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Sheaths of the spinal nerve roots

Permeability and structural characteristics of dorsal and ventral spinal nerve roots of the rat*

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Summary. The present study was carried out to investigate the permeability of normal spinal nerve root sheaths around dorsal and ventral roots in the rat. In vivo studies were performed using Evans blue-albumin and lanthanum chloride as tracers. The Evans blue-albumin complex is macromolecular in size and lanthanum ions are small and easily visible in the electron microscope. Both tracers were injected into the subarachnoid space and 15 min later samples were taken and further processed for detection of tracer. Post-mortem studies with lanthanum was also performed. Following fixation by cardiac perfusion with fixative without tracer, lanthanum chloride was added to the fixative and applied directly to exposed spinal cord including the spinal nerve roots. Macroscopical examination showed Evans blue staining of the superficial blood vessels of the spinal cord, but no staining of the parenchyma of either spinal cord or nerve roots. Fluorescence microscopy revealed, in addition to a bright red fluorescence of root sheaths, a faint longitudinally orientated red fluorescence in the endoneurium of the nerve roots, indicating the presence of the dye-albumin complex. In both in vivo and post-mortem lanthanum studies, the tracer was detected between cell layers of the nerve root sheath and in invaginations of the plasma membrane of these cells, as well as inside the nerve root parenchyma. Some of the cells of the sheaths in post-mortem animals were diffusely marked with intracellular tracer. The endoradicular lanthanum was most often seen superficially, but lanthanum could occasionally be detected deeper in the parenchyma in the post mortem studies. The results show that the spinal nerve root sheaths are permeable to both the macromolecular substance Evans blue-albumin and the small lanthanum ion. No differences were detected between dorsal and ventral roots, nor between proximal and distal parts of the roots.

Key words: Spinal nerve roots – Root sheath – Structure – Permeability

The spinal nerve roots connect the central nervous system with peripheral nerves and by electrical signals along axons transfer information to and from the spinal cord. Such a complicated function is best carried out when there is homeostasis, i.e. a constant internal milieu as pointed out by Goldstein and Betz [11], otherwise fluctuations in the concentrations of ions, various macromolecules and amino acids may result in inadequate transmission. Thus, for normal function, the axons of the nerve roots most probably depend on the composition of the extracellular fluid, the regulation of which is not well known [26, 45]. The root sheaths [39] and blood vessels [2, 28–31, 41, 48] would be expected to influence its composition and detailed knowledge of their structure and permeability is essential to understand how the internal milieu is regulated in the nerve roots.

In 1873 Key and Retzius [19] published one of the earliest important papers on the histology of the epi-, peri- and endoneurium of the peripheral nervous system. Shanthaveerappa and Bourne [42, 43] demonstrated by microdissection and light microscopy, the continuity of the pia-arachnoid membrane covering spinal nerve root with the "perineurial epithelium" surrounding nerve fascicles. They considered that the squamous cells covering the spinal roots and the "perineurial epithelium" were identical in shape, size, cell borders, nuclear character, distribution of sex chromatin and enzyme histochemical properties.

From the ultrastructural features in laboratory animals, particularly the rat, the root sheaths are regarded as consisting of an inner perineurial coat and an outer coat continuous with the pia-arachnoid [10, 12–14, 22].

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The cells of the outer coat are loosely arranged with intervening lacunae containing connective tissue elements. Junctions between the cells are intermittent and would permit the fluid of the subarachnoid space to communicate with that in intercellular spaces of the outer coat [12]. The inner coat is regarded as an extension of the peripheral nerve perineurium or at least to resemble it [10, 12–14, 22]. A basal lamina lies between the innermost cells of the inner coat and the nerve root parenchyma [10, 12, 14, 22, 23] but elsewhere in the root sheath, the basal lamina is inconstant [12, 22]. These structural arrangements of nerve root sheaths are confirmed in the rat by Radek et al. [32]. The inner coat of the root sheath is considered to end at the transitional zone close to the spinal cord [10, 13, 22].

The perineurium has a barrier function to macromolecular substances [21, 22, 26, 45] and probably acts as a metabolic diffusion barrier [42, 43]. Reports on the permeability of the spinal nerve root sheaths are scanty. Weiss and Röhlich [49] reported passage of India ink from the subarachnoid space of the rat into both sensory and motor nerve roots. Bowsher [3] using ^{35}S-containing serum protein and Klatzo et al. [20] using fluorescein isothiocyanate-labelled albumin illustrated in cats the presence of tracer inside spinal nerve roots following injection into the cerebrospinal fluid. Rydevik et al. [39] published an important study in which they reported in the pig, the transfer of [^{3}H] methylglucose from the cerebrospinal fluid into the lumbar nerve roots. They considered this pathway to be more important for the nutrition of the nerve root than nutrition from the blood supply. On the other hand Kaar and Fraher [18], on the basis of a morphological investigation using rats, claimed that the subpial, endoneurial and interradicular spaces are continous, but isolated from the subarachnoid space.

Pettersson et al. [30] observed by immunohistochemistry in normal rats that the spinal nerve roots contain immunoreactive material indicating the presence of serum albumin in the extracellular spaces. This may denote an incomplete blood-spinal nerve root barrier due to leaking of the intrinsic blood vessels [29, 31]. The purpose of the present study was to investigate the structure and permeability of the potential barrier in the root sheaths of the spinal nerves of the rat by evincing by microscopical methods, the permeation and distribution of Evans blue-albumin and lanthanum. The results show that the nerve root sheaths do not have a complete barrier function to such tracers when present in the cerebrospinal fluid.

Material and methods

Male Sprague-Dawley rats weighing 250–380 g were adjusted to laboratory environment, with free access to food and water, for a few days before use. For 12–16 h before the experiments food was not provided. Animals were obtained from Alab, Sollentuna, Sweden.

