NERVE GRAFTS: THE IMPORTANCE OF AN ADEQUATE BLOOD SUPPLY*

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The use of nerve grafts to bridge gaps is unavoidable in those instances in which the various manipulative measures for closing defects, such as the mobilization, rerouting or postural shortening of nerves with subsequent stretching, are insufficient or inadvisable. The use of autologous grafts, such as those employed by Bunnell for the repair of nerves of the hand and by Ballance and Duel for bridging gaps in the facial nerve within the Fallopian canal, has been successful in a high percentage of cases. However, clinical success has been relatively uncommon following the insertion of grafts into gaps of other large major nerves. This occasionally led to the performance of end-to-end suture of nerves under great tension, in which cases the use of grafts might have proven wiser. This point was forcefully illustrated in a recent experience in which occasions arose for suturing the digital nerve of the index finger in two patients. In one, a male aged 65 years, loss of sensation over the radial border of the index finger followed a laceration at the base of the digit. Because of the presence of intense pain at the site of the scar, operation was undertaken. The nerve ends were found to be separated by a gap of 0.5 cm. and, after the terminal neuromas were resected, a graft 1 cm. long taken from the lateral femoral cutaneous nerve was introduced into the defect of the digital nerve and sutured with 10 drops of autologous unmodified plasma. Excellent coaptation of nerves was obtained. The operation was done 6 months after the original injury was sustained. Four weeks after the operation, sensation for pin prick had returned over the formerly analgesic area of the index finger. The day after this operation was performed, the occasion arose for treating a laceration of the digital nerve of the index finger of a physician 31 years of age. The injury severed the branch of the median nerve at the base of the proximal phalanx on its radial side, exactly the same site as in the previous case. The patient, a pianist, was greatly concerned over the analgesia of the radial side of his finger. At operation 6 hours after the accident, direct end-to-end suture of the nerve was done by the combined tantalum wire-plasma clot technique using 6 drops of autologous unmodified plasma. The union appeared quite satisfactory except that it was under considerable strain. Examinations 4 weeks and again 7 weeks after operation revealed no return of sensation to the finger. The use

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of a nerve graft rather than direct suture under tension would probably have been the operation of choice in this as in the former case.

A similar conclusion follows from our experience in dogs in which after the excision of 8-cm. segments of the sciatic nerve bilaterally, a 3-cm. fresh autologous sciatic nerve graft was used on one side and on the other side the nerve ends were sutured directly under considerable tension. Roentgenographic studies in two such cases following the intravascular injection of a mixture of red lead and glue showed good vascularization of the nerve on the side of the graft but practically none distal to the suture site on the other side (Fig. 1 A, B). Histological examination of the nerve specimens revealed considerably more fibrosis at the junction on the side of the simple end-to-end suture under tension. In another animal in which this procedure was carried out, there was regeneration of nerve fibers which had become myelinated down to the paw. However, fewer nerve fibers were present on the side sutured under tension. In two other dogs in which the same procedure was carried out, there occurred separation of the nerve ends on the side of the strained suture. Under such conditions it seems fair to conclude that nerve grafts would have a definite usefulness if the conditions governing their success could be determined, and this has been the purpose of our study.

**FIXED AND FRESH GRAFTS**

Some success has been reported with the use of formalinized homologous nerve grafts in humans although evaluation of these results is difficult because of insufficient data. We have found, however, that satisfactory restoration of function may follow the use of formalinized nerve grafts in dogs but, on the whole, animal experimentation has shown that the results obtained with such grafts and also with heterologous grafts are not as good as those which follow the insertion of fresh homologous nerve grafts. Autologous nerve grafts have been attended by better results, but in the case of the principal limb nerves the use of a single autologous nerve graft would be out of the question since there would be no justification in man for sacrificing one major functioning nerve for another. The only type of autologous nerve graft that could be used to bridge a gap in a large nerve is the cable graft prepared by placing segments of small nerves that can be sacrificed without serious deficit to the patient side by side until their combined cross-sectional diameter is equal to that of the nerve to be repaired. In our earlier experiments in dogs and monkeys the use of cable grafts prepared from intercostal nerves to repair defects in the sciatic nerve did not seem promising, since microscopically a large part of the central stump proved to be in contact with the considerable amount of connective tissue derived from the epineurium of the individual strands forming the graft. It seemed to us that such a condition would hinder the downgrowth of a significant proportion of nerve fibers from the proximal stump and our experiments at this point were focussed on the use of single grafts. The most favorable type of single graft that would be available for the repair of defects of the large nerves in
Nerve Grafts: Importance of Blood Supply

Man is the homologous cadaver graft. There are good grounds for the belief that Schwann cells of the graft proliferate and guide the regenerating axis cylinders and are essential for satisfactory regeneration. Hence it seems desirable that the graft be viable.

**Thick Grafts**

The use of fresh homografts in dogs is accompanied within the first 2 to 3 weeks by a variable, usually slight lymphocytic and monocytic response. At times practically no inflammatory reaction is discernible. The graft becomes invaded by macrophages which remove the myelin and axonal remains. The proliferation of Schwann cells and the disintegration of nerve fibers proceed in a manner similar to that in the peripheral stump. The grafts are capable of becoming innervated, conducting nerve fibers to the peripheral stump and, in favorable cases, good functional recovery may follow. However, not infrequently there occurred large patches of necrosis within the graft, usually in its center, or marked resorption and fibrosis without restoration of limb function. Complete absorption occurred in 8 of 50 fresh homografts in 33 dogs which were followed 35 to 489 days after operation. Thinning of the graft of about 50 per cent occurred in 20 of the homografts, the degree of reduction in calibre of the nerve being approximately 30 per cent in the remaining grafts. Seven homografts, which had been kept in sponges soaked in serum for 24 hours, were removed at sacrifice of the dogs 64 to 195 days after operation. Three of these grafts showed a decrease in diameter of 50 to 75 per cent, the other grafts exhibiting less change in calibre. Five homografts stored in serum for 48 hours were removed from dogs 63 to 234 days following operation. There was reduction in calibre of the graft to the extent of 50 per cent in 2 grafts with less diminution in size in the other instances. Two of these animals, followed 228 and 234 days after operation, had shown an excellent functional recovery. In one dog a homograft kept in serum for 72 hours was used, and at sacrifice there was but about 30 per cent reduction in calibre of the graft. The decrease in size of the grafts refers to the approximate degree of reduction in calibre of the total number of nerve bundles. Blood compatibility was considered to be of possible importance in governing the fate of homografts but destruction and fibrous tissue replacement of grafts was found to occur even when preliminary cross-matching of the cells and serum of host and donor bloods showed no agglutination. The inconstancy of the histological and functional results associated with homografts led us to consider the possible significance of tissue compatibility, other than as expressed by cross-matching of blood, in determining the outcome following these operations. However, in one instance in which an autologous sciatic nerve graft was used to bridge a defect in a dog's sciatic nerve, examination of the graft 156 days after operation revealed reduction in its diameter to the extent of approximately 75 per cent and much of the graft

