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An Electron Microscopic Observation on Postvagotomy Necrosis of Dog Stomach Muscle

(vagotomy/degeneration and necrosis/stomach muscle)

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Postvagotomy degeneration of the dog stomach smooth muscles was studied using an electron microscope. By one week after complete vagotomy, many preganglionic axons in both Auerbach's and Meissner's plexus had degenerated. Subsequently, the affected smooth muscles associated with disintegrated axons showed various types of degenerative changes, and such occurred markedly 2 to 6 weeks after vagotomy. These changes included : loss of electron density in the intercellular matrix and the cytoplasm of muscle cells due to edematous swelling ; vacuolar dilatation of both endoplasmic reticulum and mitochondria ; lipid accumlation in the cytoplasm ; and lysis of myofilaments. Four or more weeks after vagotomy, necrotic smooth muscle cells and cellular debris considerably increased in the two muscle layers. Increased permeability of endothelial cells and openings of endothelial junctions that induced an extravascular infiltration of fibrin resulted in fibrinoid necrosis of the capillary wall and smooth muscles. Eight weeks after vagotomy, these changes were less frequently observed in the two muscle layers where there was an increase in the number of both fibroblasts and macrophages in active forms, and which were associated with an increased proportion of attenuated muscle cells. Possible mechanisms of these degenerative changes were discussed. It was emphasized that on occasion the considerable functional changes of the stomach muscles might be induced by damage to both smooth muscle cells and the microvascular system when the stomach was completely denervated.

In recent years, vagotomy is being performed at more peripheral levels of the vagus nerves for the purpose of preserving pyloroantral vagal branches. In a previous study (1), it was reported that from electron microscopic observations, an intact antral innervation is necessary for the normal antral contractile activity. In this respect, highly selective (proximal) vagotomy (2) or parietal cell vagotomy without drainage (3, 4) is receiving considerable attention. It is evident, however, from the recent reports (3, 5) that these operations are unavoidably associated with occurrence of some postoperative complications such as dumping, diarrhea, bilious vomiting, recurrent ulcers and others.

Of special interest is the finding that proximal selective vagotomy caused a necrosis of the stomach wall (6). To our knowledge, however, there is no

ultrastructural evidence of postvagotomy necrosis of gastric smooth muscles.

The present electron microscopic study was made to investigate the effect of vagotomy on smooth muscles in dog stomach. Based on these morphological data, possible mechanisms involved in the postvagotomy necrosis of gastric muscles are discussed.

MATERIALS AND METHODS

Whole stomach was examined both in normal and in vagotomized adult dogs. The electron microscopic observations were made on 10 healthy, female dogs and twenty-six vagotomized dogs, from 24 hours to 16 weeks after the truncal vagotomy. The surgical technique for the vagotomy comprised truncal vagal sections about 3 cm above the esophageal hiatus : both anterior and posterior vagal trunks were transected at two places, and the intermediate segments (0.5 to 1.0 cm) were removed. The ends of both distal and proximal nerve segments were crushed and small metal clips were attached. All communicating branches of the vagal trunk were also transected. Completeness of vagotomy was assessed by means of electrical stimulation of the vagus nerves just before arterial perfusion with the fixatives. Usually, the proximal ends of the cut were stimulated. The usual stimulus parameters were 10−14 V, 5 msec duration, and 9−10 Hz. Absence of contraction of the stomach after electrical stimulation was taken to indicate that the vagotomy was complete. In four dogs, stimulation of nerves distal to the cut still elicited contractions up to two months after vagotomy. These stomachs were not examined in the present study.

