Intercellular Bridges as Protoplasmic Anastomoses between Smooth Muscle Cells*

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PLATES 37 TO 40

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ABSTRACT

In electron microscopic studies, protoplasmic anastomoses were found to occur between smooth muscle cells in the gastrointestinal tracts of rats. The protoplasmic connections are apparently cylindrical in form and contain cytoplasm having a density similar to that of the cellular regions connected. The membranes enclosing the cytoplasmic connections are continuous with the membranes of the connected cells. These connecting structures have been called intercellular bridges, which is adequately descriptive if emphasis is placed on the interpretation that they represent protoplasmic anastomoses. To emphasize this, the term may be modified; *i.e.*, anastomotic intercellular bridges.

In some cellular connections, transverse diffuse lines are seen. These may be interpreted as either disintegrating or reforming plasma membranes which is consistent with the concept that protoplasmic continuity is transitory. Since the animals were dissected under hypothermia, reduction of muscular activity by the chilling may have helped to preserve the structure of existing anastomotic intercellular bridges.

The intercellular protoplasmic anastomoses may play a role in the conduction of action potentials from one smooth muscle cell to another.

INTRODUCTION

Whether or not protoplasmic continuity occurs between smooth muscle cells has been a subject of controversy. Some early investigators described wide end-to-end and side-to-side anastomoses between smooth muscle cells (1, 16). In opposition, others regarded the so called anastomoses as preparation artifacts (12, 23). Recently, because of negative findings in their electron microscopic studies, Caesar *et al.* (8) and Bergman (4) have questioned the occurrence of protoplasmic continuity between smooth muscle cells. However, Mark (15) has presented evidence in favor of continuity. The present report together with an earlier one (21), supports the findings of Mark and offers more convincing evidence that intercellular anastomoses do occur in smooth muscle.

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Material and Methods

For this investigation, smooth muscle tissue from 2-month-old rats was prepared as follows: Light sodium nembutal anesthesia was temporarily deepened by the use of ether to facilitate immersion of the rats in an ice water bath. Each rat remained in the ice water until visible breathing movements ceased. The colonic temperatures of the rats ranged from 5° to 9°C. at the time of excision of portions of the pyloric stomach and descending colon. These portions were immediately placed in a fixing solution of 1 per cent osmium tetroxide (pH 7.7), divided into smaller pieces, and left in the fixative for an additional 90 minutes. The tissues were dehydrated in methanol, embedded in methacrylate, sectioned with a Servall Porter-Blum microtome, and studied with a Philips electron microscope (EM 100A).

OBSERVATIONS

Protoplasmic anastomoses are demonstrated in Figs. 1 to 4. These intercellular bridges are most often seen between the lateral aspects of the cells; one example of end-to-end anastomosis was observed but electron micrographs to display this

continuity were not obtained. The intercellular bridges are links of muscle protoplasm; the plasma membranes and cytoplasm are continuous between the connected cells.

Close examination of the cytoplasm within these intercellular bridges reveals the presence of vesicles which average 50 mμ in diameter; some are centrally located (Figs. 1 and 2), others are adjacent to the plasma membranes (Figs. 2 and 3). These vesicles are similar in density and dimension to those present elsewhere in the cytoplasm proper.

The widths of the intercellular bridges range from approximately 0.1 to 0.7 microns and their lengths from approximately 0.04 to 0.5 microns. A similarity in dimensions of the intercellular bridges in micrographs of connected smooth muscle cells sectioned in transverse (Figs. 1–3), oblique and longitudinal (Fig. 4) planes, suggests that they have a tubular or cylindrical shape.

Connections between the cells in Fig. 5 are considered to be intercellular bridges fixed in the process of forming or of retracting. Transverse diffuse lines (arrows, Fig. 5) intervening between the connected cells may be either remnants of plasma membranes between the cells or stages in their reformation.

General structural features of the cells of smooth muscle tissue observed in this investigation conform to descriptions published by Mark (15), Caesar *et al.* (8), and Bergman (4).

DISCUSSION

The electron micrographs obtained in this study demonstrate the occurrence of protoplasmic anastomoses between smooth muscle cells in the walls of the pyloric stomach and descending colon of the rat. These observations corroborate the light microscopic investigations of McGill (16) and Aunap (1) and the electron microscopic studies of Mark (15) and Thaemert (21).

