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Nerve regeneration and repair
A review

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The Problem

Injuries to peripheral nerves still represent one of the most challenging surgical problems. Severance of major nerve trunks in the upper extremity causes total or subtotal loss of sensory and/or motor function of the hand. These lesions are associated with considerable disability: a hand without sensibility is a hand without function. Our possibilities to treat these conditions are limited. In spite of microsurgical techniques, we still lack methods to restore all qualities of sensibility in the hand of an adult after complete severance of a peripheral nerve.

The healing of an injured nerve represents a unique biological problem. Nerve cells do not multiply after injury – instead each severed neuron extends new peripheral processes to restore its original axoplasmic volume, lost with amputation of the original axon. This *cellular repair* contrasts brightly to the intense proliferation of fibroblasts and endothelial cells at the site of injury, as well as Schwann cells in the distal segment.

The results of peripheral nerve repair have today reached a plateau where functional recovery is still unsatisfactory, but where the surgical techniques cannot be further refined. Misdirection of regenerating axons at the site of injury is still a major problem. Therefore, increasing interest is being focused on the role of microenvironmental factors for regulation of axonal growth and direction. In this review current neurobiological concepts are discussed and related to methods for treatment of peripheral nerve injury.

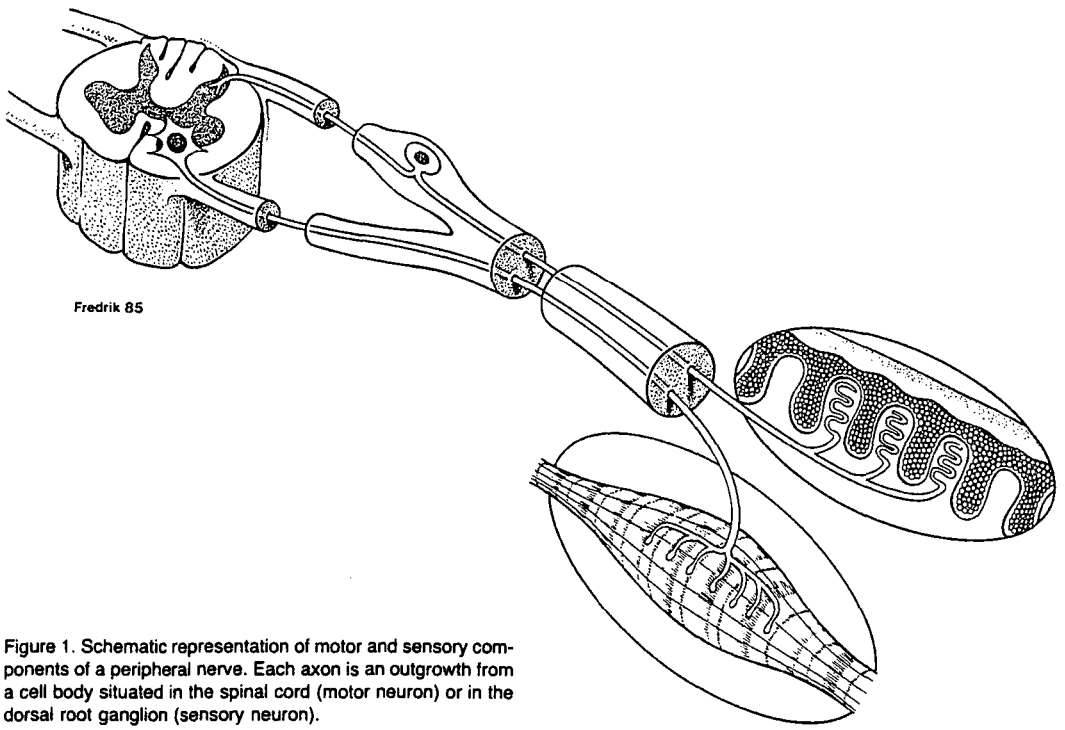


Figure 1. Schematic representation of motor and sensory components of a peripheral nerve. Each axon is an outgrowth from a cell body situated in the spinal cord (motor neuron) or in the dorsal root ganglion (sensory neuron).

Biological basis

The neuron

Each axon is an extension from a nerve cell (Figure 1), situated in the anterior horn of the spinal cord (motor neurons) or in dorsal root ganglia (sensory neurons). The relations in size between an axon and its corresponding cell body is remarkable – axons may extend over distances that correspond to many thousands times the diameter of the cell body. Because the metabolic activities of the nerve cell are situated in the cell body, the peripheral axonal parts are dependant on the continuity and integrity of the central parts: severance of an axon causes degeneration of its distal parts.

Axonal transport

The neuron has a unique system for intracellular transport, making possible an adequate supply of necessary materials to the terminal axonal parts. By *axonal transport* a wide variety of materials, synthesized in the cell body, move along the axon at different rates. Two main components of such anterograde axonal transport have been defined (Lubinska 1964, Droz 1975, Black & Lasek 1980, 1982, Grafstein & Forman 1980, Brady & Lasek 1982, McLean et al. 1983): Slow transport (1–6 mm/day),which involves subunit proteins of the cytoskeletal elements of the axon, such as microtubules, neurofilaments, and microfilaments; and secondly rapid transport (about 400 mm/day), in which mainly plasma membrane constituents are conducted: viz., glycoproteins, lipids, and transmitter storage vesicles.

There is also a constant *retrograde* transport of materials from axonal terminals towards the cell body (Lubinska 1964, Bisby 1976, 1980, 1982, Bisby & Bulger 1977, Forman et al. 1977a,b, Hammond & Smith 1977, Kristensson & Olsson 1977, Dahlin et al. 1986). Retrograde transport involves recycled neurotransmitter vesicles, but may also include various extracellular materials taken up by nerve terminals or by the cut end of a transected axon.

Neuronotrophic and neurite-promoting factors

It is believed that cell bodies of neurons are de-

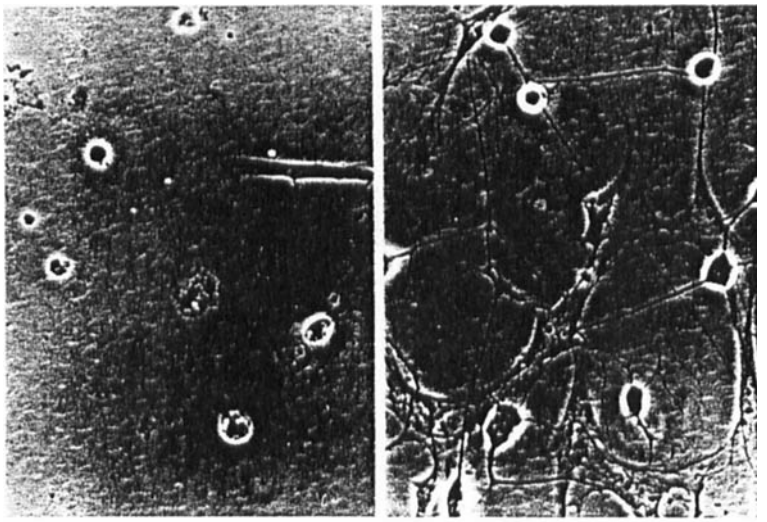


Figure 2. Effect of Nerve Growth Factor (NGF) on embryonic sensory neurons. Dissociated 8-day chick embryonic neurons were cultured in Eagle's Basal medium and fetal calf serum without NGF (A) or in the presence of NGF (B). In this system, NGF supports neuronal survivial and stimulates axonal outgrowth.

pendant on a constant supply of *neuronotrophic factors (NTF)*, which are synthesized by target organs of the neuron and/or by their corresponding Schwann cells. NTF are transmitted by retrograde axonal transport along the axon and used by the nerve cell body to sustain vital processes (Prestige 1970, Landmesser & Pilar 1978, Varon & Adler 1980, 1981, Cowan 1983, Varon et al. 1984). NTF are directed toward the regulation of the anabolic machinery of the neuron, whereas a specifying influence on, for instance, neurite outgrowth and elongation may be also based on several factors in the local microenvironment of growing axons *Neurite promoting factors (NPF)* occurring in the interstitium or bound to surfaces of cells or other structures in the microenvironment are important in this context.

