

HOW TO DO IT

Nerve

biopsy

INTRODUCTION

It used to be said that a neurologist could diagnose the cause of a peripheral neuropathy in approximately 50% of cases on the basis of the history, examination and minimally invasive investigations – ‘screening’ blood and urine tests, chest X-ray, CSF examination and nerve conduction studies. About half the remainder would then be diagnosed by more specialized and invasive investigation, notably nerve biopsy, leaving approximately 25% of the original number cryptogenic. No doubt these proportions have changed with the increasing availability of specific DNA analysis for many of the genetic neuropathies, and the expanding range of serum autoantibodies associated with immunologically mediated neuropathies. Yet nerve biopsy remains an important diagnostic tool in patients with a progressive polyneuropathy of unknown aetiology, especially (but not exclusively) if asymmetrical. The emphasis on asymmetry (on clinical and/or electrical grounds) in this statement of the prime indica-

tion for nerve biopsy reflects the diagnosis most often sought in this context, namely a multifocal neuropathy due to vasculitis, which may be tissue-specific. Many other conditions, however, can be diagnosed or suggested by the findings on nerve biopsy (Box 1). The potential usefulness of the investigation must be balanced against the risk of complications (limiting its application to those patients with significant functional disability), and the fact that biopsy findings are often less specific than the box suggests. In this article we will outline the surgical approach to nerve biopsy, techniques for processing specimens and the interpretation of biopsy findings, at each stage indicating ways of optimizing the diagnostic information obtained.

WHICH NERVE?

The sural nerve is most often biopsied because:

- it is usually readily accessible at the level of the lateral malleolus;
- its structure has been studied extensively in health and disease;

Lionel Ginsberg, Rosalind King and Richard Orrell

University Department of Clinical Neurosciences, Royal Free Campus, Royal Free and University College Medical School, University College London, Rowland Hill Street, London, NW3 2PF, UK;

Email: Lionel.Ginsberg@royalfree.nhs.uk

Practical Neurology, 2003, 3, 306–313

- the resulting sensory loss is confined to a small area on the dorsolateral aspect of the foot.

For patients with predominant upper limb involvement, the superficial radial nerve or the dorsal cutaneous branch of the ulnar nerve may be biopsied at the wrist. An alternative lower limb nerve is the superficial peroneal; this has the advantage of allowing access to the peroneus brevis muscle through the same incision. Combined nerve and muscle biopsy is recommended as a way of increasing diagnostic yield in a patient with suspected vasculitis. All the above nerves are most likely to provide useful information in patients whose neuropathy includes sensory loss. For those with a pure motor neuropathy, it may be necessary to sample a nerve to a muscle with little functional importance, e.g. the nerve to gracilis.

The selected nerve should be biopsied on the more severely affected side, as determined by symptoms, signs and electrical findings. A nerve with a well preserved sensory action potential should not be biopsied unless there is strong suspicion of a neuropathy purely and severely affecting small fibre function, otherwise the diagnostic yield will be low and the patient may have been subjected to an unnecessary invasive procedure and end up with even more sensory impairment.

HOW TO TAKE THE BIOPSY

Patient preparation

Nerve biopsy requires written, informed consent. In our unit, patients are given a printed information sheet outlining the indications, technique and complications of nerve biopsy in lay language. A nerve biopsy request form is also

BOX 1 WHERE A NERVE BIOPSY MAY BE USEFUL

Diagnostic abnormalities can be shown in:

- inflammatory neuropathies – vasculitis, sarcoidosis, leprosy;
- dysproteinaemic neuropathies – amyloidosis, paraproteinaemic neuropathy (especially IgM gammopathy with antimyelin-associated glycoprotein antibody);
- genetic neuropathies – hereditary neuropathy with liability to pressure palsies (with characteristic *tomaculous* swellings of the myelin sheath), other myelin folding defects, giant axonal neuropathy;
- metabolic disorders, with distinctive features and storage inclusions (sometimes only visible at the ultrastructural level) e.g. metachromatic leukodystrophy, Krabbe's disease, Fabry's disease, polyglucosan body disease;
- tumour infiltration;
- toxic neuropathies, with characteristic changes, e.g. amiodarone, solvent abuse.

