Acta neuropath. (Berl.) 23, 105—111 (1973)

The Perineurium as a Diffusion Barrier to Protein Tracers Following Trauma to Nerves

Yngve Olsson* and Krister Kristensson

Neuropathological Laboratory, Institute of Pathology, University of Göteborg, Sweden

Received September 10, 1971

Summary. A study was made on the permeability of the perineurium to protein tracers following crush injury to mouse and rat sciatic nerves. Albumin labelled with Evans blue (EBA) was injected around the nerves at various times after the injury and then localized by fluorescence microscopy. The following observations were made:

1. The perineurium of control nerves acted as an efficient diffusion barrier to EBA but crush injury caused an abnormal permeability of the perineurium at the site of the trauma.
2. This abnormality started within the first day after the crush and persisted in most animals throughout the period of observation i.e. 4 months. Once inside the endoneurium protein tracers spread mainly in distal direction.
3. The permeability of the perineurium distal to the trauma remained unchanged.

Accordingly, localized trauma to a nerve may for long time change the environment of the nerve fibres even far away from the primary injury by diffusion of substances into the endoneurium.

Key words: Perineurium — Diffusion Barrier — Trauma — Protein Tracers.

Introduction

The individual nerve fasciculi of the peripheral nervous system (PNS) are surrounded by a multilayered cellular membrane which forms the inner part of the perineurium. Each layer is formed by flat polygonal cells which are joined edge to edge by so-called "tight junctions" and covered with basement laminas (see Key and Retzius; Röhlich and Knoop; Shanthaveerappa *et al.*; Thomas, 1963; Cravioto; Burkel; Lieberman).

Physiological studies have shown that the sheaths surrounding peripheral nerves act as a diffusion barrier to a broad range of substances including proteins (see review by Martin). Recent ultrastructural studies with ferritin (Waggener *et al.*) and horseradish peroxidase (Olsson and Reese, 1969, 1971; Klemm, 1970) as protein tracers have provided conclusive evidence that it is the perineurium that exerts the barrier function of the nerve sheaths.

The perineurium in normal nerves separates the nerve fibres from contact with the surrounding body fluids housing them in an environment of their own. Clinical and experimental observations indicate that the perineurium in normal nerves may act as a protective membrane against various toxic and infectious agents (Kurucs and Osgyáni; Sunderland; Shanthaveerappa and Bourne, 1966; Olsson, 1966a). Accordingly, an abnormal barrier function of the perineurium

* Present affiliation: Neuropathological Laboratory, Institute of Pathology, University of Uppsala, Sweden.

produced by a pathological process would have important implications since the diffusion of such agents into the nerves would no longer be prevented. However, we have not found any report of experiments designed to determine to what extent various pathological processes actually interfere with the barrier function of the perineurium.

The present study is a link in a series of investigations on the barrier *function* of the perineurium in various pathological conditions. We have started with the effect of traumatic injury to peripheral nerve since there are some previous reports dealing with *ultrastructure* and *cytochemistry* of the perineurial cells following crush injury to sciatic nerves (Shanthaveerappa and Bourne, 1964; Thomas, 1967).

Material and Methods

Adult mice and rats were obtained from Anticimex, Stockholm. Under ether anesthesia both sciatic nerves were exposed in the upper part of the thigh through small incisions of the skin and the subcutaneous fascia. The nerves were then crushed with the tip of a jeweller's forceps which caused a marked but narrow (1 mm) impression of the nerve.

The tracer (0.04 ml of 1% Evans blue in 5% bovine albumin, abbreviated EBA) was injected around the lesion to the sciatic nerve with a micrometer syringe at various times after the nerve injury. It was left in situ for 2 or 24 h (approximately equal no. of animals). Precautions were taken to minimize surgical trauma during injection and to avoid reflux of tracer (cf. Kristensson and Olsson). The number of mice and the survival times after the nerve crush are presented in Table 1.