The stomach was perfused through an arterial cannula with 1 to 2% glutaraldehyde in Krebs solution. After excision of the stomach, twenty-four or more muscle strips (8 to 10 mm long and 5 mm wide) were prepared from both the anterior and posterior walls, and these strips were fixed in the same fixatives for an additional 2 hours, diced into 8 to 10 blocks and then postfixed for 2 hours in 2 % osmium tetroxide in phosphate buffer. After dehydration in graded ethanol, the tissues were embedded in Epon mixture. Ultrathin sections were contrasted with uranyl acetate and lead citrate, and examined with a Philips 301 electron microscope operated at 60 kV.

RESULTS AND DISCUSSION

Ultrastructural profiles of the smooth muscle in the normal dog stomach, as in other visceral muscles, exhibited variant alterations in different stages of mechanical activities. In moderate relaxation, normal cells contain a centrally located oblong nucleus with smooth-surfaced contours. The plasma membranes display a smooth surface with randomly placed pinocytotic vesicles. The cytoplasm contained relatively dense myofilaments, a moderate amount of dense bodies, a few mitochondria and Golgi bodies and a few less of sarcoplasmic reticulum. Small amounts of glycogen granules and free ribosomes were also present in the cytoplasm (Fig. 1).

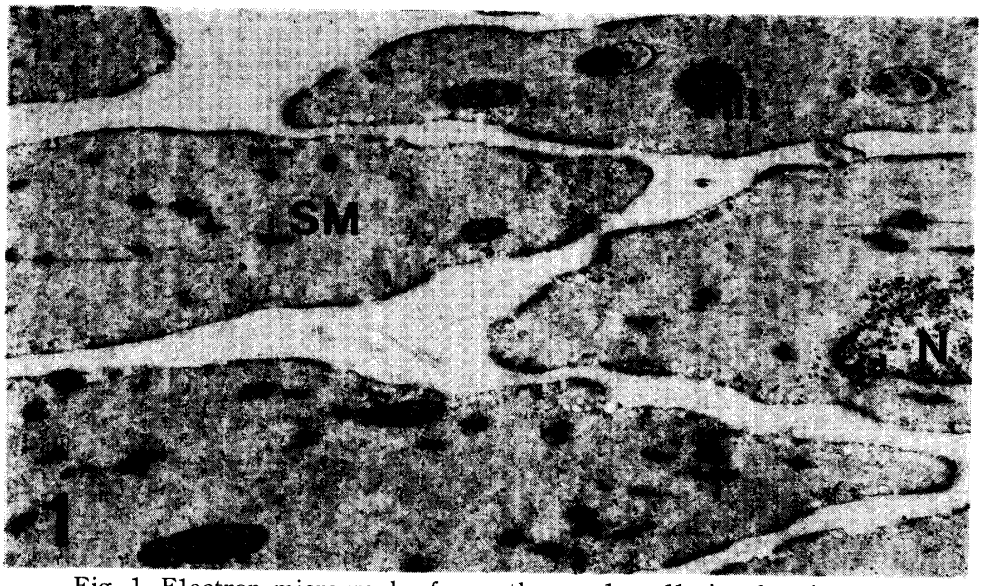

Fig. 1. Electron micrograph of smooth muscle cells in the circular muscle layer obtained from the body of a healthy dog stomach (longitudinal section). ×14,850

One week after complete vagotomy, many preganglionic, presynaptic cholinergic axons in both Auerbach's and Meissner's plexus had degenerated. These changes were reported in a previous paper (1). Subsequently, vagotomy