Suggestive abnormalities can be shown in:

- inflammatory neuropathies – chronic inflammatory demyelinating polyradiculoneuropathy;
- genetic neuropathies – demyelinating forms of Charcot-Marie-Tooth disease;
- metabolic disorders – diabetes, Refsum's disease.
- small fibre neuropathies.

Although many of the systemic and genetic disorders listed here can now be diagnosed by alternative and less invasive means, nerve biopsy remains a valuable route to the diagnosis in instances where these disorders are not obvious initially, e.g. a patient with Charcot-Marie-Tooth disease but no family history.

completed preoperatively, summarizing the clinical and electrical findings, to accompany the specimen to the laboratory.

Operative technique

An experienced surgeon (or physician) should perform the biopsy. Even the sural nerve can be hard to find if the patient is obese or has oedematous or varicose ankles. The sural nerve is a delicate structure, easily damaged during removal. Stretching, angulation or compression by the forceps can cause artefactual changes sufficient to hinder adequate histological assessment. Further artefact may arise from delay between obtaining the specimen and preserving it by freezing or fixation. This is avoided in our unit by the presence of the neuropathology technician in the operating theatre, ready to receive the sample as soon as it is taken, with no time for it to dry out.

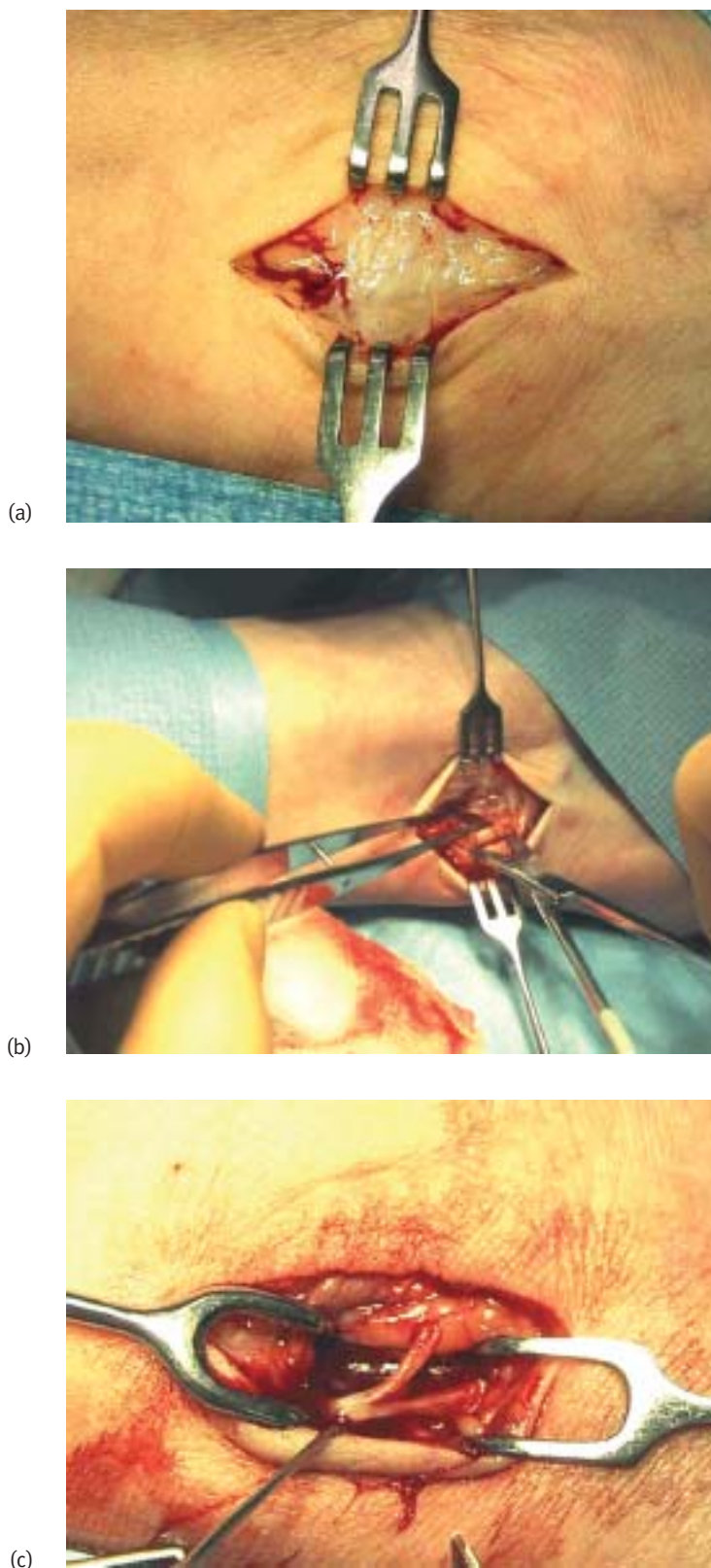


Figure 1 Technique of sural nerve fascicular biopsy. (a) A longitudinal incision has been made posterior to the lateral malleolus. The subcutaneous tissues are exposed using cat's paw retractors. (b) Gentle dissection reveals the sural nerve. (c) The nerve has been isolated from surrounding tissues using double-hooked retractors and a fascicular biopsy is in the process of being taken.

There is controversy about whether the whole nerve should be sacrificed or a 'fascicular' biopsy performed. With care, it is possible to remove a group of fascicles leaving the remainder of the nerve trunk undamaged and in continuity, thereby minimizing any sensory deficit. However, a larger sample will be required if the disease process is likely to be patchy. Thus, the whole nerve is usually taken in suspected vasculitis. The technique of sural nerve fascicular biopsy will now be described.