Permeability investigations

Evans blue tracer technique

Evans blue-albumin was used as an in vivo macromolecular tracer. Five rats were injected subcutaneously with 0.2 ml Atropin (0.5 mg/ml, ACO, Sweden) and 30 min later they were anesthetized with Mebumal (60 mg/ml, Nord Vacc, Sweden). The back of the head was incised and the atlanto-occipital membrane exposed. An opening was made in the membrane with a 22-gauge needle. The cannula was withdrawn, which resulted in leakage of small amounts of cerebrospinal fluid from the opening in the membrane. A small catheter (PE 10) was introduced through the hole for a few millimeters in a caudal direction.

A solution containing 25 µl of 1 % Evans blue and 5 % albumin in saline was injected, through the catheter, for 3 min and 15 min later the rats were killed by cardiac perfusion. The chest was opened rapidly, the right auricle incised and the blood washed out with saline through a cannula inserted into the left ventricle. The saline perfusion lasted 5 min at a pressure of 100 mm Hg and was followed by perfusion of 250 ml of 10 % formalin at the same pressure, at room temperature. Portions of the cervical enlargement including the spinal nerve roots were fixed for 1 h and 10-µm-thick sections were cut on a Leitz Kryomat and mounted in 50 % glycerin in water.

During the dissection, the spinal cord and nerve roots were examined macroscopically and the presence of blue staining recorded. Cryo-sections from five to eight ventral and dorsal roots of each rat were inspected in a Leitz fluorescence microscope for the presence of Evans blue which emits red fluorescence in ultraviolet light [44]. The spinal nerve roots of the normal rat do not exhibit any red autofluorescence disturbing evaluation of these experiments (cf. [31]).

Lanthanum tracer techniques

A solution of lanthanum ions added to a fixative provides a tracer of small size which due to its high electron density is easily visible by electron microscopy [6, 8, 15, 36, 37, 40]. Two forms of application were used.

Post-mortem lanthanum studies. Five rats were killed by perfusion as in the Evans blue experiments but the fixative used was 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M cacodylate buffer, pH 7.2. After fixation, the cervical enlargement of the spinal cord, and nerve roots were exposed. Drops of fixative containing 2 % lanthanum chloride were applied and the tissues submerged in this fixative for 40 min. Then portions of the dorsal and ventral roots were removed and put in fixative devoid of lanthanum and were post fixed in osmium tetroxide, rinsed in buffer, contrasted with 1 % uranyl acetate, dehydrated in a graded series of ethyl alcohol and embedded in Epon.

In vivo lanthanum studies. As in the Evans blue experiments five rats were treated with Atropin, Mebumal and catheter insertion into the subarachnoid fluid. A solution containing 25 µl of 2 % lanthanum chloride in saline was injected for 3 min and the rats were killed 15 min later by cardiac perfusion and fixed as in the post-mortem studies. Cervical, dorsal and ventral nerve roots were then prepared for electron microscopy, by the same procedure as in the post-mortem studies. Sections (1 µm thick) of nerve roots from post-mortem and in vivo lanthanum experiments were stained with toluidine blue and examined by light microscopy. Regions containing root sheath and underlying endoneurium were selected and sectioned for electron microscopy. These thin sections were contrasted with lead citrate [38]. Observations were made on 20 post-mortem and 20 in vivo nerve roots using a Philips electron microscope.

Structural investigations

Morphological examinations were carried out on 21 rats. Ten of them were derived from the lanthanum experiments used for permeability studies in this report and 11 from previous experiments [30, 31]. Cervical nerve roots were taken from 16, and lumbar and caudal roots from 9 rats.

Light microscopy. Light microscopy was carried out on formalin-fixed, paraffin sections from 32 roots stained by hematoxylin and eosin, and on toluidine blue-stained plastic sections from 88 roots. The toluidine blue material was from animals killed by cardiac perfusion with 2.5 % glutaraldehyde and 2 % paraformaldehyde in 0.1 M cacodylate buffer pH 7.2 with or without 2 % lanthanum nitrate added to the fixative. All the samples were then fixed in osmium tetroxide, rinsed in buffer, contrasted in 1 % uranyl acetate, dehydrated in a graded series of ethyl alcohol and finally embedded in Epon.

Electron microscopy. Ultrathin sections, contrasted with lead citrate [38], from all 88 toluidine blue-stained specimens were examined using a Philips electron microscope.

Results

Permeability properties

Evans blue experiments. Fifteen minutes after injecting Evans blue through the opening of the atlanto-occipital membrane a clear staining of the cerebrospinal fluid throughout the whole length of the cervical enlargement could be seen. Dissection of the spinal cord cervical enlargement with its spinal nerve roots was made and the samples were put into fixative. Macroscopical examination of these samples showed no clear staining of either spinal cord parenchyma, or the nerve root endoneurium but blood vessels on the spinal cord were strongly blue.

Fluorescence microscopy elicited bright red fluorescence of the sheaths of the nerve roots and longitudinally orientated, weaker fluorescence of the nerve root parenchyma probably delinating individual nerve fibers (Fig. 1). The blood vessels in the nerve roots were strongly fluorescent and fluorescence surrounded the nerve cells of the root ganglia. Adjacent parts of peripheral nerve endoneurium also showed fluorescence. These observations were made in all samples from all animals.

In vivo lanthanum experiments. Lanthanum experiments made it possible to perform ultrastructural observations on the spread of tracer from the subarachnoid space into nerve root parenchyma. In 80 % of the nerve roots investigated small amounts of electron-dense tracer were detected on the surface of the root sheath (Fig. 2A).