Observations of smooth muscle with the light microscope have resulted in conflicting interpretations as to what constitutes protoplasmic continuity between smooth muscle cells. McGill (16) and Aunap (1) described protoplasmic continuity as wide anastomoses between the ends and between the sides of smooth muscle cells. Kultschitzky (13), Klecki (11), and Barfurth (2) considered the small "twigs" of muscle protoplasm that occurred between the lateral aspects of cells as examples of protoplasmic continuity. These "twigs" were designated "intercellular bridges." McGill (16) considered these "twigs" to be connective tissue strands but stated that they may be surrounded by muscle protoplasm. Aunap (1) did not accept the "twigs" as examples of anastomosis. The anastomotic intercellular bridges observed in the present study compare favorably with the "intercellular bridges" of Kultschitzky, Klecki, and Barfurth, in both size and location. It is likely that these early light microscopists were observing protoplasmic continuity but were hampered by relatively poor resolution.

In publishing electron micrographs of smooth muscle from rat uteri, Mark (15) interpreted gaps in the sarcolemma as suggestive of continuity. As the result of their electron microscopic studies, Caesar *et al.* (8) reject the concept of anastomosis between smooth muscle cells. Their micrographs of smooth muscle from mouse urinary bladder display only cells completely separated from each other by a "cytolemma," which they describe as being comparable to the sarcolemma of striated muscle. In the present study, however, electron micrographs distinctly show that some smooth muscle cells are connected by small intercellular bridges and therefore are not always completely separated from each other.

The use of the term "intercellular bridge" by Kultschitzky (13), Klecki (11), and Barfurth (2) implied a continuity of protoplasm between smooth muscle cells. This concept was accepted by Thaemert (21), but Lewis (14) and Bergman (4) applied the term to intercellular processes of smooth muscle which did not display protoplasmic continuity. The term is also currently being used to describe connecting processes of epidermal cells which are not considered to be anastomotic. Because of the application of the term "intercellular bridge" to a variety of cellular contact relationships and the confusion which may result therefrom, it has been suggested (3) that a clarification of the terminology is needed. The term is well established and would be adequate in describing the intercellular continuity observed here if emphasis is placed upon the interpretation that this represents protoplasmic anastomosis. It is suggested that the term could be modified to emphasize this interpretation; *i.e.* anastomotic intercellular bridge.

Consideration of the evidence presented here and in publications of Lewis (14) and Bergman (4) has led to a concept of active formation and retraction of living anastomotic intercellular bridges. Transverse, diffuse lines between connected smooth muscle cells apparent in Fig. 5, may represent plasma membranes either forming or disinte-

grating. In addition, evaginations of apposed smooth muscle cells have been observed to project toward each other, some in contact, others distinctly apart. The "intercellular bridges" of Bergman (4) may have been anastomotic intercellular bridges in the process of formation or of retraction. Lewis (14) indicated that "intercellular bridges" connecting living smooth muscle cells would withdraw on slight provocation, such as the direct application of glycerin or certain other chemicals. Withdrawal reactions of irritated cells may account for the failure of certain investigators to find connections between smooth muscle cells. The animals from which the tissues were obtained for the present study were subjected to low temperatures before the tissues were excised. This technique has been used by van Breemen and Marx (22) in the preparation of tissues for electron microscopy. Chilling reduces enzyme activity, muscular contraction, and, possibly, the withdrawal of existing anastomotic intercellular bridges.

Current electrophysiological concepts of action potential propagation within smooth muscle tissue suggest that action potentials are transmitted from muscle cell to muscle cell (4–7, 17–20). Nevertheless it is generally agreed that smooth muscle cells are separated from each other by plasma membranes which, together with the intercellular space and its contents, would seem to provide sufficient resistance to prevent the conduction of action potentials from one cell to another. Several hypotheses have been developed to explain by what means action potentials are conducted from one cell to the next. Bozler (5–7) believed that conduction occurs through the wide anastomoses between smooth muscle cells described by the light microscopists. Prosser *et al.* (17–20), who concluded that protoplasmic continuity does not occur between smooth muscle cells, explained conduction of action potentials as occurring across the intercellular space in overlapping muscle cells. They refer to this as ephaptic conduction.

A third hypothesis has been advanced by Bergman (4), who expressed the belief that the foregoing hypotheses should be modified in view of the structural relationships which he observed, namely highly organized "intercellular bridges" between smooth muscle cells. Intrabridge membranes were considered to form cell boundaries between the connected cells; therefore protoplasmic continuity was not accepted. The "intercellular bridges" were considered by Bergman to be well adapted for the conduction of action potentials because of what he considered to be a consistency between this type of structure and reported electrophysiological and pharmacological data (9, 10). He maintained that the features of his specialized structures "are more consistent with the reported uniform conduction velocities and highly organized rhythmic contractions than can be explained by an ephaptic relationship. As the muscle cells are contractile (and thus variable in shape) and not rigidly bound together, the uniform spread of action currents across an intercellular space of inconsistent dimension and without specialized structures intervening appears to be improbable."