Unless the patient is a small child or an uncooperative adult, the biopsy may be performed under local anaesthesia. It is best carried out under sterile conditions in an operating theatre. The skin over the biopsy site is first cleaned and the surrounding area draped. The operating field is then infiltrated subcutaneously with lignocaine (1%). A 4-cm longitudinal incision is made midway between the lateral malleolus and the posterior border of the ankle (the skin overlying the Achilles tendon), with its distal limit at the level of the malleolus (Fig. 1a). As the subcutaneous tissue is gently dissected, the first structure identified is usually a moderate-sized vein. The nerve can then generally be found posterior and deep to the vein (Fig. 1b). The nerve's glistening white appearance with a pattern of alternating longitudinal lighter and darker bands helps distinguish it from veins and other tissues. Further dissection allows the nerve to be isolated and exposed (Fig. 1c). To reduce patient discomfort, the nerve is then infiltrated with lignocaine, using a fine needle proximal to the proposed resection site. With the nerve gently supported on a small hook, the biopsy is taken, using a sharp (No. 11) scalpel blade, and passed immediately to the waiting technician on a saline-soaked swab. The specimen should be at least 2 cm long and preferably 3 cm. Once haemostasis has been achieved, the wound is closed with absorbable subcutaneous stitches and 4–6 silk sutures to the skin.

Aftercare

With lower limb nerve biopsies, patients are nursed overnight on the ward and can be discharged the next day after being allowed to mobilize. They are advised to keep the wound dry for 48 h and avoid strenuous activity for 2 weeks. Skin sutures may be removed after 10 days.

Complications of nerve biopsy

Nerve biopsy is associated with various complications including minor wound infections,

wound dehiscence and neuroma formation. Up to 10% of patients experience persistent pain or paraesthesiae at the biopsy site. Focal sensory loss is almost inevitable, assuming the patient had some sensory function in the distribution of the nerve before the biopsy. However, the sensory loss resolves in 90% of patients by 18 months due to collateral reinnervation.

PROCESSING THE BIOPSY SPECIMEN

Nerve biopsy pathology should only be analysed in centres with appropriate expertise. Little useful information is obtained if the sample is processed solely by haematoxylin and eosin staining in the general pathology laboratory of a district hospital, yet this still happens.