Table 1. *Survey of the examined material. EBA applied around both sciatic nerve for 2—24 h at various times after crush lesion of mouse sciatic nerves*

Group no.	Survival time after crush (days)	No. of mice	No. with abnormal perineurial permeability
1 (control)	—	4	0
2	1	5	5
3	7	5	3
4	14	3	3
5	28	5	4
6	120	9	6

The permeability of the perineurium distal to the crush was also tested in a separate group of rats. In this species the nerve was long enough to permit the application of tracer about 1 cm below the lesion through a separate opening in the covering muscles. Groups of three rats were subjected to bilateral crush lesions of the sciatic nerves and EBA was applied for 2 h, 1, 7, and 30 days thereafter.

The animals were sacrificed by decapitation and the sciatic nerves were fixed in 5% formalin over night. Ten microns thick frozen longitudinal sections of the nerves were mounted in glycerin and viewed under a fluorescence microscope equipped with dark field condensor. The filter combination and other details of this method have been presented elsewhere (Steinwall and Klatzo; Olsson, 1966b). The method is based on the fact that EBA emits a red secondary fluorescence which permits detailed histological localization of the tracer (Steinwall and Klatzo; Hamberger and Hamberger).

Results

EBA applied around the sciatic nerve of the control (non-traumatized) mice for 2—24 h caused an intense red fluorescence of the epineurium and the perineurium. The red fluorescent EBA stopped abruptly at the inner part of the perineurium and the tracer could not be detected in the underlying endoneurium where the myelin sheaths emitted a blue autofluorescent light (Fig. 1). This is in accordance with previous findings in various adult animals (Olsson, 1966b; Kristensson and Olsson).

However, when EBA was applied around the nerve immediately after the crush injury the tracer also passed into the endoneurium (Fig. 3). 2 h after the injury the tracer was seen only in the part of endoneurium that was immediately adjacent to the traumatized region. As in the control nerves the tracer had spread along the nerve in the epineurial and perineurial connective tissue but there were no signs of passage of tracer into the endoneurium distal or proximal to the traumatized part.

Twenty-four h after the crush lesion the tracer was seen not only at the traumatized region but also in the endoneurium of the distal part of the sciatic nerve (Fig. 2). The tracer appeared as narrow intensely fluorescent lines arranged in longitudinal direction along the nerve. There was also accumulation of tracer in some endoneurial cells which appeared as intensely red fluorescent oval bodies. In two mice the tracer had accumulated predominantly beneath the perineurium.

EBA injected around the sciatic nerve 1 or 2 weeks after the trauma also passed into the endoneurium of almost all animals (Table 1). Two animals did not show any endoneurial penetration of EBA; in these animals the injection had resulted in a minimal amount of the tracer also in the connective tissue sheaths around the nerve. In all the other animals the tracer was seen in and distal to the lesion of the endoneurium where numerous yellow autofluorescent products also had been formed. Here EBA was diffusely distributed but several cells had also accumulated the tracer in their cytoplasm.

When the EBA tracer was injected around the sciatic nerve 4 weeks after the crush injury 4 out of 5 mice showed a similar endoneurial penetration of EBA as in the preceeding groups. The amount of tracer particularly distal to the lesion appeared to be considerably smaller than in the acute experiments. One mouse did not show any abnormality with regard to the localization of the tracer. Four months after the injury 6 out of 9 mice showed abnormal perineurial permeability with endoneurial penetration of the tracer.

The permeability of the perineurium in the distal part of crushed nerves was also tested in a separate series of rats. In this species the nerve was long enough to permit a localized application of tracer distal to the lesion with only a minor diffusion into the crushed area. No rats in this series (1—30 days after the lesion) showed any abnormal penetration of EBA into the endoneurium beneath the site of application. However, in three nerves there was a faint penetration of EBA into the crushed area.