In more than half of the nerve roots presenting lanthanum on their surface, tracer was seen between the layers of cells of the root sheath. This was most often found superficially but also in inner parts of the sheaths (Fig. 2A). Vesicles filled with tracer were found occasionally in both the inner and the outer coat (Fig. 2A,B). In the endoneurium of the nerve roots, which showed

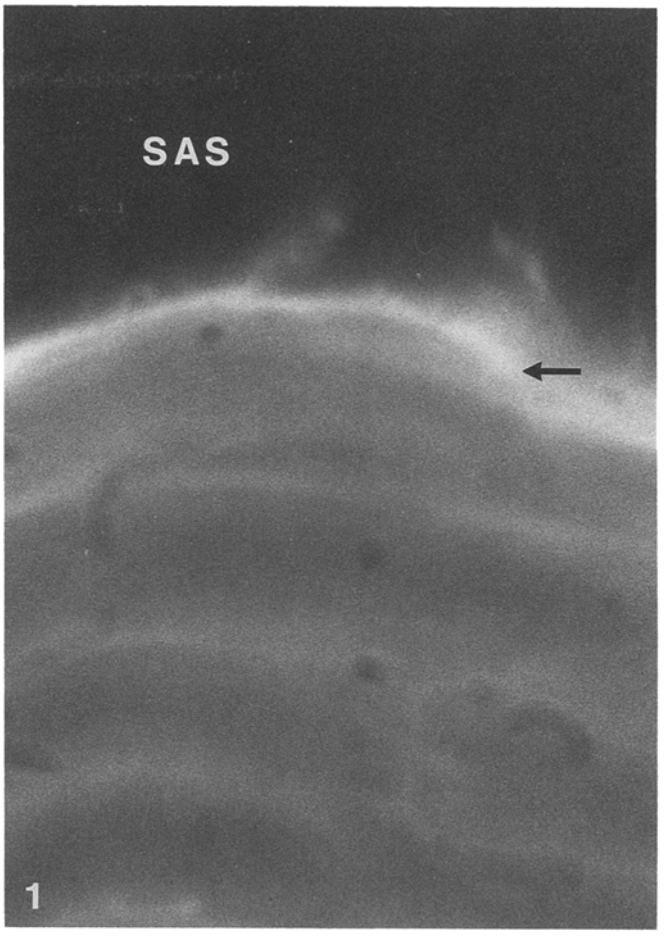


Fig. 1. Fluorescence micrograph from a dorsal spinal nerve root of a rat after 15 min of administration of Evans blue-albumin in the subarachnoid space, showing longitudinally orientated intraparenchymal fluorescence and a brighter fluorescence of the nerve root sheath (*arrow*). *SAS,* Subarachnoid space

lanthanum on their surface, there was a spot-like distribution of lanthanum crystals in about two thirds of these roots. This was even true for three roots which did not contain any tracer between different cellular layers of the root sheath. These deposits were in almost all cases restricted to regions immediately beneath the root sheath (Fig. 2B).

Post-mortem lanthanum experiments. Lanthanum was seen always in large amounts on the surface of the roots in all samples (Fig. 3A,D,E). Tracer was detected between the different cell layers of the root sheath and most pronouncedly between the outer layers but to a lesser degree also between deeper layers (Fig. 3A–C).

Some invaginations of the plasma membranes present in both the perineurial and the pia arachnoidal cells of the root sheath were filled with lanthanum (Fig. 3B,C) and occasional "vesicles" containing tracer were also seen. However, such "vesicles" may well be obliquely cut invaginations of the plasma membrane since closure of invaginations to form real vesicles would be impossible in the fixed tissue. Often great parts of the outer layers showed diffusely distributed electron-dense material in