The specimen is first divided into pieces for the various investigations. A 3–4 mm length from one end of the biopsy is frozen rapidly in liquid nitrogen. Cryostat sections may be prepared from this segment, suitable for lipid and immunostaining. The bulk of the sample (approximately 1.5 cm long) is then fixed in glutaraldehyde and subsequently osmium tetroxide, dehydrated through increasing ethanol concentrations into dried absolute ethanol and embedded in epoxy resin. Semi-thin (0.5 µm) sections can be cut from these plastic blocks, allowing much greater resolution than that provided by specimens embedded in paraffin. Tissue differentiation under the light microscope is further aided by choice of an appropriate stain, e.g. thionin counterstained with acridine orange. Most of the structural information about myelin, axons and other nerve tissue components is obtained from viewing resin sections by light microscopy but additional techniques include the following:

- **Teased fibres.** If there is sufficient material, and depending on the clinical indication, a portion of the nerve (at least 1 cm long) is preserved (fixed but not embedded) for teased fibre preparation. Separating individual nerve fibres for examination is very time consuming but potentially helpful if there is difficulty distinguishing a remyelinating from a regenerating process, or in rare cases of myelin sheath folding defects.
- **Morphometry.** Image analysis provides quantitative information on the myelinated fibre population, i.e. its density, whether there is preferential loss of small or large fibres, and whether there is a trend towards hypo- or hyper-myelination (as determined by meas-

urement of the ratio of axon diameter to total fibre diameter).

- **Immunostaining and electron microscopy** (see below).
- Any remaining biopsy tissue can be formalin-fixed for paraffin histology and storage. Paraffin sections are reserved for specific histochemistry or immunohistochemistry techniques that are not possible on resin sections.

WHAT TO LOOK FOR IN THE BIOPSY SECTIONS

Inspection of a transverse resin section of a sural nerve fascicular biopsy under the light microscope at low power will reveal several fascicles (usually 2–7), each bounded by its perineurium and embedded in epineurial connective tissue. At higher magnification, details of the endoneurial architecture within each fascicle become clearer (Fig. 2). It is possible to discern whether the biopsy has suffered from surgical and/or laboratory mishandling (Fig. 3). The following aspects should be examined systematically (and commented upon in the nerve biopsy report).

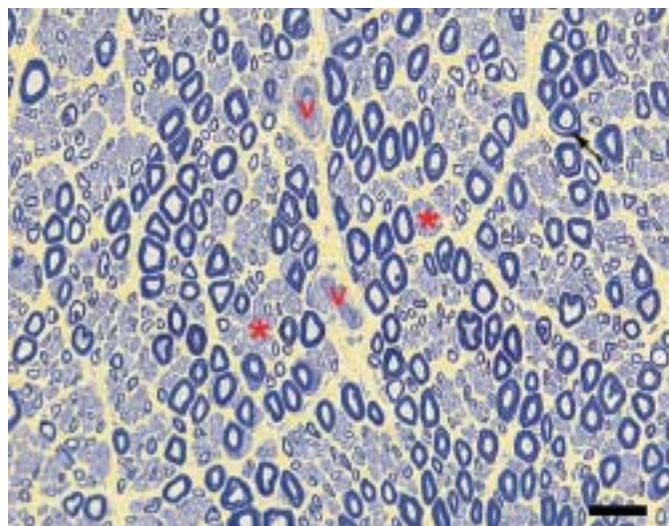


Figure 2 Normal nerve appearances in resin section – sural nerve from a 29-year-old-woman (Thionin and acridine orange, bar = 20 µm). Endoneurial collagen stains as a pale buff colour with cellular elements staining blue and myelin a darker blue. There is the expected variation in fibre size with both large and small myelinated fibres. The unmyelinated axons are patchily distributed (asterisks). There are two small blood vessels (v). Some of the myelin sheaths are apparently split in two (arrow) due to the effect of the processing on Schmidt-Lanterman incisures.

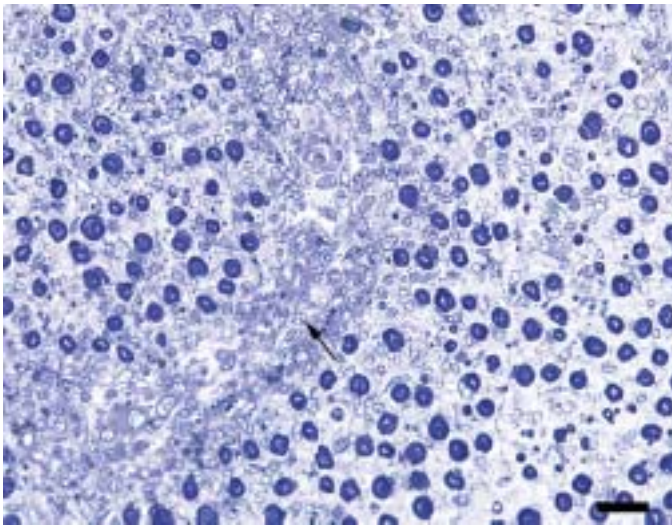


Figure 3 Artefactual appearances. The myelinated fibres have lost their annulated structure due to poor handling. There is also poor preservation of nuclear chromatin (arrow) due to delayed fixation post-biopsy. This sural nerve specimen was taken (at another centre) from a patient with borderline lepromatous leprosy, hence the cellular infiltration. (Thionin and acridine orange, bar = 20 μ m).