Discussion

The application of the fluorescent protein tracer technique (Steinwall and Klatzo, 1966; Hamberger and Hamberger, 1966) makes it possible to detect

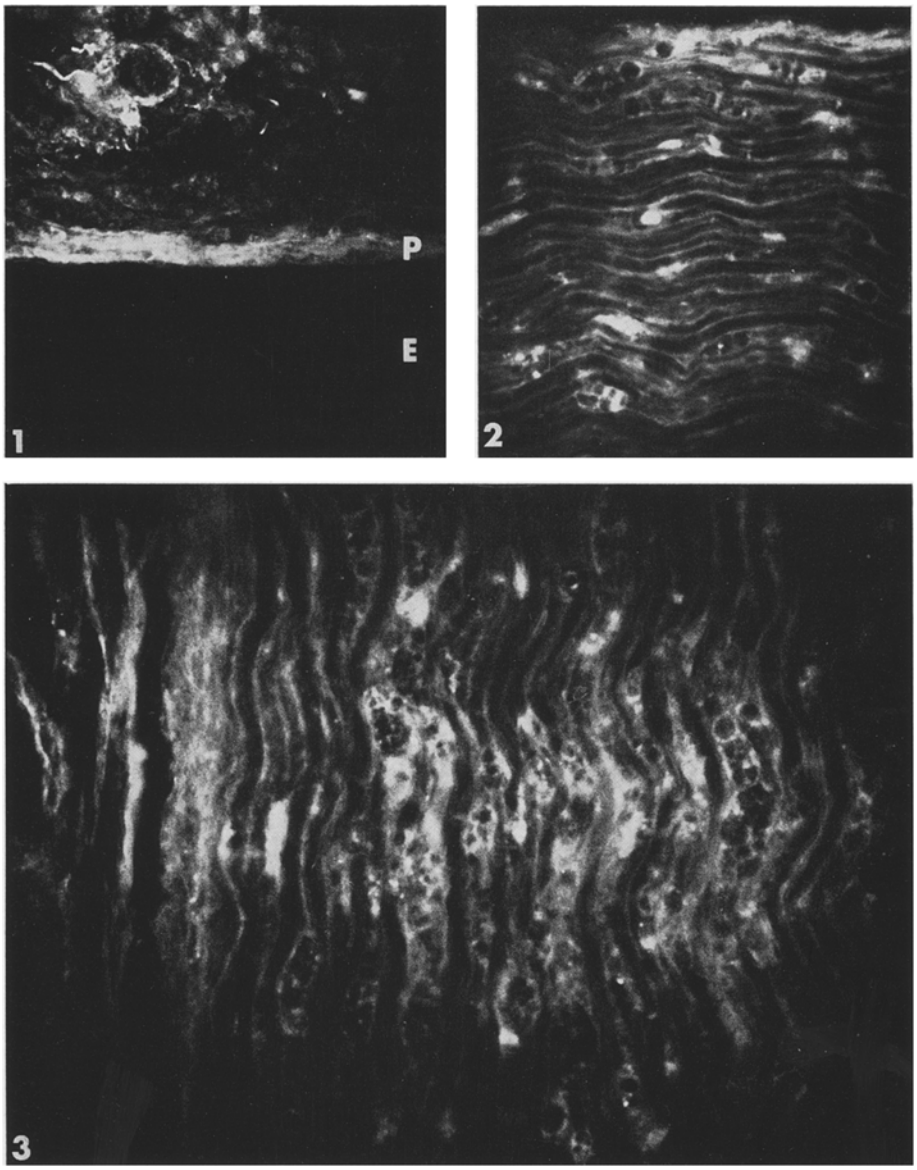

Fig. 1. Normal mouse sciatic nerve. Fluorescent albumin (white) was injected around the nerve. The tracer has spread to the innermost part of the external nerve sheaths but its diffusion into the endoneurium (*E*) is prevented by the perineurium (*P*)

Fig. 2. Distal portion of a sciatic nerve crushed 24 h earlier. The fluorescent tracer has spread into the endoneurium of the distal part and appears as fluorescent lines arranged in longitudinal direction

Fig. 3. Immediately after the crush injury the tracer has diffused into the endoneurium of the traumatized area but not into the central (upper part) or into the distal part of the nerve (lower part)

variations in perineurial permeability caused by a pathological process since in normal mature animals the perineurium completely restricts the diffusion of protein tracers like EBA and peroxidase into the endoneurium (Olsson, 1966b; Olsson and Reese, 1969, 1971; Klemm, 1970; Kristensson and Olsson, 1970). The present experiments showed that trauma to a nerve induces an abnormal permeability of the perineurium even though the anatomical continuity of the nerve trunk is not interrupted. It was also shown that this abnormality remains over a long period of time following the nerve injury.