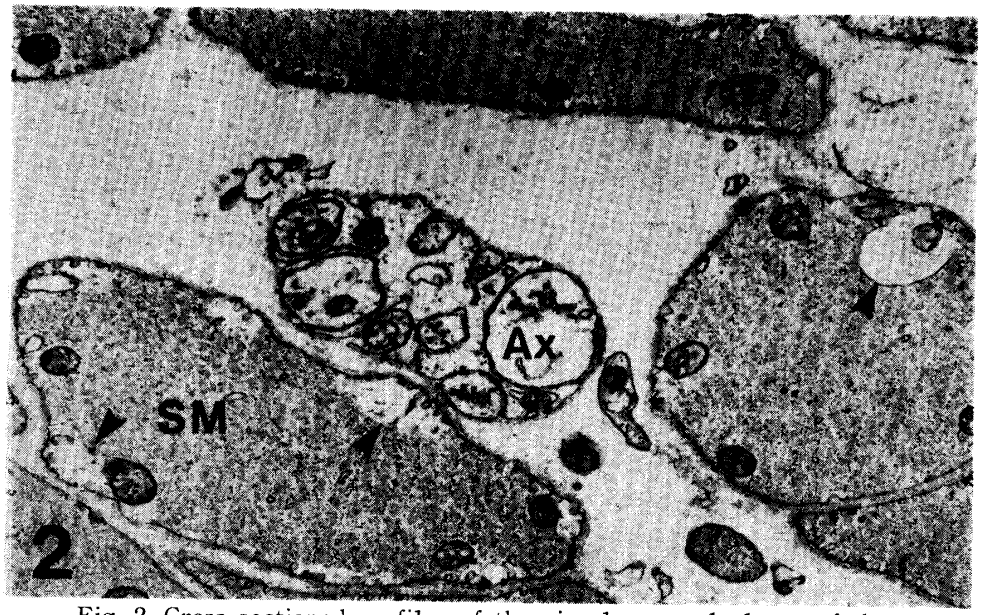

Fig. 2. Cross sectioned profiles of the circular muscle layer of the body, Corpus ventriculi ; both smooth muscle cells and axons showing early changes of postvagotomy degeneration ; affected smooth muscle cells exhibit vesicular swelling in the subplasmmalemal regions, probably containing smooth endoplasmic reticulum (arrowheads) ; intercellular ground substance shows edematous swelling and loss of electron dense materials. 2 weeks after vagotomy. ×15,000

Abbrevations : SM, smooth muscle cell ; N, nucleus ; mit, mitochondria ; End, endothelial cell ;

affected both smooth muscles and terminal axons in the longitudinal and the circular muscle layer ; the affected smooth muscles showed various types of degenerative changes, which occurred most markedly 2 to 6 weeks after vagotomy. These roughly included : a focal decrease in the optical electron density of the myoplasm ; focal edematous swelling of small peripheral regions in the cell ; and vacuolar swelling of mitochondria (Fig. 2). Focal edematous changes were particularly prominent both in the subplasmalemmal and perinuclear sites, probably due to the swollen endoplasmic reticulum. In advanced stages, extensive vacuolar swelling occurred in the cytoplasm, where there were complete lysis of myofilaments and loss of cell organelles (Fig. 3).