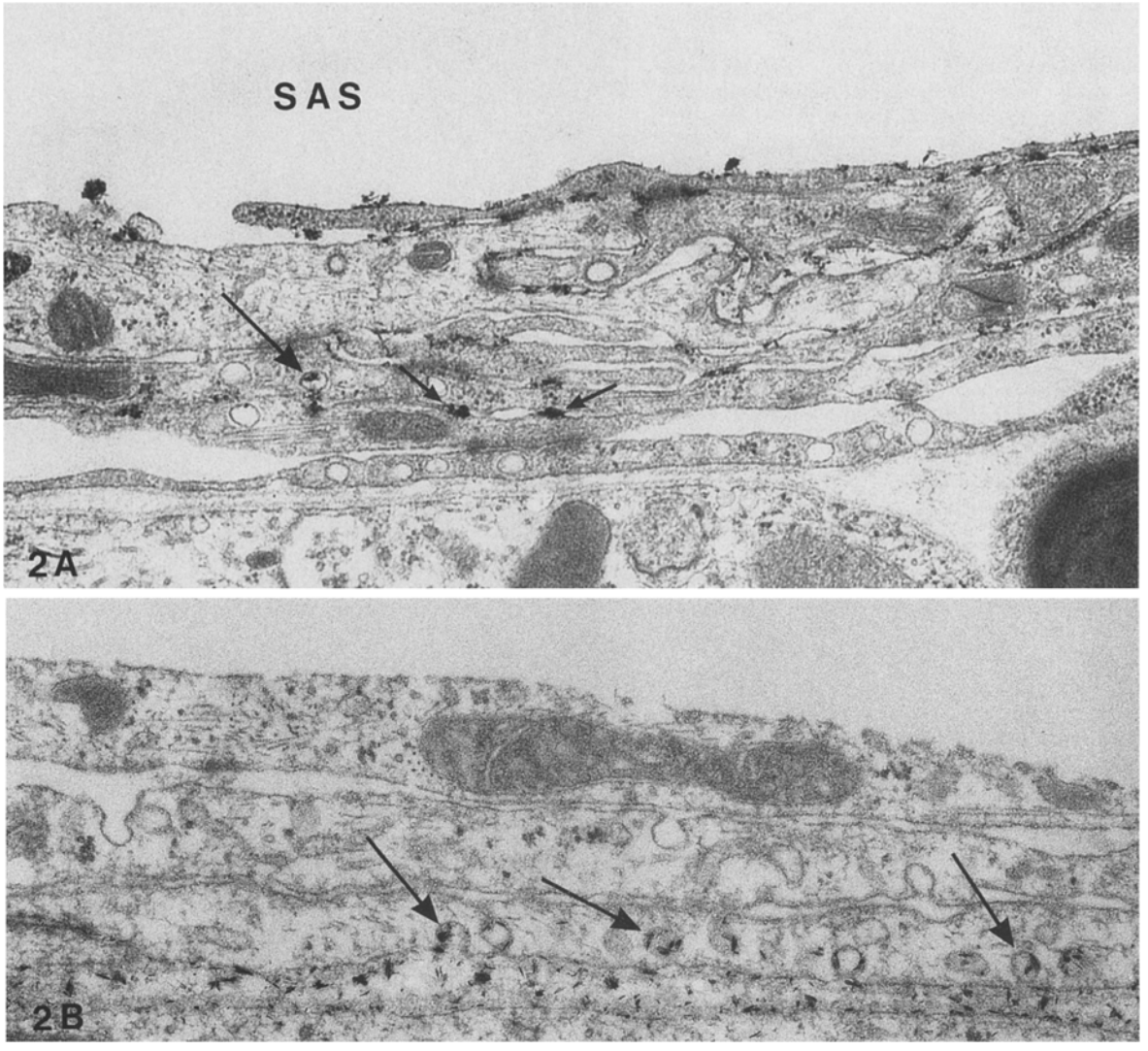


Fig. 2A,B. Electron micrographs from spinal nerve roots, at the level of the cervical enlargement, of rats subjected to lanthanum chloride injection into the subarachnoid space in vivo. **A** Small amounts of lanthanum can be seen at the surface of the nerve root sheath. The tracer is also detected between different cell layers of the root sheath. This is most pronounced between outer layers, but also seen deeper in the sheath (*small arrows*). Some vesicles, predominantly in cells of the inner coat, contain lanthanum deposits (**A,B** *big arrows*). **B** shows a spot-like distribution of lanthanum crystals in the nerve root parenchyma immediately beneath the root sheath. *SAS,* Subarachnoid space **A,B.** Dorsal root, distal part, **A** × 37 200; **B** × 55 800

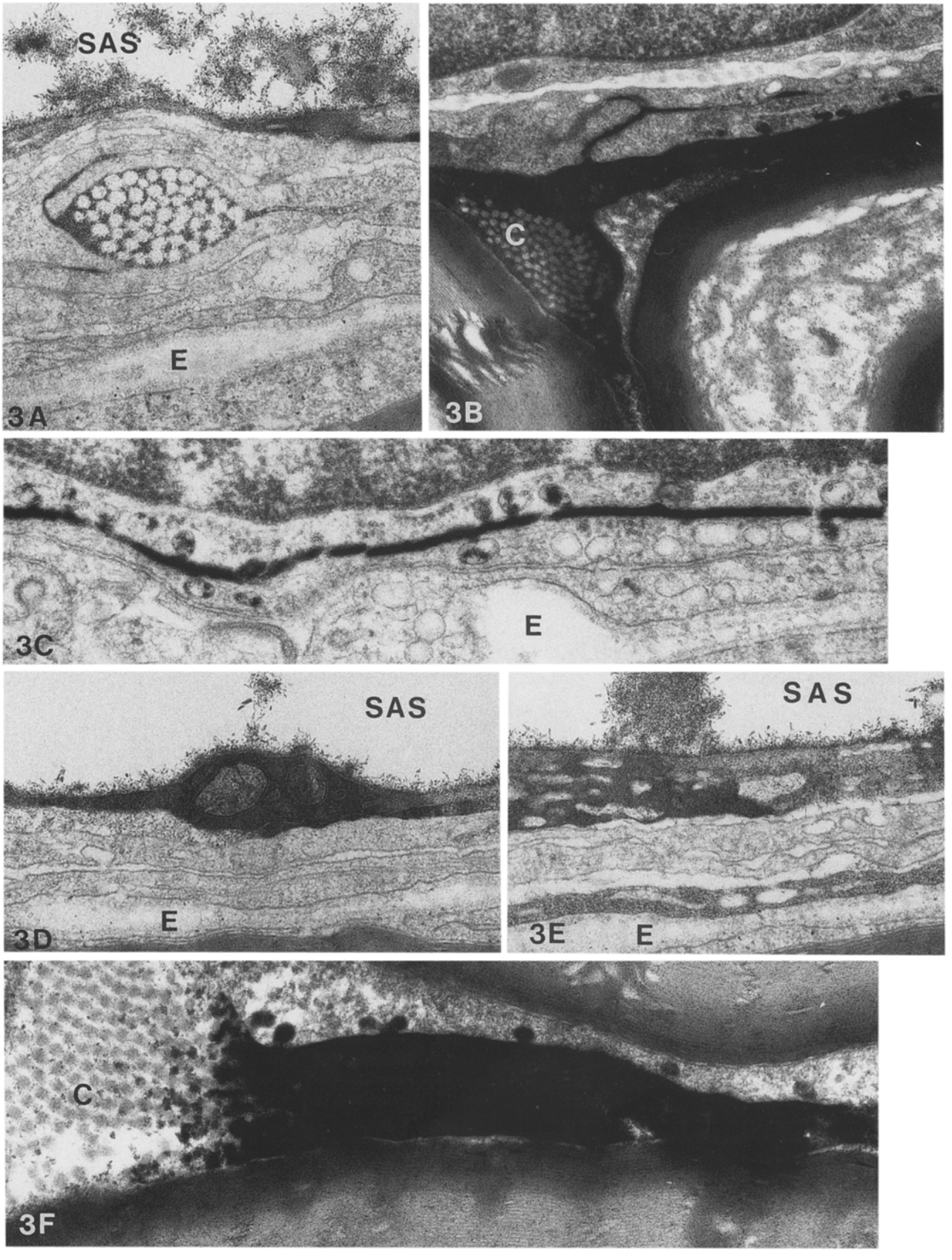
the cytoplasm of their cells. This phenomenon was also seen in deeper layers, though less frequently (Fig. 3A,D,E). No such intracellular deposits of lanthanum were ever detected in the in vivo experiments.

