cellular reaction around the blood vessel on the right. Bodian and Masson. (316d.8.2.)

Fig. 4. Portion of peroneal nerve 1 cm. below the lower junction of a homograft to show myelin remains. Flemming's fluid, Weigert stain. (198b.10.8.)

Fig. 5. Upper junction of autograft. The graft lies to the right. Bodian and Masson. (277d.4.13.)

Fig. 6. Portion of an autograft, for comparison with fig. 2. There are many Schwann nuclei, nerve fibres and macrophages. Note swollen blood vessel. Bodian. (198d.5.13.)

Fig. 7. Portion of an autograft in which the myelin remains considerably disturb the architecture of the nerve. There is also some lymphocyte reaction around the blood vessels. Bodian. (71h.11.3.)

Fig. 8. Portion of an autograft to show normal break-up of myelin but somewhat delayed removal of remains (compare fig. 4). The new fibres in the graft are already becoming medullated. Flemming's fluid, Weigert. (126e.9.3.)

Plate 2

Fig. 9. Lower junction of an autograft inserted 15 days previously. The graft is to the left. A smooth union has been made although no nerve fibres have yet arrived. Bodian-Masson. (59e.12.8.)

Fig. 10. Lower junction of an autograft inserted 30 days previously, showing Schwann bands running directly from the graft (on right) to the host nerve. Masson's stain. (399a.1.1.)

PLATE 2 (*continued*)

Fig. 11. Portion of autograft to show normal break-up of myelin and medullation of the new fibres which have entered the graft. Flemming-Weigert. (126e.9.2.)

Figs. 12–16 are of the upper junctions of various homografts. All have been taken at the same magnification and in all the graft occupies the lower portion of the picture.

Fig. 12. Homograft, showing a small amount of cellular reaction. Bodian. (316d.10.10.)

Fig. 13. Homograft showing a very extensive reaction around the graft. Bodian-Masson. (102d.6.12.)

Fig. 14. Homograft stored for 7 days in Ringer's solution at 2° C. before insertion. Small reaction. Bodian Masson. (376c.14.8.)

Fig. 15. Homograft stored for 14 days. Very little reaction. Bodian. (198a.6.2.)

Fig. 16. Homograft stored for 21 days. Very little reaction. Bodian. (398a.6.14.)

Fig. 17. Portion of homograft showing very extensive lymphocytic reaction and some necrosis. Bodian-Masson. (316d.8.2.)

Fig. 18. Portion of homograft near upper junction to show break-up of myelin and medullation of the new fibres invading the graft. The upper end is to the left and the first myelin is being deposited about the level of the middle of the photograph. Formol-Weigert. (233a.7.5.)

Fig. 19. Lower portion of the same graft as fig. 18, showing fibres whose sheaths have not become broken up even after 25 days.

PLATE 3

Fig. 20. Homograft stored for 7 days before insertion. Lower junction. The graft is at the upper end. There has been little reaction and the junction is very well made. Bodian. (385b.1.2.)

Fig. 21. Portion of homograft stored for 21 days before insertion, showing a single Schwann cell, which has presumably survived the period of storage, and several macrophages. Bodian-Masson. (398a.6.2.)

Fig. 22. Fresh homograft, showing lymphocytic reaction around a blood vessel. New nerve fibres have entered the graft. Bodian. (102d.7.)

Fig. 23. Homograft stored for 14 days before insertion. Compare with fig. 22. There are no lymphocytes around the blood vessels but there is a considerable macrophage reaction. The ingrowing nerve fibres are abnormally thick. Bodian. (198a.6.2.)

Fig. 24. Homograft stored for 21 days before insertion. Compare with figs. 7, 3, 17. Note proliferation of Schwann nuclei, absence of lymphocytes, presence of numerous macrophages and some nerve fibres. Bodian. (397a.5.15.)

Fig. 25. Homograft stored for 14 days before insertion, showing normal break-up of myelin and some very thinly medullated new fibres. Flemming-Weigert. (198b.10.8.)

Fig. 26. Heterograft of dog nerve in rabbit. Upper junction, the graft is to the right. Note very great lymphocytic reaction and no proper union. Bodian-Masson. (273b.13.10.)

Fig. 27. Lower junction of the same heterograft as fig. 26; graft to the left.

Fig. 28. Portion of heterograft of rat into rabbit. Axons not broken up even after 25 days, no proliferation of Schwann nuclei, no macrophage or lymphocyte reaction. Bodian-Masson. (368a.8.9.)

