Pathology of Peripheral Nerves

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Preface

The purpose of writing this book is to present a synopsis of established ideas and recent progress in peripheral nerve pathology. We have also attempted to make the book into a practical manual for pathologists, neurobiologists and neurologists who wish to examine biopsy or autopsy specimens of nerve from patients or experimental animals.

Chapter 1 is a brief historical outline of the development of ideas about the structure, function and pathology of peripheral nerves. Many of the names of workers mentioned in this chapter have survived as eponymous terms. The second chapter is concerned with techniques of nerve biopsy and histological preparation; we have attempted to fit the methodology to the tissue available and to the information required. In addition to the basic methods of tissue preparation and staining, there is a section illustrating the common histological artefacts seen in peripheral nerves. Chapter 3 deals with the anatomical, histological, ultrastructural and physiological aspects of normal peripheral nerves. This is followed by an account, in Chapter 4, of the general pathological reactions of peripheral nerves. It is upon an analysis of general pathological features that many of the pathological diagnoses are made. The difficult field of clinico-pathological correlation in peripheral neuropathies is approached, in Chapter 5, mainly from the pathologist's point of view. However, an attempt is made to equate the histological and ultrastructural changes seen in specific peripheral nerve diseases with the symptomatology and electrophysiological changes. The final chapter is devoted to the histology and ultrastructure of peripheral nerve tumours and their histogenesis. The electron microscope has played a significant role in clarifying the nosology of peripheral nerve tumours, and this is one of the points that is stressed in Chapter 6.

An Appendix provides a brief guide to the examination of peripheral nerve biopsies and a summary of the main pathological features in the major peripheral nerve diseases.

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R.O.W.
J.C.-N.

Contents

1

Historical Review

Elucidation of the structure, function and pathology of peripheral nerves has proceeded in a series of steps, usually associated with advances in histological and physiological techniques. Many of the workers who made these discoveries have given their names to different structures in the peripheral nerve. Although eponymous terms are decreasing in number, many are still retained and they do appear extensively in the older literature. It seems appropriate, therefore, to give a brief account of the development of ideas about the peripheral nerve.

The gross appearance of nerve trunks was well recognized by early anatomists but a whole new field of study was opened up by the introduction of the microscope. Leeuwenhoek (1632–1723) is credited with the first description of single myelinated nerve fibres (Ranvier, 1878). In his Opera II he described nerve tubes full of liquid. The more detailed structural studies of peripheral nerves did not bear fruit, however, until the 1830s. At this time Johannes Müller had three memorable figures among his pupils in Berlin: Jacob Henle, Theodore Schwann and Robert Remak. The last two were intimately connected with the original descriptions of cellular components of peripheral nerves. Conditions of study were, apparently, far from ideal, as there were few microscopes available; Remak describes how he would pray for sunshine during the short winter days in Berlin as microscopy was not possible on dull, cloudy days (Kisch, 1954). In 1838 Remak published his thesis entitled 'Observationes anatomicae et microscopicae de systematis nervosi structura' in which he described non-myelinated nerve fibres (fibriae organicae) and the cells (Remak cells) closely associated with them. During his dissections he noticed that the myelinated fibres (tubuli primitivi) each contained 'fibra primitiva'; this structure was subsequently renamed the axis cylinder by Purkinje in 1839 (Causey, 1960). Remak's other important observation was that non-myelinated fibres arose from ganglion cells. He also noticed that cephalopods, including squids, have huge non-myelinated axons (1 mm diameter); these axons have subsequently been used in experiments on the physiology of nerve conduction (Hodgkin, 1958).

The year after Remak's thesis, Schwann (1839) published a treatise on the

microscopical structure of plant and animal cells. In this work he describes how '... each nerve fibre is, throughout its entire course, a secondary cell, developed by the coalescence of primary nucleated cells'. (Translated by Henry Smith, 1847.) Schwann considered that the long chains of nucleated cells, now called Schwann cells, coalesced to form a syncytium with the formation of a continuous band of protoplasm down the centre. This polygenist concept whereby the axon was formed by the fusion of a series of separate short lengths was not shared by Remak, but it still received support until the publication of Cajal's classical studies in 1913. Schwann also observed that the nuclei of his primary cells were abundant in young developing nerve fibres but were only occasionally seen along the lengths of fully developed myelinated fibres.