Lanthanum was present in the nerve root parenchyma of most nerve roots. Massive spread of lanthanum was seen between the collagen fibrils (Fig. 3B,F). Differences were not detected in any of the lanthanum experiments between the dorsal and ventral roots nor between proximal and distal parts.

Structural characteristics

Light microscopy. Longitudinal sections of the paraffin-embedded lumbar and caudal spinal nerve roots stained

Fig. 3A–F. Electron micrographs from nerve roots, arising from the cervical enlargement, subjected to lanthanum administration following fixation (post-mortem studies). The figures show: great amounts of lanthanum on the surface of root sheaths (**A,D,E**); lanthanum between different cell layers of root sheaths (**A–C**), in **A** the tracer surrounds collagen fibrils between cells of the outer coat); lanthanum containing invaginations of plasma membranes in root sheath cells (**B,C**); diffusely distributed lanthanum particles in the cytoplasm of root sheath cells of the outer (**A,D,E**) and inner (**E**) coats; massive distribution of lanthanum in the endoneurial connective tissue space, between collagen fibrils, immediately beneath the root sheath (**B**) and deeper in the nerve root parenchyma (**F**), in **F** lanthanum is also seen in invaginations of the plasma membrane of a Schwann cell. *SAS*, Subarachnoid space; *E,* endoneurium of nerve root; *C* collagen fibrils. **A,D,E** Dorsal root, proximal part, **B,F** dorsal root, distal part, **C** ventral root, proximal part. **A,C–F** × 55 800; **B** × 37 200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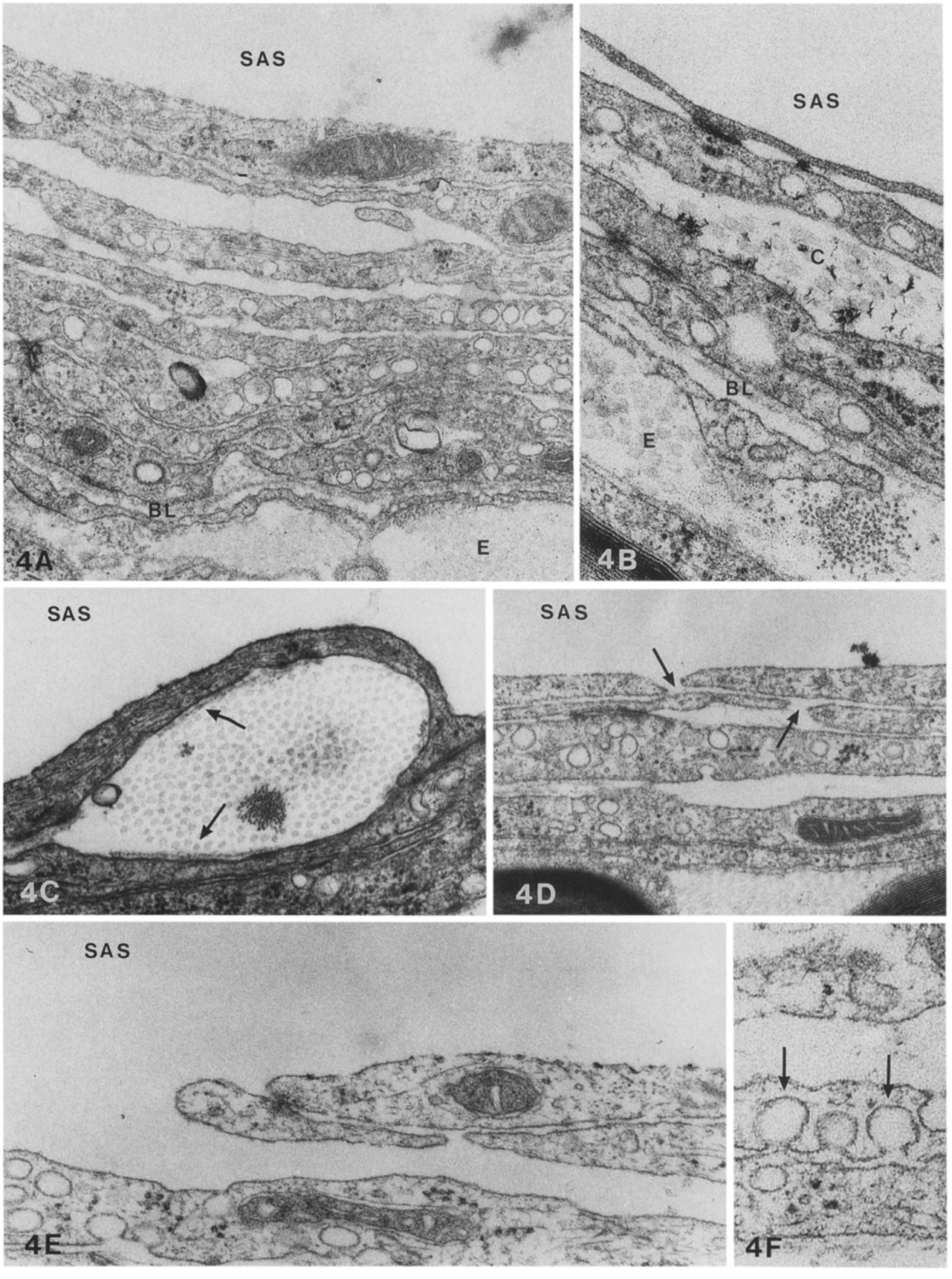
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by hematoxylin and eosin showed that the root sheath consisted of a very thin layer of cells with elongated nuclei. Epon sections of roots stained by toluidine blue confirmed this observation and showed sleeves of root sheath cells surrounding the extrinsic blood vessels.

