NERVE BIOPSY

BY

W. HOLMES and R. B. ZACHARY

From the Departments of Zoology and Orthopaedic Surgery, Oxford

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IN peripheral nerve surgery exploration of the site of injury will often reveal at once whether surgical repair of the nerve is necessary. But if the nerve has been incompletely divided, or shows some local pathological change without any naked-eye evidence of division, the decision between conservative treatment and resection is often a difficult one. In these cases the most reliable guide in determining the correct treatment is an accurate assessment of the progress of spontaneous reinnervation of the nerve distal to the site of injury.

The methods at present available for estimating the progress of reinnervation are:

1. Clinical examination; the return of power to muscles supplied by the damaged nerve and of sensibility in its autonomous zone of distribution.

2. The electrical reactions of the paralysed muscles; before return of voluntary power it is sometimes possible to detect reinnervation of a muscle by the alteration in the strength-duration curve of the muscle. (Adrian, 1916; Ritchie, 1944.)

3. Electrical conductivity in the affected nerve; percutaneous or direct stimulation of the affected nerve may sometimes give a response in the muscles when no voluntary power can be detected.

4. Electro-myography; motor-unit action potentials can always be picked up in muscles before there is any sign of voluntary activity, and a progressive increase in number and distribution of these action potentials is a fairly reliable index of recovery.

5. Muscle biopsy; this method of assessing reinnervation has been described by Bowden and Guttman (1944). In addition to information about the state of the muscle fibres, the method also reveals the condition of the terminal branches of the nerve.

All these methods depend on the reinnervation of the paralysed muscles (or skin), and, therefore, can be of little value when there has been insufficient time for regeneration to have proceeded so far. In view of the importance of early repair of a damaged nerve, it is not usually justifiable to wait for these signs before exploring any serious nerve injury or deciding on its repair, particularly when the lesion is a high one. When there is some doubt as to the advisability of resecting a lesion, or when an early prognosis is required, a picture of the condition of the nerve just distal to the lesion may be decisive.

It is the purpose of this paper to present a technique for biopsy of a nerve trunk, with illustrations of the application of the method in several groups of cases in which it has proved helpful.

Technique

THE SAMPLE. Two methods have been employed. The first is to sacrifice a small sensory or motor branch, and the second is to excise a funiculus from the main trunk. The first method is applicable only in certain regions, where an unimportant sensory or motor branch is available, e.g. one of the sensory branches of the radial nerve in the musculo-spiral groove, or one of the branches to flexor carpi ulnaris in an ulnar lesion above the elbow. The value of such a sample depends on whether it represents the state of affairs in the main trunk. If there is a lateral neuroma and the resected branch arises on the same side as, and close to the lesion, a fairly true picture of the extent of regeneration through the nerve is likely. Where the biopsy is more distant, the intraneural mixing of fibres between the funiculi will probably mean that a proportion of undamaged fibres will be present in the branch and the sample will, therefore, be less reliable.

When an unimportant branch is not available a funiculus from the main trunk may be taken. Clearly, when the lesion affects mainly one side of the nerve the same principle applies as in the case of the branch biopsy, and the funiculus should be taken close to the most damaged part of the nerve and from the same sector of its circumference. The epineurium is incised longitudinally over a distance of about two centimetres; one funiculus is gently lifted up by blunt dissection and resected with a sharp knife or scissors. Provided the site of biopsy is not close to the distribution of the nerve, the removal of one funiculus is not likely to have any markedly adverse effect on the quality of recovery.

HISTOLOGICAL TECHNIQUE. The specimens are laid on small pieces of postcard; they are attached to the card by pressure with forceps at both extremities, but should not be touched elsewhere. The cards with the attached specimen are immediately placed into bottles of saline formaldehyde solution in which they remain for two days or more. A reliable method of myelin staining, and also a silver technique for axon impregnation, should be employed for each specimen; we have used a modified Weigert technique with alum carmine as a nuclear counterstain, and a silver-on-the-slide method respectively; these techniques are described elsewhere (Holmes). Some sections from each block are also stained by Masson's light green trichrome. Transverse

sections are best for the demonstration of myelin sheaths, and longitudinal for axons.

