FINE-STRUCTURE CHANGES IN ACHALASIA OF THE ESOPHAGUS

II. Esophageal Smooth Muscle

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Achalasia of the esophagus is characterized by an abnormality of esophageal motility manifested by failure of the lower esophageal sphincter to relax during swallowing and by an absence of peristalsis in the body of the esophagus. Relaxation of the isolated inferior esophageal sphincter is dependent in part on the integrity of the esophageal wall itself, since in experimentally denervated preparations of the lower esophagus, relaxation of the terminal portion does not occur when the esophagus is directly stimulated above the site of circular myomectomy but does occur in response to direct stimulation below the line of incision.[1]

Although there have been many pathologic descriptions of the esophagus in achalasia, only brief references have been made to the morphologic features of the muscular wall itself. The muscle has been described as atrophic,[2] hypertrophic,[3,4] and normal[4,5] when studied by light microscopy. The significance of these muscular alterations has been speculated upon but remains unclear. We have attempted to define more precisely the smooth muscle changes in achalasia with the use of the electron microscope in a group of patients with this disorder.

Material and Methods

Specimens of esophageal smooth muscle were obtained from 10 patients with esophageal achalasia in whom the diagnosis was proved by esophageal motility studies, and from 8 control patients who did not have lower esophageal disease. The specimens were removed from the lower 5 to 8 cm of the esophagus in achalasia patients (Text-fig. 1) during modified Heller procedures.[6] Multiple specimens were obtained from each patient along the course of the esophagomyotomy incision, and the level of removal was recorded. Esophageal smooth muscle specimens in the control series were obtained by direct biopsy of the muscular layer of the lower esopha-

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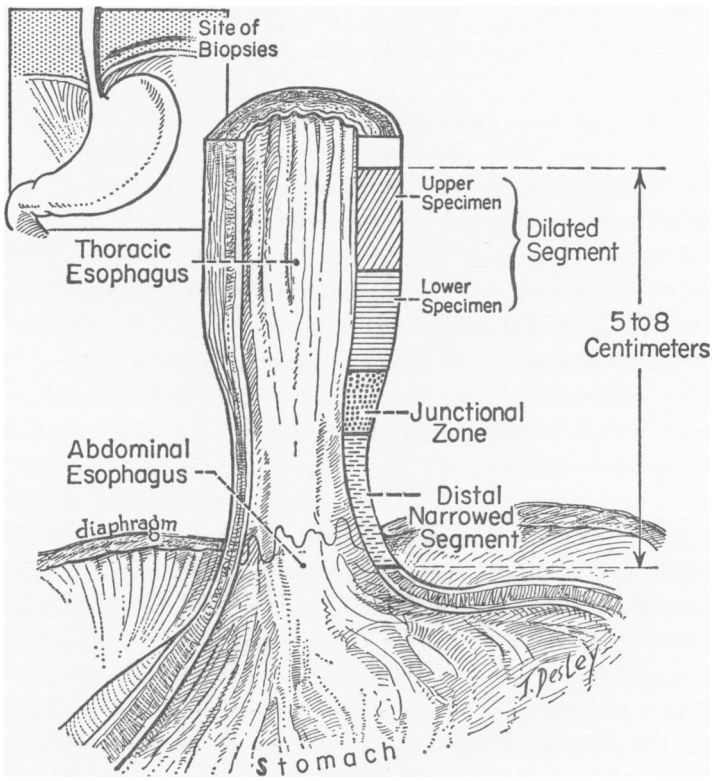
gus in patients undergoing thoracotomy for the treatment of pulmonary and esophageal carcinoma.

All smooth muscle specimens were fixed in Dalton's solution,[7] dehydrated in graded alcohol solutions, embedded in both butylmethyl methacrylate (4:1) and Epon 812[8] and stained with uranyl acetate[9] after sectioning and mounting. The material was then examined with an RCA EMU-3E electron microscope.



TEXT-FIG. 1. Site of esophageal smooth muscle biopsies in achalasia.

RESULTS

Normal Esophageal Smooth Muscle. Esophageal muscle fibers were found to be organized into continuous sheets of loosely arranged cells measuring between 4.5 and 8.0 μ in diameter in the central portion of the cell.