In parallel with the investigation of normal nerve fibres, the effects of nerve injury were also being studied. Although Burdach (1837) observed no alteration in peripheral nerve structure 1 week after ligation, Steinbruck (1838) recorded that the nerve became more slender and atrophic. Loss of irritability and fragmentation of fibres following nerve section were recorded by Guenther and Schoen (1840). Similarly, fragmentation of the degenerating nerve fibres was observed by Nasse (1839). It was with this background of early work in Germany that Augustus Waller (1850), working in London, followed the sequence of axonal (Wallerian) degeneration in the severed glossopharyngeal and hypoglossal nerves of the frog. Having found that cutting the nerves of both sides results in the animal's death, he cut only one side and was able to observe decreased power and sensation on the affected side of the tongue. During the subsequent 12–15 days he observed the breakdown of the medullary (myelin) sheath; he described not only the granular appearance under the microscope but also the change in response of the nerve fibre components to distilled water, alkalis and ether. Two years later Waller (1852) made some further fundamental discoveries. He observed the regeneration of glossopharyngeal nerve fibres into the tongue 3–4 months after section. Furthermore, he showed that when a spinal sensory nerve is cut below the ganglion, the degeneration is not carried back to the ganglion. If, however, the ganglion itself is extirpated, the nerve degenerates. These observations supported Remak's thesis that axonal processes arose from neurones.

Although Waller (1852) considered that the nerve elements disappeared totally after degeneration, Remak (1862) observed that very thin regenerating nerves grow into old Schwann tubes which still contained degenerating myelin debris. Much of the early work on peripheral nerve was performed on unstained specimens but, about 1870, Max Schultze introduced osmic acid into histology. A 1 per cent solution of osmium tetroxide (osmic acid) is colourless but it reacts with myelin to form a black osmium compound. Using this technique to stain sections of nerve and individual teased fibres, Ranvier (1878) was able to study peripheral nerves in greater detail. Much of the two volumes of his book *Leçons sur l'histologie du Système Nerveux* is devoted to the structure of peripheral nerves. Probably Ranvier's most noteworthy contribution was his description of regular constrictions or discontinuities in the myelin sheath along the length of the fibre (*Figure 1*). Thus, he showed how the myelin is divided into segments, whereas the axon is continuous. The constrictions or 'étranglements annulaires' are now known as nodes of Ranvier and represent the short gap between segments of the myelin sheath formed by

