Perineurium: Evidence for Contractile Elements

Abstract. *Electron-microscopic study of mouse sciatic nerve reveals that perineurial cells contain filaments and associated opaque regions similar to those observed in smooth muscle. This finding is consistent with observations which suggest that nerve might have a contractile property. In addition to their function in maintaining the connective tissue stroma of perineurium, as well as being a selective diffusion barrier, perineurial cells may serve the nerve in a contractile capacity.*

On the basis of electron-microscopic studies (*1*, *2*), it is evident that perineurium cannot be regarded as a typical connective tissue. The perineurial cells bear an intimate appositional relationship to one another. Moreover, unlike typical connective tissue cells, the perineurial cells are separated from other cellular and extracellular elements by well-defined basement laminae.

Perineurial cells closely resemble the sheathlike peritubular contractile cell component of the testis in mice (*3*) and rats (*4*). In the testis, the peritubular cell has cytoplasmic features which account for contractile activity observed in the seminiferous tubules. A contractile property might also be associated with nerve. This property is most apparent when a nerve is sectioned. Under these circumstances, the cut ends of the nerve retract from one another (*5*). Moreover, in specimens of sectioned material observed with the light microscope, nerve exhibits a wavy or undulating appearance suggestive of a shortening of the nerve (*6*), though perhaps not of the individual axons. Neither light- nor electron-microscope studies have revealed the basis for these phenomena.

An electron-microscope study of perineurium was undertaken to determine whether there are any structural components within the perineurium which might be the basis for a contractile property. Sciatic nerves of adult mice were excised and fixed in a 3 percent glutaraldehyde solution buffered with phosphate, further fixed with 1 percent OsO_4 (in phosphate buffer), dehydrated in ethanol to which uranyl acetate was added, and embedded in Epon. Thin sections were stained with lead citrate and were then examined and photographed with a Siemens Elmiskop I electron microscope.

The perineurial component of the nerve consists of three to five layers of squamous-type cells, each layer being separated from the adjacent layer by an acellular space containing collagen fibrils. A well-defined basement lamina separates the perineurial cells of each layer from this acellular space, as well as from the endoneurial and epineurial connective tissue; the basement lamina on the epineurial side of the perineurium is thicker than the other laminae. The perineurial cells within each layer are in close apposition to one another, thereby forming an uninterrupted single-layered cellular sheath. Other elongate cells are regularly observed in close proximity, either external or internal, to the perineurium. These cells, however, are not bound by a basement lamina; moreover, they have features typical of connective tissue cells. Those cells internal to the perineurium are regarded by us as endoneurial fibrocytes; those external to the perineurium are regarded as epineurial fibrocytes.