Individual cells had well-defined surface membranes which were composites of two membranes separated by an interspace (Fig. 1*a*). The outermost or basement membrane was of variable thickness. The inner or plasma membrane featured electron-dense plaque-like areas separated by less dense areas containing small, presumably pinocytotic vesicles. Uncommonly, portions of surface membranes projected into an

intercellular space in which circular profiles containing cytoplasmic material were present.

Two types of contact between smooth muscle cells were noted in the present study (Figs. 1 *a* and *b*); these have been previously described.[10] The more common type, the so-called complex bridge, was between the electron-dense plaque-like areas of adjacent cells. The intervening basement membranes were either closely approximated or fused in these areas. A second type, the so-called protoplasmic bridge (Fig. 1 *b*), was infrequently seen.

The bulk of the cytoplasm in the smooth muscle cells was made up of thin myofilaments which coursed longitudinally. The myofilaments appeared to aggregate at small, scattered cytoplasmic condensations and at the electron-dense plaque-like areas of the surface membranes. Dispersed throughout the normal smooth muscle cell were small numbers of mitochondria and dark granules having the appearance of ribosomes.

The central area of the cells was occupied by the nucleus, which characteristically featured small, shallow indentations at its surface. The intercellular space contained collagen and amorphous material. Axons of autonomic nerves were only rarely encountered and were always outside the surface membranes of the cells.

Esophageal Smooth Muscle in Achalasia. The surface membranes of the smooth muscle in achalasia showed several morphologic changes. The electron-dense plaque-like areas were often elongated. Large masses of ribosomes were commonly seen adjacent to the vesicular areas of the plasmalemma, while the vesicles themselves did not differ from those seen in control specimens (Fig. 2 *a*).

Conspicuous alterations of cell size and myofilament structure were seen in all specimens. Single cells or groups of cells (Fig. 2 *b*) were encountered with detachment of myofilaments from their surface membranes, which in turn had lost many of their electron-dense plaques. The overall caliber of cells with detachment myofilaments did not differ from that in controls. Other cells had decreased transnuclear caliber (Fig. 2 *c*) measuring less than 3.5 μ. At higher magnification (Fig. 3 *a*), cells of decreased caliber contained fragmented myofilaments, among which were small numbers of ribosomes. Uncommonly, normal-appearing cells of increased size (more than 8.0 μ.) were found. The distribution of these changes is shown in Table I. The junctional zone between the narrowed and the dilated portion of the esophagus was the area most altered morphologically.

The nucleus of smooth muscle cells in achalasia in some specimens appeared segmented when viewed in cut profile (Fig. 3 *b*). Nuclei commonly contained a solitary nucleolus.

The intercellular space contained focal increases in the amounts of collagen (Fig. 3 *c*). This was seen more commonly in specimens obtained

TABLE I

SITE OF SMOOTH MUSCLE CELL CHANGES IN ACHALASIA *

	Myofilament detachment	Size less than 3.5 microns	Size more than 8.0 microns
Dilated segment			
Upper specimen	+†	+	+
Lower specimen	+++	+	—
Junctional zone	++++	++	++
Distal narrowed segment	+	++	+

* 10 patients.

† +, few isolated cells; ++, many isolated cells; +++, many cells in small groups; ++++, many cells in large groups (sheets); —, not found.

from the dilated portion of the esophagus. In some specimens the intercellular space appeared broadened by collections of isolated smooth muscle and connective tissue cells and circular profiles containing cytoplasmic material (Fig. 3 *d*).

Routine hematoxylin and eosin stains on muscle tissue obtained from all patients with achalasia featured no light microscopic abnormalities.

COMMENT

The functions of the various components of the smooth muscle cell are a matter of speculation at present. The electron-dense areas of the plasmalemma have been proposed as representing attachment sites for myofilaments [10,11] as well as intercellular transmission sites.[12] Pinocytotic vesicles may function in acetylcholine accumulation or in exchange metabolism between cells.[10] The large vacuolar vesicles described by others [13,14] were so uncommonly seen in the present study that no statement regarding their structure or significance can be made.

The paucity of nerve endings among large areas of smooth muscle cells is noteworthy. Similar findings in the small intestine of the rabbit led Richardson [15] to conclude that it was unlikely that each muscle fiber received a nerve ending. The interrelationships of smooth muscle cells and their integrity as individual units would appear to be of paramount importance in normal neuromuscular excitation.

