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Title - Direct Innervation of Capillary Endothelial Cells in the Lamina Propria of the Ferret Stomach[1]

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Introduction

Innervation of blood vessels and their surrounding smooth muscle layers have received much attention for many years, and while direct innervation of the endothelial cells has not previously been described with the electron microscope, Rauber-Kopsch (2) suggested in 1941 that nerves penetrated into the endothelium. A more recent review of cutaneous blood vessels by Weddell (5) states that nerve axons have not been observed by electron microscopy to enter smooth muscle cells, or any other cell forming the wall of the blood vessels. Rhodin (4) has since shown, however, that axons do make membranous contact with smooth muscle cells of arterioles, terminal arterioles, and precapillary sphincters. Accordingly, some of the nerves penetrated the smooth muscle layers and came into close apposition with the endothelial cells, and a basement membrane remained between the two structures and no specialized ending was observed making contact with the endothelial cells. Rhodin further states that "axons in the adventitia near the smooth muscle surface are often free of Schwann cell cytoplasm and as they traverse the connective tissue space, they are surrounded by a basement membrane." In the present study single neurons were also seen separated from the nerve bundle, surrounded by a basement membrane and unaccompanied by any Schwann cell cytoplasm. Specialized finger-like projections were observed at the end of the neurons which penetrate the basement membrane of the capillary endothelium and come into membranous contact with the external plasma membrane of the endothelial cells.

Materials and Methods

Forty young adult ferrets obtained from the Gilman Marshall Ferret Ranch (North Rose, N.Y.) were available for the study. They were maintained on a diet of canned dog food (Ken-L-Ration) prior to sacrifice by an overdose of

ether. The stomach was removed from the animal during anesthesia and cut along the greater curvature and washed quickly in physiologic saline to remove debris. Small slices of tissue $\frac{1}{2}$ x 2 x 3 mm were fixed in 1% OsO_4 buffered to a pH of 7.4 with Veronal acetate containing 7.86% sucrose. Other slices were fixed in 5% glutaraldehyde solution buffered with cacodylate to a pH of 7.4. Tissue fixed in glutaraldehyde was subsequently washed in several changes of cacodylate buffer containing 7% sucrose prior to refixation in 1% OsO_4. All tissues were rapidly dehydrated in a graded series of ethanol and embedded in Maraglas (1). Sections were cut on a Porter-Blum MT2 ultramicrotome, placed on uncoated copper grids, stained with lead citrate (3) and observed with a Philips 200 electron microscope.

Results

Many small amyelinated nerve bundles were observed in the lamina propria underlying the gastric mucosa of the ferret stomach. Nerves are surrounded or partly surrounded by the cytoplasm of an associated Schwann cell (Fig. 1). A small percent of these neurons contain dense granules as well as small, smooth-membraned vesicles (Fig. 1). Individual neurons vary greatly in diameter and may contain mitochondria (Figs. 1 & 2). Occasionally a single axon may be observed separated from the main group of nerves, not associated with any Schwann cell cytoplasm but surrounded by a basement membrane (Fig. 2). The small nerve bundles frequently parallel fenestrated capillaries which also possess numerous pinocytotic vesicles (Figs. 1, 3, & 4). A prominent basement membrane is located along the outer surface of the capillary endothelial cells (Figs. 1, 3, & 4). A capillary pericyte may be close to the endothelium but with a basement membrane between it and the endothelium (Fig. 1). Many pinocytotic vesicles are seen along the plasma membrane on the side of the pericyte which is away from the capillary (Fig. 1).

Attenuated portions of interstitial cells are also seen close to the capillaries and nerves (Fig. 1).

Occasionally one observes a very small fascicle of nerves consisting of two or three neurons and a small amount of Schwann cell cytoplasm. In Figures 3 and 4 such a fascicle of nerves has been cut in serial section with several sections missing between the two figures. There are three neurons in the bundle; one has clearly left the Schwann cell and come into close contact with the external plasma membrane of the capillary endothelium. Finger-like projections extend from the end of the neuron and penetrate through the basement membrane to make a membranous contact with the capillary (Figs. 3 & 4).

Discussion

Direct innervation of endothelial cells has not been previously described at the electron microscope level. While endothelial cells have been shown to possess filaments similar to the myofilaments of smooth muscle (4) they have not been shown to contract actively.

We regard the neurons making membranous contact with the endothelium as afferent sensory fibers which possibly respond to stimuli contained in the blood. It should be noted that the capillary endothelium in the lamina propria of the stomach is fenestrated and possesses numerous pinocytotic vesicles as well and thus presumably the nerve endings would be presented quickly with chemical or hormonal changes in the blood. Since it is well known that gastric secretion is markedly influenced by hormones produced lower in the digestive tract, this type of detection mechanism might be important in regulating a reflex mechanism responsible for such gastric control. Another possibility is that the nerve endings described are pressure-sensitive and are linked via a reflex to the precapillary sphincters permitting a feedback to the sphincter.