The best known NTF is Nerve Growth Factor (NGF), described by Levi-Montalcini & Hamburger (1951, 1953) and Levi-Montalcini (1966, 1983), which has been reported to enhance the outgrowth of neurites selectively from neurons of sympathetic and embryonic sensory (dorsal root ganglion) cells. NGF, being a protein displaying numerous analogies with insulin (Frazier et al. 1972, Varon & Adler 1981), has acquired an outstanding place in modern neurobiology as a model for neuronotrophic factors. In in vitro systems, NGF promotes survival of sympathetic and embryonic sensory cells, but also promotes elongation of their neurites (Figure 2). Also in vivo, NGF is required for early development of sympathetic and dorsal root sensory neurons. By supplementing NGF levels in neonatal animals, a considerable increase in sympathetic ganglion volume and neurite growth can be induced, whereas immunosympathectomy by direct antibody administration to pregnant animals results in destruction of dorsal root ganglion cells and sympathetic cells (Levi-Montalcini 1966, 1983, Kessler & Black 1980, Johnson 1983).

Other neuronotrophic factors have been demonstrated in, for instance, *conditioned media*, i.e., media collected after exposure to cell cultures. Of special interest is the demonstration that conditioned media from Schwann cell cultures contain NTF for ciliary, dorsal root and sympathetic ganglionic neurons (Varon et al. 1981), an observation indicating a possible role of Schwann cells as a source of NTF also in vivo.

The nerve trunk

Nerve fibers are collected in fascicles, embedded in loose connective tissue – the *epineurium* (Figure 3). The epineurium serves as a protective layer for the fascicles during the movements of the extremity and protects the fascicles from external trauma. The relative

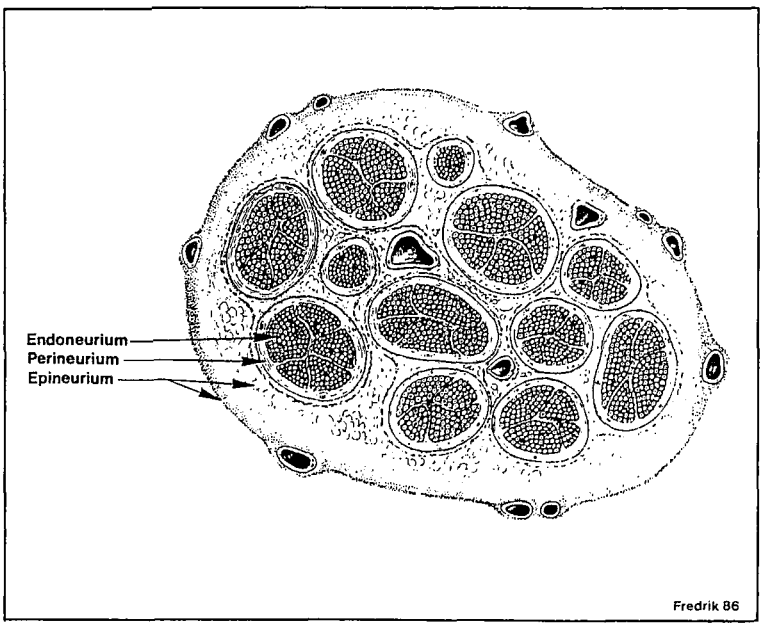


Figure 3. Microanatomy of a peripheral nerve.

amounts of epineurium vary between nerves and levels of the extremity; around joints it is often more abundant than elsewhere. In the epineurium, fibroblasts, macrophages, and mast cells are present in a pattern suggesting no difference from loose connective tissue (Low 1976).

Superficially, the epineurial connective tissue is condensed into a sheath, whereas deeper layers of the epineurium separate the fascicles and keep them loosely together. The fascicles are surrounded by a mechanically strong multilamellar sheath – the *perineurium* (Key & Retzius 1876). The perineurium has a role as a mechanical protection to external trauma, and also acts as a diffusion barrier, helping to preserve a specialized intrafascicular microenvironment (Martin 1964, Olsson & Kristensson 1971, 1973). The perineurium consists of a number of lamellae comprising flattened cells, the number of lamellae varying with the diameter of the fascicles.

Intrafascicularly, the fibers are embedded in an *endoneurium*, a loose collagenous matrix presenting large extracellular spaces. The endoneurium has a special relationship to the nerve fibers since endoneural collagen fibrils are closely packed around each nerve fiber to form the supporting walls of so-called endoneurial tubes (Sunderland 1978).

Nerve fibers are usually defined as axons, which are surrounded by a chain of Schwann cells arranged end to end. Nerve fibers can be myelinated or nonmyelinated. In the myelinated fiber, one axon is associated with only one Schwann cell at any level, whereas one Schwann cell of nonmyelinated fibers contains a large number of axons.

Vascularization

Conduction, as well as axonal transport, is dependent on a local energy supply provided by an intraneural vascular system. The peripheral nerve trunks are well vascularized comprising separate, however, extensively anastomosing microvascular systems in the epineurium, perineurium, and endoneurium (Blunt 1957, Edshage 1964, Smith 1966, Lundborg & Brånemark 1968, Lundborg 1975, 1979, Bell & Weddell 1984a,b).

Large, longitudinal vessels in the epineurium send frequent anastomoses to a vascular plexus between the lamellae of the perineural sheath. Deep epineural vascular branches supply bundles of fascicles in a segmental way

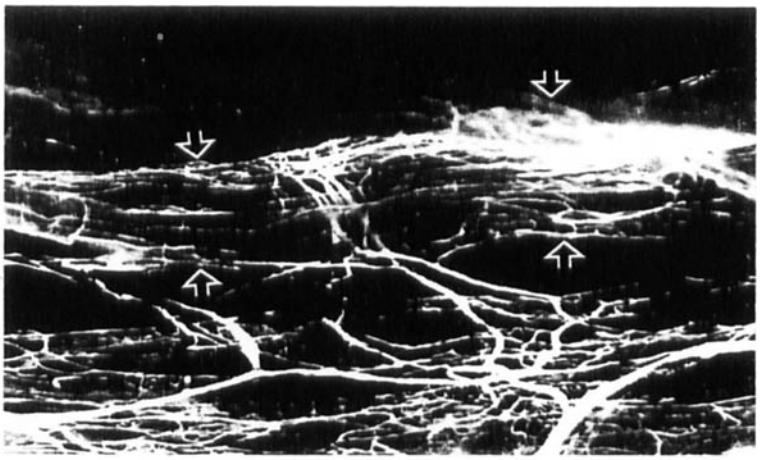
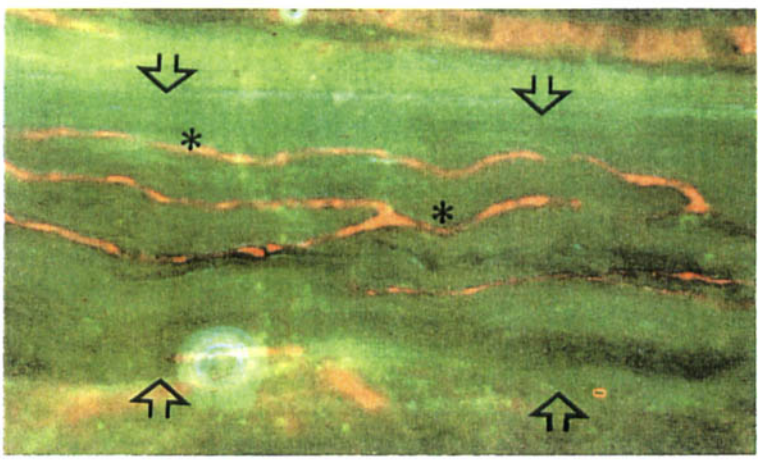


Figure 4. Microangiogram, illustrating the intrinsic vascularization of a human median nerve from the carpal tunnel region. Arrows indicate the outline of one fascicle. Reproduced with permission from Lundborg 1979. *J. Hand Surg.* 4, 34–41.