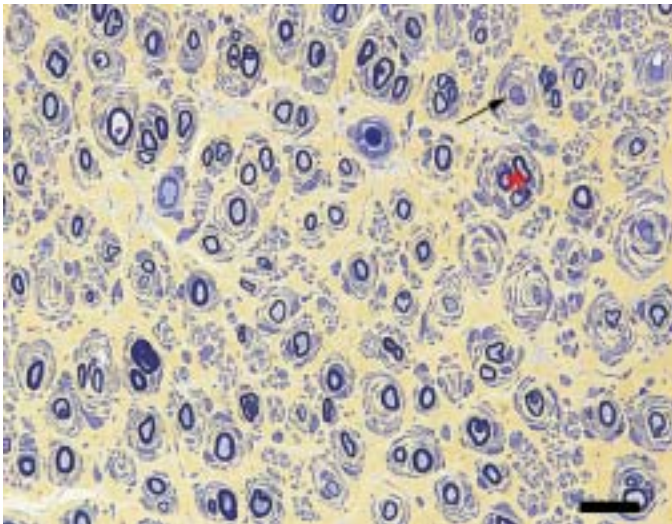


Figure 4 Hypertrophic neuropathy in Charcot-Marie-Tooth disease type 1A – sural nerve biopsy from a 39-year-old woman (Thionin and acridine orange, bar = 20 μ m). There is extensive 'onion bulb' formation due to Schwann cell multiplication. The axons within the onion bulbs vary from being demyelinated (arrow) through thin myelin to thickly myelinated fibres. There are occasional regenerative clusters (asterisk). Endoneurial collagen is increased.

Myelinated fibre population

Most neuropathies involve loss of myelinated fibres. This may be so severe that few or no fibres remain. With milder degrees of depletion, it may be possible to decide whether there has been selective damage of smaller or larger myelinated fibres, potentially narrowing the range of causes of the neuropathy. The distribution of fibre loss within and between fascicles may also be determined. Patchy depletion suggests an acquired, i.e. usually inflammatory process. Unmyelinated axons cannot be examined in detail by light microscopy (Fig. 2).

Myelin sheath calibre

Fibres may be inappropriately thinly myelinated for their axon diameter, or the myelin sheath may appear too thick (Fig. 4). Tomaculous (sausage-shaped) myelin swellings are seen in genetic neuropathies with myelin folding defects, but also occur in a range of other inherited and acquired demyelinating neuropathies. Thin myelin sheaths pose a problem in interpretation as they may result from either remyelination or regeneration, hence the occasional need for teased fibre preparations.

Demyelination/remyelination

Rare demyelinated axons are easily missed by light microscopy, as is active demyelination by macrophage stripping, necessitating ultrastructural studies (see below). There may be circumstantial clues to a demyelinating process, e.g. the presence of fibres with intramyelinic oedema. Remyelination may be suggested by the presence of isolated thinly myelinated fibres. More convincing evidence of repeated episodes of demyelination and remyelination is the proliferation of multiple Schwann cell layers around axons, resulting in classical 'onion bulb' formation (Fig. 4).

Degeneration/regeneration

Axonal degeneration is more readily detected at the light microscope level than demyelination. Regeneration is indicated by the presence of clusters of small thinly myelinated fibres, grouped closely together, arising by a process of axonal sprouting (Fig. 4).

Cellular infiltration

Debris-laden macrophages may be found in the endoneurium in both demyelinating and axonal neuropathies. Inflammatory cell infiltrates are visible in thionin and acridine orange stained

resin sections (Fig. 3), and indeed in haematoxylin and eosin sections, but are better characterized by specific immunostaining (see below).