Fig. 29. Heterograft of rat into rabbit. Upper end of graft stained osmium tetroxide to show myelin not broken up. (281e.24.4.)

PLATE 4

Fig. 30. Heterograft of rabbit in dog to show invasion by macrophages and complete destruction of graft structure except for isolated Schwann cells. Masson. (Dog 5a.18.3.)

Fig. 31. Alcohol-fixed (Nageotte) graft made 15 days previously; upper junction. The grafted material appears on the left. On the right can be seen new fibres growing from the central stump through a bed of macrophages which are attacking the graft. Bodian. (59b.8.7.)

Fig. 32. Alcohol-fixed (Nageotte) graft inserted 25 days previously, showing how new fibres grow out through the bed of macrophages which replaces the graft. The central stump appears above and from it proceed bundles of new fibres. The graft itself appears below but its upper and lateral portions have been removed already. Bodian. (60d.10.6.)

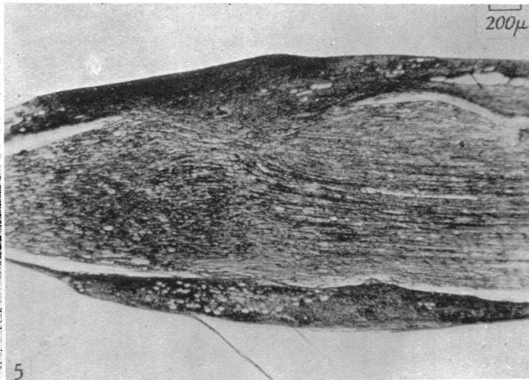
PLATE 4 (*continued*)

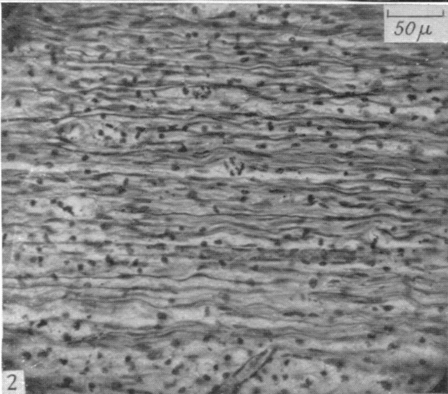
Figs. 33, 34 and 35 are portions of an alcohol-fixed (Nageotte) graft recovered 61 days after insertion. Fig. 34 shows the middle where the graft has been replaced by a mass of macrophages, in which a few collagen fibres are appearing. To the left of the graft are numerous fibrous bundles along which some Schwann cells have grown from the stumps. Fig. 35 is nearer to the central end and here the replacement of the macrophage bed by fibrous tissue is more advanced. In fig. 33 it is almost complete. Mallory. (137a. 3. 10.)

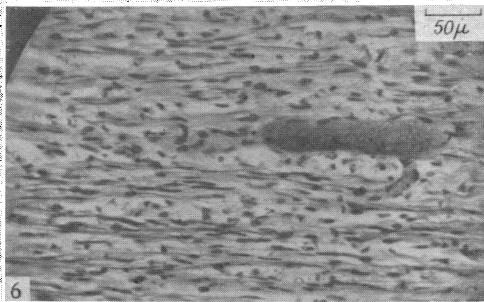
Fig. 36. Formol and alcohol-fixed spinal cord graft (Gosset). Upper junction. The graft has been enclosed in a fibrous capsule, and is itself being attacked by giant cells. From the central stump some outgrowth is proceeding alongside the graft. Bodian-Mallory. (223c. 10. 7.)

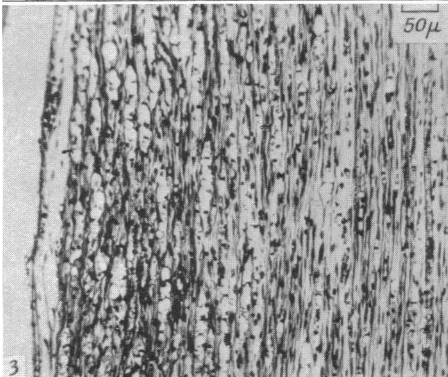
Fig. 37. Lower junction of the same graft as fig. 36. Some outgrowth from the peripheral stump alongside the graft.