INTERPRETATION. The interpretation of the biopsy presents some difficulties. The recognition of normal nerve fibres and of those which have undergone complete Wallerian degeneration is as a rule simple, but some uncertainty arises in attempting to assess the significance of the number and diameter of regenerating fibres when these are present. The rate of advance of regenerating axon tips should be of the order of 2.5 or 3 millimetres a day, but there is some delay of varying degree before the axons pass the level of injury, and further development is influenced by many factors which affect the increase in diameter of the axons and the myelination of the regenerating fibres. In spite of these difficulties in interpretation, we think it is possible to judge within fairly wide limits whether satisfactory reinnervation is in process. The following examples illustrate the application of the method in several different types of nerve injury.

Results

GUNSHOT WOUNDS

There are some gunshot wounds in which exploration of the site of the nerve lesion is undesirable or unnecessary, but an early prognosis is still required. The following cases are illustrative.

CASE 1

History.—A., aged 30, on Jan. 23, 1944, received a gunshot wound of the right side of the neck, with partial brachial plexus paralysis. By June 23 there was paralysis of the upper trunk of the plexus except for a flicker in the biceps and some sparing of sweating. At operation on July 19 no lesion was visible on the upper trunk; the injury must have been more proximally situated, in the region of the intervertebral foramina. At biopsy a specimen was taken from the trunk of the sixth cervical nerve, and a small branch from the fifth.

Biopsy report.—Both specimens were too small for subdivision, so that only one staining method could be applied: the myelin method was chosen. The nerve bundles in the specimen from the trunk of the sixth cervical nerve showed the appearance of degeneration followed by regeneration. Many of the Schwann tubes contained small myelinated nerve fibres (Fig. 1), none of which were larger than 4μ in diameter. Although many of the tubes did not contain fibres large enough to have a stainable sheath, the distribution of the larger visible fibres indicated a satisfactory spontaneous reinnervation.

The condition of the specimen from the branch of the fifth cervical nerve was similar, but the myelinated fibres were more numerous and somewhat larger; a few of them were $8-10\mu$ in diameter, and were probably fibres that had escaped division by the injury. The condition of both biopsies indicated that a satisfactory spontaneous regeneration was in progress.

Treatment.—Intensive physiotherapy was, therefore, continued for the next 18 months. By Dec. 20, 1945, all muscles except the brachioradialis and extensor carpi radialis longus had recovered, and there was a return of light touch sensibility on the arm.

CASE 2

History.—B., aged 33, on Jan. 31, 1944, received a gunshot wound of the right shoulder, involving the joint. There was considerable sepsis, and brachial plexus paralysis. By July 14 there was complete median and

ulnar and medial cutaneous nerve palsy. The wound in front of the shoulder joint was still discharging pus, but a wound in the upper part of the arm, which might have been responsible for the damage to the nerves, was healed. At an operation on Aug. 9, the median and ulnar and medial cutaneous nerves in the arm were explored, but no serious lesion was found. Since all three nerves were passing through the region of the wound in close proximity to one another, it was thought that a biopsy of the medial cutaneous nerve would be a reliable guide to the condition of the others. Portions of the nerve were removed at 12, 15, and 23 cm. below the coracoid process.

Biopsy report.—Each specimen was subdivided and examined by the myelin and axon methods. At all levels the nerve showed the normal appearance of Wallerian degeneration, together with evidence of regeneration. At the proximal level there were many small myelinated fibres (Fig. 2), and although many of the Schwann tubes were empty in the myelin-stained sections, the longitudinal sections stained with silver showed an abundant and probably complete reinnervation by nerve axons. At the middle and distal levels the number of myelinated fibres and of axons became progressively smaller, as was to be expected. The condition of the myelin-stained sections at the distal level is represented in Fig. 3. It was clear from the sections that spontaneous regeneration was in progress in the medial cutaneous nerve, and spontaneous recovery seemed likely in the median and ulnar nerves on the presumption that intraneural conditions were similar.