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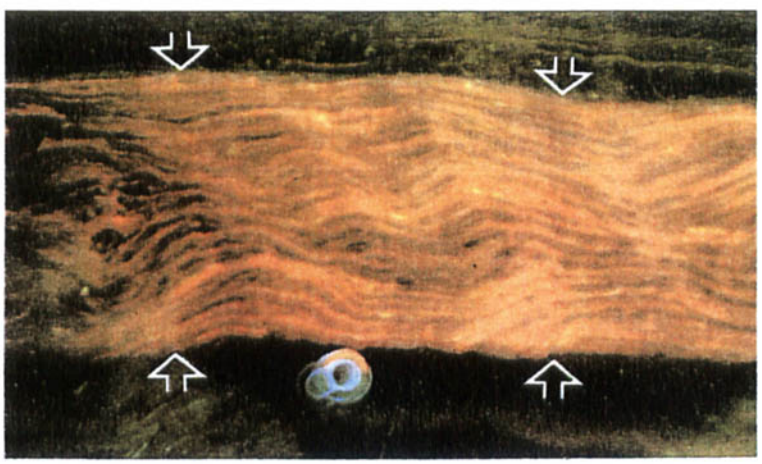

B

Figure 5. Fluorescence micrograph, illustrating the distribution of intravenously infused Evan's blue-albumin (EBA) in rabbit tibial nerve. EBA has been circulating for 30 min. (A) Control specimen. Red-fluorescence EBA is confined to the lumen of endoneural capillaries. The outline of one fascicle is indicated by arrows. Nerve fibers exhibit a green autofluorescence. (B) Endoneural edema following a crush lesion.

Table 1. Classification of nerve injuries

Seddon	Sunderland type	Functional disorder	Anatomic/ Pathophysiologic basis	Prognosis/ Recovery
Neurapraxia	1	Local conduction block.	Local myelin damage with preserved axonal continuity. No degeneration.	Reversible within weeks or months.
Axonotmesis	2	Loss of conduction at injury level and within distal nerve segment.	Axonal degeneration, endoneural tubes preserved.	Axonal regeneration will occur without misdirection. Correct end organs will be reinnervated.
	3	"	Axonal degeneration, loss of endoneural tube continuity. Perineurium intact.	Endoneural pathways deranged. Axonal misdirection will occur. Intraneural scar may be formed. Poor prognosis.
	4	"	Axonal degeneration, endoneural tubes and perineurium deranged. Epineurium intact.	Total disorganization of all guiding elements of the nerve trunk. Poor prognosis.
Neurotmesis	5	"	Rupture or transection of entire nerve trunk.	Surgical repair required.

(Figure 4). The perineural vascular plexus intimately anastomoses with an endoneurial microvascular system comprising mainly longitudinally oriented capillaries (Figure 5). The well-developed longitudinal collaterals of the "intrinsic" microvascular system of peripheral nerves permit a great deal of mobilization before the capillary nutritive intrafascicular blood flow is disturbed (Lundborg 1970, 1975). The endothelium of endoneurial capillaries constitutes a barrier against certain substances circulating in the blood, including macromolecules (Figure 5). The "blood-nerve barrier" corresponds functionally and structurally to the "blood-brain barrier" of the central nervous system.

Pathophysiology of nerve injury

Stages of nerve injury

Trauma to a nerve trunk may be followed by various degrees of functional loss depending on the pathoanatomic basis of the lesion. Slight local compression, causing obliteration of intraneural microvessels, may induce a rapidly reversible *local metabolic conduction block* because of local anoxia. The situation corresponds to loss of nerve function following cuff inflation around the upper arm to suprasystolic pressure (Lewis et al. 1931, Gilliatt 1980) or when a "foot goes to sleep" after crossing one leg over the other. A more long-standing local conduction block, not immediately reversible, can be induced by pressure of higher magnitude or stretching. Based upon experience from the Second World War (Seddon 1943, 1972), such a local conduction block, reversible within weeks or months, was called *neurapraxia* (nonaction). The term refers to a *local conduction block* with preserved continuity of axons and preserved excitability of nerve structures, as well as muscle tissues *distal* to the lesion (Table 1).

A classic neurapractic lesion is *Saturday night palsy*, i.e., paralysis of the extensor muscles of the wrist and fingers due to compression of the radial nerve where it passes in the groove around the humerus of the upper arm. Another example is *tourniquet palsy*, sometimes seen after inflation of tourniquets around the upper arm to too high pressure (Bruner 1951, 1970, Moldaver 1954, Wheeler & Lipscomb 1964, Hamilton & Sokoll 1967, Prevoznik 1970, Flatt 1972, Calderwood & Dicke 1972, Fry 1972, Middleton & Varian 1974, Rudge 1974, Bolton & MacFarlane 1978, Saunders et al. 1979, Rorabeck & Kennedy 1980, Lundborg & Rydevik 1982, Aho et al. 1983, Muirhead & Newman 1986). These lesions are due to local myelin damage induced by mechanical pressure from the cuff (Denny-Brown & Brenner 1944, Fowler 1972, Ochoa et al.

1972, Rudge et al. 1974). Neurapraxia corresponds to a Type I lesion according to another classification introduced by Sunderland (1951, 1978) and based on the pathoanatomy of the various tissue components of the nerve trunk (Table 1).

A severe compression or stretching lesion may result in loss of axonal continuity at the level of the lesion, however, still with intact endoneurial tubes. This lesion is called *axonotmesis* (Seddon 1943, 1972) or a Sunderland Type II lesion. In these cases the axons degenerate, but the prognosis is good because of the preserved endoneurial tubes: axonal regeneration is required, but there is no axonal misdirection at the level of injury.

Neurotmesis (cutting) includes lost continuity also of some or all the remaining elements of the nerve trunk including endoneurial tubes, perineurium, and epineurium. Usually neurotmesis represents a *complete* severance of the nerve trunk, but a nerve in continuity – damaged and disorganized by scar tissue – may also make spontaneous regeneration possible. Sunderland names these lesions Types III, IV, and V corresponding to involvement of the endoneurium, perineurium, and epineurium, respectively.