The principal smooth muscle changes in esophageal achalasia were myofilament detachment, an increase in the number of ribosomes and alterations in cell size. The cause and significance of these changes are unknown. There is some evidence, however, to suggest that these alterations are the result of esophageal denervation. Recent denervation

experiments in the rat have shown by electron microscopy that skeletal muscle undergoes similar myofilament destruction and reduction in cell size when the nerve supply is interrupted.[16] The accumulation of ribosomes in the cytoplasm of disintegrating cells with myofilament fragmentation suggests an attempt to compensate for the destruction of intracellular protein, and is consistent with known cellular function in protein metabolism. Cells of increased size represent true cellular hypertrophy; since only isolated cells of this type were found, however, it is unlikely that the characteristic gross hypertrophy (organ hypertrophy) can be explained on the basis of their increased size. It is likely that gross hypertrophy of the esophageal wall is due in some degree to increases in connective tissue. Although multiple nuclear profiles were occasionally seen, the role of hyperplasia in the production of gross hypertrophy was not clarified in the present study.

Summary

A study of the fine structure of esophageal smooth muscle in achalasia was made in 10 patients. Myofilament detachment, ribosomal activity and changes in cell size were the major morphologic alterations found. The area most involved was the junctional zone between the narrowed and dilated segments of the esophagus. It is suggested that the smooth muscle changes result from esophageal denervation.

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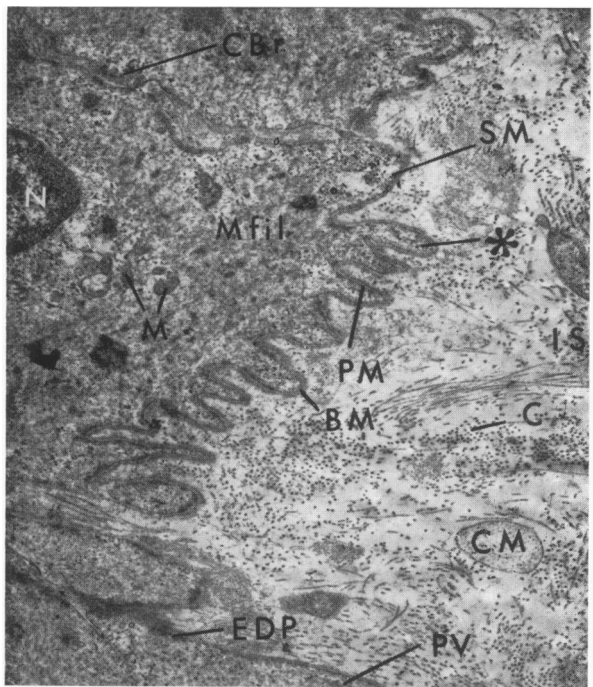
LEGENDS FOR FIGURES

FIG. 1. Normal esophageal smooth muscle. *a.* Transverse section. Surface membrane (SM) is a composite of an outer basement membrane (BM) and an inner plasma membrane (PM). The plasma membrane contains electron-dense plaque-like areas (EDP) and rows of small pinocytotic vesicles (PV). Portions of surface membrane (*) project into intercellular space (IS). Complex bridge, CBr; cytoplasmic material, CM; nucleus, N; mitochondria, M; myofilaments, Mfil; collagen, C. × 9,780.

b. Transverse section. Shown is a protoplasmic bridge (PBr). Cytoplasmic condensations, CC; complex bridges, CBr; myofilaments, Mfil; indented nuclear profiles, N. × 10,000.



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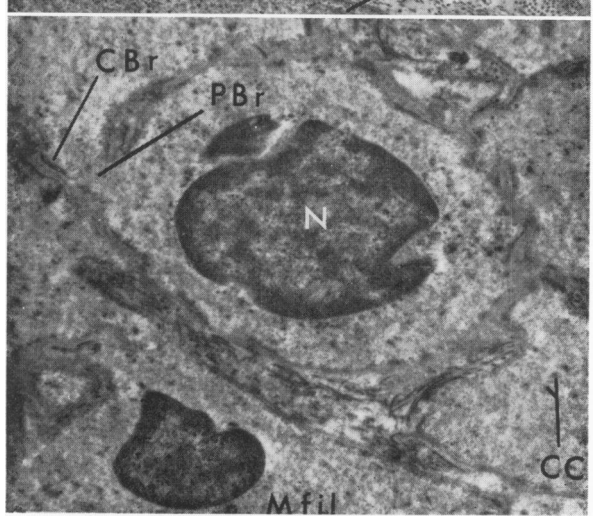