*Grafts present in infected wounds were discarded from all series.
had undergone fibrous tissue replacement. In fact complete absorption of the graft occurred in 4 of 50 autografts in 33 dogs followed for periods ranging from 33 to 380 days after operation. A reduction in calibre of the graft of about 50 per cent occurred in 3 grafts, the diminution appearing to be less than this in the remaining dogs. This experience seemed to indicate that some factor other than tissue compatibility plays the major role in the fate of a nerve graft.

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*Fig. 1. Contact prints from x-ray films of sciatic nerves of dogs in which a mixture of red lead and glue was injected intraarterially. Autologous sciatic nerve grafts 3 cm. long were used in all instances and sutured by the combined silk-autologous plasma clot technique. A, Well-vascularized graft. B, Opposite sciatic nerve of the same dog in which, following excision of a 3-cm. segment, the nerve ends were sutured directly under tension. Note the virtual absence of injected blood vessels in the distal segment.* The injection was done 50 days after operation. C, Well-vascularized graft 26 days after operation. D, Graft to which fat-areolar tissue flaps were sutured. Eleven days after operation the flaps were completely freed from the nerve graft on one side, just before the animal was sacrificed and injected. Note decrease in vascularization of the graft on the freed side (E). F, Graft to which a fat-areolar tissue flap was applied 11 days before operation, when ligatures were tied around the suture sites, thus cutting off longitudinally coursing vessels. The graft remained well-vascularized, an indication that it receives a rich vascular supply from the surrounding tissues. G, Graft to which a fat-areolar tissue flap was sutured. The flap was completely freed from the graft 10 days after operation, just before sacrifice and injection of animal. No decrease in blood supply of the freed graft is apparent, indicating that a free anastomosis between longitudinal and regional blood vessels has already been established. H, Graft surrounded by tantalum foil. The animal was sacrificed and injected 15 days after operation. Note sparseness of vascularization of the graft in zone of tantalum wrapper. I, Well-vascularized graft 15 days after operation.

*The relative avascularity of the distal segment of the nerve is of interest in relation to the observations of Hight and Sanders (The effects of stretching nerves after suture. Brit. J. Surg., 1948, 30: 355–369). They resected 2.5 to 4.0 cm. of the external popliteal nerve in dogs and performed end-to-end suture of the nerve with the limb in the flexed position. Following extension of the limb, considerable histological damage to the nerve occurred, the stretching force having fallen more heavily on the distal segment. “Adhesions cause the suture site to act as a fixed point, so that when the suture line is made close to a joint, the peripheral stump is subjected to greater tension than the central.” In their experiments, rapid stretching did not appear to cause any more damage than slow stretching (over a period of 14 days) except that separation at the suture line occurred more frequently.
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METHOD OF STUDYING VASCULARIZATION OF GRAFTS AND
TECHNIQUE OF SUTURE

In order to test the importance of the vascular supply of nerve grafts in governing their take, studies were undertaken in dogs to determine the manner of revascularization of nerve grafts and then attempts were made to improve their vascular supply by the application of pedicled muscle or fat-areolar tissue flaps to them. The blood vessels were studied by histological methods as well as radiographically following the intravascular injection of a mixture of red lead and glue. This latter technique was elaborated by one of us (J. A. E.) and consists of the injection of the mixture directly into the abdominal aorta. The red color of the injection medium facilitates gross dissection of the blood vessels and x-rays reveal the blood supply with considerable clarity (Fig. 1). The small capillaries of the nerves were rarely penetrated by the injection mass but these were studied in microscopical preparations. By these combined methods adequate studies of the blood supply of nerve grafts can be made.*

Homologous nerve grafts, removed from the cadaver within 2 hours post mortem and kept in a sponge soaked in autologous serum or in Ringer's solution at room temperature for

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* Some of the operations and injections were done by Dr. F. Kantrowitz, to whom the authors are grateful.
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Fig. 3. A, Proximal suture site of a fresh cadaver nerve graft 3 cm. long 10 days after suture by the combined tantalum wire-autologous plasma clot technique. Blood vessels may be seen proliferating into the graft (below suture site) from the host stump. Hematoxylin and eosin, X60. B, Proximal suture site of autologous graft 57 days after suture by the same technique. Practically no inflammatory reaction is seen at the suture site and nerve fibers pursue a fairly straight course. Hematoxylin and eosin, X100.

periods up to 4 hours, as well as autologous grafts were used in our experiments. The success of a nerve graft depends to a large extent on the accuracy with which the ends of the graft are brought into apposition with those of the central and distal stumps. Since more precise contact of nerve ends with less trauma to them may be obtained with plasma clot than with the conventional thread suture, all nerve junctions were secured by the technique of autologous plasma clot (Figs. 2, 3 and 4). When some degree of strain existed at the suture site, then tantalum wire or silk tension sutures were used in addition to the plasma clot. The grafts were obtained from the sciatic nerve and were thick enough to cover the transverse area of the host stumps. Unless this is done some of the nerve fibers from the central stump having no path along which to grow may be lost in the tissue surrounding the graft.