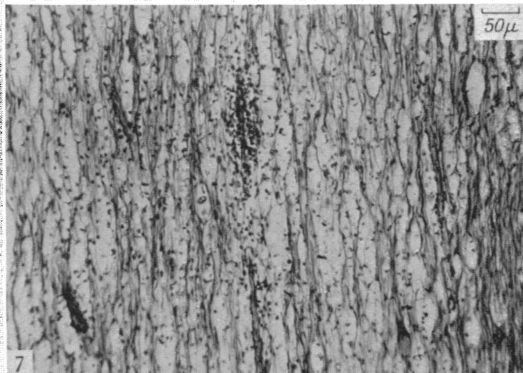
Fig. 38. Same graft as figs. 36 and 37, detail of giant cells attacking the graft. Bodian-Mallory. (223c. 9. 9.)

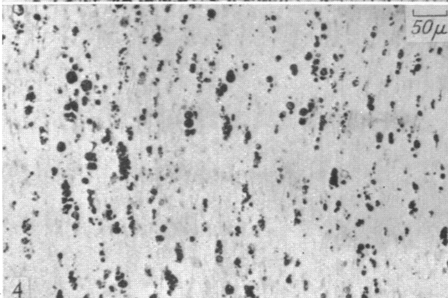


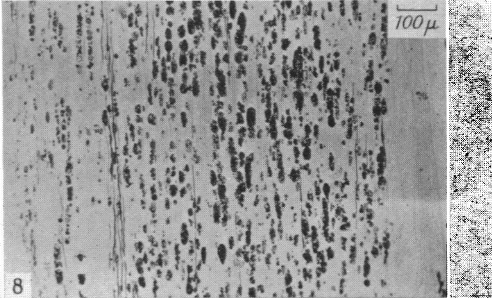




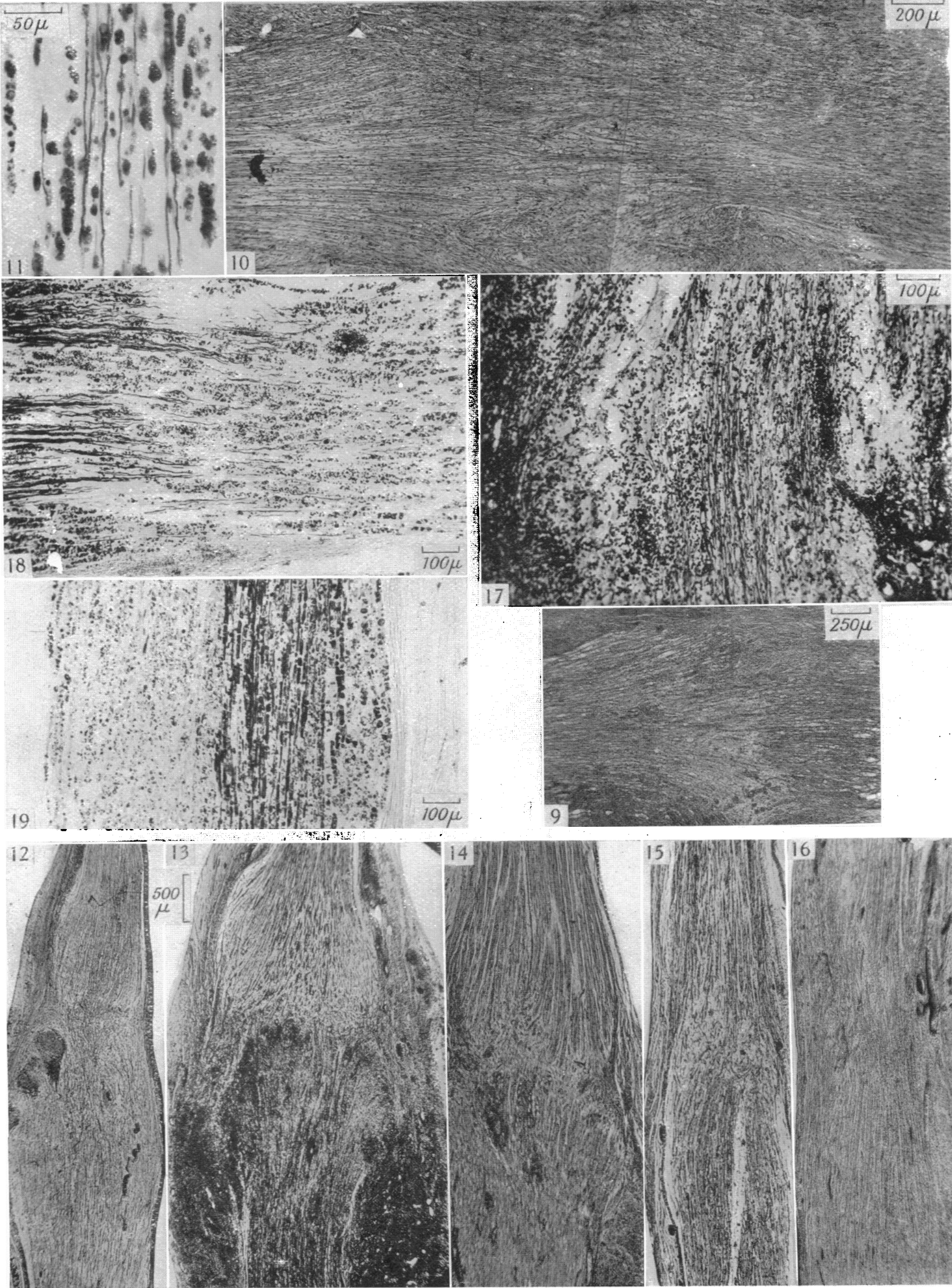




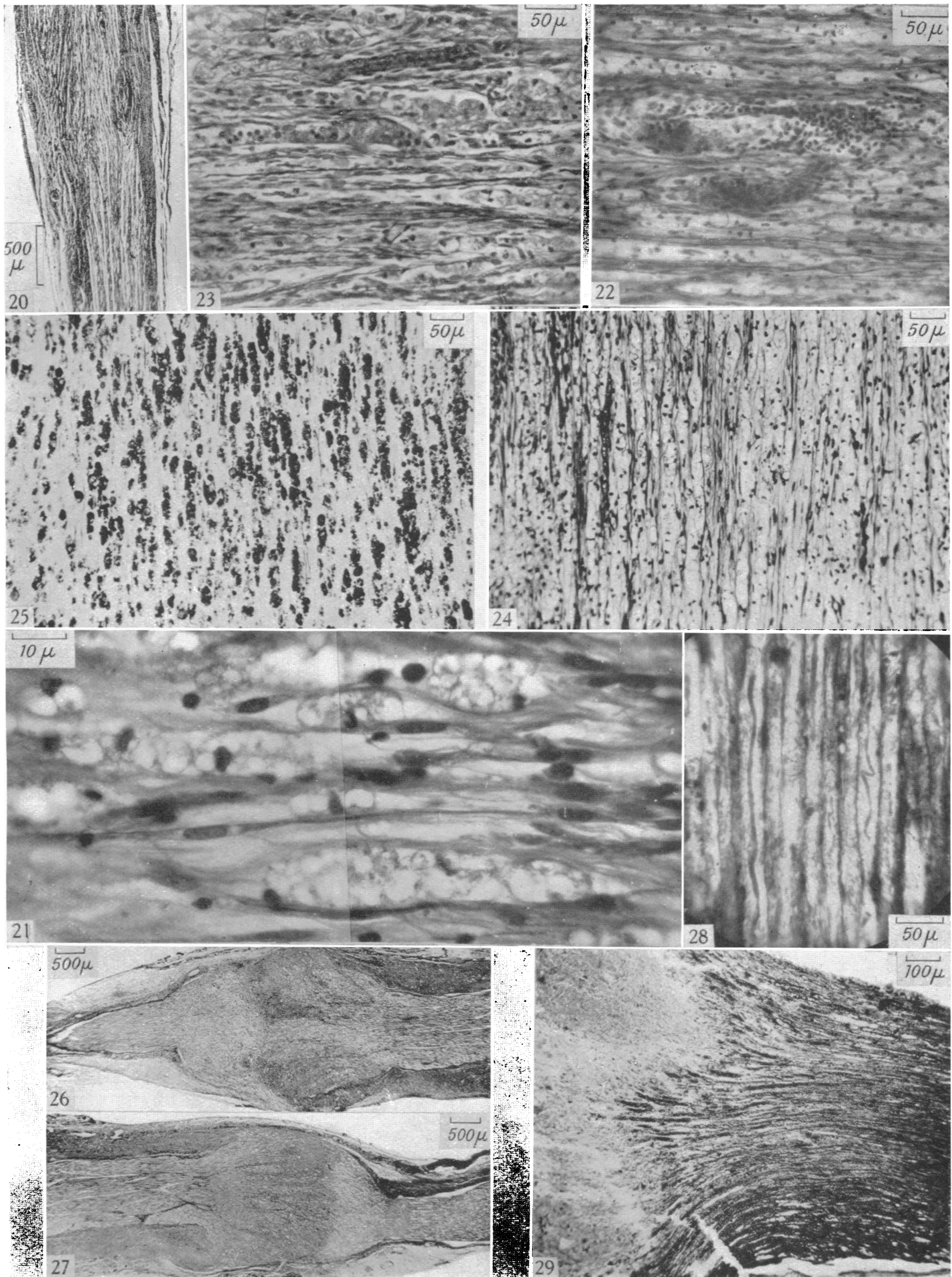