Recovery.—By Oct. 16 the proximal median and ulnar muscles had recovered; there were flickers of voluntary power in the intrinsic muscles of the hand, and touch and pain sensibility had returned to the median and ulnar areas.

CASE 3

History.—C., aged 31, on July 17, 1944, received a gunshot wound of the left buttock with complete lateral popliteal palsy. There was no recovery by Jan. 31, 1945. Exploration of the nerve in the buttock was not undertaken because late suture of such lesions had not previously given satisfactory results; however, a prognosis was needed to decide whether the patient should continue treatment or not. A specimen was taken from the lateral popliteal nerve in the thigh.

Biopsy report.—The specimen was examined by the myelin and axon methods. The sections contained one nerve bundle showing a typical state of Wallerian degeneration with an oedematous endoneurium and without abnormal collagenization. There was no evidence of nerve fibres in the myelin-stained sections, but in the longitudinal sections stained with silver it was found that the Schwann tubes were abundantly innervated by regenerating axons of small diameter (Fig. 4).

Treatment.—The patient was advised to continue electrotherapy for the paralysed muscles, since it would be many months before they were reinnervated. By Feb. 11, 1946, recovery had started in the tibialis anterior; and by May 20 there was considerable recovery in the tibialis anterior, extensor digitorum longus, and peronei.

When a nerve is explored after a gunshot wound and is found to be damaged but in continuity, the question arises whether a complete or partial repair is likely to give a better result than would occur spontaneously. The appearance and consistency

of the lesion are often useful indications; a lesion involving less than one-third of the nerve, or one which is quite soft, is best left alone. In the more severe type of lesion with a firm scar on one aspect of the nerve, careful section of the scarred portion will reveal how much of the nerve is grossly involved and will thus give a useful guide to procedure. There are, however, a number of cases in which funicular biopsy is preferable to trial section, since there may be serious intrafunicular damage, even though the bundles are continuous through the injured segment.

CASE 4

History.—D., aged 24, on Dec. 3, 1943, received a gunshot wound of the right axilla. There was gross hæmorrhage from the wound and paralysis of the hand. At an emergency operation the median nerve was accidentally crushed, with hæmostats. By April 26, 1944, there was complete median and musculo-cutaneous paralysis. At a further operation the median nerve was found to be enveloped in a mass of scar; after neurolysis a funicular biopsy was taken 4 cm. below the lesion.

Biopsy report.—The specimen was very small and in consequence it was impossible to employ both axon and myelin stains: the myelin stain only was used. The sections showed that the biopsy contained a part of one nerve bundle; in it the endoneurium showed the general moderate œdema often found in Wallerian degeneration (Fig. 5), but there was no abnormal fibrosis. There was a general and uniform reinnervation of the specimen with myelinated nerve fibres, of which the largest had a total diameter of from 4.5μ to 5μ . The nerve fibres present were all regenerating, since they were small in diameter; nevertheless they were sufficiently large and numerous to indicate a successful reinnervation of the trunk. It was concluded that a good spontaneous recovery could be expected.

Progress.—By June 2, 1944, the proximal median muscles had recovered, and by July 19 there was perfect recovery of the median nerve.

Where there are multiple wounds, so that the level of nerve injury is uncertain, the most likely site is explored first; and if the findings are inconclusive a much more extensive search has to be contemplated. A biopsy at the site of an inconclusive exploration will reveal whether there is a degenerative lesion at a more proximal level, as in the following case.

CASE 5

History.—E., aged 26, on Sept. 19, 1944, received a gunshot wound of the right knee which resulted in complete sciatic paralysis. By April, 1945, there was recovery in the lateral popliteal distribution, but on Sept. 12, 1945, medial popliteal paralysis was still complete. On Sept. 19 the patient was operated on at the site of the large wound behind the knee. There was no evidence of serious damage to the nerve in the popliteal fossa. A funiculus of the medial popliteal nerve was removed.