THE MANNER OF REVASCULARIZATION OF NERVE GRAFTS

As early as 3 to 4 days after introduction of the nerve graft, either autologous or fresh homologous, endothelial-lined blood vessels appear at the ends of the graft adjacent to the stumps to which they have been sutured. At this time no intact blood vessels are seen elsewhere in the graft. Five days after the transplantation, the blood vessels that enter the graft from proximal and distal nerve stumps become more prominent and an occasional vessel from the epineurial sheath may be seen in the outermost portion of the adjacent nerve substance. The ingrowth of blood vessels from the surrounding tissues into the epineurium and thence into the nerve tissue of the graft is
quite apparent 6 to 8 days after suture of the graft, and by this time further vascularization has developed at the graft ends (Figs. 3A and 4B). In these vascularized zones the cells of the graft are more numerous and stain more distinctly than elsewhere in the graft where the cells may be almost completely absent. A day or two later this manner of vascularization of the graft by the ingrowth of blood vessels from the nerve ends and also from the tissues around the graft is emphasized, and thrombosis of some of the blood vessels of the graft with recanalization of them becomes apparent (Fig. 5A). Some of the thin bundles of nerve fibers appear completely vascularized at this time, whereas the thick bundles show on cross section many blood vessels with intact cells peripherally and with few or no blood vessels and cells in the central portion of the graft. In other words, the blood vessels in the mid-segment of the graft enter it from the surrounding tissue and grow centralward. Other vessels which appear earlier are derived from the stumps of the host nerve and grow from the ends of the graft toward its midportion. Ten to 15 days after insertion of the nerve graft, an increase in the number of blood vessels occurs and further evidence of thrombosis and recanalization of them appears.
Myelophages are seen in the nerve graft and, like the normal cell constituents of the nerve, they occur first in those areas supplied with blood vessels and their subsequent appearance runs pari passu with the latter (Fig. 5 B, C). Approximately 3 weeks after their introduction grafts 3 cm. in length may become well-vascularized (Fig. 1 C, I). The behaviour of fresh homologous cadaver grafts, kept in a sponge soaked in Ringer’s solution at a temperature of 5°C up to 6 hours after death of the donor animal, is essentially the same as that of single autologous nerve grafts except that somewhat more inflammatory changes (infiltration with monocytes and lymphocytes) are apt to occur in the former. At times, however, no histological differences are discernible in the two types of grafts and no inflammatory reaction occurs.
During the period required for vascularization of the graft the cells of the non-vascularized portions of the graft, many of which appear quite intact for several days after its introduction (Fig. 5 D), probably absorb their nutrition from the surrounding tissue. Some portions of the graft undergo fibrosis over large areas (Figs. 6, 7 and 8), whereas other portions become richly supplied with blood vessels and innervated. Complete fibrosis of autologous and homologous grafts has been found and, in some instances, similar grafts on the other side of the same animal were well-vascularized and innervated. The variability in the histological and functional results of nerve grafts is probably largely dependent upon variations in their blood supply. In certain cases, the bed in which the nerve graft lay may be more favorable for the development of adequate vascularization than in others.

Following carefully performed suture of nerve grafts by means of the plasma clot technique slight fusiform enlargement of the proximal and distal suture lines has been found (Fig. 2). Microscopically very little connective tissue has been present at the junctures, no more distally than at the proximal suture site. Axis cylinders have been found to cross the suture lines and in well-made unions they pursue a fairly straight course into the trans-
plant, through the distal suture line and into the peripheral segment. Nerve fibers have been found to cross the proximal suture line as soon as 10 days after introduction of a graft and in a 5-week specimen they had traversed the distal suture site of a graft 3 cm. in length. Studies undertaken to determine the rate of growth of nerve fibers through grafts have not yet been completed but there seems to be no doubt from our observations and prior ones reported by Young, Holmes and Sanders that the rate is not as fast as that following direct end-to-end suture of nerves.

THE PATTERN OF BLOOD VESSELS IN NERVE GRAFTS

Roentgenographic studies of transplanted sciatic nerves following the intravascular injection of a mixture of red lead and glue indicate that an important source of blood supply for grafts is their surrounding tissue. Blood vessels enter the graft from the host stumps a few days sooner than from the tissues around the graft and vascularization then proceeds from both sources. Within approximately 3 weeks a rich blood supply of the graft has developed (Fig. 1 C, I). At this time it may be seen roentgenographically, and confirmed by histological examination, that the blood vessels that traverse the suture sites, although they may be numerous, are usually small in contrast to the large vessels that enter the graft from the regional tissues. Even weeks after introduction of the nerve graft the principal longitudinal vessels often do not cross the junctions.
That the graft, within a period of a few weeks after its introduction into the recipient, is dependent for its vascular supply largely upon the surrounding tissues was demonstrated by the following experiment. Three-cm. segments of the sciatic nerve were excised, cross-switched and sutured by means of the combined silk-autologous plasma clot technique. In addition, the surrounding fat and areolar tissue was sutured to the scarified epineurium of the nerve graft in an attempt to improve the vascularization of the latter. The same procedure was carried out on both sides of the animal. Eleven days later both grafts of the animal were exposed and on one side the graft was freed from the surrounding tissue. On the next day x-ray studies following the intravascular injection of the radio-opaque medium revealed a striking contrast in vascularization of the grafts on the two sides, the graft freed from the surrounding tissue being to a large degree deprived of blood vessels (Fig. 1 D, E). This experiment was repeated after a post-operative period of 19 days, 26 days and at longer intervals with the result in these instances that practically no difference in vascularity was apparent on the two sides of the animals (Fig. 1 G). In other animals the initial operation was the same as that described but at the time of reoperation the junctions between the graft and host nerve were crushed by silk ligatures tightly applied on one side of the animal, the other limb serving as the control. Very little difference in the blood supply of the grafts on the two sides was apparent after x-ray studies were made for the visualization of blood vessels (Fig. 1 F), thus indicating that a rich vascular supply enters the graft from the surrounding tissues. It must be stated that the small capillaries that may be seen microscopically entering the nerve graft from the host stumps are not clearly visualized by the radiographic technique employed. They doubtless play an important role in the vascularization of the nerve graft. The relative importance of the blood supply of the graft has been examined by injection of radioactive substances into the blood stream and the subsequent X-ray demonstration of the distribution of the material in the tissues of the animal. The results of these experiments will be presented in a later communication.