The anastomotic intercellular bridges observed in the present investigation would seem to be specialized structures that would provide the means whereby action potentials could be conducted from one cell to another, permitting the production of conduction velocities of a uniform nature.

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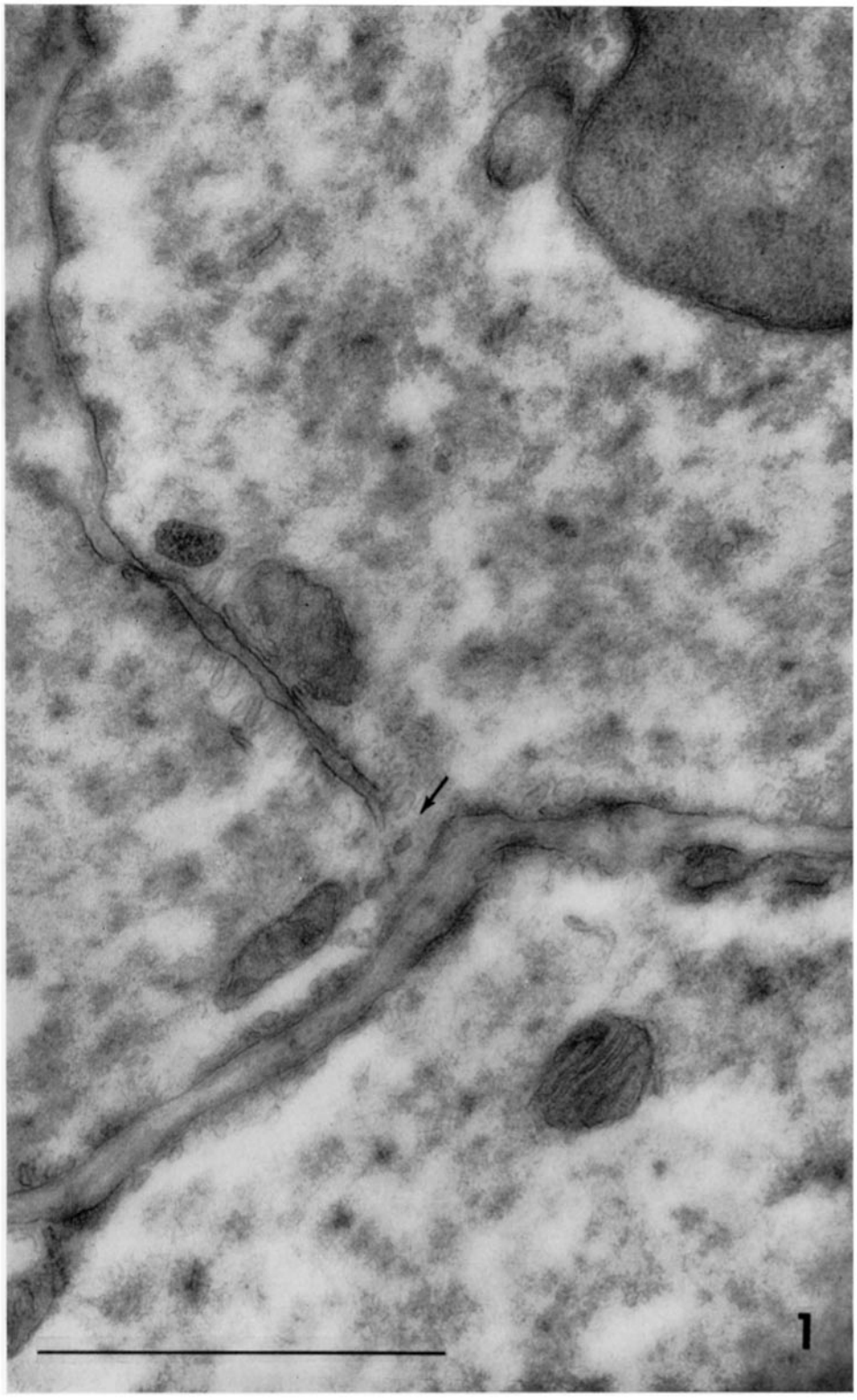
Explanation of Plates

The line in the lower left corner of each figure represents one micron.