Proximal nerve segment

Following a nerve lesion the corresponding nerve cell bodies undergo characteristic structural and functional changes (Lieberman 1971, Grafstein 1975, Grafstein & McQuarrie 1978). There is an increase in cell-body volume, displacement of the nucleus to the periphery, and changes in structure of the cytoplasm (chromatolysis). The reaction reflects metabolic preparation for replacement of the lost axoplasmic volume (amputated distal axonal segment), expressed as a concentration of RNA-containing material in the segment leading to increased protein synthesis (Brattgård et al. 1957). The cell-body reaction may vary with proximity of the lesion, proximal lesions sometimes leading to death of the cell-body. The intracellular changes peak in the second and third week after injury (Ducker et al. 1969, Kreuzberg 1981).

Sprouting

Within the first days or weeks after transection of the nerve trunk, axons in the proximal stump produce a large number of collateral and terminal sprouts that advance distally. At an early stage, regenerating units – representing clusters of a large number of nonmyelinated sprouts, originating from the same parent axon – are characteristic features in the regenerating nerve. In time, the average number of axonal sprouts continuously decreases. The temporal reduction in the number of sprouts occurs when some of them ("pioneer fibers") make peripheral connections and then mature at the expense of disappearing sprouts, which had not established peripheral contact (Sanders et al. 1946). Obviously, there is competition for targets, reflecting a need for trophic substances supplied by the end organs.

The growth cone

At the distal-most part of each sprout, there is a *growth cone* – a swelling from which several microspikes or filopodia arise. These filopodias palpate and explore the environment displaying constant movements. Considerable amounts of actin have been demonstrated in the filopodia, indicating an important role for their mobility (Yamada et al. 1971, Wolosewick & Porter 1976, Pleasure 1980). In vitro the filopodias may extend and retract in a matter of minutes. Growth cones prefer substrates with a certain adhesiveness (Letourneau & Wessels 1974, Letourneau 1975, 1979), and it is known from in vitro studies that the physical and chemical properties of the substrate is essential for the advancement of the growth cones. In addition, *contact guidance* may be provided by surfaces of cells or axons, polarized structures like stress in a fibrin clot, and even irregularities and scratches in a culture dish (Weiss 1941, 1945).

Distal nerve segment

Following transection of a nerve trunk, the distal axon segments undergo Wallerian degeneration. There is disintegration of the axoplasmic cytoskeleton as a result of proteolytic break-

down Hasler (1979). This process is calcium-dependant, because neurofilament disruption is mediated by a calcium-activated enzyme in the axoplasm (Schlaepfer & Hasler 1979). The myelin is phagocytized by Schwann cells and macrophages. Physical changes are, to a great extent, completed within the first few weeks, but one to several months may be required for disposal of all myelin debris.

Loss of axonal continuity is followed by an intense proliferation of Schwann cells in the distal axonal segment, with the cells lining up in columns (band of Büngner). Using autoradiographic techniques, it has been shown that the highest values of Schwann cell multiplication is reached 3 days after axonal severance and then continues with decreasing frequency for about 2 weeks after injury (Bradley & Asbury 1970, Aguayo et al. 1976, Aguayo & Bray 1980). There is also a progressive increase in the collagen content of the distal segment resulting in reduction in size of the endoneurial tubes. The largest endoneurial tubes may be reduced to 10–20 per cent of their original diameters during the first 3 months after denervation (Sunderland & Bradley 1959, Sunderland 1978).

Sprouts will grow from the proximal nerve stump across the zone of injury towards the distal segment. This is a critical moment in the regenerative process to a great extent determining success or failure. Some sprouts may grow into the connective tissue in the nerve trunk or extraneurally forming neuromas. Other sprouts may approach a Schwann cell column, and may hereby be guided to the periphery. Because an excess number of sprouts are invading the distal Schwann cell columns, the total number of axons in the distal segment may initially considerably exceed the number of axons in the proximal stump. However, with time the number of axons decreases as sprouts, not making periphery connections disappear. Misdirection of sprouts at the level of injury is a major problem, the final result depending on the extent of linking up with original tubes.

Animal experiments have shown that the rate of growth in the distal segments is 2–3.5 mm per day after transection (neurotmesis) and 3.0–4.5 mm per day after crush (axonotmesis) (Gutmann et al. 1942, Black & Lasek 1976, Danielsen et al. 1986). Axonal growth in man is nonlinear, with a gradually decreased regeneration rate in distal parts. The average outgrowth rate has been estimated to be at most 1–2 mm per day (Seddon 1972, Buchthal & Kühl 1979).

Nerve regeneration

Mechanisms regulating axonal growth and orientation

Earlier, it was believed that regenerating nerve fibers tend to grow towards certain targets rather than at random (Forssman 1898, Ramon y Cajal 1928). The term "chemotaxis" (neurotropism) was introduced, implying a preferential growth of axons towards a distant source of chemical emanation: it was found that axons growing out from cat peroneal nerve found their way to distal nerve segments or grafts, even if these segments were dislocated and at some distance from the proximal part. However, Weiss (1941) felt that this phenomenon was an expression of randomized axonal growth, where some axons strike the peripheral nerve stump by accident, whereas others grow in wrong directions and disappear. Weiss observed that in a medium lacking orientation of the stroma nerve processes follow random courses, whereas oriented interphases could offer "contact guidance." For instance, when nerve fibers regenerated into a blood plasma clot under some tension, the fibers followed the stretch lines, hereby presenting polarized and directed growth. Weiss studied the growth of axons from a severed sciatic nerve of rat introduced into the main stem of a Y-shaped chamber system (transplanted aorta bifurcation from a donor rat). When a piece of nerve or tendon was introduced into the distal outlets, no preferential growth was observed towards the nerve segment when compared with the tendon.

With development of new techniques and refined methods for assessment of axonal growth, opinions have, however, changed. Observations from both in vitro and in vivo experiments indicate that chemotaxis or "neurotropism" may be an important mechanism regulating growth of regenerating axons under

certain circumstances (Charlwood et al. 1972, Ebendal & Jacobsen 1977, Letourneau 1978, Lumsden & Davies 1983). A dramatic example of chemotactic response to NGF in tissue culture was presented by Gundersen & Barrett (1979, 1980) and Gundersen (1985). In these experiments, NGF was applied close to axons from embryonic sensory cells by means of a micropipette. The pipette was moved directionally opposite the initial direction of axonal growth. The presence of NGF was found to dramatically affect the axonal growth; the axons rapidly altered their direction in response to the concentration gradient of NGF.

There are several indications that axons also in vivo grow preferentially towards nerve tissue rather than other types of tissues. Y-chamber systems, made of silicon, have been used to study the growth of axons given a choice to grow towards various types of distal tissues placed in competition with each other (Politis et al. 1982, Ochii 1983, Williams et al. 1984b, Lundborg et al. 1986, MacKinnon et al. 1986). In these experiments, axons grew preferentially towards nerve tissue rather than towards tendon or granulation tissue. The phenomenon was obvious even when millipore filters were placed proximal to the distal nerve piece, indicating that diffusible substances rather than outgrowth of cells formed the base for the attraction of axons.

There are reasons to believe that the Schwann cell proliferation in the distal segment is associated with production of some diffusible substances that may attract axons. It is known that proliferating Schwann cells in a degenerating nerve segment synthesize and release an acidic 37 kDa protein, the production increasing for 2 weeks after injury and thereafter slowly declining (Müller 1981, Skene & Shooter 1983, Skene 1984). Chemical inhibition of protein synthesis in a degenerating distal nerve segment inhibits axonal growth in rat sciatic nerve (Kanje et al. 1986).