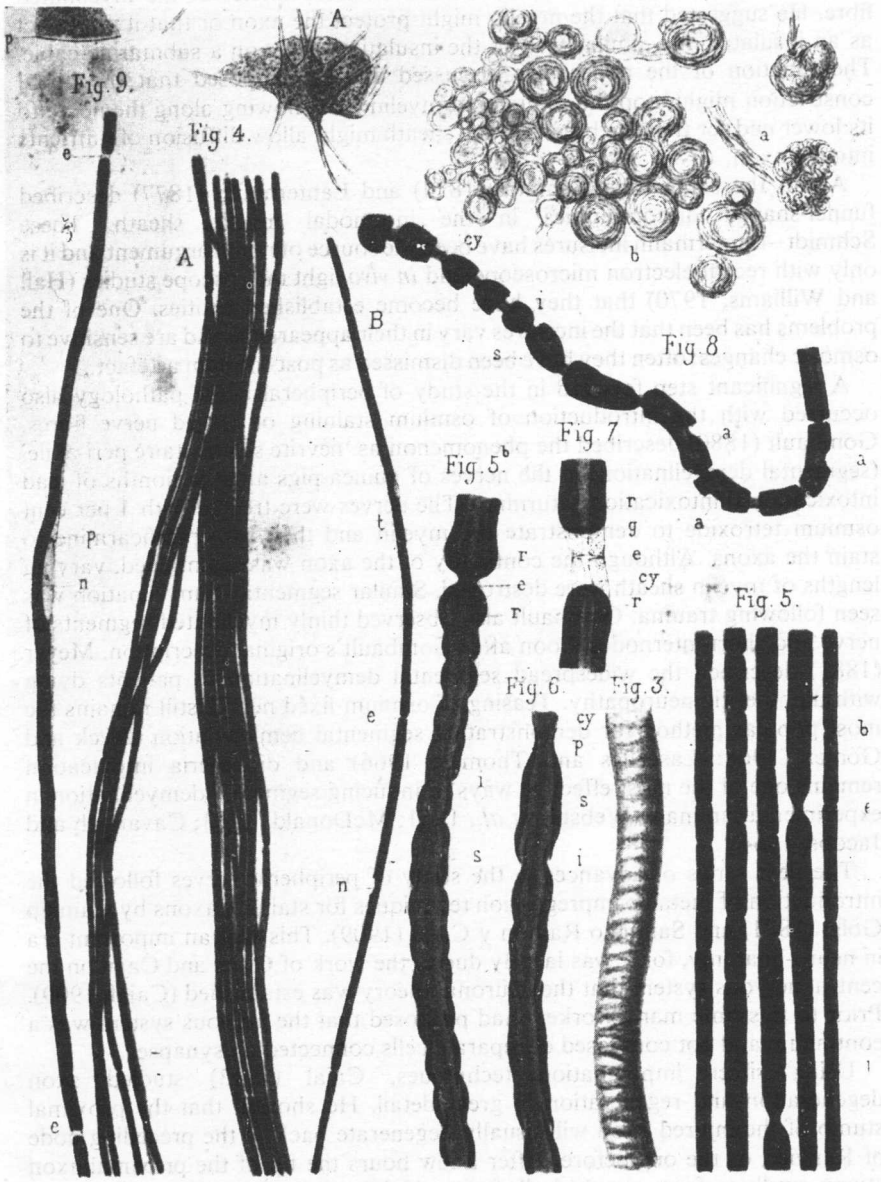

Fig. 1. An illustration from Ranvier's Leçons sur l'histologie du Système Nerveux *(1878), showing 'nodes of Ranvier' (e)*

consecutive Schwann cells. Ranvier also noted that the distance between each node (internodal length) was proportional to the diameter of the myelinated fibre. He suggested that the myelin might protect the axon or that it might act as an insulator in a similar way to the insulating cover on a submarine cable. The function of the nodes was discussed and he proposed that the nodal constriction might stop the semi-liquid myelin from flowing along the nerve to its lower end, or that the breaks in the sheath might allow diffusion of nutrients into the axon.

About the same time, Schmidt (1874) and Lantermann (1877) described funnel-shaped discontinuities in the internodal myelin sheath. These Schmidt–Lantermann incisures have been the source of much argument and it is only with recent electron microscope and *in vivo* light microscope studies (Hall and Williams, 1970) that they have become established entities. One of the problems has been that the incisures vary in their appearance and are sensitive to osmotic changes; often they have been dismissed as post-mortem artefact.

A significant step forward in the study of peripheral nerve pathology also occurred with the introduction of osmium staining of teased nerve fibres. Gombault (1880) described the phenomenon as 'névrite segmentaire péri-axile' (segmental demyelination) in the nerves of guinea-pigs after 8 months of lead intoxication (l'intoxication saturnine). The nerves were treated with 1 per cent osmium tetroxide to demonstrate the myelin and then with picrocarmine to stain the axons. Although the continuity of the axon was maintained, varying lengths of myelin sheath were destroyed. Similar segmental demyelination was seen following trauma. Gombault also observed thinly myelinated segments of nerve and short internodes. Soon after Gombault's original description, Meyer (1881) described the widespread segmental demyelination in patients dying with diphtheritic neuropathy. Teasing of osmium-fixed nerves still remains the most popular method for demonstrating segmental demyelination (Dyck and Gomez, 1968; Lascelles and Thomas, 1966) and diphtheria intoxication remains one of the most effective ways of inducing segmental demyelination in experimental animals (Webster *et al.*, 1961; McDonald, 1963; Cavanagh and Jacobs, 1964).

The next series of advances in the study of peripheral nerves followed the introduction of metallic impregnation techniques for staining axons by Camillo Golgi (1881) and Santiago Ramon y Cajal (1909). This was an important era in neuro-anatomy, for it was largely due to the work of Golgi and Cajal on the central nervous system that the neuronal theory was established (Cajal, 1909). Prior to this time many workers had proposed that the nervous system was a continuum and not composed of separate cells connected by synapses.