Electron microscopy. The root sheaths consisted of two to eight layers of flattened cells (Fig. 4A,B,D). Collagen fibrils occurred between these layers and in lacunae formed by the cells, especially in outer root sheath layers (Fig. 4B,C). The cells in the inner coat of the sheath were more compactly arranged than in the outer coat, in which cells were not always closely apposed, and in the outermost layer cells did not always completely encircle the nerve root (Fig. 4A,B,D,E).

A basal lamina covered the innermost aspect of the root sheath. In other parts the basal lamina was intermittent and occurred only rarely in outer layers (Fig. 4A–D). The root sheath cells of both layers contained vesicles and invaginations of their plasma membranes (Fig. 4A,B,D,F). Some of them were elongated and very big, occupying half and sometimes even more of the depth of the cell (Fig. 4F). These structures were more numerous in the inner cell layers (Fig. 4A).

Discussion

In the central nervous system, the generation and transmission of electrical impulses function best when there is homeostasis [11] since fluctuations in the concentration of ions, macromolecules and amino acids influence neuronal activity. This is probably also true for the peripheral nervous system. In the brain and spinal cord the composition of the extracellular fluid is controlled by the blood-brain and blood-cerebrospinal fluid barriers [4, 5, 7, 11, 24, 27, 33, 47]. In peripheral nerve fascicles, the blood-nerve barrier and perineurium are the two chief components for the regulation of the internal milieu [21, 25, 26, 34, 45].

Fig. 4A–F. Electron micrographs illustrating morphological features of cervical spinal nerve root sheaths in the rat. All photos are from animals used for in vivo lanthanum experiments. **A** A root sheath built up of eight layers of flattened cells with a basal lamina (*BL*) facing the endoneurium of the nerve root. Cells of the inner coat of the root sheath are more closely arranged than cells of the outer coat. Multiple invaginations of plasma membranes and vesicles are seen predominantly in the inner coat. Dorsal root, distal part. **B** Collagen fibrils (*C*) between cells of a root sheath build up of four layers of cells. A continuous basal lamina (*BL*) is seen at the innermost surface of the root sheath. Ventral root, proximal part. **C** A collagen containing lacuna in the outer coat of a root sheath. Note the intermittent basal lamina (*arrows*), which only occasionally is found in this outer coat. Ventral root, proximal part. **D** Interspace between cells of the same layer in the outer coat (*arrows*). Dorsal root, proximal part. **E** Incomplete outermost cell layer. Ventral root, distal part. **F** Very big invaginations of the plasma membrane (*arrows*), occupying two thirds of the cell depth, in cells of the inner coat of a root sheath. Ventral root, distal part. *SAS*, Subarachnoid space; *E*, endoneurium of nerve root. **A** × 37200; **B** × 55800; **C,D** × 37000; **E** × 57700; *F* × 86800

Compared with the brain and peripheral nerves there have been few investigations on the control of the internal milieu of spinal nerve roots and most have concentrated on vascular permeability [2, 28–31, 41, 48]. Reports on vascular permeability within dorsal root ganglia confirm that they lie outside the territory of the blood-brain barrier [1, 17]. Since the root sheaths are very thin and lie in the subarachnoid space, it is plausible that substances in the cerebrospinal fluid influence the internal milieu of the roots and that as in edematous conditions, substances produced in the roots are transferred to the cerebrospinal fluid. This would explain why changes in the protein composition are recorded in the cerebrospinal fluid in inflammatory diseases of the roots, such as the Guillain-Barré's syndrome [46].

When injected into the subarachnoid space, analgetics act more rapidly and in smaller doses than when injected epidurally or alongside peripheral nerve trunks [10]. Thus, the root sheath seems to provide less insulation than a true perineurium, which could be of great significance for the regulation of the internal milieu in the nerve roots.

While the present investigation was in progress, Rydevik et al. [39] in a study on pigs reported important observations on the diffusion of [^{3}H methylglucose from the cerebrospinal fluid into spinal nerve roots. [^{3}H] Methylglucose was injected both intravenously and directly into the cerebrospinal fluid, and samples of nerve roots, cerebrospinal fluid, blood and peripheral nerves were taken. They showed that two routes of transport are available for the small solutes into the roots, namely from the blood vessels and cerebrospinal fluid. The authors proposed that the contribution of nutrients from the cerebrospinal fluid to the nerve roots is greater than the contribution from the blood.

In the present experiments two tracers were used: Evans blue-albumin, which is a macromolecule, and lanthanum, which is a very small molecule and easily visible in the electron microscope. The tracers were injected into the cerebrospinal fluid and 15 min later their distribution was determined by microscopy. Lanthanum was also used after fixation by cardiac perfusion to record if differences occurred in the permeability of lanthanum in live and fixed cells. Post-mortem studies can establish if there are open pathways through or between the cells.

The results show that, in the rat, substances applied to the outside of the sheaths of the spinal nerve roots can be detected inside the nerve root parenchyma already after 15 min. This is different than in peripheral nerves, the perineurium of which is an efficient diffusion barrier [21, 26, 34, 45]. The findings are in line with results in other animal species or with other compounds [3, 10, 20, 39, 49]. On the other hand Kaar and Fraher [18] showed in the rat that the subarachnoid and endoneurial spaces are morphologically isolated from each other. According to our data and that of others [3, 10, 20, 39, 49], this isolation is rather structural than functional. To my knowledge this is the first demonstra-

tion of the transfer of a visible tracer from the cerebrospinal fluid into nerve roots in experimental animals.