Fig. 8. A, Axis cylinders just above ankle in limb of animal in which graft shown in Fig. 7 was introduced. Good innervation has occurred. Gros-Bielschowsky impregnation, X70. B, Myelinated nerve fibers at approximately the same level as in A. Osmic acid stain, X100.
vessels entering the nerve graft from the surrounding tissue has been further tested by cross-switching 3-cm. segments of the sciatic nerve in 3 dogs and surrounding the graft on one side with tantalum foil. Very little vascularization of the graft under the tantalum foil occurs (Fig. 1 H), and in one instance complete necrosis of the graft under the foil was found 25 days after the operation. These experiments leave no doubt that the circulation of a nerve graft is derived to a large extent from blood vessels of the surrounding tissue. After a period of approximately 3 weeks a free anastomosis between the longitudinal and regional blood vessels develops (a condition that exists in the normal peripheral nerve), so that cutting off the blood supply from either source results in no diminution of the vascularization of the graft as demonstrated by the radiographic technique described.

The pattern of blood vessels of the nerve graft becomes similar to that of normal nerves. Several large blood vessels run longitudinally along the graft and here and there occur horizontal connecting offshoots which tend to produce a step-ladder effect. In addition there is a rich meshwork of small blood vessels running in various directions. The similarity of vascular pattern between normal nerves and nerve grafts suggests that revascularization of the latter may occur along the preexistent vascular channels. That this may actually be true is supported by the histological observations that blood vessels within the graft at times become thrombosed within the first few weeks after introduction of the graft and then recanalization of them may occur.

ATTEMPTS TO INCREASE THE VASCULARIZATION OF NERVE GRAFTS

Since the vascularization of nerve grafts seems to be one of the most important factors concerned in success following their use, various methods have been tried to improve the vascular supply of nerve grafts and thus prevent the resorption and fibrosis of them which sometimes occurs.

A. The Use of Collodion Tubes for the Preparation of a Bed of Granulation Tissue for Nerve Grafts. In an attempt to produce a well-vascularized bed for the reception of nerve grafts, tubes of collodion were inserted into defects in the sciatic nerve. The walls of the collodion tubes were 1 mm. thick, and contained 1 mm. perforations, 1 mm. apart. At intervals varying from 8 to 21 days after introduction of the tubes, the wounds were reopened and the collodion tubes removed. The tubes were found to be enveloped in a membrane of connective tissue and it was planned to place the nerve graft in this envelope at the time of reoperation to determine whether it would lead to greater vascularization of the graft than on the control side, where the excised segment of nerve was not replaced by a collodion tube. Eight such operations were carried out and the membrane that formed around the tube was found in each case to consist of a few layers of connective-tissue cells and fibers which formed its inner lining. Moderate numbers of blood vessels were observed under the fibroblastic layer. The vascularization of the tissue surrounding the collodion tube was not strikingly greater than on the
control side so that the procedure did not appear promising and these experiments were discontinued.

The occurrence of an infection around a nerve graft was found to be associated with a well-marked increase in vascularization of the distal stump in one instance. This finding led us to consider various methods of producing inflammation and thereby increasing the blood supply of nerve grafts. However, in other instances of inflammatory reactions of various grades, which usually were associated with infection, no increased vascularization of the nerve grafts was observed so that it was considered unwise to undertake experiments of this type.

B. The Use of Pedicled Flaps of Fatty Areolar or Muscle Tissue Applied to Nerve Grafts. The sciatic nerve lies in a bed of fatty and areolar tissue and the flaps were made by undercutting this layer at its lateral margins and then suturing the 2 flaps to the middle 1.5 cm. of the 3 cm. single autologous or fresh homologous cadaver-sciatic nerve graft. The sutures were carried through the scarified epineurium of the graft. In later experiments the undercutting procedure was omitted since it was believed that vascularization of the flaps might be impaired as a result of the severance of some of its blood vessels. The loose tissue on each side of the nerve was merely brought directly over the graft and sutured to it.

Our early experiments dealing with the application of pedicled muscle flaps fashioned from the biceps femoris muscle to the nerve grafts resulted occasionally in necrosis of the muscle flap and this was attributed to inadequate vascularization. Studies were then undertaken to determine the blood supply of this muscle and it was observed that the blood vessels are present mainly at its proximal and distal extremities. It then became possible to prepare a more favorable type of muscle flap for suture to the nerve graft. Since the biceps femoris runs fanwise from above downwards, it is possible to cut the flap mainly in the direction of its muscle fibers, with a broad base distally and its apex near the origin of the muscle. The large blood vessels may thus be avoided and the flap retains a rich vascular supply. The muscle flap is then sutured to the epineurium of the nerve graft with its raw surface applied to the latter. The pedicle of the muscle flap was cut long enough to preclude the possibility of tugging on the nerve graft as a result of muscle contraction.