Plate 37

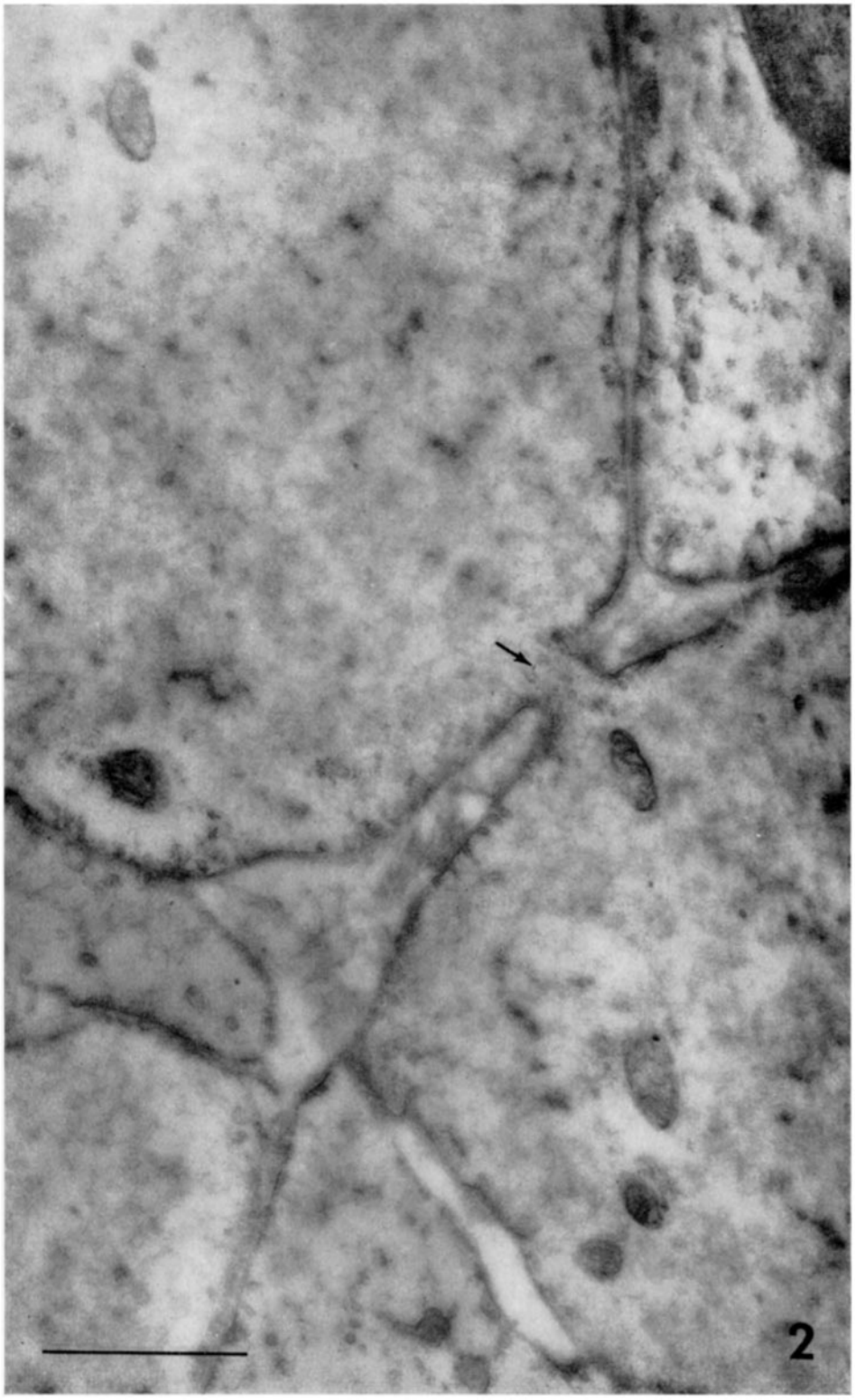
Fig. 1. Portions of transversely sectioned smooth muscle cells from the wall of the pyloric stomach of a rat. An anastomotic intercellular bridge is indicated by arrow. × 60,000.



(Thaemert: Anastomotic intercellular bridges)

PLATE 38

FIG. 2. Portions of transversely sectioned smooth muscle cells from the wall of the pyloric stomach of a rat. An anastomotic intercellular bridge is indicated by arrow. × 30,000.