Biopsy report.—The specimen was divided into two pieces, of which one was treated for an axon stain, the other for myelin. Sections showed that the biopsy contained three small nerve bundles, all entirely devoid of nerve fibres, since both the silver and myelin stains gave negative results. It was concluded that the nerve had suffered damage at a level proximal to the suspected level

of injury, and that no spontaneous regeneration had taken place.

Progress.—At an operation on Oct. 10, 1945, the nerve was found to be completely divided in the upper part of the thigh, where there was a minute entrance wound of which the patient knew nothing and which might easily have been the scar of a small boil. A suture was performed.

TRACTION LESIONS

These injuries vary considerably in severity; many are mild and the nerves recover rapidly; others cause permanent paralysis. Moreover, the damage is often spread over a long segment of nerve, and when recovery occurs it is sometimes late. Macroscopically the nerve is usually in continuity and presents a twofold problem of prognosis and immediate action. Any information about the severity of the intraneural changes or about the progress of regeneration is valuable. The following examples give the indications for nerve biopsy in traction injuries.

CASE 6

History.—F., aged 36, on Nov. 3, 1943, fractured the pelvis and dislocated the hip; there was sciatic palsy. On Aug. 17, 1944, the lesion was found to be complete except for slight sparing of sweating and sensibility in the medial popliteal area. At an operation on Aug. 19 the nerve was found to be in continuity, but the sheath was thickened and the nerve looked gelatinous. Stimulation produced no response. A specimen was taken.

Biopsy report.—The sections were stained by the myelin method. The biopsy was found to contain several nerve bundles, and all of them were very fully reinnervated by myelinated fibres, some of which had reached a diameter of 6μ (Fig. 6). There was no evidence of excess collagenization of the endoneurium or of the other abnormalities sometimes found in nerves damaged by traction. It was concluded that spontaneous regeneration was well in progress and that a good recovery could be expected without further operative intervention.

Progress.—By July 9, 1945, all muscles except the intrinsic muscles of the foot had recovered. There was considerable recovery of sensibility on the foot.

CASE 7

History.—G., aged 24, in a 'plane crash on Feb. 20, 1945, received a simple fracture of the right humerus with paralysis of the right arm. By Aug. 2 the fracture was united, but there was complete paralysis of radial, median, ulnar, musculo-cutaneous, and circumflex nerves. The patient was thought to have suffered a traction lesion.

At an operation on Aug. 8, the median, ulnar, and musculo-cutaneous nerves were explored. There was some local scarring, indicating circumscribed trauma, but a traction lesion could not be excluded. A specimen of the median nerve was removed.

Biopsy report.—The sections showed that the nerve had undergone Wallerian degeneration, but was fully reinnervated. None of the fibres had a total diameter greater than 4μ , but the myelin and axon stains showed that the reinnervation was as complete as would be expected after a simple axonotmesis. An excellent spontaneous recovery seemed likely.

Progress.—In view of this report it was thought very likely that the radial nerve might have sustained a

similar injury, and there was a strong indication for exploration with a view to neurolysis. At an operation on Sept. 19 the radial nerve was found to be firmly bound to the site of the fracture. Neurolysis was performed.

MISCELLANEOUS NERVE LESIONS

Permanent motor and sensory paralysis sometimes occurs without division of a nerve, and in such cases the intraneural changes cannot be assessed by inspection, whereas a small sample will often assist in establishing the cause of the lesion and help in prognosis.

(a) Lesions due to Local Application of Toxic Substances

The intramuscular route for the injection of drugs is in fairly common use, and the administration of the sulphonamides by this method has been the cause of many nerve injuries, some of which have been reported by Elkington (1942). Spontaneous recovery may follow the injection of small quantities, but permanent paralysis due to local necrosis is by no means rare and, as the macroscopic changes are slight, some guide to the severity of the intraneural changes is needed.