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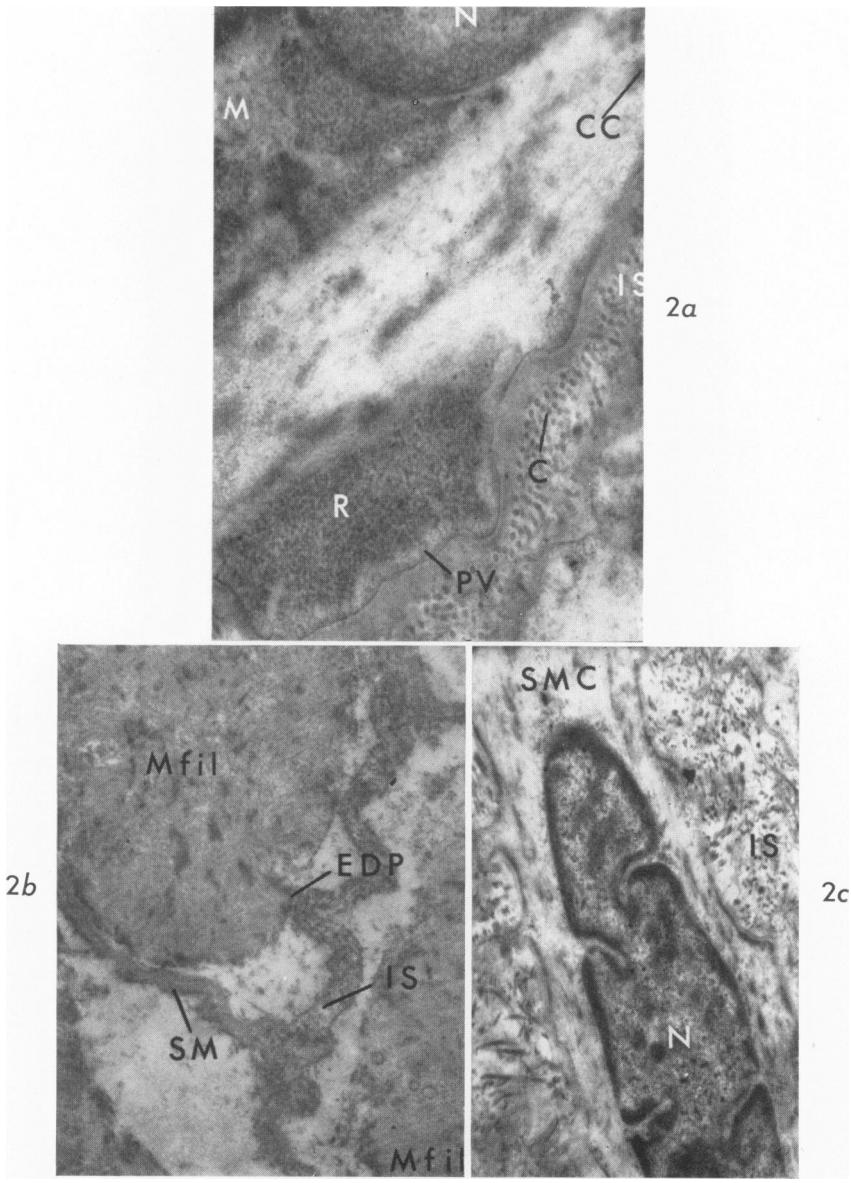

FIG. 2. Esophageal smooth muscle in achalasia. *a.* Oblique section. Large masses of ribosomes (R) are seen adjacent to vesicular rows (PV) and near the nucleus (N). Mitochondria, M; cytoplasmic condensation, CC. An interspace (IS) contains collagen (C). × 28,500.

b. Three adjacent cells exhibit detachment of their myofilaments (Mfil) from their respective surface membranes (SM). Electron-dense plaques (EDP) are less distinct. The intercellular space (IS) has not widened. × 9,900.

c. Longitudinal section through a cell (SMC) with decreased transnuclear caliber. Nucleus, N; intercellular space, IS. × 9,790.