Thus, the tissue specificity described above seems well confirmed. An important question is whether such specificity also includes a topographic specificity, i.e., a preferential growth of axons belonging to a certain fiber component of the nerve towards the corresponding parts distally. A phenomenon of this nature was observed by Politis et al. (1982, 1985) and Seckel et al. (1986). These authors introduced cut peroneal stumps in the proximal inlet of the Y-chamber system and peroneal and tibial nerve segments, respectively, in both outlets. The results indicated a preferential growth of peroneal axons to the outlet associated with distal stumps of peroneal rather than tibial nerves. However, with *inversion* of the distal stump graft (so that the proximal nerve segment still was facing an analogous native distal stump, but the transverse surface of *another level*), the frequency and extent of native preference was much less pronounced in these experiments.

The regeneration chamber

The axonal growth in suture gap is regulated and influenced by a pattern of local factors of cellular and biochemical origin. Because axonal misdirection at this level is a major problem, a special model has been designed to address the biological basis for axonal growth and direction and to influence axonal growth by external manipulation. In this model both ends of the cut nerve are enclosed to keep them apart. By extending the microscopic space in a suture gap from microns to several millimeters, the spatial-temporal progress of nerve regeneration can be studied in detail, and the effects of extrinsic factors introduced into the chamber can be assessed.

The original nerve regeneration chambers for experimental purpose were created in a two-stage procedure to constitute a wall made of flattened autologous mesothelial-like cells (Lundborg & Hansson 1979, Lundborg et al. 1981). A solid silicone rod surrounded by a metal spiral was inserted subcutanously into rats. After 3 weeks a flattened monolayer of cells, resembling mesothelial cells, formed a pseudosheath around the rod. After removing the rod a mesothelial-lined tube remained expanded owing to the spiral in the wall. Such a *mesothelial tube* could be designed to any length and diameter hereby satisfying various experimental requirements. These experiments have inspired others to develop tubes of various biological and synthetic materials, such as biodegraded polyester (Seckel et al. 1984, da Silva 1985), collagen membranes (Co-

lin & Donut 1984), polyglactin (Molander et al. 1982). Silicone tubes have proved to be a useful tool for detailed exploration of nerve regenerative mechanisms: tissue fluid accumulates within these chambers around the regenerating nerve structure, making possible sampling of factors occurring in the microenvironment of regenerating axons (Lundborg et al. 1982a-d, Longo et al. 1983a,b, Williams et al. 1983, 1984b).

Following introduction of both stumps of a severed sciatic nerve of rat into a mesothelial or silicone tube, a new structure, macroscopically and microscopically resembling a peripheral nerve trunk, is formed between both nerve ends (Figures 6, 7). The nerve fibers traversing the space are organized into small fascicles, each of them surrounded by a new perineurium. Regeneration occurs as long as the distance between the nerve ends does not exceed 10 mm (Lundborg et al. 1982a, Danielsen et al. 1983). Regeneration does not occur if the distal nerve stump is omitted or if skin or tendon is introduced into the distal opening (Lundborg et al. 1982a, c, Williams et al. 1984b). In successful regeneration the gap between the proximal and distal nerve stumps is initially bridged by a cylindric fibrin matrix that is invaded by Schwann cells, fibroblasts, and capillaries (from proximal and distal nerve stumps) during the second week. The migrating Schwann cells are followed by axons, growing from the proximal stump. Fluid, collected in the chamber, can be sampled and transferred to in vitro systems. This fluid has shown high neuronotrophic activity (Figure 2) directed towards sensory neurons (maximal activity 3 hours after transection of the nerve), as well as sympathetic and spinal motor cells (maximal activity 1 and 3 days, respectively, after transection) (Lundborg et al. 1982, Longo et al. 1983a,b, Varon & Lundborg 1983). By employing immunofluorescence techniques, fibronectin could be demonstrated in the original fibrin stroma after 7 days, whereas laminin was abundant after 14 days parallel to ingrowth of the Schwann cells. Laminin is known to strongly promote the growth of neurites in vitro (Longo 1984).

The results obtained in chamber experiments illustrate cellular and biochemical

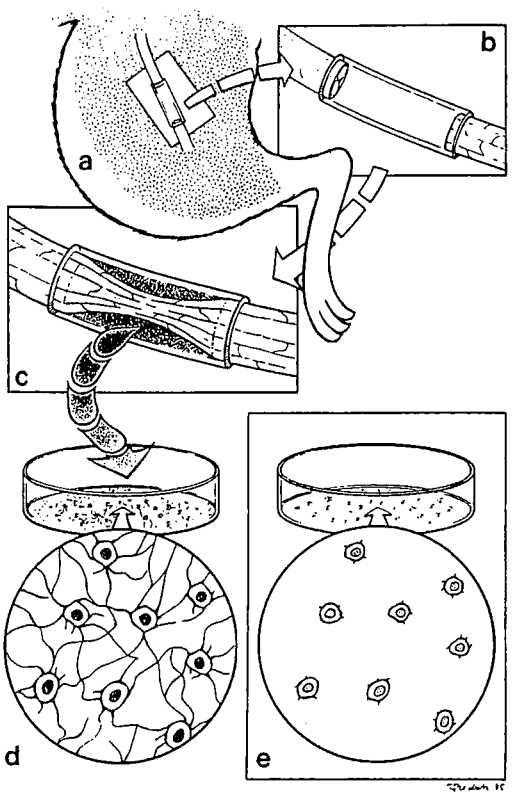

Figure 6. The combined in vivo-in vitro system for nerve regeneration. The ends of a transected rat sciatic nerve are introduced into a silicone tube (a,b); the gap (10 mm) becomes bridged by a new nerve structure (c). The tissue fluid, accumulating in the chamber, is transferred to an in vitro system for sensory, sympathetic, or motor neurons. The fluid contains strong activity for all these types of neurons (d). (e) illustrates a control system without addition of tissue fluid.

events occurring in the gap between cut nerve ends under ideal conditions, but, of course, may represent the pattern of cellular and biochemical events occurring in the microscopic gap between two sutured nerve ends. The fibrin matrix originally formed, containing fibronectin, is not sufficient for axonal elongation, but is adequate for immigration of fibroblasts and Schwann cells from the proximal and distal segments. Presence of Schwann cells is necessary for advancement of axons, possibly because of production of neuronotrophic substances and occurrence of laminin in their basal laminae. The *early* occurrence of neuronotrophic factors in the chamber showing peaks of activity towards *different* types of neurons after *varying* periods of time postoperatively indicate that a complicated pattern of

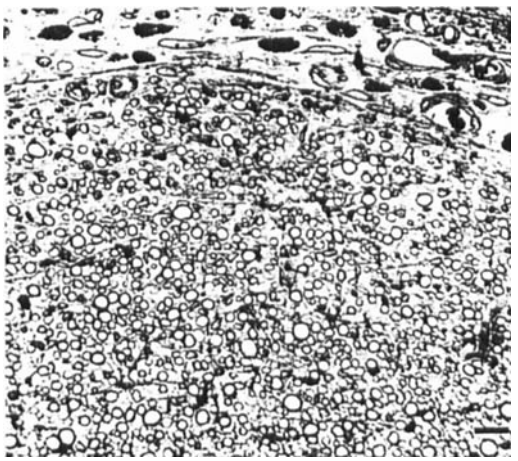

Figure 7. Transverse section showing the histological appearance of the regenerating nerve structure in chamber (see Figure 6). Scale bar = 10 microns. Reproduced with permission from Lundborg & Hansson. *J. Hand Surg.* (1980) 5, 35–38

chemical factors influences the regenerative process. The phenomenon might express an early need for stimulus of the nerve cell-body to prepare for axonal sprouting, whereas the later occurrence of neurite-promoting factors in the stroma might help to support axonal growth.