Using silver impregnation techniques, Cajal (1913) studied axon degeneration and regeneration in great detail. He showed that the proximal stump of the injured axon will usually degenerate back to the preceding node of Ranvier, or the one before. After a few hours the tip of the proximal axon stump swells to form an end bulb from which axons will sprout on about the second day. Meanwhile the axons and myelin of the distal stump have started to fragment and the debris lies mainly in the Schwann cells. Büngner (1891) described how the Schwann cells in the distal stump form rows along the nerve (Zellbänder, or bands of Büngner). However, Büngner belonged to the 'polygenist' school and proposed that, during regeneration, the axoplasm appeared in the centre of each Schwann cell and finally fused to form a

continuous axon. Cajal was able to show that each axon regenerated by growing from the proximal stump along the bands of Büngner and retained its continuity with the neuronal cell body throughout.

The induction of chromatolysis in the neurone cell body by axon section had been observed by Nissl in 1892. He evulsed the facial nerve from the stylomastoid foramen and then stained sections of the facial nucleus with cresyl violet. Some days after the axotomy the motor neurones swelled, the nuclei became eccentric and the staining intensity of the cytoplasmic 'Nissl substance' decreased.

During the last 30–40 years there has been a parallel acquisition of information about the structure and function of peripheral nerves using several techniques. Schmidt and Bear (1939) really opened this modern era with their X-ray diffraction and polarized light studies of myelin. They reached the conclusion that myelin is composed of concentric sheets of protein interspersed with layers of lipoid so as to form structures which are repeated periodically in a radial direction. This structure is basically a stack of membranes with the bimolecular lipid form proposed by Gerter and Grendell in 1926, and Davson and Danielli (1943). Subsequent electron microscope studies of fresh myelin (Fernández-Moran, 1950) have confirmed its lamellar structure. Molecular models for myelin were proposed by Finean (1953) on the basis of X-ray diffraction and electron microscope data. He arranged the polar phospholipids as bimolecular leaflets with the polar ends of the molecules associated with hydrophilic protein layer. Cholesterol was incorporated into the hydrophobic part of the structure. Variations on this model have been put forward by Vanderheuvel (1965).

As with most other tissues, electron microscope studies have added an immense amount of detailed information about the structure of peripheral nerves. Geren (1954) showed, in developing chick nerves, that the myelin sheath is formed from the compaction of layers of Schwann cell membrane arranged in a spiral around the axon. Fine-structural studies of permanganate-fixed material led Robertson (1961) to formulate a 'unit membrane' theory. He found that cell membranes exhibited a trilaminar structure, the outer layers of which fused during the compaction of a myelin sheath. Gradually the complex structural characteristics of the node of Ranvier and paranodal regions emerged (Elvin, 1961; Landon and Williams, 1963; Williams and Landon, 1963). Following the introduction of glutaraldehyde as a primary fixative (Sabatini, Bensch and Barrnett, 1963), the structure of the axon and its high content of microtubules and filaments could be more adequately studied.

Advances in peripheral nerve physiology occurred in various fields. Weiss and Hiscoe (1948) observed that axons became swollen proximal to the compression of the nerve by an arterial sleeve and proposed that axoplasm flowed from the cell body of the neurone to the distal end of the nerve. The introduction of radioactive isotope techniques and histochemically or ultrastructurally identifiable markers (Barondes, 1967) has led to a better understanding of the rates of axoplasmic transport and the mechanisms involved (Ochs, 1972; Kristensson and Olsson, 1973).

There have also been major advances in the electrophysiological investigation of isolated nerve fibres and of intact nerve trunks in the last 30 years. Young (1936) rediscovered the very large non-myelinated nerve fibres in cephalopods. The giant axon of the squid can be emptied of cytoplasm so that

both the inside and the outside of the limiting membrane can be studied. In this way, the mechanisms involved in membrane depolarization and impulse propagation could be investigated (Hodgkin, 1958). The basic differences between the continuous passage of an impulse along the non-myelinated nerve and the saltatory conduction of myelinated fibres was emphasized by Huxley and Stämpfli (1949) in their studies on isolated frog sciatic nerve.