The effect of the application of muscle or of fat-areolar tissue flaps to nerve grafts was studied in 30 dogs. Bilateral fresh homologous sciatic nerve cadaver grafts 3 cm. long were used to bridge defects following the excision of 2-cm. segments of the sciatic nerve in 11 dogs. In the remaining 19 dogs, 3-cm. segments of the nerve were excised and then cross-switched. Fat-areolar tissue flaps were applied unilaterally to the nerve grafts in 19 dogs and muscle flaps were sutured to one of the grafts in each of 11 dogs. In 22 of these animals, in which the survival period varied from 3 to 125 days, roentgenographic studies of the nerve were made following the injection of a mixture of red lead and glue. Also histological preparations of these nerves
were studied. In 4 of these animals the combined studies indicated better vascularization and less fibrosis of the grafts to which the flaps were applied. In 3 instances the graft was thinner and less vascularized on the side to which the flap was applied and in the remaining 15 dogs there was no difference whatever in the grafts on the two sides. The 8 remaining dogs of this series were observed for periods ranging from 164 to 321 days. Autologous cross-switch grafts were used in 5 of these animals and cadaver grafts in 3. There occurred complete absorption of one cadaver graft to which a muscle flap was applied, while the other cadaver graft lacking the flap showed a 50 per cent reduction in calibre. The other muscle flap in this series had been applied to an autologous graft and in this instance too the graft had undergone complete absorption while its fellow, to which no flap was applied, but which received many blood vessels via spontaneously formed adhesions to the surrounding tissue, was essentially unreduced in size. In the other 6 animals fat flaps were used and practically complete absorption of the grafts occurred in one of them. In 3 of these dogs the grafts were somewhat thicker on the side of the fat-areolar tissue flaps and in the remaining 2 dogs there was no difference in appearance of the grafts on the two sides. The muscle flaps in most instances had either become converted to fibrous tissue or had disappeared. These experiments indicate clearly the futility of attempting to improve the vascularization of nerve grafts by the use of flaps such as described.

C. Tunnelling Grafts Through Muscle. In a series of 6 dogs fresh homografts 4 to 5 cm. in length were introduced into sciatic nerve defects following excision of 2-cm. segments of the nerve. On one side of these animals the graft was placed in a muscle tunnel made by forcing a hemostat through the biceps femoris and enlarging the opening so that it would accommodate the nerve without constricting it. The middle 3-cm. segments of the grafts were thus in contact with raw-surfaced muscle which it was thought might favor vascularization of the graft. These animals were followed for periods of time ranging from 89 to 161 days after operation, when they were reoperated upon just before sacrifice. At reoperation the nerves were stimulated with faradic current at various levels above and below the graft and the muscle responses were compared on the two sides. Such tests yielded no striking differences in the two limbs of the animals. There was moderate thinning (30 to 50 per cent) of both grafts in 3 animals, more reduction in calibre of the tunnelled graft (50 as against 30 per cent) in 2 other dogs, and in the remaining dog the tunnelled graft was essentially unreduced in thickness while its fellow showed a thinning of about one third. Again the conclusion follows that the desired objective of preventing the reduction in calibre of grafts by tunnelling them through raw-surfaced muscle was not attained.

CABLE GRAFTS

Microscopic study of nerve grafts has shown that thin bundles of nerve become vascularized and innervated more readily than thick bundles and it was therefore decided to repeat our earlier experiments on the use of cable grafts. In these earlier studies autologous cable grafts were used, the strands
being taken from the intercostal nerves. Many strands (8 to 10) were required to form a cable the calibre of the dog’s sciatic nerve and 4 cm. in length. The considerable amount of connective tissue derived from the epineurium and perineurium of the filaments of the cable was thought to be disadvantageous in that the downgrowth of axis cylinders from the central stump might be impeded. Moreover this procedure necessitated the excision of 5 to 6 intercostal nerves and the dogs and monkeys did not tolerate this operation well—the mortality rate was high. For these reasons, chiefly the second, it was decided to use cable grafts formed from thicker strands of dog cadaver nerve. The cable was formed from 4 to 5 strands of brachial nerves (twice or more as thick as the intercostal nerves). Only the ends of the cable were glued together with plasma, and the cable was made long enough so that the intervening segments could be spread about in the tissues to favor the development of vascularization. The cable grafts were 4 to 5 cm. in length and were sutured into defects resulting from the excision of 2 to 3 cm. of the sciatic nerve in dogs. This experiment was controlled against a single sciatic homograft removed from the same cadaver and introduced into the defect in the opposite sciatic nerve. The grafts were removed 1 to 3 hours post mortem and were kept in a sponge soaked in serum. They were used within 3 hours later except in two instances in which a period of 24 hours had elapsed before the grafts were sutured in place. These operations were done in 7 dogs. The animals were reoperated upon at periods varying from 65 to 180 days after introduction of the grafts. At reoperation faradic stimulation of the sciatic nerve at various levels was carried out and the muscle responses were compared on the two sides. The animals were then sacrificed and the nerves studied histologically.

Complete recovery from foot drop had occurred in 4 animals, in 2 of which the grafts had been stored in serum for 24 hours. No striking differences in gait* or in muscle responses following faradic stimulation were ob-

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* Functional studies of recovery were carried out in most animals. These were done by Dr. Walter Bornstein. Position sense was tested by the ability of the animal to correct an upside-down position of the foot. Touch sensation was tested by thrusting the animal’s foot over the edge of the table to determine whether the foot would be returned to its original position, the placing reaction described by Rademaker and by Bard (MacLeod’s Physiology in modern medicine. P. Bard, Ed. St. Louis: C. V. Mosby Co., 1938, 8th ed., p. 148). Pain sense was tested by pin-prick or by pinching the foot and following the progressive recession of the zone of anesthesia. Motor functions were studied by observing the animal’s gait and posture. The presence of foot drop, the result of paralysis of the extensors of the foot, was considered to be one of the most important signs following section of the sciatic nerve. An attempt was made to evoke foot drop under as many different conditions as possible, as, for example, by allowing the animals to walk on a slippery floor, or incline, upstairs and downstairs and by suddenly stopping or turning the running animal. Paresis of the plantar flexors of the foot resulted in an inability of the animal to raise the heel on the affected side so that the animal stood or walked on the whole sole of his foot rather than on his toes. In several animals there was found gross discrepancy between the results of these functional studies and the observations at autopsy. Recovery from foot drop was recorded in animals in which autopsy revealed practically complete absorption of the nerve graft and in which the muscles of the foot could not have become reinnervated. In such instances synergic muscles must have assumed the function of the extensor muscles of the foot and given the appearance of recovery of function of these muscles when such recovery had in fact not taken place. The conclusions based on the functional tests described were considered tentative until checked by histological studies to determine the extent of regeneration. These studies are still in progress.
served on the two sides of these animals. It must be stated that the comparisons of muscle movements, following application of the electrical current to the nerves, were of a crude sort consisting merely of visual observation of the extent of contraction of various parts of the limb.