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References

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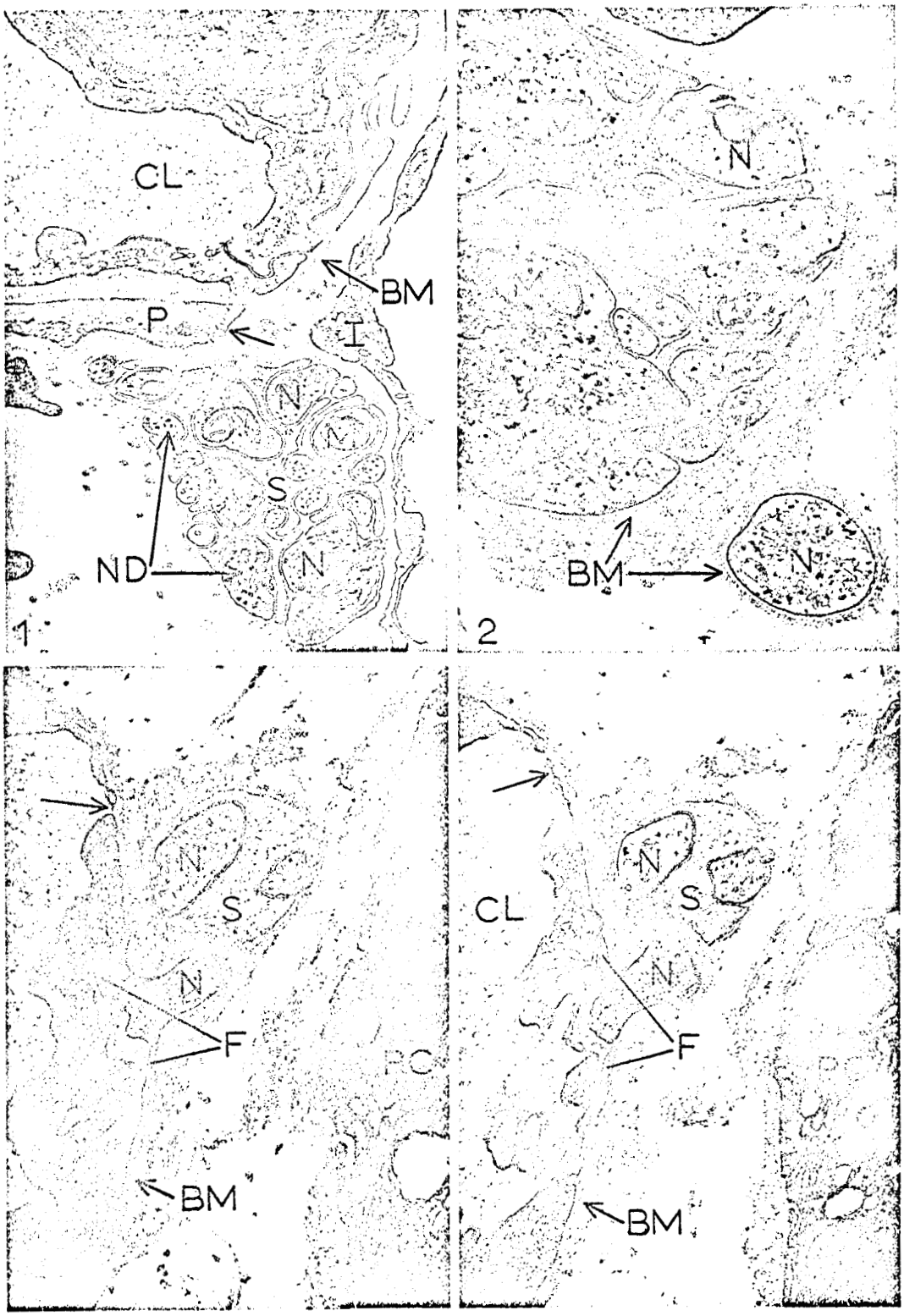
Fig. 1. A small bundle of amyelinated neurons (N) separated in folds of cytoplasm of a Schwann cell (S). Some of the neurons (ND) contain dense granules while others have only small smooth-membraned vesicles. A basement membrane (BM) surrounds the capillary endothelium and a pericyte (P) is seen close to the capillary. Note pinocytotic vesicles along the plasma membrane of the pericyte on the side distal to the capillary. x 20,000.

Fig. 2. A single neuron (N) is seen at the lower right, separated from the rest of a small fascicle of nerves. Note the basement membrane (BM) surrounding the single neuron as well as the entire bundle. Schwann cell cytoplasm (S) is seen in the bundle but does not accompany the single neuron. Mitochondria (M). x 34,000.

Figs. 3 and 4. Serial sections of three neurons (N), one of which has separated from the Schwann cell (S) and come into close contact with the endothelium of a capillary. Note the finger-like projections at the end of the neuron which penetrate the basement membrane (BM). The arrows indicate sites of capillary fenestration. A parietal cell (PC) of the gastric mucosa is seen at the left edge of the micrograph. x 22,000.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