The regeneration process is, to a large extent, regulated and modified by the *distal nerve segment*. Outgrowth of Schwann cells, as well as chemotactic action of diffusible factors must be considered in this context. In square-formed mesothelial chambers where the proximal and distal stumps of a severed sciatic nerve of rat were introduced into diagonally opposite corners, a directed axonal growth, passing obliquely through the chamber toward the distal nerve segment, has been observed (Lundborg & Hansson 1981). If the distal nerve segment is omitted, axonal growth becomes totally disorganized and occurs only over a few millimeters.

Factors influencing axonal growth

It is well known that nerve repair results in superior function in children as compared with adults (Ønne 1962, Almqvist & Eeg-Olofsson 1970, Almqvist et al. 1983). Probably, this phenomenon is primarily based on better brain plasticity in children, including a superior ability for cerebral adaptation to a new afferent impulse pattern presented by misdirected axons. Another factor is the generally shorter distances axons have to grow in young individuals to reinnervate target organs. In addition, motor axons seem to regenerate at a faster rate in young animals than in adults (Gutmann et al. 1942, Black & Lasek 1979), but such differences in elongation rate were not demonstrated in sensory axons (Gutmann 1942).

Conditioning lesions, i.e., a priming lesion 2 weeks before a testing lesion, contribute to more rapid axonal outgrowth (McQuarrie & Grafstein 1973, McQuarrie et al. 1977, Grafstein & McQuarrie 1978). McQuarrie et al. (1977) demonstrated a 23 per cent increase in outgrowth of the most rapidly growing sensory axons in the sciatic nerve of rat after a conditioning lesion 2 weeks earlier. Acceleration of axonal growth has been reported also after an interval of only 2 days (Forman et al. 1980). The effects of a conditioning lesion is probably attributable to increased protein synthesis in the cell-body, reflected by the cell reaction after injury.

Various *hormones* also influence nerve regeneration. Thriiodothyronine administration was reported to stimulate the protein synthesis in the nerve cell-body (Cook & Kiernan 1976), the rate of axonal outgrowth (Cook & Kiernan 1973), and maturation of regenerating axons (Stelmack & Kiernan 1977, Berenberg et al. 1977). L-thyroxinsodium (T_4) treatment for 3 months increases the ability of axons to pass extended gaps (Danielsen et al. 1986). Also adrenocorticotrophic hormone (ACTH) has been reported to increase the number of regenerating fibers after a crush lesion, although it has not been possible to confirm enhanced outgrowth *rate* by such treatment (Biljsma et al. 1983a,b, Verghese et al. 1982).

Pulsating electromagnetic fields (PEMF) have been reported to stimulate nerve regeneration in certain experimental models. Improved nerve regeneration in limbs treated with PEMF to support bone healing was reported as a side observation by Kort & Basset (1980). Reports in the literature are, however, conflicting. In vitro, there is a preferential neurite outgrowth from nerve explants towards

the cathode of extracellularly applied electric fields (Marsh & Beems 1946, Sisken & Smith 1975, Jaffe & Pow 1979). Patel & Pow (1982) and Sisken & Smith (1975) observed increased survival of neurons under such conditions. Raji & Bowden (1983) reported dramatic effects of PEMF on axonal outgrowth following repair of severed peroneal nerve of rat. Orgel et al. (1983), studying the effects of PEMF on repaired transected peroneal nerve, could not verify such effects. These authors, however, found an increased number of motor neurons that reestablished appropriate connections to the periphery, i.e., improved axonal orientation at the suture site under influence of the field. The results from these two studies could not be immediately compared because the nature, as well as the application, of the field was different.

Inhibition of local scar formation has been tried to improve nerve regeneration. Topical application of triamcinolon-acetate has been reported to improve nerve regeneration in squirrels and monkeys. Graham et al. (1973), and Pleasure et al. (1974) reported that treatment with cis-hydroxyproline resulted in an increased content of myelin sulfatide in regenerating nerves of experimental animals. Penicillamine has not been reported to improve nerve regeneration (Bucko et al. 1981). Nachemson et al. (1986) treated rats systematically with estrogen-progesterone, methylprednisolon-acetate (Depomedrone®) or cis-hydroxiproline after severing the sciatic nerve. No improvement in axonal outgrowth could be found, but the velocity of motor nerve conduction across the suture site was increased in groups treated with cis-hydroxiproline and Depomedrone®.

Gangliosides are normal membrane constituents located in the lipid layer of the plasma membrane. Exogenous gangliosides in vivo seem to promote the *sprouting process*, but no effects on axonal *outgrowth rate* have been reported (Gorio et al. 1980, 1983, 1984, Sparrow & Grafstein 1982).

Cyclic AMP (cAMP) was found to have a stimulatory effect on neurite growth in vitro (Roisen 1972): when cultured dorsal root ganglia from chick embryo were treated with cAMP, there was an increase in both axonal numbers and length. Also, an in vivo stimulatory effect of cAMP was reported by Pichichero et al. (1973), expressed in rapid recovery of reflex function in the hind limb of rats after the sciatic nerve had been crushed. Others have not been able to demonstrate a similar effect (McQuarrie et al. 1977, Black & Lasek 1979). In frogs, forskolin (an activator of adenylatcyclase), used experimentally to produce an increase in neuronal cAMP, was found to induce a 40 per cent increase in outgrowth of sensory axons (Kilner & Carlsen 1984).

There are indications that local treatment with certain hormone-related substances might increase the rate of axonal outgrowth. IGF (somatomedin-C) has been demonstrated in large amounts in Schwann cells of injured nerves close to the lesion site, whereas this substance occurs in normal undamaged nerves (Hansson et al. 1986). Preliminary data indicate that local application of IGF-1, provided by an osmotic pump, may increase the outgrowth rate of the sensory axons in the sciatic nerve of rat following crushing (Kanje et al. 1986). Among other factors reported to influence nerve regeneration positively are treatment with inhibitors of proteolytic enzymes or calcium inactivators to minimize degeneration in axon segments (Hurst et al. 1984), as well as the local use of irrigation fluids imitating axoplasmic composition as much as possible (de Medinaceli et al. 1982, 1983a,b, de Medinaceli & Church 1984, Freed 1985).

Nerve repair – clinical aspects

Nerve repair regularly results in a great deal of axonal misdirection at the suture site. Among various consequences, incorrect peripheral reinnervation leads to a new pattern of sensory impulses in afferent fibers and to a new cortical projection of peripheral cutaneous areas. The brain has to adapt to the new language spoken by the hand. Thus, although the complexity of peripheral factors influencing axonal regeneration is striking, the *central nervous component* of the problem is equally important; sensory reeducation, implying a detailed program for cerebral adaptation to the new situation, represents an important com-

ponent of rehabilitation following peripheral nerve injuries (Dellon 1981).

With respect to the complex biology of nerve regeneration, the role of the surgeon is by necessity limited (Lundborg 1984). However, several factors could be controlled by the surgeon, and the *primary treatment* of nerve injuries is often essential, determining the final function. The management of peripheral nerve lesions is extensively covered in several textbooks and reviews, e.g., Omer & Spinner (1980), Green (1982), and Frykman (1981). In the following discussion, principles for treatment of nerve injuries are reviewed as they may currently be interpreted in biological terms.