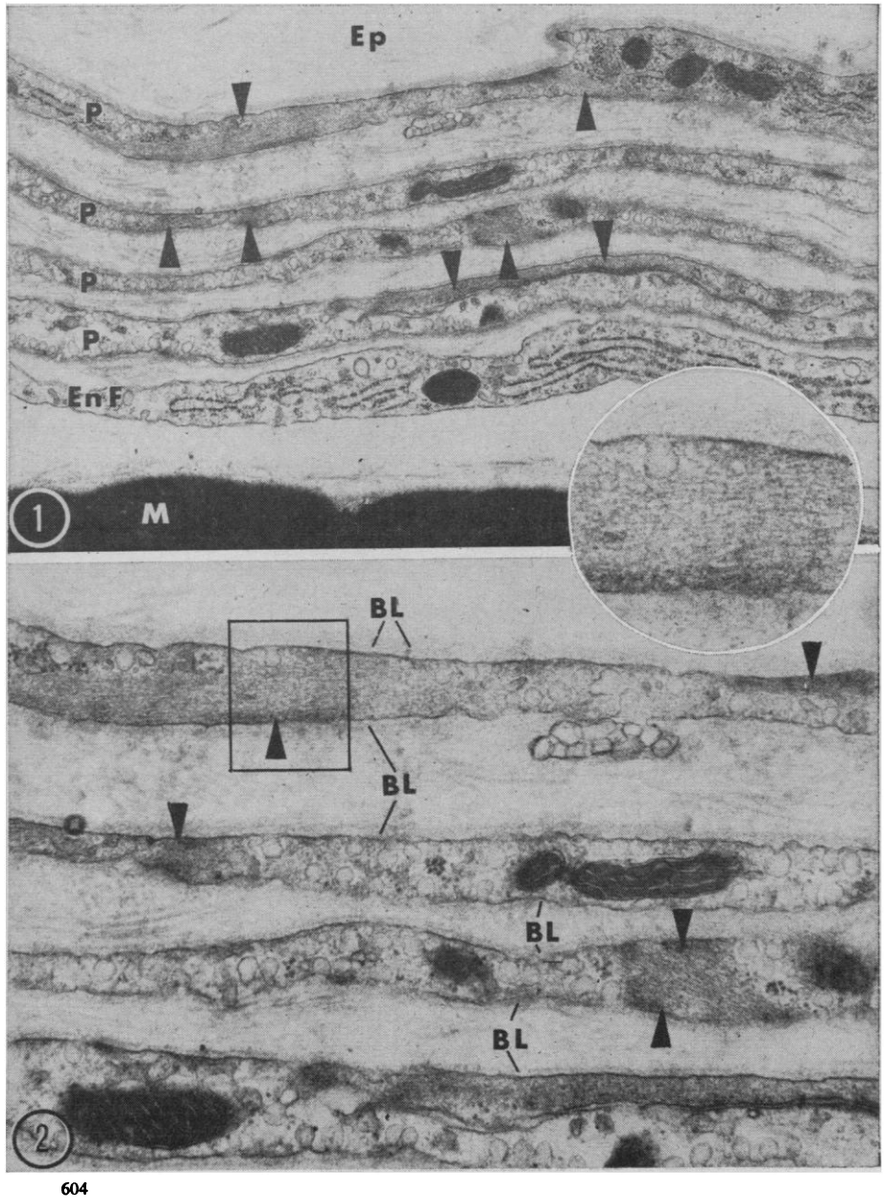

Fig. 1. Electron micrograph showing (from top to bottom) epineurium (*Ep*) and portions of perineurial cell layers (*P*), part of an endoneurial fibrocyte (*EnF*), and the myelin (*M*) of a nerve fiber. The regions of electron-opaque appearing cytoplasm (arrow heads) are within the perineurial cells. The endoneurial fibrocyte is devoid of basement lamina (× 15,000).

Fig. 2. Higher magnification of part of Fig. 1 showing the perineurial cells, their basement laminae (*BL*) and the filamentous nature of the opaque-appearing cytoplasm. Within these regions are the small localized opacities (arrow heads). The opacities and associated fine filaments within the enclosed area are shown at higher magnification in the circular inset (× 30,000; inset × 60,000).

A typical specimen of the nerve (Fig. 1) reveals four perineurial cell layers and their basement laminae. Between the perineurium and the myelin at the bottom of the figure is the elongate profile of a portion of an endoneurial fibrocyte. This cell is devoid of basement lamina and contains several profiles of rough endoplasmic reticulum as typically seen in fibrocytes.

Among the significant features of Fig. 1 are the regions of relatively electron-opaque cytoplasm within the perineurial cells. Such regions are not found in the endoneurial or epineurial fibrocytes. When viewed at higher magnification (Fig. 2), it is evident that the localized areas of greater opacity within the perineurial cytoplasm are due to the presence of closely aggregated filaments. The filaments are unlike those ordinarily encountered in fibroblasts (*7*). The filaments of fibroblasts are more dispersed and do not seem to be organized with respect to other cytoplasmic elements. The perineurial cell filaments are comparable to the myofilaments seen in smooth muscle cells, both in appearance and in the nature of their organization. Also present are small electron-opaque regions adjacent to the cell membrane, associated with the filaments (Figs. 2 and 3). These localized regions are similar in appearance to the "attachment devices" (*8*) or "dense bodies" (*9*) of smooth muscle. The presence of both aggregated filaments and electron-opaque regions, associated as they are in the perineurial cells, suggests that they are comparable to the contractile elements of smooth muscle.

The cytoplasmic matrix of perineurial cells also contains other organelles, some of which are characteristic of fiber-forming cells. Figure 3 shows a region which contains both the possible contractile elements and profiles of rough-surfaced endoplasmic reticulum and free ribosomes in polysomal aggregates. Small vesicular invaginations of the cell membrane are also a common feature of the perineurial cell. These micropinocytotic vesicles are most numerous in the more attenuated regions of the cell (Fig. 3) and may constitute the only structural element present in these sites. In those parts of the cell in which the filaments occur, the micropinocytotic vesicles are either absent or few in number. Novikoff (*10*) likened these vesicles to those seen in smooth muscle, especially with respect to their similar nucleoside phosphatase activity. He raised the possibility that these cells might be considered modified smooth muscle, though he did not observe myofilament-like structures or electron-opaque regions of the plasma membrane.