CASE 8

History.—H., aged 30, on Sept. 3, 1943, had cerebrospinal meningitis. On Sept. 5 intramuscular injection of sulphapyridine caused severe pain in the sciatic distribution, resulting in complete sciatic palsy. At an operation on April 12 it was found that 6 cm. below the greater trochanter the nerve narrowed over a distance of 6 cm. and the epineurium was thickened. The consistency was normal. A biopsy was taken at both ends of the lesion.

Biopsy report.—The distal specimen showed the appearances of simple Wallerian degeneration without reinnervation. The myelin-stained sections showed no nerve fibres and only a few patches of myelin remains (Fig. 7). It was concluded that the nerve fibres had suffered complete division above the level of biopsy and that no spontaneous regeneration was in progress.

Progress.—At an operation on May 17 the sciatic nerve was resected and sutured. Histological examination of the resected segment of the nerve showed that it consisted largely of a dense mass of collagen with no trace of nervous structure; at various points there were necrotic remains of nerve fibres. It was clear that spontaneous regeneration could never have taken place through this mass of scar.

(b) Vascular Lesions

In Volkmann's ischaemia the paralysis of the intrinsic muscles of the hand and the sensory loss are due to ischaemic degeneration of the nerve, in which the proliferation of collagen within the nerve may be so extensive as to preclude regeneration (Holmes, Highet, and Seddon, 1944). Less severe

degrees of collagenization which permit the down-growth of axons are sometimes met with, but the naked-eye examination of the nerve is not an adequate guide to the state of affairs within it, and several cases have warranted a biopsy of the nerve trunk.

CASE 9

History.—J., aged 29, on Nov. 19, 1943, had a motor cycle accident which caused a closed fracture of the radius and ulna. The fracture was reduced and the arm plastered. On Nov. 20 there was severe pain in the arm, and the plaster was bivalved. The next day median and ulnar paralysis was noticed. By March 30 there was ischaemic contracture of the long flexor muscles, and paralysis of the intrinsic muscles except for a flicker in the intrinsic hypothenar muscles. There was sensory loss on the digits. At an operation on May 17 the median nerve appeared to be avascular; stimulation gave no response in the thenar muscles. A funiculus was taken as biopsy.

Biopsy report.—Transverse sections showed that fibroblast proliferation had caused an abnormal thickening of the endoneurium, but that the Schwann tubes had not been obliterated, and all contained small regenerating nerve fibres (Fig. 8). It was concluded that the ischaemic change in the nerve was of a moderate degree, and that some spontaneous recovery of function could be expected.

Progress.—By April 5 there was almost complete recovery of muscle power, and complete return of sensibility in median and ulnar areas.

Conclusion

Nerve biopsy may be a useful aid to diagnosis and a valuable guide for treatment and prognosis in the following circumstances:

1. Local traumatic nerve injuries, when there is doubt about the advisability of resecting the lesion or when it is difficult or inadvisable to expose the nerve lesion.
2. Traction injuries in order to establish a prognosis.
3. Toxic or ischaemic nerve lesions.

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FIG. 1.—Case 1. Biopsy in transverse section. Weigert-carmine. Scale=20 μ .

FIG. 2.—Case 2. Biopsy in transverse section at a proximal level. Weigert-carmine. Scale=20 μ .

FIG. 3.—Case 2. Biopsy in transverse section at a distal level. Weigert-carmine. Scale=20 μ .

FIG. 4.—Case 3. Biopsy in longitudinal section. Axon stain. Scale=20 μ .

FIG. 5.—Case 4. Biopsy in transverse section. Weigert-carmin. Scale=20 μ .

FIG. 6.—Case 6. Biopsy in transverse section. Weigert-carmin. Scale=20 μ .

FIG. 7.—Case 8. Distal biopsy in transverse section. Weigert-carmin. Scale=20 μ .

FIG. 8.—Case 9. Biopsy in transverse section. Axon stain. Scale=20 μ .