We also observed that the protein tracer passed into the nerve at the site of the primary injury. From this area the protein tracers diffused into the endoneurium from the surrounding tissue. Previous electron microscopical observations by Haftek and Thomas (1968) also show that crush injury to a nerve destroys perineural cells at the site of the lesion and that the continuity across the compressed area is maintained inter alia by the basement membranes of the perineurial cells. It therefore appears reasonable to assume that the deficient barrier function of the perineurium at the site of the compression is caused by the destruction of the perineurial cells allowing diffusion of the protein tracer through or between these cells.

Once inside the endoneurium the protein tracer spread into the distal portion of the crushed nerve. This is in line with previous observations on a proximodistal flow of colored and radioactive substances in normal nerves (Weiss *et al.*) and of the spread of protein-rich edema in crushed rat sciatic nerves (Olsson, 1966).

Our information about the behaviour of the perineurium in the distal part of crushed nerves is rather limited. The characteristic enzymehistochemical pattern of the individual perineurial cells in the distal part of sectioned nerves will not change over time (Shanthaveerappa and Bourne, 1964). On the other hand, O'Daley and Imaeda (1967) found that in subcutaneous nerve branches distal to a crush lesion performed three days earlier the perineurial sheeth is no longer continuous since gaps are formed between perineurial cells. After six days signs of restitution started and finally the perineurium did not show any ultrastructural abnormalities. We found that in the distal part of crushed sciatic nerves the perineurium maintains it's property as a diffusion barrier when tested 1, 7 and 30 days after the lesion. Since the permeability of the perineurium is intimately connected with the presence of tight junctions between the perineurial cells (Olsson and Reese, 1969, 1971; Klemm, 1970; Kristensson and Olsson, 1971) it would be interesting to reinvestigate to what extent perineurial gaps actually are formed in the distal parts of large peripheral nerves following a crush. The perineurium in a nerve like the sciatic differs from that in cutaneous nerve branches in being multilayered and it is possible that a limited number of gaps may be without significance for the barrier function as tested in our experiments. In this connection it should also be recalled that the barrier function of the perineurium at the site of the lesion was abnormal in most animals even four months after the injury. This would then indicate that some abnormality of the perineurial cell contacts remain in this area even though an early ensheathment with perineurial cells have been reported in crushed nerves (Haftek and Thomas, 1968).

Even a localized trauma to a nerve may therefore for long time change the environment of the nerve fibres far away from the primary injury by diffusion of substances from the tissue surrounding the traumatized region. This may have implications in certain pathological conditions since it can be assumed that various toxic and infectious agents in this way may enter the endoneurium which otherwise would be prevented by the impermeable perineurium.

Acknowledgements. Supported by grants from the Swedish Medical Research Council, Project No. B72-12X-3020-03A.

References

Burkel, W. E.: The histological fine structure of perineurium. Anat. Rec. **158**, 177—190 (1967).

Cravioto, H.: The perineurium as a diffusion barrier: Ultrastructural correlates. Bull. Los Angeles neurol. Soc. **31**, 196—208 (1966).

Denny-Brown, D.: Importance of neural fibroblasts in the regeneration of nerve. Arch. Neurol. Psychiat. (Chic.) **55**, 171—215 (1946).

Haftek, J., Thomas, P. K.: Electron-microscope observations on the effects of localized crush injuries on the connective tissues of peripheral nerve. J. Anat. (Lond.) **103**, 233—243 (1968).

Hamberger, A., Hamberger, B.: Uptake of catecholamines and penetration of trypan blue after blood-brain barrier lesions. Z. Zellforsch. **70**, 386—392 (1966).