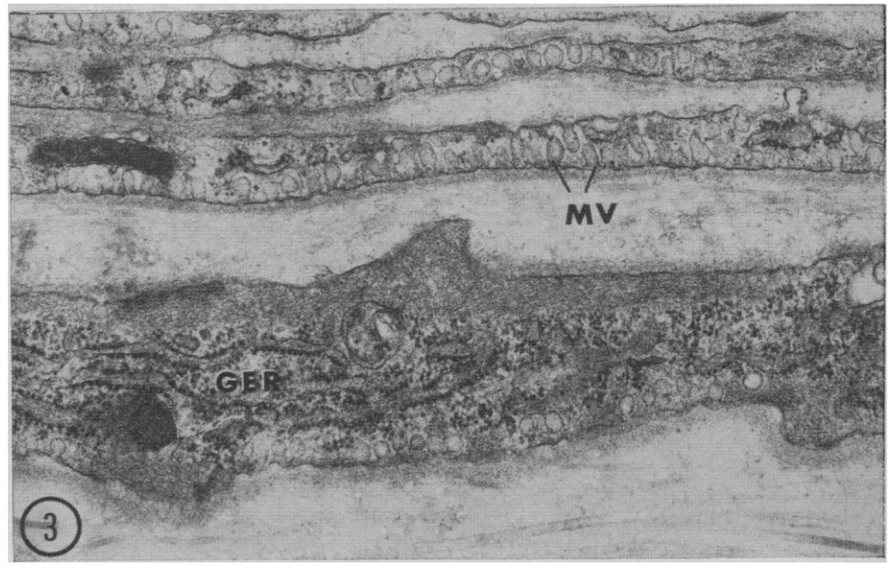

Fig. 3. A portion of a perineurial cell which contains, in addition to the filaments, profiles of rough endoplasmic reticulum (*GER*) and numerous free ribosomes. The chief constituents in the two perineurial cells in the upper part of the figure are micropinocytotic vesicles (*MV*) (× 27,000).

The nature and combination of the aggregated filaments and their associated opaque regions, the relatively numerous profiles of endoplasmic reticulum, the free ribosomes, and the pinocytotic vesicles are indicative of a cell having morphological characteristics of a fibrocyte as well as of a smooth muscle cell. In this respect, these cells are remarkably similar to the peritubular contractile cells in the testis of rat and mouse (*3, 4*).

Our present views with regard to the structure and function of perineurium may have to be revised. Despite the early observation of Key and Retzius (*11*) and Ranvier (*12*), perineurium has been regarded primarily as a connective tissue component of nerve. The ultrastructural features of the perineurial cell justify the retention of the concept that these cells function, at least in part, in maintaining the connective tissue stroma within the perineurial lamellae. The absence of other cell types within the perineurium also points to the perineurial cells as performing this function. The role of perineurial cells as a selective diffusion barrier has also been suggested (*2*). Ultrastructural features are consistent with such an interpretation.

Perineurial cells may also have the ability to contract. A contractile perineurium may contribute to the retraction of nerve, when sectioned, as well as to the wavy appearance of nerves seen in histological sections. It may well be that the contractile apparatus described is analogous to the contractile system that has been described in invertebrate nerves (*13*), and it may serve to shorten the nerve to adjust for body movement. Such a function has been suggested for nerve of invertebrates (*14*).

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Antigen Combining Activity Associated with Immunoglobulin D

Abstract. *The titers of antibodies to the benzylpenicilloyl antigenic determinant are increased with specific antiserum to immunoglobulin D, as shown by the enhancement method. This increase in titer is blocked by purified immunoglobulin D protein but not by immunoglobulins of the other four classes.*

Immunoglobulin D (γD) was discovered by Rowe and Fahey during studies of myeloma proteins (*1*). They demonstrated that γD was present in normal human serum and was structurally similar to the other classes of immunoglobulins but possessed unique antigenic determinants associated with the heavy chain. Also γD-containing cells were present in human spleen sections and resembled plasma cells (*2*). However, in spite of these similarities to the other immunoglobulins, antibody activity in γD has been difficult to demonstrate.