When the catheter is inserted through the atlanto-occipital membrane there is always the risk of rupturing the spinal cord: hence during dissection, careful examination is essential. If there was the slightest suspicion of rupture of the spinal cord, the animal was excluded from the experiment. All rats used in this investigation should, therefore, be considered as normal. In all the Evans blue experiments, the cerebrospinal fluid around the cervical enlargement was distinctly blue. Therefore, all nerve roots investigated have been exposed to the tracer and could be considered as valid for further investigations. In the lanthanum studies, macroscopic examination was invalid but after examination by electron microscopy, all specimens which did not present lanthanum on the surface of the nerve root sheath were excluded from the investigation.

The routes whereby substances move from the cerebrospinal fluid into the nerve roots are unknown but it must be stressed that the nerve root sheaths are very thin compared with the perineurium of peripheral nerves. The inner cell layers of the root sheaths show basal lamina and have the characteristics of perineurial cells, but only the innermost basal lamina adjacent to the endoneurium seems to be complete. The remaining cell layers (outer coat) are supposed to be formed by pia-arachnoid, and only occasional fragments of basal lamina occur on the surface of these cells. Substances in the cerebrospinal fluid may first diffuse between cells of the outer coat to reach the inner perineurial cell layers. In this connection, it should be recalled that the pia-arachnoid of the brain and spinal cord is permeable to horseradish peroxidase but not to erythrocytes or Indian ink [9, 16, 35].

The further passage of tracers from the extracellular space of the outer coat into the nerve root parenchyma is a mystery. Tracers may enter the roots at the termination of the perineurium close to the spinal cord [10, 13, 22] or at the lateral recess [14], the lateral extension of the subrachnoid space between the dorsal root ganglia and the ventral nerve root. This region is thought to communicate with the endoneurium of the corresponding nerve [14]. Tracers which enter the endoneurium at the lateral recess may diffuse in a distal-proximal direction into the nerve roots [22, 23]. Other possible routes are through the perineurial cells by active transport or pinocytosis. It should be stressed that in this study we have found that vesicles are more numerous in the inner than in the outer coat of the root sheaths and that some of these vesicles, predominantly in the inner coat, contained lanthanum particles. An additional possible pathway for the trasfer of tracer from the subarachnoid space to the parenchyma of spinal nerve roots is by uptake in radicular or meningeal blood vessels and further transport into nerve root endoneurium due to vascular permeability in the roots [2, 30, 31, 41, 48].

Our observations that the sheath of the spinal nerve roots of the rat consists usually of 3 to 4 and sometimes up to 8 layers of flattened cells, are in accordance with the findings of Haller and Low [12] who found usually 3 to 4 but variations between 2 to 12 layers of cells. We never counted to more than 8 layers. This may be because we used rats, while in addition they used other animals, and the greater number of cell layers may occur in another species of animal. Both the present study and the study of Haller and Low [12] report the presence of collagen between these cells, and a basal lamina which seems to be continuous at the innermost aspect of the root sheath. This arrangement of the sheaths is also in accordance with the findings of Radek et al. [32]. Haller and Low [12] divide the root sheath into an inner (perineurial) and an outer (pia-arachnoidal) part. The cells of each part show somewhat different morphological characteristics. This was not that obvious in this study, but the number of cytoplasmic vacuoles and pinocytotic vesicles was, in agreement with Haller and Low [12], higher in the inner coat than in the outer coat.

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References

1. Arvidson B (1979) Distribution of protein tracers in peripheral ganglia. Acta Univ Ups 344:1–72
2. Arvidson B, Tjälve H (1986) Distribution of ^{109}Cd in the nervous system of rats after intravenous injection. Acta Neuropathol (Berl) 69:111–116
3. Bowsher D (1957) Pathways of absorption of protein from the cerebrospinal fluid: an autoradiographic study in the cat. Anat Rec 128:23–39
4. Bradbury M (1979) The concept of a blood-brain barrier. John Wiley & Sons, New York
5. Bradbury MWB (1985) The blood-brain barrier. Transport across the cerebral endothelium. Circ Res 57:213–222
6. Brightman MW, Reese TS (1969) Junctions between intimately apposed cell membranes in the vertebrate brain. J Cell Biol 40:648–677
7. Crone C (1986) The blood-brain barrier; a modified tight epithelium. In: Suckling AJ, Rumsby MG, Bradbury MWB (eds) The blood-brain barrier in health and disease. Harward, Chichester, pp 17–40
8. Doggenweiler CF, Frenk S (1965) Staining properties of lanthanum on cell membranes. Proc Natl Acad Sci USA 53:425–430
9. Feurer DJ, Weller RO (1991) Barrier functions of the leptomeninges: a study of normal meninges and meningeomas in tissue culture. Neuropathol Appl Neurobiol 17:391–405
10. Gamble HJ (1976) Spinal and cranial nerve roots. In: Landon DN (ed) The peripheral nerve. John Wiley & sons, New York, pp 330–354
11. Goldstein GW, Betz AL (1986) The blood-brain barrier. Sci Am 225:70–79
12. Haller FR, Low FN (1971) The fine structure of the peripheral nerve root sheath in the subarachnoid space in the rat and other laboratory animals. Am J Anat 131:1–20
13. Haller FR, Haller AC, Low FN (1972) The fine structure of cellular layers and connective tissue space at spinal nerve root attachments in the rat. Am J Anat 133:109–124
14. Himango WA, Low FN (1971) The fine structure of a lateral recess of the subarachnoid space in the rat. Anat. Rec 171:1–20