Extracellular deposits

Loss of nerve fibres is accompanied by an increase in endoneurial collagen. Amyloid deposition is readily apparent on resin sections (Fig. 5) but special techniques are required to confirm its presence, e.g. Congo red staining and viewing for birefringence under polarized light, electron microscopy looking for amyloid fibrils and immunostaining for the different forms of amyloid.

Intracellular inclusions

Specific stains may be needed, depending on the clinical indication, such as Ziehl–Neelsen or auramine for mycobacteria in leprosy, oil red O or Sudan black for lipid, cresyl violet for metachromatic material and periodic acid Schiff (PAS) for polysaccharide (e.g. polyglucosan bodies).

Blood vessels

Resin sections may show thickening of the basal lamina of endoneurial blood vessels in diabetes or as an age-related phenomenon. More florid abnormalities are seen in vasculitis and the inflammatory infiltrate surrounding and invading vessels may be confirmed by immunostaining (Fig. 6).

Perineurium

Isolated perineuritis may occur. Subperineurial oedema suggests an inflammatory neuropathy.

Of these various aspects, some are more useful than others in narrowing the differential diagnosis, notably the presence of demyelination, tomacula, cellular infiltrates and inclusions.

Two additional techniques are regularly used to complement the resin section findings:

Immunostaining

Immunostaining for immunoglobulins, different types of amyloid, macrophage and lymphocyte markers is an important technique (Fig. 6). Because frozen sections can be processed relatively rapidly compared to resin sections, they may be useful in providing preliminary information where an urgent diagnosis is required, e.g. suspected vasculitis. Care must be taken to examine all the sections stained with each marker because the inflammatory process may be patchy.

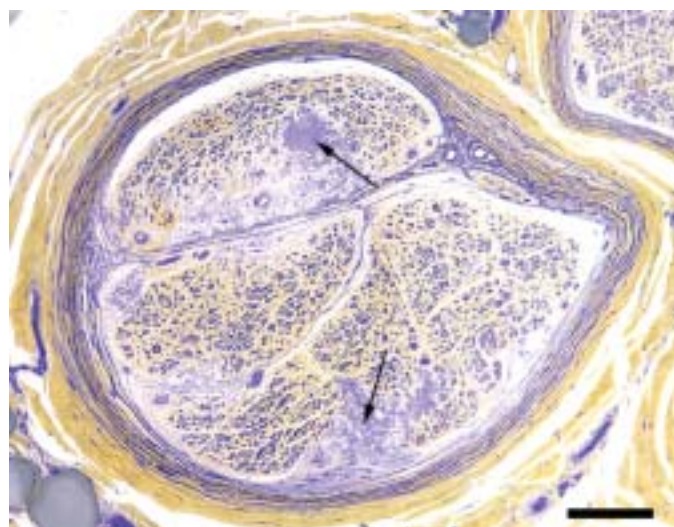


Figure 5 Amyloid neuropathy – radial nerve biopsy from a 73-year-old man (Thionin and acridine orange, bar = 100 μ m). There is preferential loss of the smallest fibres. Amyloid deposits are arrowed.

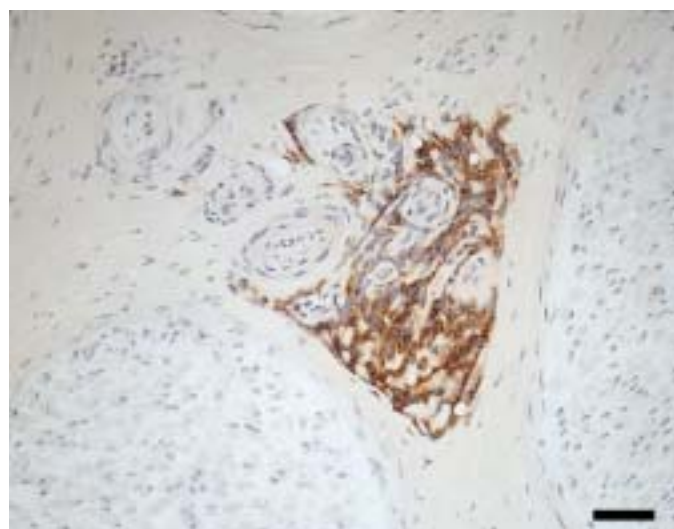


Figure 6 Immunostaining for B lymphocytes (CD 22) in a sural nerve biopsy frozen section from a 61-year-old woman with vasculitic neuropathy. There is a dense patch of positively staining cells around a group of small blood vessels. The presence of B lymphocytes indicates a systemic process. Lymphocytes in a tissue-specific vasculitis or chronic inflammatory demyelinating polyradiculoneuropathy stain only with T cell markers (CD 4 or CD 8). (Bar = 50 μ m).