At autopsy the individual strands of the cable graft were found to have become agglomerated into a cord rather resembling the single graft. In some instances, however, the segments of cable graft were grossly identifiable. Microscopically the strands proved to be embedded in a vascular connective-tissue matrix.

In one dog complete absorption of the single graft had occurred while the cable graft was essentially unreduced in size. Microscopically very little fibrosis of the strands was evident. In 3 of the remaining 6 dogs histological preparations revealed more fibrous-tissue replacement of the single grafts with fewer nerve fibers within them, the condition being reversed, however, in the other 3 animals. Axis cylinders were found to have grown through the grafts to the end of the nerve and medullation had taken place. Actual counts of nerve fibers have not yet been made but osmic acid preparations through various levels of the two types of grafts revealed no consistent differences. Although the results in this small series of experiments are somewhat better with the cable grafts, their superiority is certainly not striking.

All homografts used after 3 hours post mortem were kept at a temperature of approximately 5°C either in a sponge soaked in serum or immersed in test-tubes containing serum. These conditions were considered suitable for maintaining the viability of nerves as a result of some experiments carried out with Dr. Lorente de Nó at the Rockefeller Institute. The ability of nerves to transmit impulses was employed as an index of their viability and hence their suitability as nerve grafts. On the basis of these studies it seemed that homologous serum is superior to either Ringer’s or normal saline solution as a medium for preserving the viability of fresh cadaver nerves and that a temperature of approximately 5°C is preferable to room temperature. The receptacle in which the graft is kept is loosely stoppered so that air might enter in order to supply the nerve with its necessary oxygen requirement.

DISCUSSION

The incidence of complete absorption of sciatic nerve grafts was found to be approximately twice as high with fresh homografts as with autografts, i.e., 16 per cent as against 8 per cent. Also the degree of reduction in calibre of nerve at the expense of a loss of nerve fibers is apt to be somewhat greater with homografts. It is of interest, however, that of 13 stored homografts, which were kept in serum for periods of time varying from 24 to 72 hours, complete absorption did not occur in a single instance and, in general, the degree of thinning of the graft was rather moderate. Complete recovery of function took place in 2 dogs with 48-hour-old homografts. This experience seems confirmatory of that of Gutmann and Sanders that “stored homografts gave a distinctly better recovery than fresh homografts. . . .” These
authors stored the grafts in Ringer's solution. The better degree of recovery following stored homografts was considered to result from the reduction of the undesirable cell reactions brought about by storage. The degree of recovery obtained by Gutmann and Sanders "after stored homografts was definitely superior to that obtained after fresh homografts, although slightly inferior to the results of autografts and sutures." Our results are in agreement with this statement and would seem to favor the use of stored rather than fresh homografts. However, further work seems indicated to test this point.

In some instances at autopsy the grafts have been found encapsulated in a fibrous tissue envelope. Sanders and Young have found this tendency toward capsulation of the graft in rabbits greater in the case of fresh homografts than with stored homografts or with autografts. Our results in dogs appear inconclusive on this point. Adhesions between the nerve graft and its bed occurred in all cases. This would appear desirable since the necessity for blood vessels to enter the graft from the surrounding tissues during its early period in the host has been indicated by the results of our experiments. Such a condition might, however, conceivably lead later to delay in functional recovery as a result of interference with the free mobility of the nerve and the exertion of tension upon it coincident with movement of the part. If this does occur, then reoperation with freeing of the graft from its adhesions to the surrounding tissue and even transposition of it to a relatively unscarred bed, if possible, might be advisable. Injection experiments with red lead and glue in dogs following separation of the graft from its neighboring tissue has indicated that a free anastomosis develops between the longitudinal and regional blood vessels in approximately 3 weeks. At this time then, or preferably several weeks later, separation of the regional vessels from the graft would probably do no harm and might hasten restoration of function to the innervated part. However, this supposition remains to be tested. Such a neurolysis might be justifiable clinically under conditions where, after months or a year or longer, recovery has either not occurred or has failed to reach a satisfactory level.

The extent of functional recovery following the use of sciatic nerve grafts in dogs has varied and it seems likely that the extent of the vascularization of the graft represents the most variable factor involved. It has been shown that the vascularization of nerve grafts proceeds from two sources: (1) the host stumps and (2), the bed in which the nerve graft is placed. With very short grafts it is possible that adequate vascularization may be derived from the nerve stumps within a short time and satisfactory regeneration follow. In fact, rapid vascularization with very little fibrosis of grafts 5 to 7 mm. long has been found to occur in dogs. However, such grafts are hardly ever of practical value and with grafts several cm. long satisfactory vascularization from the nerve stumps cannot be expected to take place. Without blood vessels entering the graft from the surrounding tissue the mid-portion of these grafts would surely undergo necrosis with subsequent fibrosis and in-
terference with the downgrowth of nerve fibers. In view of this fact the use of impermeable membranes, either of animal or metallic origin, to enwrap the nerve for the purpose of "protecting" it is to be condemned. This practice prevents the ingrowth of blood vessels to the graft from the surrounding tissue and massive necrosis may follow.

Revascularization of a sciatic nerve graft 3 cm. in length in a dog requires approximately 3 weeks. During this period some of the cells become pale, pyknotic and disappear and the architectural pattern of the nerve graft becomes indistinct. Such changes become more marked near the center of the graft where small areas of necrosis tend to occur. The occurrence of some degree of necrosis and fibrosis in grafts together with the fact that two suture lines are present when they are used, renders conditions for functional restoration less favorable than in the case of direct end-to-end suture of nerves. But when the latter procedure cannot be done without great strain at the suture line, then the use of a nerve graft is in order. Very satisfactory recovery of function may follow the use of 3-cm. cadaver grafts in dogs and it is unnecessary to resect the distal suture line in cases where accurate coaptation of the graft with the host stumps has been obtained and the junctions made secure by means of autologous plasma clot suture with or without the aid of silk or tantalum wire tension sutures.

