A PERINEURAL EPITHELIUM

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INTRODUCTION

In 1876 Key and Retzius (2) demonstrated what appeared to be a cellular sheath made up of endothelial-like cells associated with nerve trunks. Studies made in our laboratory on several mammalian species and on frogs have demonstrated that this sheath surrounds not only the common nerve trunks but also their divisions, the nerve fasciculi of peripheral nerves. This sheath, which is made up of flat squamous epithelium, is many layered in mammals and only two layered in frogs. This epithelial layer is found surrounding each nerve fasciculus completely, lying immediately under the perineurium of peripheral nerves.

MATERIALS AND METHODS

This work was carried out on the sciatic nerve of rat, cat, guinea pig, and frog, the trigeminal nerve of rat, and also on spinal nerve roots as soon as they emerge from the spinal cord. The material was removed under intraperitoneal Nembutal anaesthesia in the mammals, and from pithed frogs.

The nerves were dissected under a binocular dissecting microscope, with the help of fine watchmaker's forceps, scissors, fine needles, and foreign body spud. The epineurium was removed first and the nerve fasciculi were isolated. Each nerve fasciculus was cut into 2 mm pieces, and each 2 mm piece was meticulously cleared of perineural connective tissue and care was taken not to injure the epithelial covering. At this stage of isolation the nerve fasciculus is seen to be surrounded by a delicate, semi-transparent, thin shining membrane. A tip of a fine pair of scissors was introduced into one end of this block of fasciculus, and the membrane was slit, leaving it flat on the glass slide, and the nerve fasciculus with endoneurium was removed (Fig. 1). Various staining techniques were then applied. The isolation of spinal root along with its membranous covering was also carried out under a dissection microscope. The outlines of the cellular borders were visualized by incubating fresh pieces of nerves in 5 per cent lead nitrate, 0.2 M acetate buffer (pH 4.9) at 37°C for 8 days, isolating as described above and then treating with dilute yellow ammonium sulphide (Lehmann, 3) and mounting on Apathy's medium. The cellular borders can also be demonstrated by keeping the fresh nerves in 0.75 per cent silver nitrate in the dark for 8 to 12 hours, and then isolating the epithelial sheet as described above and mounting on glycerin, sealing the edges of the coverslip with nail polish and then exposing to sunlight (to reduce the silver nitrate) till the mounted tissue becomes brown.

Standard techniques of hematoxylin and eosin and of pyronin/methyl green were applied also to Zenker-fixed material, isolation being carried out as above. Phosphatase reactions were obtained by standard histochemical techniques.

RESULTS

By these methods we were able to see, with alterations of the fine adjustment on the microscope, up to five layers of this epithelium in rat, cat, and guinea pig sciatic nerves but we were able to photograph only three of these layers (Fig. 4), whereas in frog sciatic nerve we were able to see only two layers. The layers consist of flat squamous epithelial cells with serrated borders (Fig. 3). In H & E preparations the nuclei are well stained, the cytoplasm is pale, and the cellular borders and outlines cannot be made out (Fig. 2). Sex chromatin is found in adult female rats and cats in the pia-arachnoid membrane and in the perineural epithelium but is absent from male animals. Pyronin/methyl green–stained preparations of perineural epithelium showed a well defined capillary network (Fig. 5).

Preparations of spinal roots, as soon as they emerge from the spinal cord, were seen to be covered by the pia-arachnoid membrane (Fig. 6). We were able to demonstrate the continuity of this membrane starting from the spinal root to the nerve fasciculus in serial isolations. These squamous epithelial cells of the pia-arachnoid and the perineural epithelial cells are identical in shape, cellular borders, nuclear character, and sex chromatin distribution. These epithelial layers are so thin that they are always missed in trans-

verse sections of nerve fibers and confused with the fibroblasts of the perineurium.

We conclude that the leptomeninges continue on the nerve roots of the spinal cord and surround the nerve trunks, and divide along with the main division of nerve fibers and surround the nerve fasciculus. The number of epithelial layers goes on decreasing as the nerve fasciculus advances and divides into finer divisions.