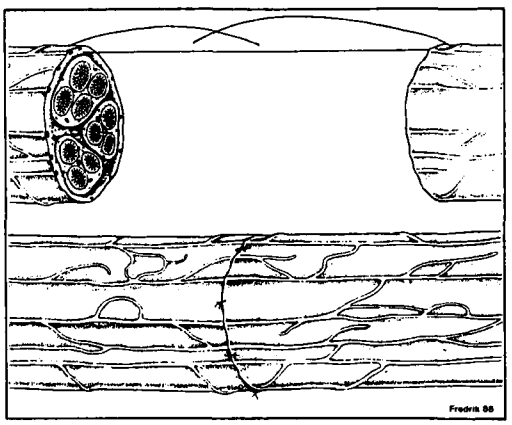

Figure 8. Principles for epineurial suture.

Timing

With reference to the effects of a conditioning lesion, there is a theoretical basis for delayed repair of acute nerve lesions. However, clinical realities favor an immediate, primary repair of a nerve injury. In the initial stage it is usually possible to achieve an exact orientation of the cut nerve ends with the aid of local landmarks, like epineurial vessels and a well-preserved fascicular pattern in the transverse cut surfaces of the nerve. A coaptation of the ends can be achieved without tension. At a later stage, the nerve segments have often retracted; scar tissue at the stumps has to be resected; and it may not be possible to suture the nerve ends without considerable tension. In addition, the distal nerve segment undergoes fibrotic alterations with time, and atrophy of distal targets starts at an early stage. Thus, considering the total situation, the nerve repair should be carried out as a primary procedure.

This conclusion is supported also by experimental and clinical data (Grabb 1968, Grabb et al. 1970, Müller & Grubel 1981). Denervated muscles rapidly atrophy; after 2 years muscle fibers may fragment and disintegrate (Wilgis 1982). A delay of 18–24 months induces irreversible change in the muscle cells with little hope for recovery of motor function. It has been reported that sensory organs are more resistant to denervation than muscles and that ultimate sensory recovery has little correlation with the time between injury and nerve repair (Ønne 1962). However, a poor result can sometimes be explained on the basis of atrophied skin receptors in spite of good reinnervation of digital nerves (Carlstedt et al. 1986).

Suture techniques

Repair of nerves by placing sutures in the epineurium represents a classical method of nerve repair (Edshage 1964, Moberg 1964, Wilgis 1982). The epineurial suture technique (Figure 7) is relatively atraumatic, and easy to perform, but it does not ensure correct matching of the fascicular internal structures of the nerve. Edshage (1964) demonstrated that an apparantly perfect epineurial suture could be associated with considerable malalignment and displacement of fascicles. With the development of microsurgical techniques, the concept of fascicular repair, or more accurately *group* fascicular repair (Figure 8), was introduced to achieve an optimal orientation and adaptation of fascicles or groups of fascicles (Smith 1964, Bora 1967, Hakstian 1968, 1973, Grabb et al. 1970, Millesi 1973, 1981, Yamamoto 1974, Bora et al. 1976, Cabaud et al. 1976, 1980, Ito et al. 1976, Orgel & Terzis 1977, Terzis 1979, Ikeda 1980, Tupper 1980, Kline et al. 1981, Kutz et al. 1981). With this technique, fascicular groups are dissected under high magnification; epineurial tissue is resected over a short distance; and corresponding fascicular structures in both nerve ends are sutured individually to achieve matching of cor-

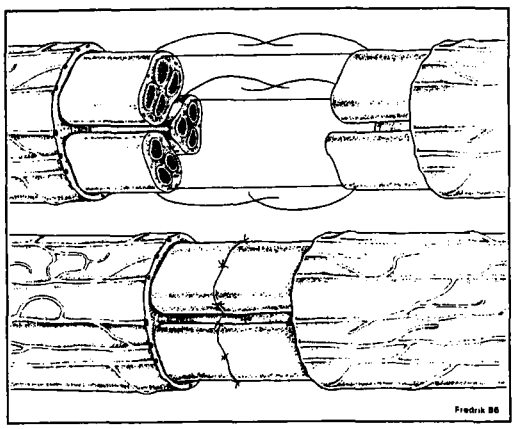

Figure 9. Principles for group fascicular repair of a severed peripheral nerve. Groups of fascicles in both stumps are isolated and sutured individually.

responding nerve elements (Figure 9). The fascicular pattern in both nerve ends should be inspected under the microscope to enable coaptation of the corresponding structures. Stitches are placed in the connective tissue immediately outside the perineurial lamellae. Fascicular sutures do not resist any tension and could usually be carried out only as a primary procedure. The advantage is improved matching of corresponding fascicular components. However, the procedure includes a considerable amount of surgical dissection with consequent potential risks in terms of microbleedings, edema, and scarring.

Epineurial vs. perineurial suture techniques

Attempts have been made to experimentally assess whether perineurial suture techniques have any advantages over epineurial techniques or vice versa. No consistant superiority of one technique over the other has been found in these studies (Wise 1969, Grabb 1970, Yamamoto 1974, Bora et al. 1976, Cabaud 1976, 1980, Kline et al. 1981). However, improved specificity of muscle reinnervation after fascicular suture of rat sciatic nerve has been demonstrated (Brushart et al. 1981, 1983). These authors analyzed the retrograde transport of horseradish peroxidase (HRP) in the nerve to study the location of motor neurons of peroneal and tibial muscles in the spinal cord. Changes in normal nerve cell distribution reflected peripheral axonal misdirection after suture. It was found that such inappropriate reinnervation of a peroneal muscle by tibial motor neurons was minimized by individual fascicular sutures.

Clinically, the indications for performing epineurial sutures and fascicular sutures, respectively, have been much debated. It is generally agreed that each case should be judged individually, and that there is a place for both techniques in selected situations. In general, a clean-cut, fresh nerve injury is best treated by a simple epineurial suture, especially at levels where fascicles are closely packed with minimal interfascicular epineurial tissue. However, if the transectional area of the nerve is dominated by epineurial tissue, epineurial tissue should be resected and fascicular groups adapted by individual stitches. At proximal levels of an extremity, separate fascicles contain a mixture of fibers belonging to several peripheral branches. In this situation exact coaptation of separate fascicular groups would have no advantages over an epineurial suture. However, at more distal levels, branches representing unique functions are well defined in separate fascicles within the main trunk. In these situations a microsurgical approach might be motivated, especially regarding the median and ulnar nerves at the wrist level, where motor and sensory components are separated and well defined.

Nerve grafting

Experimental and clinical data indicate that *tension* at a suture line increases scar tissue formation and prohibits axonal regeneration (Millesi et al. 1972, 1976, Miyamuto 1981, Miyamuto & Tsuge 1981, Millesi & Meissl 1981). Even slight tension might considerably compromise intraneural microcirculation (Lundborg & Rydevik 1973, Lundborg 1975, Miyamuto 1981, Miyamuto & Tsuge 1981). Accordingly, interposition of a nerve graft has been proposed as the method of choice when nerve suture is not possible to perform without undue tension (Millesi et al. 1972, 1976, 1980, 1981, 1984). There is a great deal of disagreement regarding the critical length of a defect

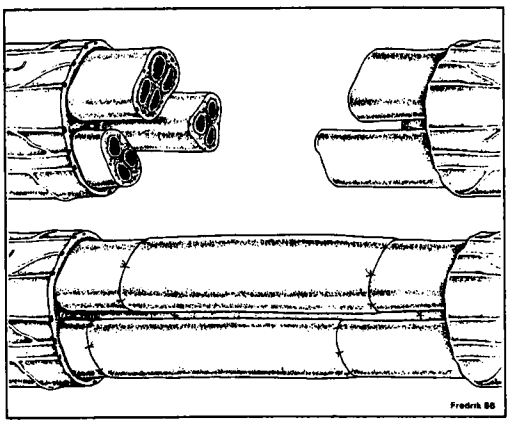

Figure 10. Principles for nerve grafting. Groups of fascicles in both stumps are isolated and severed at different levels. The gap between individual fascicular groups is bridged by a nerve graft.

that indicates the use of a nerve graft instead of direct suture under slight tension: at a panel discussion involving a number of experts of the field, opinions varied from 1.5 to 7 cm (Millesi 1977). A nerve graft serves as a useful mechanical guideline for advancing axons, but also provides an optimal microenvironment constituted by the columns of Schwann cells (Büngner bands). It has been demonstrated by isotope techniques that most transplanted Schwann cells in a thin graft survive, multiply and form Büngner bands (Aguayo et al. 1976, 1979). Initially, the graft survives by diffusion from surrounding tissues. Revascularization starts on the third postoperative day (Almgren 1974). Thick grafts might have difficulties in surviving during the first few days because the diffusion distances are too long.