15. Hirano A, Kato T (1988) Ultrastructural methods to identify extracellular spaces in the central nervous system. In: Boulton AA, Baker GB, Walz W (eds) The neuronal microenvironment. Humana press, Clifton, pp 105–126
16. Hutchings M, Weller RO (1986) Anatomical relationships of the pia mater to cerebral blood vessels in man. J Neurosurg 65:316–325
17. Jacobs JM (1980) Vascular permeability and neural injury. In: Spencer PS, Schaumburg HH (eds) Experimental and clinical neurotoxicology. William & Wilkins, Baltimore, pp 102–117
18. Kaar GF, Fraher JP (1986) The sheaths surrounding the attachments of rat lumbar ventral roots to the spinal cord: a light and electron microscopical study. J Anat 148:137–146
19. Key A, Retzius G (1876) Studien in der Anatomie des Nervensystems und des Bindegewebes. Samson and Wallin, Stockholm
20. Klatzo I, Miquel J, Ferris PJ, Prokop JD, Smith DE (1964) Observations on the passage of the fluorescein-labelled serum proteins (FLSP) from the cerebrospinal fluid. J Neuropathol Exp Neurol 23:18–35
21. Klemm H (1970) Das Perineurium als Diffusionsbarriere gegenüber Peroxydase bei Epi- und Endoneuraler Applikation. Z Zellforsch 108:431–445
22. Low FN (1976) The perineurium and connective tissue of peripheral nerve. In: Landon DN (ed) The peripheral nerve. John Wiley & sons, New York, pp 159–187
23. McCabe JS, Low FN (1969) The subarachnoid angle: an area of transition in peripheral nerve. Anat Rec 164:15–34
24. Neuwelt EA (1989) Implications of the blood-brain barrier and its manipulation, vols 1 and 2. Plenum Medical Book Corporation, New York London, pp 1–403; 1–633, respectively
25. Olsson Y (1984) Vascular permeability in the peripheral nervous system. In: Dyck PJ, Thomas PK, Lambert EH; Bunge R (Eds) Peripheral neuropathy, vol 1, 2nd edn. WB Saunders, Philadelphia, pp 579–598
26. Olsson Y (1990) Microenvironment of the peripheral nervous system under normal and pathological conditions. CRC Crit Rev 5:265–311
27. Pardridge WM (1986) Blood-brain barrier: interface between internal medicine and the brain. Ann Intern Med 105:82–95
28. Parke WW, Watanabe R (1985) The intrinsic vasculature of the lumbosacral spinal nerve roots. Spine 10:508–515
29. Pettersson CÅV, Olsson Y (1989) Blood supply of spinal nerve roots. An experimental study in the rat. Acta Neuropathol 78:455–461
30. Pettersson CÅV, Salahuddin TS, Olsson Y (1990) Presence of plasma proteins in spinal nerve roots. An immunohistochemical study in the rat. Acta Neurol Scand 82:21–27
31. Pettersson CÅV, Sharma HS, Olsson Y (1990) Vascular permeability of spinal nerve roots. A study in the rat with evans blue and lanthanum as tracers. Acta Neuropathol 81:148–154
32. Radek A, Thomas PK, King RHM (1986) Perineurial differentiation in interchange grafts of rat peripheral nerve and spinal root. J Anat 147:207–217
33. Rapoport SI (1976) The blood-brain barrier in physiology and medicine. Raven Press, New York
34. Rechthand E, Rapoport SI (1987) Regulation of the microenvironment of peripheral nerve: role of the blood-nerve barrier. Prog Neurobiol 28:303–343
35. Rennels ML, Gregory TF, Blaumanis OR, Fujimoto K, Grady PA (1985) Evidence for a paravascular fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. Brain Res 326:47–63
36. Revel J-P, Hamilton DW (1969) The double nature of the intermediate dense line in peripheral nerve myelin. Anat Rec 163:7–16
37. Revel JP, Karnovsky MJ (1967) Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. J Cell Biol 33:C7–C12
38. Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J Cell Biol 17:208–212
39. Rydevik B, Holm S, Brown D, Lundborg D (1990) Diffusion from the cerebrospinal fluid as a nutritional pathway for spinal nerve roots. Acta Physiol Scand 138:247–248
40. Schatzki PF, Newsome A (1975) Neutralized lanthanum solution: a largely noncolloidal ultrastructural tracer. Stain Technol 50:171–178
41. Seitz RJ, Heininger K, Schwendemann G, Toyka KV, Wechsler W (1985) The mouse blood-brain barrier and blood-nerve barrier for IgG: a tracer study by use of avidin-biotin system. Acta Neuropathol (Berl) 68:15–21
42. Shantaveerappa TR, Bourne GH (1962) The "perineural epithelium", a metabolically active, continous, protoplasmic cell barrier surrounding peripheral nerve fasciculi. J Anat 96:527–537
43. Shantaveerappa TR, Bourne GH (1966) Perineural epithelium: a new concept of its role in the integrity of the peripheral nervous system. Science 154:1464–1467
44. Steinwall O, Klatzo I (1966) Selective vulnerability of the blood-brain barrier in chemically induced lesions. J Neuropathol Exp Neurol 25:542–559
45. Thomas PK, Olsson Y (1984) Microscopic anatomy and function of the connective tissue components of peripheral nerve. In: Dyck PJ, Thomas PK, Lambert EH, Bunge R (eds) Peripheral neuropathy, vol 1, 2nd edn. WB Saunders, Philadelphia, pp 97–120
46. Thomas PK, Landon DN, King RHM (1984) Diseases of the peripheral nerves. In: Adams JH, Corsellis JAN, Duchen LW (eds) Greenfields neuropathology, 4th edn. Edward Arnold, London, pp 807–920
47. van Deurs B (1980) Structural aspects of brain barriers with special reference to the permeability of the cerebral endothelium and choroidal epithelium. Int Rev Cytol 65:117–191
48. Waksman BH (1961) Experimental study of diphtheritic polyneuritis in the rabbit and the guinea pig. III. The blood-nerve barrier in the rabbit. J Neuropathol Exp Neurol 20:35–77
49. Weiss M, Röhlich P (1954) Significance of the interstice of the peripheral nerve. Acta Morphol 4:309–317