Isolation of the perineural epithelium in fine nerve divisions is almost impossible. In rats intravitally stained by methylene blue, and counterstained by cresyl violet, we have seen nuclei surrounding finer divisions of nerve fibers and endings at the motor end-plates which are identical with perineural epithelial cell nuclei. Phosphatase preparations showed that this membrane contained an active ATPase and creatine phosphatase.

DISCUSSION

Electron micrographs of published papers by Robertson (6) show a protoplasmic layer surrounding the finer division of nerve fibers. Robertson says that this material is "endoneurium" (see Figs. 3 and 4, his paper). It seems much more likely that this protoplasmic layer is an extension of the perineural epithelium and that the nuclei seen in our intravital studies are those to which this protoplasmic material belongs.

Couteaux (1) points out that the terminal bell mouth of Henle's sheath ("The sheath that

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FIGURE 1

Rat sciatic nerve, Zenker fixed. Isolation of the perineural epithelium under binocular dissection microscope. Black mass is the nerve fasciculus (arrow *f*) lying by the side of the isolated perineural epithelium (arrow *p*). × 11.

FIGURE 2

Trigeminal nerve of rat. Perineural epithelium isolated under dissection microscope Stained by H & E. The perineural epithelium (*p*) lying by the side of the nerve fasciculus (arrow *f*). Note with H & E method the cellular borders are not stained. Cytoplasm is pale, and nuclei are well stained. × 213.

FIGURE 3

Sciatic nerve of cat. Perineural epithelium isolated under dissection microscope. Treated with lead nitrate, acetate buffer, visualized by dilute yellow ammonium sulfide. Note the squamous nature of the cells, serrated borders of epithelial cells (arrow *s*), and some nuclei of the deeper layers crossing the superficial cell layer borders. × 213.

FIGURE 4

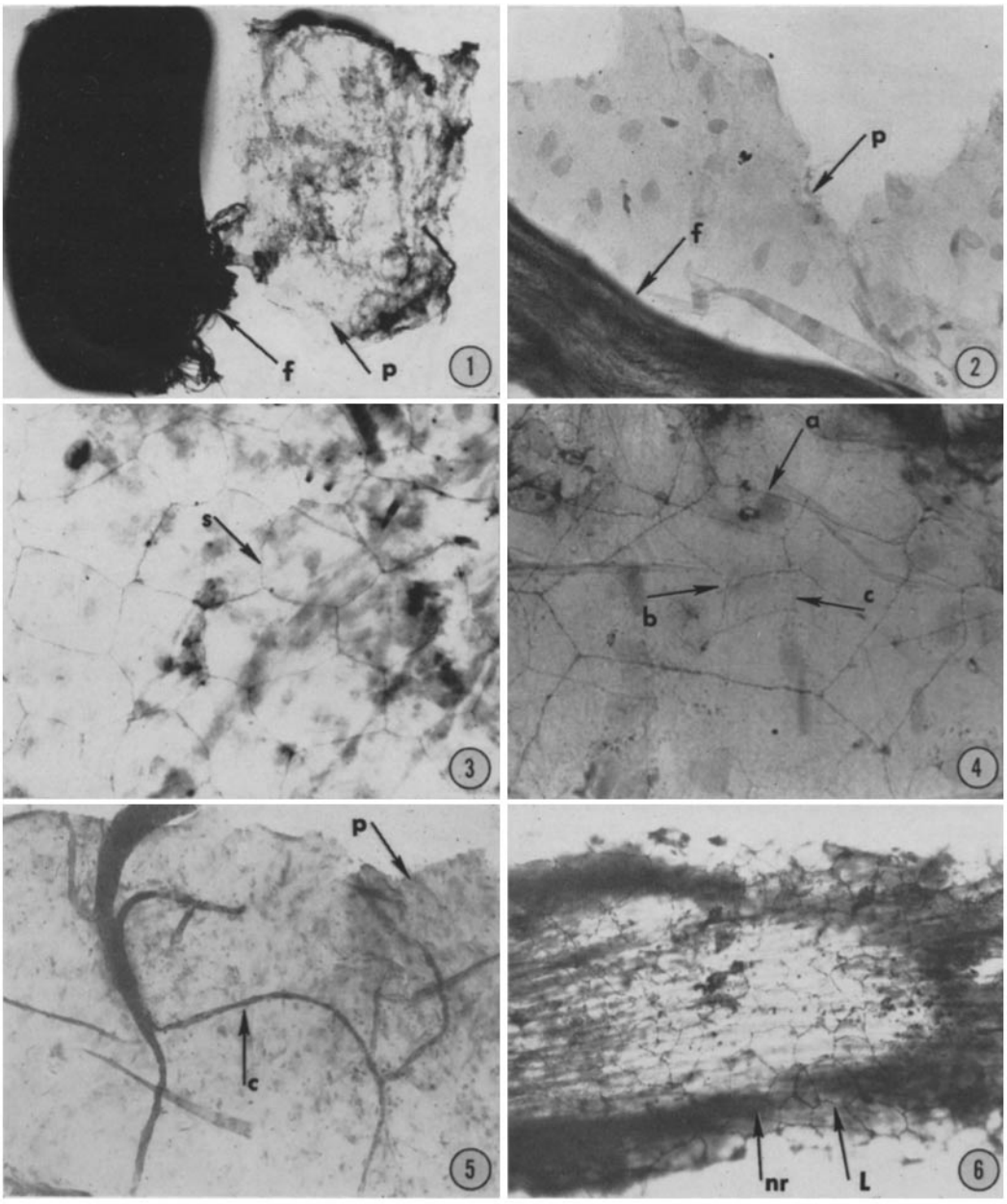
Rat sciatic nerve. Method same as for Fig. 3. Note the clear cut three layers of flattened squamous epithelial (perineural epithelium) cells, represented by arrows *a b, c*, which indicate each layer of cells. × 213.