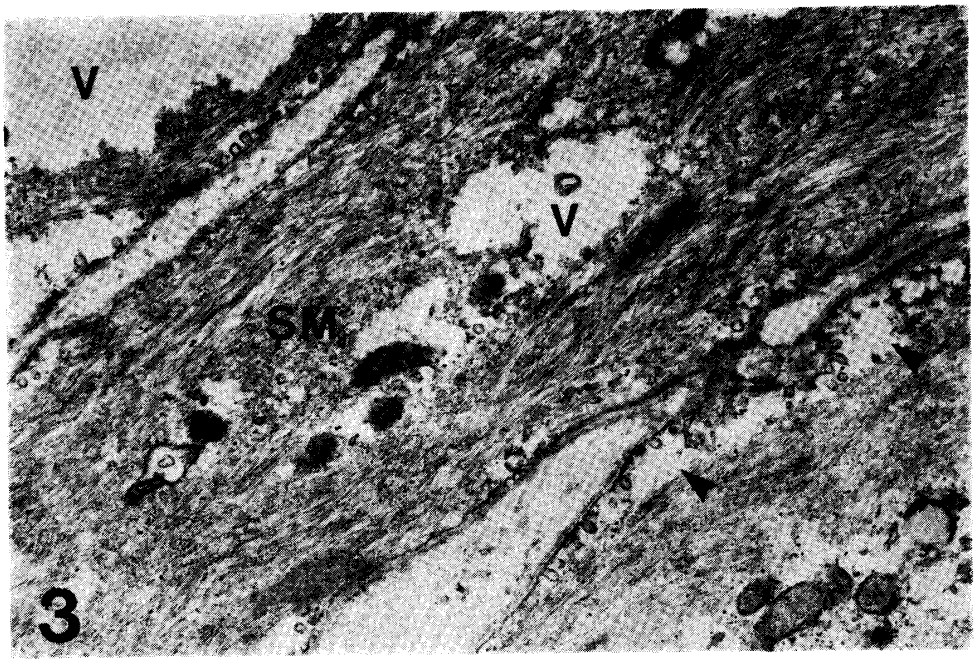

Fig. 3. Degenerated smooth muscle cells in the circular muscle layer in long section, showing occurrence of peripheral vesicular swelling (arrowheads), vacuole (v), vacuolar mitochondria and lysis of myofilaments. 3 to 4 weeks after vagotomy. ×15,000

These changes are assumed to be the result of deterioration in the ion-transport by plasma membranes. Furthermore, progressive changes along this line led ultimately to necrosis of the muscle cells where the increased activities of the fibroblasts and macrophages were found (1).

Accumulation of lipid droplets, indicating impaired fatty acid oxidation by mitochondria (7), also appeared as relatively early changes in the degenerating muscle cells (data not included).

Four or more weeks after vagotomy, an occasional blood capillary in the muscle layers was affected. This capillary which was near the necrotic cells, cellular remnants and degenerated axons, often showed an increase of pinocytotic vesicles and focal vacuolar swelling (Fig. 4). Openings of the endothelial junctions, where they had close apposition contacts, were occasionally observed (Fig. 5). Around and apart from the blood capillary, infiltration of leucocytes,

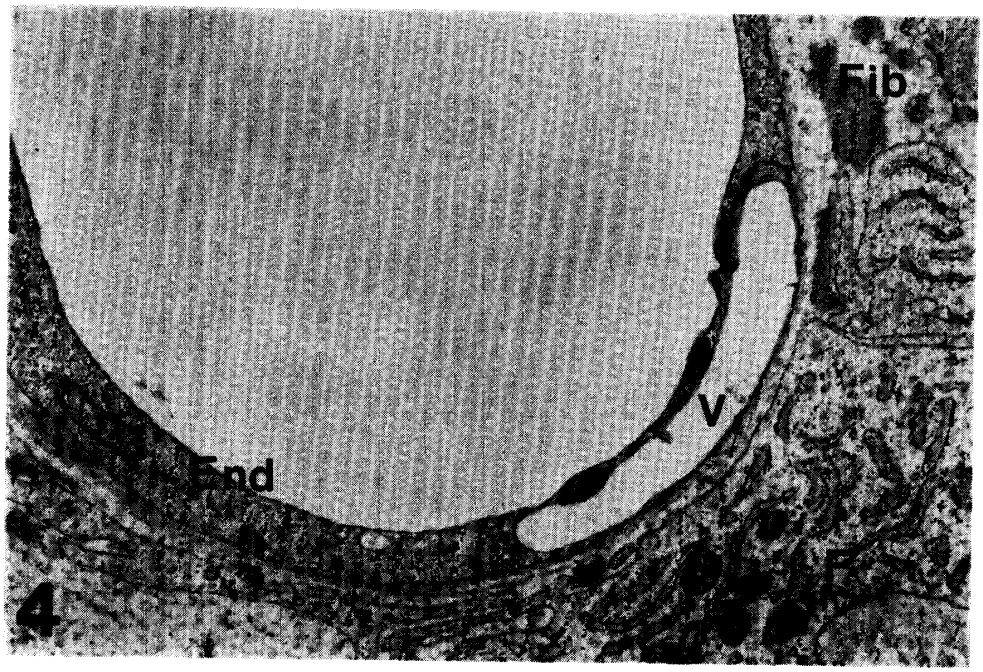

Fig. 4. Degenerating endothelial cell of a venule, which appeared in the circular muscle layer, showing occurrence of intraendothelial vacuole (v), and fibrin deposits (Fib) with active forms of fibroblast (F) in the perivascular region. 4 weeks after vagotomy. ×15,000