Within the first few days after introduction of a graft, before blood vessels have entered it, the cells of the graft (many of them do survive) probably derive their nutrition by the process of osmosis from the surrounding tissue. Further evidence that a part of the graft remains viable is the observation that there occurs thrombosis of some of its blood vessels with recanalization later. With the entrance of blood vessels into the graft, its cells multiply and large phagocytic cells (myelophages) make their appearance with the newly formed blood vessels. Less necrosis occurs in thin than in thick grafts because of the more rapid vascularization of the former and it is for this reason that cable grafts would appear to be more promising than single thick grafts for the repair of defects in nerves of large calibre. However, with the use of cable grafts a certain number of downgrowing axis cylinders from the central stump encounter epineurium and perineurium derived from the individual strands of the cable and many of these fibers become lost from the functional standpoint. On the other hand, with single thick grafts many of the nerve fibers are doubtless obstructed by areas of necrosis and subsequent fibrosis of the graft resulting from inadequate vascularization. These changes develop to some extent in all grafts but are more marked in thick ones. They occur in the center of the graft, that is, in those areas that are remote from the sources of its blood supply. Some degree of sacrifice of the proliferating axis cylinders seems inevitable in either case. In those instances where minimal necrotic and fibrotic changes take place in thick grafts the microscopical appearance of the graft is very favorable, the number of downgrowing nerve fibers, which become myelinated, is plentiful and the functional result in the dog is excellent and was not bettered in our experiments by the use of the ca-
ble graft. However, it seems that the risk of absorption is somewhat greater with the single graft which would tend to favor use of the cable graft. The use of thick (cadaver) grafts would appear to present an advantage in that one can choose a homologous nerve (sciatic nerve graft for repair of a gap in the sciatic nerve) taken, in fact, from the same level of the nerve. Thereby one can to some extent at least match fasciculi of the graft and the host stumps in a rough way. However, as an actual fact, much distortion of the internal relationships occurs within the graft as a result of its nerve fiber degeneration and fibrous proliferation so that the theoretical advantage is thereby largely negated.

The use of single thin grafts for the repair of defects in the digital nerves is associated with satisfactory results but clinical experience with single thick grafts has been disappointing. If single thick grafts (usually obtained from cadavers or amputation stumps) are used, it seems wise to split the graft longitudinally into several bundles or more if this can be done easily and without significant trauma to the nerve. This suggestion was first made to us by Lt. Commander T. I. Hoen. The procedure combines the advantages of the more unobstructed suture line of a single graft with the thinner, more readily vascularized intermediate segments of the cable graft. However, this plan cannot always be followed since the bundles of fibers within a nerve do not regularly pursue a parallel course but may be interwoven so that separation of the graft into bundles would, in these instances, result in the severance of a large number of nerve fibers with subsequent misdirection of many axis cylinders into the surrounding connective tissue. If the bundles of a single thick nerve graft are loosely connected and are easily separable, this measure should be taken and the strands spread apart in the bed of the graft.

The use of cable grafts seems somewhat preferable to single grafts for the repair of defects in large nerves for two reasons: firstly, the thin strands of nerve appear to be more readily vascularized than the thick single graft, and secondly, autologous nerves can be obtained for the purpose of forming the cable grafts and the possibility for a favorable take is greater with an autologous than a homologous nerve graft. However, some degree of necrosis and fibrosis appears inevitable with all grafts. For this reason the functional results following the use of grafts for the repair of defects in the major nerves cannot be expected to equal those associated with direct end-to-end suture of nerves. The loss of nerve fibers in transit through the graft or the deflection by barriers of fibrous tissue is considerable except, probably, when very thin grafts are used. In some cases it becomes possible to join nerve ends directly after rerouting a nerve and acutely flexing a neighboring joint, some degree of damage to the nerve follows the subsequent gradual extension of the joint and stretching of the nerve. The results in some such cases after extensive resections (10 cm. or more of the lateral popliteal nerve) have been a complete failure and it is possible that the use of nerve grafts under such conditions might be preferable. However, the clinical results in many cases where less extensive resection is necessary appear to be better.
than one might anticipate if nerve grafts were used. Satisfactory return of function to the hind limb has followed the introduction of grafts into sciatic nerve defects in dogs in spite of the great reduction in the number of nerve fibers that follows. A similar gratifying recovery may occur with the repair by grafts of defects in some of the nerves in humans. However, repair of the ulnar or median nerve by operations involving the use of grafts is not likely to be so satisfactory. These nerves contain a great number of small funiculi supplying muscles that carry out delicate and complex functions. Misdirection of axis cylinders and reduction of their number in passage through a thick graft occur in spite of the branching of axis cylinders from the central stump that accompanies regeneration. This would probably impair considerably the range of movements and the accuracy of proprioceptive sensation required for precise coordinative activity. However, grafts should be used only when methods of achieving end-to-end union of nerves are impossible or inadvisable and, under such conditions, they are worthy of trial.

When cable grafts are used, a natural question is the optimal calibre of the strands that should be used to form the cable. If the decision is made to use an autologous graft, then one will be obliged to choose cutaneous or possibly intercostal nerves or both, depending on the nerve to be repaired and the size of the defect. If the less desirable choice of a cadaver nerve is made, then one has the option of selecting a nerve from a greater range of sizes. The fewer the strands comprising the graft, the less the obstruction of nerve fibers at the suture site but the greater the likelihood of their blockage by the fibrotic areas developing within the thick strands of the graft. On the other hand, if thinner nerves are used, more strands will be required to form a cable the calibre of the nerve to be repaired and greater hindrance to the nerve fibers at the suture site will be offered by the connective-tissue sheaths of the individual filaments. But there will be less possibility that within the thin strands of the graft fibrosis will appear with impedance to the downgrowing axis cylinders as a result. Some degree of compromise is necessary in the selection of the calibre of the nerve to be used for the graft and it is difficult to know just where the happy medium lies. For the repair of the dog's sciatic 4 to 5 strands were used and necrosis within some of the segments of the graft occurred. It seems, then, that thinner strands might be preferable for a nerve the size of a dog's sciatic—6 to 8 strands rather than 4 to 5 would probably be more desirable from the standpoint of minimizing the fibrosis and loss of nerve fibers that tend to occur within the graft. It is important to direct the individual strands comprising a cable graft in a parallel course, thereby attempting to reduce, so far as one is able to do this, the likelihood of misdirection of axis cylinders from central to peripheral segments of the nerve.