The introduction of techniques for measuring nerve conduction in intact animals and human patients was a major advance in the study of normal physiology and peripheral nerve diseases. In the 1930s electromyography involved the recording of electrical activity in resting and active muscles (Gilliatt, 1966). Hodes, Larrabee and German (1948), however, introduced techniques for measuring nerve conduction. They measured conduction velocities in motor nerves by stimulating various peripheral nerves and recording the response with electrodes placed over the relevant muscles. Subsequently Dawson and Scott (1949) measured conduction velocities in sensory nerves with electrodes placed on the skin. It was soon found that conduction velocities were reduced in regenerating nerves following injury (Hodes *et al.*, 1948), in polyneuropathies (Henriksen, 1956) and in nerve compression (Simpson, 1956); the most dramatic slowing, however, is associated with segmental demyelination (McDonald, 1963). These findings added impetus to the renewed interest in the study of teased fibres, where the relationship between internodal lengths and fibre diameters was established in developing and regenerating nerve fibres (Vizoso and Young, 1948; Thomas and Young, 1949; Lascelles and Thomas, 1966). Many neuropathies are now studied by combined clinical electrophysiology and peripheral nerve biopsy. Techniques have been developed also for the measurement of conduction velocities and compound action potentials in isolated lengths of nerve biopsy which are subsequently studied histologically (Dyck, Lambert and Nichols, 1971).

Sophistication of biochemical and histochemical techniques for lipids and proteins (Adams, 1965; Davison and Peters, 1970) during the last few decades has led to a more complete understanding of the metabolism of normal and diseased peripheral nerves. Similarly, the advances in the pharmacology of neurotransmitters and the physiology of neuromuscular transmission are too many to enumerate individually. These fields have benefited greatly from the introduction of intracellular electrode and iontophoretic techniques whereby minute quantities of pharmacologically active agents or radioactive material can be administered at physiologically active sites (Globus, Lux and Schubert, 1968).

No attempt has been made in this chapter to give an exhaustive history of the development of ideas about the nerve fibre. Opinions differ about what is important in the growth of knowledge, just as many of the discoveries recounted here took many years to gain acceptance. The historical background to various structures, functions and disease processes discussed in the rest of the book will not be emphasized, as more prominence will be given to modern concepts and recent developments.

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2

Techniques of Peripheral Nerve Biopsy and Histological Preparation

INTRODUCTION

There are three main sources of peripheral nerve tissue usually available to the pathologist: elective biopsies from patients, peripheral nerve material obtained at autopsy and specimens from experimental animals. All sources have their advantages but should be approached differently if the maximum amount of information is to be obtained from the tissue. Patients with peripheral neuropathies should be thoroughly investigated clinically before a biopsy is done so that any special techniques or fixatives that are required can be prepared. Great care must be taken with the biopsy technique, as the nerve can be easily damaged by rough handling either by the surgeon or by the pathologist. The introduction of artefact in this way has been responsible in the past for several misleading reports of histological changes in peripheral neuropathies. If possible, part of each nerve biopsy should be embedded in epoxy resin for the preparation of 1 μm sections and electron microscopy. Other parts of the biopsy can be preserved for teasing and paraffin embedding. Biopsy material is ideal for enzyme and lipid studies, by both biochemical and histochemical techniques. Although the amount of tissue available at autopsy is usually very much greater than that obtained at biopsy, the methods suitable for studying autopsy tissue are limited. What this means in practical terms is that fine structural details of peripheral nerves and labile biochemical processes must be studied in biopsy material, whereas the distribution throughout the body of histologically recognizable peripheral nerve damage can be documented in autopsy material. For example, the cytological identification of the cells forming the 'onion-bulb' whorls in hypertrophic neuropathy was only possible by the electron microscopical study of biopsy material. The distribution of vascular lesions in peripheral nerves, however, is only possible

when long lengths of nerve are examined at autopsy (Dyck, Conn and Okazaki, 1972).

Some neuropathies only affect motor nerves or nerve roots; it is therefore not possible to study the changes extensively in biopsy specimens. A few successful post-mortem studies have been carried out at electron microscope level, but usually the tissue preservation is far from ideal and the findings may be misleading.

Study of the basic pathological processes in peripheral nerves is done most satisfactorily in experimental animals. The time intervals between the pathological insult and the study of the results can be strictly regulated. Ideal fixation may be attained by whole body perfusion with either buffered formalin or Susa fixative for light microscopy, or with glutaraldehyde for electron microscopy. If material is required for biochemical estimations, it can be obtained in a very fresh state. By correlating the information obtained from human biopsy and autopsy material with findings in experimental animals, a more complete picture of the pathology of peripheral neuropathies has emerged.