We have analyzed human serums containing antibody reactive with the benzylpenicilloyl antigenic determinant. Fresh type O, Rh-positive erythrocytes were sensitized with penicillin G, as described by Levine and his associates (*3*). Serums from subjects allergic to penicillin G were diluted to determine the lowest concentration necessary for agglutination of sensitized erythrocytes. The cells were washed, and the titer was again determined in the presence of buffer or specific goat antiserum to γD. An increase in titer in the presence of this antiserum is referred to as "enhancement," and presumably reflects the presence of γD antigenic determinants on the erythrocytes. This antiserum was produced in a goat by immunization with purified γD from myeloma serum, the protein being injected in complete Freund's adjuvant. The antiserum was rendered specific for γD by absorption with γG, with lambda-type Bence Jones protein, and with normal human serum deficient in γD protein. The absorbed antiserum formed a single band with γD myeloma serum in Ouchterlony double-diffusion experiments and contained 160 μg of antibody nitrogen per milliliter, as judged by quantitative precipitin analysis with purified γD protein. In addition, the absorbed antiserum to γD did not form precipitin bands in Ouchterlony double-diffusion with immunoglobulins of the other four classes. Serums from allergic subjects which contained detectable γD and which agglutinated penicillin-sensitized erythrocytes in high titer were screened to determine whether their titers were enhanced by antiserum to γD. Three such serums containing from 3.4 to 6.5 mg of γD per 100 ml were found and studied further with antiserum to γD and various inhibitors. The direct agglutination reactions were specific for the benzylpenicilloyl antigenic determinant in that they were inhibited by the hapten, α-D-benzylpenicilloyl-ε-aminocaproate, $1 \times 10^{-2}M$, but not by the unrelated hapten, dinitrophenyllysine, $2 \times 10^{-3}M$ (Table 1). The marked enhancement of the titer, 32-fold, in the presence of antiserum to γD is blocked by purified γD but not by immunoglobulins of the four other classes. Similar results were obtained with serums from two other subjects whose titers were enhanced 8-fold and 16-fold. The enhancement of titer by antiserum to γD was demonstrated several times with each serum.

These results indicate that serums from some subjects allergic to penicillin G contain γD-globulins specific for the benzylpenicilloyl antigenic determinant. Although we have not studied serums before and after exposure to antigen to demonstrate a rise in the concentration of reactive γD proteins, these results do suggest that γD has antibody activity. Ritchie (*4*) has reported antibody activity against cell nuclei which is associated with γD; Heiner and his associates (*4*) have shown that antibody activity to diphtheria toxoid and bovine γ-globulin is associated with γD. Levine and Redmond (*5*) also found γD on erythrocytes from patients with hemolytic anemia caused by penicillin G. However, blocking of these reactions by purified γD protein or absorption of activity with antiserum to γD was not demonstrated.

Table 1. Enhancement of titer by antiserum to γD. Normal erythrocytes and erythrocytes (RBC) sensitized with penicillin G were incubated with dilutions of serum in buffer [dextran, fetal calf serum, tris-buffered saline (*3*)] and washed thrice with 3.0 ml of 0.15*M* NaCl, 0.02*M* tris(hydroxymethyl)aminomethane, *p*H 8.2. Cells were resuspended in 0.2 ml of buffer, anti-γD, or anti-γD and inhibitor and incubated for 45 minutes at 24°C; the titer was then determined. Reactions were graded as described by Levine and his associates (*3*). Anti-γD was used at 1.3 μg of antibody nitrogen per tube, while the inhibitors were used in amounts from 24 to 29 μg of nitrogen per tube. Anti-γD and the various inhibitors were absorbed with normal and sensitized erythrocytes to remove naturally occurring agglutinins. γA, γM, and γD were purified myeloma proteins free of other immunoglobulins by immunodiffusion analysis while Cohn fraction II was used as γG. As a source of γE, a serum was used which contained γE (2 g/100 ml) but was deficient in γD protein (*6*). As additional controls, sensitized and normal erythrocytes were incubated with all the inhibitors and their reactions were tested directly and after addition of antiserum to γD. Moreover, normal goat serum did not produce enhancement of the titer. Finally, pooled normal human serum containing 5 mg of γD per 100 ml and treated with an insoluble benzylpenicilloyl immunoabsorbent to remove penicilloyl antibodies did not directly agglutinate sensitized or normal erythrocytes, and after being washed the cells were not agglutinated by specific antiserum to γD.

RBC incubated with	$10^3 \times$ Reciprocal dilution of patient's serum								
	Normal RBC	Sensitized RBC							
	4	4	16	32	64	128	256	512	1024
Buffer	0	1+	Tr	0	0	0	0	0	0
Anti-γD	0	—	1+	1+	1+	1+	1+	Tr	0
Anti-γD + γD	0	—	Tr	0	0	0	0	0	0
Anti-γD + γG	0	—	1+	1+	1+	1+	1+	Tr	0
Anti-γD + γM	0	—	1+	Tr	1+	1+	1+	Tr	0
Anti-γD + γA	0	—	—	—	1+	1+	1+	Tr	0
Anti-γD + γE	0	—	1+	1+	1+	1+	1+	Tr	0

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