When forming a cable graft it is advisable to glue the nerve strands together at their extremities with plasma, then trimming off a thin slice by means of the special nerve holder and the razor blade described. A flat cut surface is thus obtained and one may proceed to carefully coapt the stumps.
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of the host nerve to the ends of the graft, utilizing the latex mold in which the plasma clot suture is then carried out. The strands are united with plasma at their ends only so as to allow spreading of the intervening segments in the tissue bed in order that vascularization might more readily develop.

The state of nutrition and the activity of the animal following the use of nerve grafts appeared to have been factors in determining the speed and degree of functional recovery that occurred. In those dogs that had remained in good health and vigorous, the grafts seemed to have shown less reduction in size and the animals appeared to have achieved a greater degree of functional restoration than the animals that were emaciated and relatively inactive. Although this correlation was not invariable, it does seem probable that the greater activity of the more healthy dogs was associated with an improved state of circulation and that vascularization of the nerve grafts was thus promoted. Various nutritional factors as well as the age of the animal probably play a role in governing the result following the use of nerve grafts. The compatibility of tissues of donor and host also must be considered as a possible factor in determining the take of homografts. An attempt was made to test this hypothesis by cross-matching the blood cells and serum of host and donor dogs but, out of more than a score of animals tested, marked red cell agglutination was found in but one pair of animals. Pronounced blood incompatibility between dogs appears, therefore, to be uncommon in non-immunized animals. The graft from this donor dog whose blood was incompatible with that of the recipient did show complete absorption, but absorption of grafts also occurred in animals in which no blood incompatibility was detectable. It is possible that incompatibilities of nerve tissues which are not reflected by blood incompatibility may still be important in relation to the fate of homografts. Even more important is the existence of certain latent incompatibilities which may be shared by blood and tissues and which become manifest only after isoimmunization has occurred. This may explain the instances of skin homografts that seem to take well at first but, after a fortnight or so, undergo dissolution. However, the fact that varying degrees of absorption of autografts is common points to the primacy of some other factor, which in all likelihood is the vascularization of the graft. This must be kept in mind not only during the operation of nerve grafting (and a vascular bed chosen for the graft when this is possible) but also in the period of post-operative care. Massage, heat, exercise and, in short, all measures designed to improve the circulation of the part harboring the graft, and all means of improving the general health of the individual, should be used in an attempt to favor adequate vascularization and take of the graft. Moreover autologous grafts should be used whenever possible in order to reduce the possibility of tissue incompatibility affecting the result. Bunnell and Boyes advocate the use of the sural nerve for grafts since the resultant area of anesthesia is small and seldom annoying. They have been able to obtain as much as a foot of nerve from each calf, thus "allowing ample material
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even for a cable graft into a fairly thick nerve.” However, there is some evi-
dence that the size of the Schwann tubes within a peripheral nerve stump
controls the diameter of the downgrowing nerve fiber.9 It is thus possible, as
expressed by Gutmann and Sanders,7 “that some of the alleged failures of
long autografts in man may be due to the inability of the nerve fibers within
small tubes of cutaneous nerve bundles in the graft to enlarge beyond a cer-
tain diameter and so conduct impulses with proper time relations to bring
about a restoration of the more delicate functions.” If this is so then it would
appear unwise to use cable grafts made up of cutaneous sensory nerves to
bridge gaps in the major mixed nerves in man. On this basis the use of inter-
costal nerves which contain a great number of motor fibers with large
Schwannian tubes would seem preferable. However, it is probable that
further light will be thrown on the importance of the size of the Schwannian
tube in governing the maturation of a regenerating nerve fiber as a result of
studies being carried out in various laboratories and clinics. At present, al-
though the evidence is suggestive, the issue is undecided. Contrary to the
findings of Ballance and Duel,1 Bunnell and Boyes3 as well as Sanders and
Young13 reported no significant difference in the rate of growth of nerve
fibers through fresh and predegenerated autografts.

CONCLUSIONS

1. Very good recovery of sensory and motor function may follow the in-
roduction of fresh homologous cadaver grafts into sciatic nerve defects in
dogs. Satisfactory restoration of function has in fact occurred with the use of
homografts stored in serum at a temperature of approximately 5°C for 24 to
48 hours. However, complete absorption occurred in 8 of 50 fresh sciatic
homografts and in 4 of 50 sciatic autografts. In all thick grafts varying de-
grees of necrosis and fibrosis appear to be inevitable.
2. Adequate vascularization is a most important factor in determining
the take of a nerve graft.
3. Revascularization of nerve grafts develops by the ingrowth of blood
vessels from the host stumps and also from the surrounding tissue. Massive
necrosis of a graft may occur if blood vessels present in the surrounding tissue
are prevented from entering it. There is, then, no justification for the prac-
tice of interposing an impermeable membrane between the graft and the sur-
rounding tissue.
4. Although it is imperative that the bed for a nerve graft consist of well-
vascularized tissue it has proven futile to attempt to increase the blood sup-
ply of nerve grafts by the application of fat-areolar tissue or muscle flaps to
them or by tunneling the grafts through muscle.
5. In our small series of experiments on the use of cable homografts com-
pared with single thick homografts the results were only slightly better with
the former. Nevertheless cable grafts seem preferable to single grafts for the
repair of defects in large nerves since autologous nerves would be available
for forming these grafts. On the other hand, if single thick grafts were used
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Cadaver material would be required. Since necrosis and subsequent fibrotic changes are more apt to occur in homografts than in autografts, the latter should be used whenever possible.

6. When cable grafts are used, its strands should be bound together with plasma only at their ends so that the intervening segments may be separated in the bed of the graft in order that they may more readily acquire an adequate blood supply.

7. The use of plasma clot is essential for suturing cable grafts since considerable damage to the strands accompanies the use of thread suture and, in all, better unions are obtainable with the plasma clot technique.

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