The technical details of nerve grafting (Figure 9) have been described in many reviews (Millesi et al. 1972, 1976, 1981a-c, 1984, Wilgis 1982). Separate fascicular groups in both nerve stumps are dissected and isolated (Figure 10). The epineurium is excised and the fascicular groups are cut at different levels. The transectional surfaces are studied under high magnification and corresponding fascicular groups in both ends are identified. Nerve grafts are interposed between corresponding groups and secured in place by sutures or fibrin glue (Kuderna 1985). The sural nerve is commonly used as a graft because of its accessibility and appropriate thickness. Other suitable choices are the lateral or medial antebrachial cutaneous nerves (MacFarlane & Myers 1976), and in more rare instances the superficial radial or lateral femoral cutaneous nerves.

The use of free vascularized nerve grafts have been suggested in selected situations where the recipient bed is heavily scarred and nonvascularized nerve grafts will not become optimally vascularized. Improved regeneration rate and axonal maturation have been reported in vascularized nerve grafts in rats as compared with free nonvascularized nerve grafts (Kushima et al. 1981). The clinical use of vascularized nerve grafts has been discussed by, among others, Taylor & Ham (1976), Breidenbach & Terzis (1984), Bonney et al. (1984), Rose & Kowalski (1985). Among possible advantages of vascularized nerve grafts that are used in a scarred recipient bed are the ability of these grafts to act as vascular carriers of nonvascularized nerve grafts (Breidenbach & Terzis 1984).

Appropriate matching of corresponding fascicles in the cut ends of a nerve is possible only if the pattern of the fascicular groups is identical in the respective surfaces. With a sharp transection this is not a problem. However, the intraneural fascicular topography rapidly changes from one level to another along the course of a peripheral nerve trunk (Sunderland 1945, 1978), and if there is a loss of nerve substance over some length, exact matching of corresponding fascicular groups may be difficult or impossible to perform. Sunderland found the longest section of a nerve with a constant fascicular pattern to be 50 mm, although considerable changes in topography in some instances were found over only 0.25 mm.

Sunderland's findings have been confirmed by others (Jabaley et al. 1980), although it appears that the fascicular topography in certain instances is more constant than has previously been realized; changes in fascicular topography might express random wandering of epineurial septa rather than true changes in positions of fibers. The concept that functional units of fibers remain in constant individual sectors of the nerve over long distances has been confirmed in axonal transport studies. Brushart (1985) mapped the location of digital

nerve axons within the median nerve of primates from the wrist to the brachial plexus by studying the retrograde intraaxonal transport of horseradish peroxidase (HRP). It was found that axons could be traced over long distances within the same quadrant of the nerves. Shady et al. (1980) used intraneural microstimulation techniques combined with the localizing ability of the brain to identify cutaneous projections of individual fascicles at various levels along the course of human peripheral nerves in the arm. Most rearrangements of the spinal root fibers into terminal nerve branch groupings occurred proximally at plexus level, whereas minimal additional regrouping occurred in more distal portions of the nerve trunks. Together, these findings indicate that individual nerve fibers remain within the same sector of nerve trunks over long distances, and that grafting with the attempt to restore continuity between *corresponding sectors* at different levels therefore, is justified and a well-motivated procedure.

Identification of sensory and motor fascicles

When adaptation of fascicular groups are performed, peroperative identification of motor and sensory fascicles, respectively, in the proximal and distal stumps of the severed nerve would facilitate correct orientation and matching of corresponding fascicular groups. Intraoperative neurophysiologic stimulation and recording techniques have been used for this purpose (Hakstian 1968, Vandeput et al. 1969, Grabb et al. 1970, Terzis & Strauss 1978, Terzis et al. 1980), but require special expertise and equipment. The principle is based upon stimulation of fascicles of the distal and proximal nerve segments. Fascicles of the distal stumps are tagged as sensory if they do not give a motor response when stimulated. A central sensory response is induced on a conscious and cooperative patient when sensory fascicles are stimulated in a proximal segment, whereas no similar response occurs if a proximal motor fascicle is stimulated. Because nerve fibers ceased to conduct within 72 hours after injury (Sunderland 1979), the test cannot be performed after this time.

Histochemical staining techniques have been used by some authors to differentiate between motor and sensory fibers. The occurrence of acetylcholineesterase in motor fibers can be demonstrated by such techniques (Freiliger et al. 1975a,b, Gruber et al. 1976, Engel et al. 1979). The principle has, however, limited use because samples from the nerve ends must be removed within 22 hours after injury, and 25–30 hours incubation is required for the staining to occur. Carbonic anhydrase activity in sensory neurons has been used as a possible means of distinguishing them from motor axons: staining of the axoplasm was reported as being a predominant feature in sensory fibers, whereas staining of the myelin sheath characterized motor axons (Riley & Lang 1984), the staining procedure being completed within 3–4 hours after receiving the tissue.

The use of histochemical staining for differentiating motor fascicles from sensory fascicles might have limited value because so-called motor nerves contains a large number of afferent fibers: the deep motor branch of the ulnar nerve of monkeys contains over 60 per cent sensory fibers from deep-tissue structures (Terzis & Dykes 1977).

Sensory reeducation

Because of axonal misdirection at the suture sites, peripheral cutaneous receptor fields generally project into new cortical areas following nerve repair. A new pattern of impulses in afferent fibers forces the brain to adapt to the new situation if discriminative sensibility is to be restored. This is not much of a problem in children, but in adults the difficulties are apparent, corresponding to difficulties in learning to speak a new language without a foreign accent. Sensory reeducation – i.e., a specialized rehabilitation program with the purpose of learning to recognize the shape and form of items and surface structures – has proved to considerably facilitate recovery of useful sensibility in the hand (Dellon 1981). The documented effects of sensory reeducation emphasizes the total perspective of nerve injuries: the problems involve the peripheral, as well as the central nervous system.

Concluding remarks

Healing of nerve injuries is a complicated process, regulated and influenced by local and general factors that are not fully known. Axonal misdirection at the suture site is a major problem because inappropriately innervated cutaneous receptor fields will project into new cortical areas. In optimal experimental situations, axonal growth is influenced by the distal nerve segment, possibly by a combination of cellular and chemotactic mechanisms. There is strong evidence that the Schwann cells of the distal segment synthesize factors that stimulate axonal growth and influence axonal directionality. Microsurgical techniques have contributed considerably to improved results in selective situations; but in the overall perspective, factors operating in the microenvironment probably play a key role for the end result. Because surgical techniques have already been optimally refined, future development of improved methods for nerve repair must include a further analysis of microenvironmental factors regulating axonal growth and orientation.

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