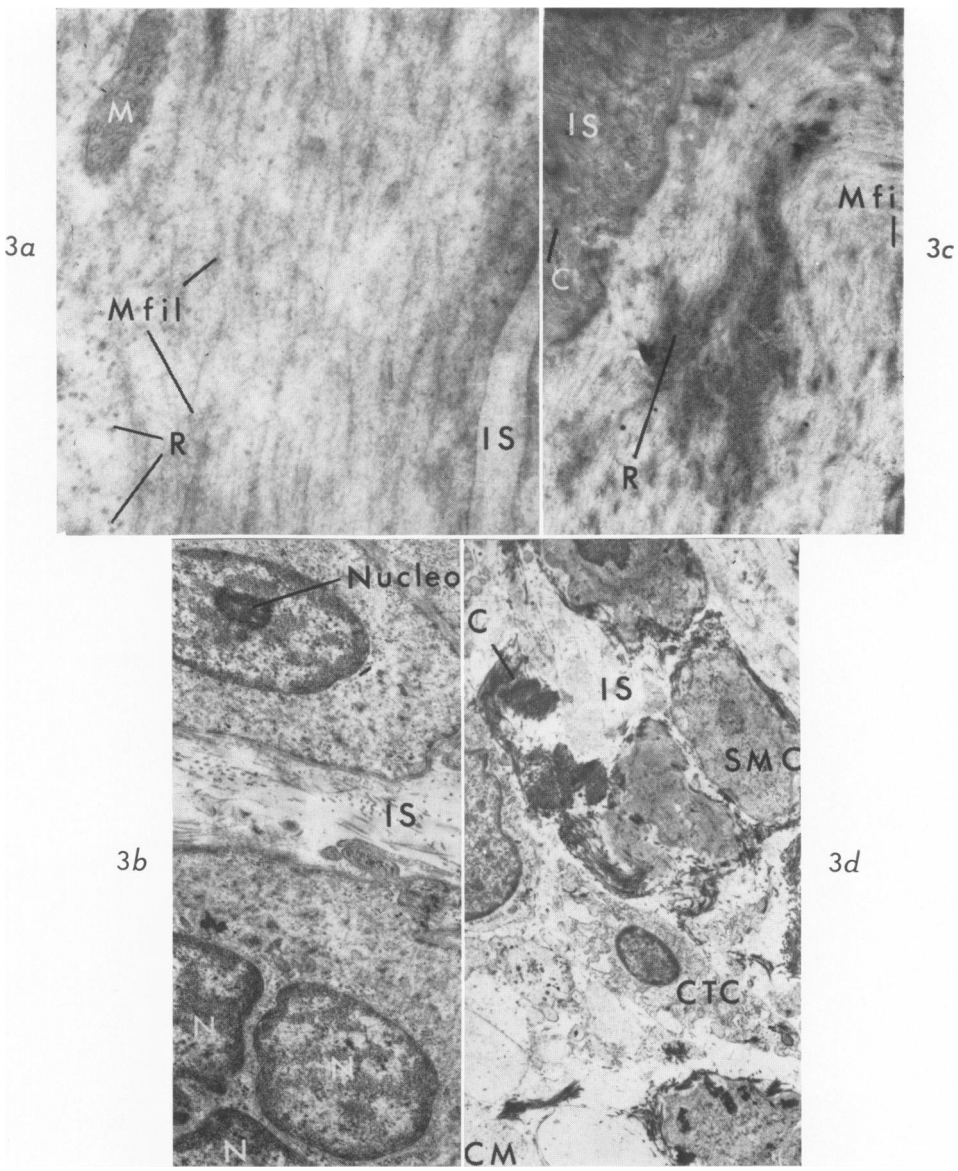

FIG. 3. Esophageal smooth muscle in achalasia. *a.* Higher magnification of a cytoplasmic area in a cell showing fragmentation of myofilaments (Mfil). Small rounded profiles appear to be ribosomes (R). Mitochondrion, M; intercellular space, IS. × 19,000.

b. Oblique section. Three nuclear profiles (N) appearing in one cell suggest segmentation. Distinct nucleolus, Nucleo; intercellular space, IS. × 9,780.

c. Oblique section. The intercellular space (IS) is widened by collections of collagen (C). Ribosomes, R; myofilaments, Mfil. × 9,780.

d. The intercellular space (IS) contains isolated smooth muscle cells (SMC), connective tissue cells (CTC) and circular profiles containing cytoplasmic material (CM). Collagen, C. × 2,570.