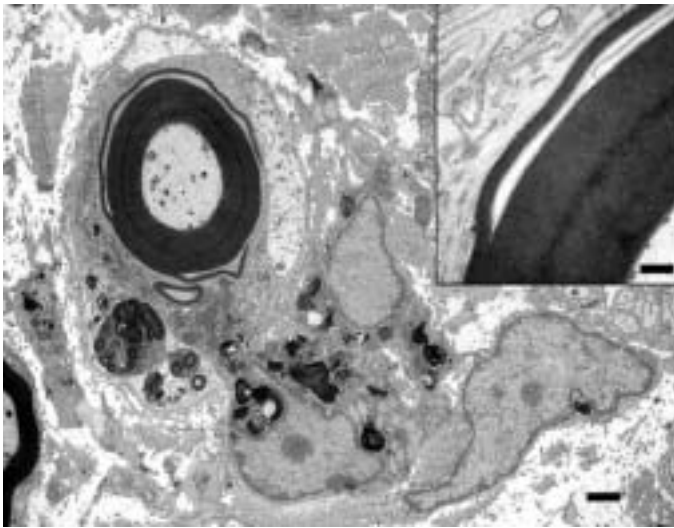


Figure 7 Electron micrograph showing active myelin stripping in a sural nerve biopsy from a 60-year-old woman with chronic inflammatory demyelinating polyradiculoneuropathy. A debris-laden macrophage has penetrated the Schwann cell basal lamina and is engulfing myelin lamellae (bar = 2 μm). Inset shows myelin being lifted by a tongue of macrophage cytoplasm (bar = 0.5 μm). (Uranyl acetate and lead citrate).

Electron microscopy

Electron microscopy (EM) can be used when light microscopy does not provide enough detail, e.g. to examine unmyelinated axons and in cases of leprosy, amyloidosis, paraproteinaemic and demyelinating neuropathies (Fig. 7). EM sections may also show abnormalities of neurofilaments and other organelles in various toxic, metabolic and inherited neuropathies.

ACKNOWLEDGEMENTS

We thank John Muddle and Jane Workman for longstanding scientific and technical support, including assistance in the preparation of figures for this article.

FURTHER READING

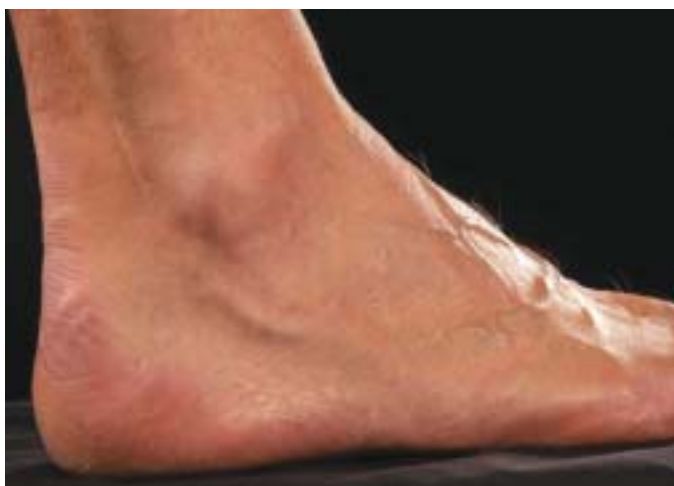
Thomas PK (1995) Biopsy of peripheral nerve tissue. In: *Peripheral nerve disorders 2* (eds Asbury AK & Thomas PK), pp. 281–300. Butterworth, Oxford.
King RHM (1999) *An atlas of peripheral nerve pathology*. Arnold, London.