FIGURE 5

Rat sciatic nerve, isolated perineural epithelium (arrow *p*), Zenker fixed. Pyronin/ methyl green–stained. Showing capillary network (arrow *c*) in the perineural epithelium. × 85.

FIGURE 6

Rat spinal cord, from which the nerve root as soon as it emerges from the spinal cord is isolated under a dissection microscope. Lead nitrate, acetate buffer treated, visualized by dilute yellow ammonium sulfide. Note the complete nerve root (arrow *nr*) is covered by the leptomeninges (arrow *L*) as soon as it emerges from the spinal cord and continues on the peripheral nerve. × 85.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prolongs the system of the lamellar nerve sheath to the level of the isolated nerve fiber") extends over the motor end-plate covering it completely and has its edges attached to the muscle fiber. Couteaux mentions that it is formed from an endothelium. Renaut in 1899 (5) stained the boundaries of these flat uninucleated endothelial cells. Nuclei similar to those of perineural epithelium cells can also be seen in association with motor end-plates in our own preparations and in various published figures of other authors. We consider that these flat uninucleated endothelial-like cells at the bell mouth of Henle's sheath at the motor end-plate are the extended perineural epithelium.

Pease and Quilliam (4), in their electron microscope studies of the Pacinian corpuscle of adult cats and a newborn kitten, showed lamellae consisting of cytoplasmic sheets surrounding the central nerve fiber of the corpuscle. These lamellae appear to us to be the flat squamous epithelial cells, which are a continuous extension of the perineural epithelium. Further work with the electron microscope is being carried out in order to verify this suggestion (see Figs. 1 to 12 from Pease, 4).

SUMMARY

The perineural epithelium in rat, cat, guinea pig sciatic nerve is made up of up to five layers of flat squamous epithelial cells; in frog sciatic nerve it is made up of 2 layers. We have demonstrated that this layer is the extension of the leptomeninges. On the basis of electron micrographs published by Robertson (6, see his Figs. 3 and 4) on myoneural junctions and by Pease and Quilliam (4, see their Figs. 1 to 12) on cat Pacinian corpuscles, we believe that this layer extends up to the nerve terminations and sensory and motor end organs. Thus, the nerve fibers appear to be isolated from body fluids by this membranous squamous epithelial sheet from their point of origin at the spinal cord to their termination. This epithelium, which contained considerable ATPase and creatine phosphatase activity, though it showed little oxidative enzyme activity, is, therefore, equipped to act as a metabolic barrier.

The work described in this preliminary communication will be published in full elsewhere.

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