Fig. 5. A profile of extravasation of red cells from capillary, which appeared in the circular muscle layer. One erythrocyte is passing through the interendothelial gap (End), while the other is present in the extravascular space (Ery), where there are also cellular remnants with amorphous profiles. 4 weeks after vagotomy. ×12,000

fibrins and occasional erythrocytes were observed (Figs. 4 to 6), and fibrinoid necrosis could also be seen in these affected muscle tissues (Fig. 7). Phago-

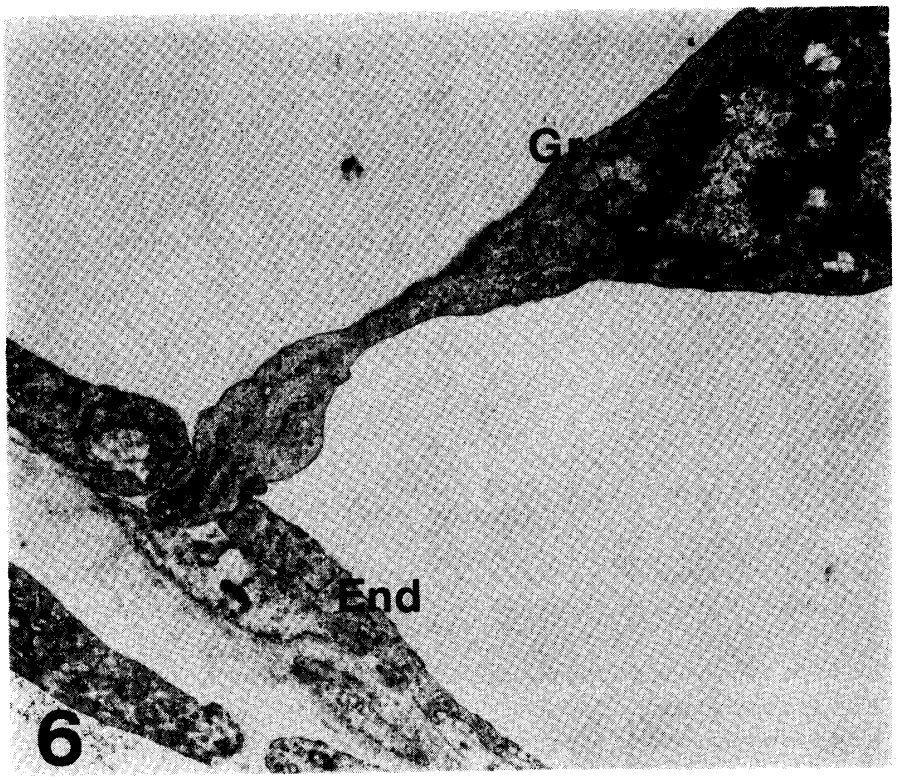

Fig. 6. Blood granulocyte in transvascular migration ; the granulocyte (Gr) inserts a cytoplasmic pseudopodium between two endothelial cells. 4 weeks after vagotomy. ×6,000