Key, A., Retzius, G.: Studien in der Anatomie des Nervensystems und des Bindegewebes Stockholm: Samson & Wallin 1876.

Klemm, H.: Das Perineurium als Diffusionsbarriere gegenüber Peroxydase bei epi- und endoneuraler Applikation. Z. Zellforsch. **108**, 431—445 (1970).

Kristensson, K., Olsson, Y.: The perineurium as a diffusion barrier to protein tracers. Differences between mature and immature animals. Acta neuropath. (Berl.) **17**, 127—138 (1971).

Kurúcz, J., Osgyáni, J.: Contributions to the pathomorphology of pulmonary innervation. Acta morph. Acad. Sci. hung. **4**, 227—232 (1954).

Lieberman, A. R.: The connective tissue elements of the mammalian nodose ganglion. An electron microscope study. Z. Zellforsch. **89**, 95—111 (1968).

Martin, K. H.: Untersuchungen über die perineurale Diffusionsbarrieren an gefriergetrockneten Nerven. Z. Zellforsch. **64**, 404—428 (1964).

O'Daly, J. A., Imaeda, T.: Electron microscopic study of Wallerian degeneration in cutaneous nerves caused by mechanical injury. Lab. Invest. **17**, 744—771 (1967).

Olsson, Y.: The effect of the histamine liberator, Compound 48/80 on mast cells in normal peripheral nerve. Acta path. microbiol. scand. **68**, 563—574 (1966a).

— Studies on vascular permeability in peripheral nerves. 1. Distribution of circulating fluorescent serum albumin in normal, crushed and sectioned rat sciatic nerve. Acta neuropath. (Berlin) **7**, 1—15 (1966a).

— Reese, T. S.: Inaccessibility of the endoneurium of mouse sciatic nerve to exogenous proteins. Anat. Rec. **163**, 722 (1969).

— — Permeability of vasa nervorum and perineurium in mouse sciatic nerve studied by fluorescence and electron microscopy. J. Neuropath. exp. Neurol. **30**, 195—221 (1971).

Röhlich, P., Knoop, A.: Elektronmikroskopische Untersuchungen an den Hüllen des N. ischiadicus der Ratte. Z. Zellforsch. **53**, 299—312 (1961).

— Weiss, M.: Studies on the histology and permeability of the peripheral nervous barrier. Acta morph. Acad. Sci. hung. **5**, 335—347 (1955).

Shanthaveerappa, T., Bourne, G. H.: Enzyme histochemical properties of the perineurial epithelium following nerve transection. Anat. Rec. **150**, 35—47 (1964).

— — Perineural epithelium. A new concept of its role in the integrity of the peripheral nervous system. Science **154**, 1464—1467 (1966).

— Hope, J., Bourne, G. H.: Electron microscopic demonstration of the perineural epithelium in rat peripheral nerve. Acta anat. (Basel) **52**, 193—201 (1963).

Steinwall, O., Klatzo, J.: Double tracer methods in studies on bloodbrain barrier dysfunction and brain edema. Acta neurol. scand. ,Suppl. **41**, 591—595 (1965).
Sunderland, S.: The connective tissues of peripheral nerves. Brain **88**, 841—854 (1965).
Thomas, P. K.: The connective tissue of peripheral nerve: an electron microscope study. J. Anat. (Lond.) **97**, 34—44 (1963).
Waggener, J. D., Bunn, S. M., Beggs, J.: The diffusion of ferritin within the peripheral nerve sheath: an electron microscopy study. J. Neuropath. exp. Neurol. **24**, 430—443 (1965).
Weiss, P., Wang, H., Taylor, A. C., Edels, M. V., Jr.: Proximo-distal connection in the endoneurial spaces of peripheral nerves demonstrated by colored and radioactive (isotope) tracers. Amer. J. Physiol. **143**, 521—540 (1945).

Yngve Olsson, M.D.
Institute of Pathology
Box 553
75122 Uppsala
Sweden