REFERENCES

Gabriel CM, Hughes RAC, Howard R *et al.* (2000) Prospective study of the usefulness of sural nerve biopsy. *Journal of Neurology, Neurosurgery and Psychiatry*, **68**, 442–6.
Pollock MP, Nukada H, Taylor P, Donaldson I & Carroll G (1982) Comparison between fascicular and whole sural nerve biopsy. *Annals of Neurology*, **13**, 65–8.

CONCLUSIONS

- In most patients with a peripheral neuropathy, the cause can be established on the basis of history, examination and initial ‘screening’ investigations, without the need to resort to nerve biopsy.
- Nerve biopsy should be reserved for patients with a progressive polyneuropathy of unknown aetiology, which is causing significant functional disability.
- Diagnostic yield is likely to be highest when the neuropathy involves sensory impairment and when there is asymmetry.
- The nerve to be biopsied should generally have an absent or markedly reduced sensory action potential.
- The interpretation of biopsy findings is subject to surgical and laboratory artefact.
- The analysis of resin sections may be supplemented by additional special techniques, most importantly immunostaining and electron microscopy. The full range of investigation of biopsy specimens is only likely to be available in specialist centres.
- Despite careful patient selection, nerve biopsy may only reveal changes of a chronic axonal neuropathy, with nonspecific features.
- With these provisos, nerve biopsy remains a useful diagnostic tool in a minority of neuropathy patients.



BOX 2 ME AND MY NERVE BIOPSY

Richard Hughes

Department of Clinical Neurosciences, Guy's, King's and St Thomas' School of Medicine, London, UK

I have always felt that if you are going to ask a patient to undergo a procedure you should be able to say that

you would be prepared to have it done to you. I was also aware that many senior investigators were walking around with tell-tale scars at the back of their calves. So when I needed a piece of really normal nerve to compare with my patients' nerves there was no escape. Ignoring the unkind comment from a 'friend' that it was presumptive to assume that any nerve of mine would be normal, I asked my surgeon to perform a partial thickness biopsy of the right sural nerve, the opposite side from an old L5-S1 disc prolapse. We discussed ethical committee approval but agreed at the time that it was unnecessary. Halcyon days! I slipped surreptitiously into the day case surgery unit and, disdaining to take off my trousers, rolled my trouser-leg as high as I could. The surgeon painted my leg mahogany brown with iodine and injected lignocaine so that the procedure was painless. He protested about the excessive number of veins, which had been produced by my trouser-leg tourniquet. I allowed myself a taxi home instead of the train and kept my leg elevated with an impressive pressure bandage round the ankle as much as I could for the next day.

As the local anaesthetic wore off I became aware of loss of feeling along the lateral border of the foot from the heel as far as the base of the little toe associated with a warm tingling feeling. I took the stitches out myself after 12 days, by which time I was on an exchange visit to the Johns Hopkins Medical School. To my embarrassment I had to seek reassurance when the wound became inflamed a few days later. It probably was not infected because there was no pus and a wound swab grew nothing but I gave myself the precautionary course of antibiotics I had brought with me from the UK against this eventuality. As the days went by I became aware of a more or less continuous burning feeling in the numb area and also, to my surprise, up the back of the calf proximal to the scar. This was uncomfortable but was never severe enough to require analgesia. This discomfort persisted for several months and slightly for a couple of years, becoming more apparent when I was tired or stressed, a genuine Achilles' heel. Gradually the discomfort wore off but I still had a sharp pain radiating down the lateral border of the foot when I tapped the wound scar. Having had the biopsy did not have any discernible effect on my ability to run long distances but gave me a convenient excuse for not winning races.

Now 15 years later I have a barely detectable 3 cm long scar just above and behind the right lateral malleolus. I no longer have a Tinel's sign. I am not spontaneously aware of any anaesthesia but when I deliberately test the area I am just aware of an approximately 4 cm area of slightly reduced light touch sensation below the scar, between the lateral malleolus and the heel. I asked for a fascicular biopsy because of the theoretical expectation that this would be followed by less deficit than a full thickness biopsy, an expectation which was not fulfilled in the only direct comparison of the two procedures (Pollock *et al.* 1982). Having a nerve biopsy was not too bad but biopsies can cause persistent discomfort and the wound can become infected and break down, especially if a patient is being treated with steroids (Gabriel *et al.* 2000). A nerve biopsy is therefore an investigation of last resort.