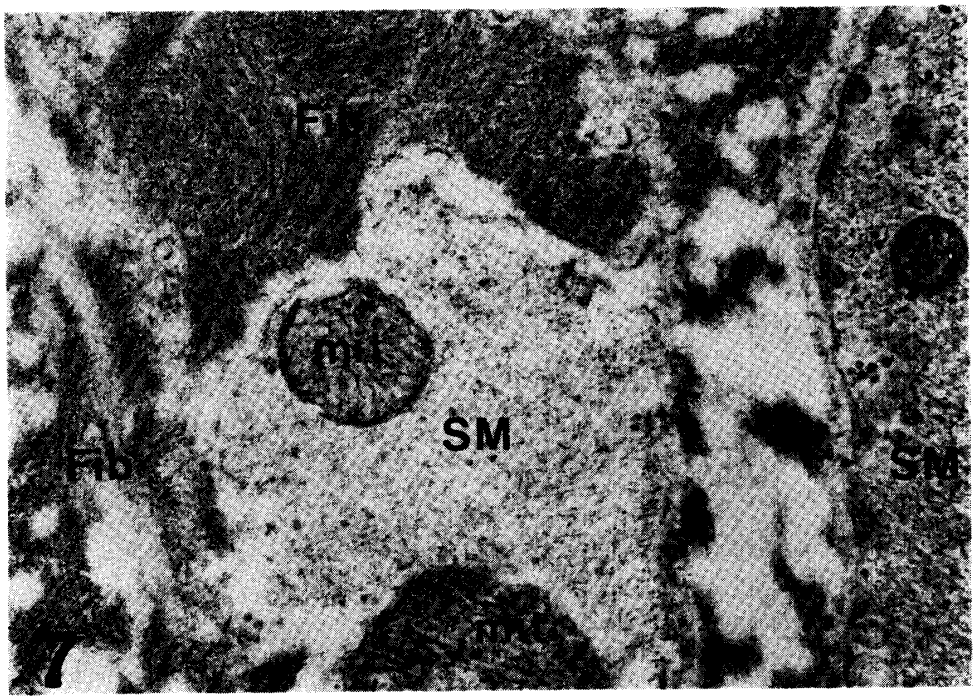

Fig. 7. Fibrinoid necrosis of circular muscle cells ; fibrin meshworks (Fib) in intercellular matrix invade smooth muscle cells, one of which shows amorphous profiles containing two mitochondria (mit). 5 weeks after vagotomy. ×50,000

cytic leucocytes and macrophages were also present in these degenerated muscle layers. These degenerative changes became less frequent 8 weeks after vagotomy. After this stage, an increase of the intercellular connective tissue components including active fibroblasts was observed between the affected cells, where there was a decrease in cell number per unit area associated with an increased proportion of attenuated smooth muscle cells. No actual increase of regenerated muscle cells was observed.

The mechanism of degenerative changes in smooth muscles following vagotomy is so far essentially unknown. In the present study, degenerative changes of the smooth muscles appeared to be primarily responsible for the presence of degeneration in the local nerve elements, both axons and Schwann cells. Microvascular changes were also evident. This possibility has been discussed for the skeletal muscles, where fibrinoid necrosis of capillaries was assumed to be related to toxic products released from the degenerating cells (8). However, it seems likely that there is no single hypothesis concerning the mechanism of both increased microvascular permeability and loosening of interendothelial junctions. We can only speculate that these degenerative changes may be related to the disruption of some direct trophic function of nerves or some indirect trophic control by nerves through release of gastrin. Alternatively, toxic substances, such as vasoactive polypeptides from degenerated elements, may cause these changes in muscle cells. Prostaglandins are known to function as a cytoprotector in preserving stomach tissues from damaging agents such as 5-HT and other ulcerogenic chemicals or stimulants (9) ; and, they also act as a vasodilator (10). Creaghe and coworkers, however, reported that various types of vagotomy did not alter prostaglandins-levels in the stomach (11). In conclusion, early degenerative changes in the vagotomized dog stomach wall, including both smooth muscles and microvascular system, may be initiated through the loss of axons, even when the stomach is selectively denervated. Thus, functional changes in the stomach may be unavoidable due to the disintegration of the smooth muscles and microvascular system.

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