TECHNIQUES OF PERIPHERAL NERVE BIOPSY

Choice of nerve

The most suitable nerves for biopsy are those which are moderately involved in the neuropathy; nerves that are severely involved may be too damaged to reveal useful information about the original disease process. Ideally, the nerve should be either purely motor or purely sensory; it should be constant in its anatomical site and easily accessible so that, if necessary, a 4–5 cm length of nerve may be obtained. Some nerves are often damaged by entrapment—for example, the ulnar, median and lateral popliteal nerves—and they should be avoided if the purpose of the biopsy is to study a polyneuropathy. Electrophysiological studies should be carried out before the biopsy, and it is very useful to have physiological and histological data on the same nerve.

Impairment of neurological function in peripheral neuropathies is usually more pronounced in the distal nerves in the lower extremities. For this reason together with those mentioned above, the sural (sensory) and the deep peroneal (motor) nerves are often chosen for biopsy. The sural nerve is more commonly used, as its removal causes only minor sensory loss and paraesthesiae over the lateral side of the foot; these symptoms usually disappear after a few months (Dyck and Lofgren, 1966). Biopsies of motor nerves may be difficult to justify, as the patient may be left with a permanent weakness of the denervated muscle. A more practical solution is to biopsy a muscle in its motor end-plate region and to examine the intramuscular nerves. An estimate of the extent of denervation can also be obtained from the muscle biopsy. Other nerves are accessible to biopsy; for example, the occipital nerve and the lateral cutaneous nerve of the forearm. One of the disadvantages of biopsies from these less usual sites is that control data are not as readily available. The sural nerve, on the other hand, is well documented both in normal individuals at varying ages

(Lascelles and Thomas, 1966; Dyck *et al.*, 1968; Ochoa and Mair, 1969) and in a variety of pathological conditions.

Technique of nerve biopsy

Unfixed peripheral nerves are very susceptible to damage produced by crushing during excision; myelin sheaths are particularly vulnerable, as they are almost liquid in consistency in the unfixed state. Extreme care must, therefore, be taken by the surgeon during the removal of the nerve if artefact is to be avoided. For this reason peripheral nerve biopsies should be performed by an experienced surgeon after full consultation with the pathologist and preferably in his presence.

The sural nerve contains sensory fibres which supply the skin of the lateral side of the foot and heel; it passes behind and below the lateral malleolus, where it breaks up into several cutaneous branches. The patient lies prone on the operating table with the ankle supported and slightly everted. A 5–6 cm incision is made under local anaesthesia in the furrow just in front of the tendo achilles ending just above and behind the lateral malleolus. Bleeding points should be tied and no diathermy should be used. By careful sharp dissection the lesser saphenous vein is exposed deep to the deep fascia. The sural nerve at this point is a bundle of fascicles tightly bound together in an elliptical white nerve trunk 2–3 mm in diameter. It is usually behind and deep to the lesser saphenous vein and bound to the vein by loose connective tissue. Branches of the vein crossing the nerve should be divided and the connective tissue between the vein and the nerve carefully incised. As little adipose tissue as possible should be left attached to the nerve, but vigorous cleaning or even touching the nerve should be avoided completely. It is important that haemostasis should be rigorously maintained by clipping and tying even small vessels, as dabbing the nerve with a swab produces crush artefact. The whole width of the nerve trunk may be taken as a biopsy or just part of the nerve. In this latter technique of fascicular biopsy (Dyck and Lofgren, 1966) only part of the width of the nerve is cut across and this is gently separated by sharp dissection from the main trunk of the nerve. Fascicular nerve biopsy reduces the degree of neurological deficit and is suitable for most purposes, but it may not be possible to examine the epineurial arteries adequately by this technique.

For most purposes of histological investigation a 3–4 cm length of nerve is sufficient. If conduction studies are performed on isolated nerve segments, however, 5–8 cm is required (Dyck and Lofgren, 1966).

Directly the nerve has been excised, it should be handed with the forceps directly to the histologist and placed on dental wax. If the nerve is placed on gauze, it will stick firmly and damage will occur as it is picked free. It is useful at this time to note whether the nerve is a normal white colour or whether it is thin and grey with the tough texture of long-standing axonal degeneration. The thickened nerves from a patient with hypertrophic neuropathy may have a distinctive grey and gelatinous appearance. The nerve is gently stretched on a piece of dry card so that the minute transverse ridges in the epineurium disappear; then the very ends of the nerve are pressed on to the card so that they adhere. In this way the nerve is kept straight during fixation and it is easier to prepare and to examine histologically. Alternatively, the nerve can be