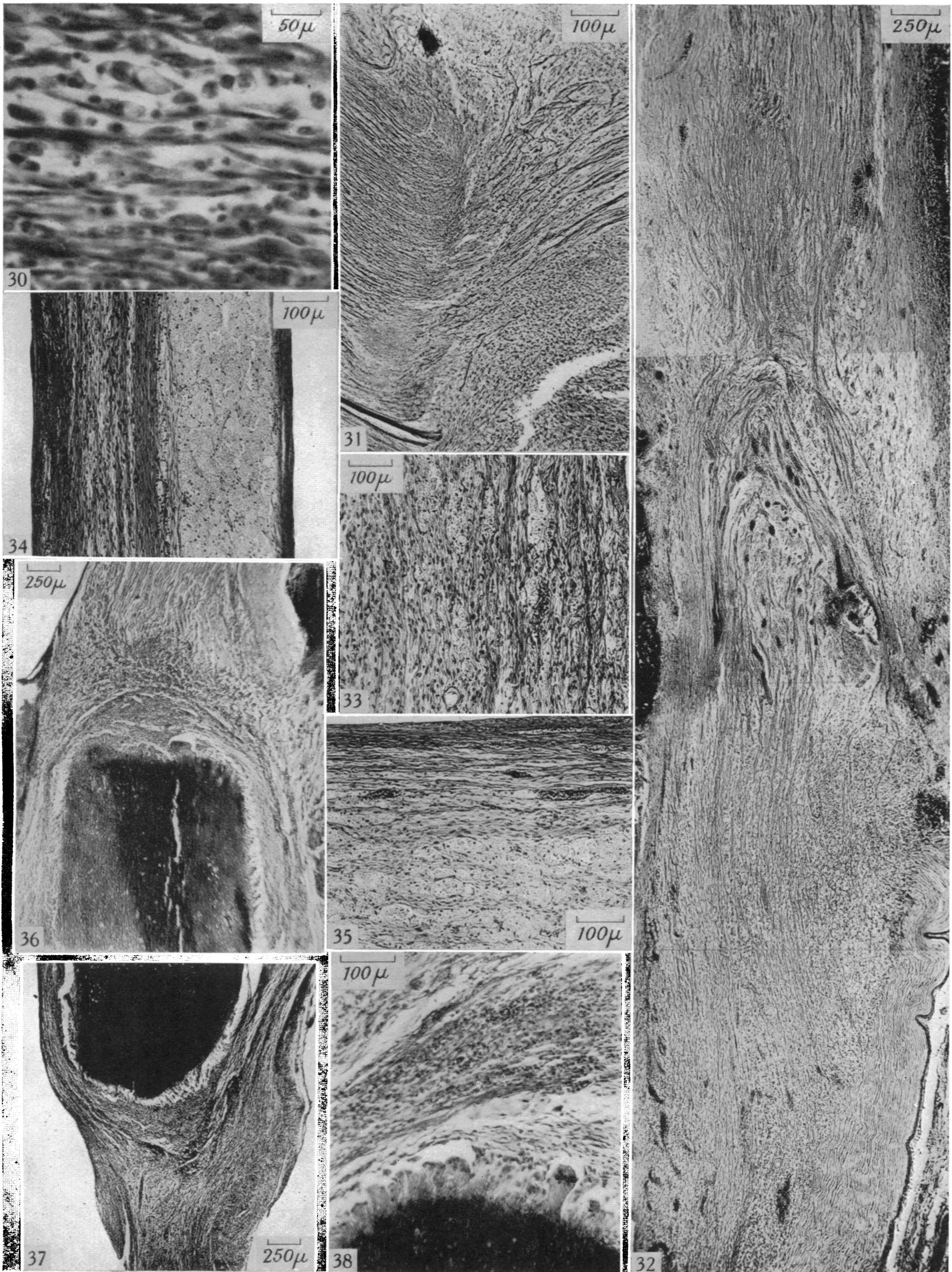
SANDERS AND YOUNG—NERVE GRAFTS



SANDERS AND YOUNG—NERVE GRAFTS



SANDERS AND YOUNG—NERVE GRAFTS



SANDERS AND YOUNG—NERVE GRAFTS