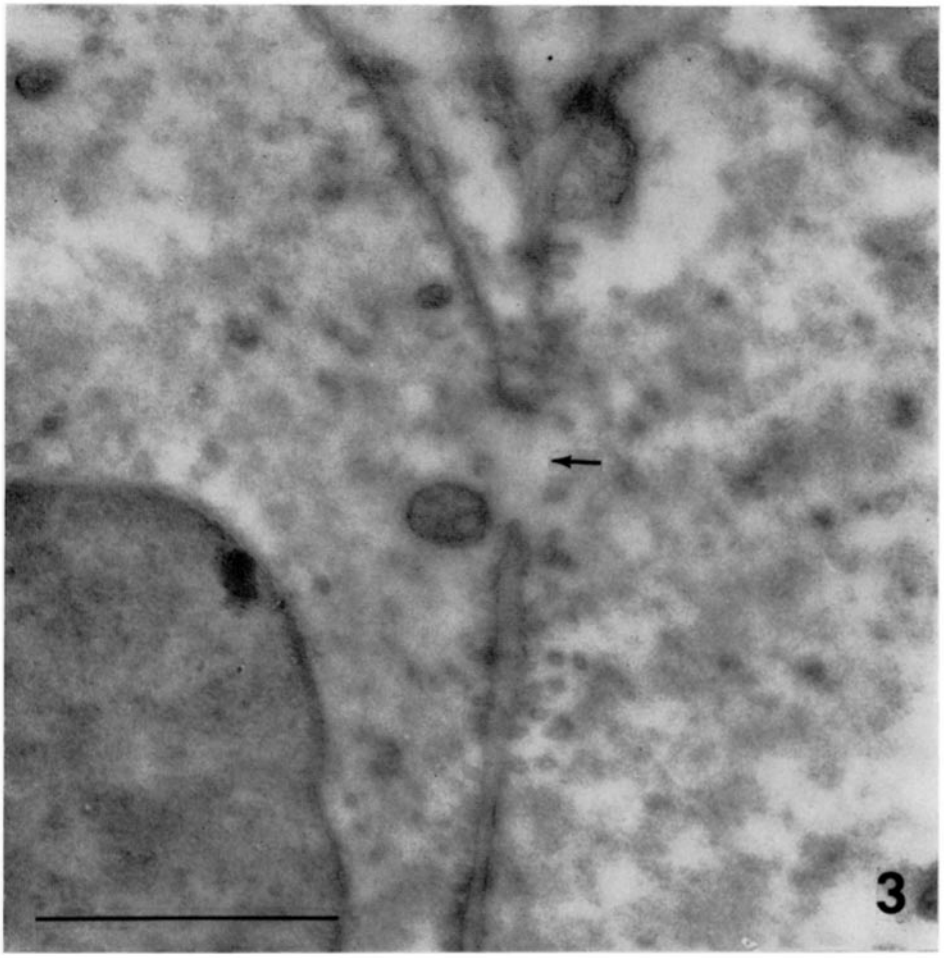


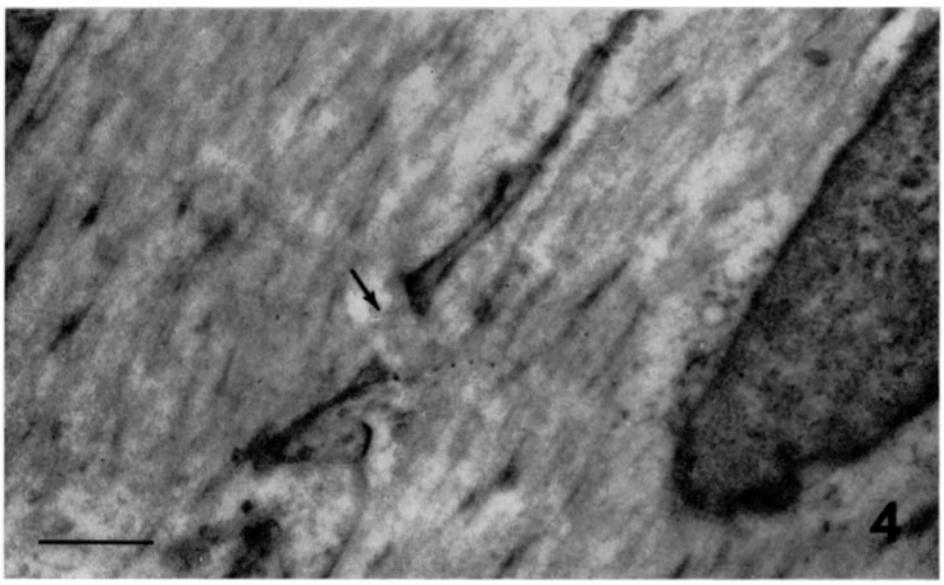
(Thaemert: Anastomotic intercellular bridges)

PLATE 39

FIG. 3. Portions of transversely sectioned smooth muscle cells from the wall of the pyloric stomach of a rat. An anastomotic intercellular bridge is indicated by arrow. × 40,000.

FIG. 4. Portions of longitudinally sectioned smooth muscle cells from the wall of the pyloric stomach of a rat. An anastomotic intercellular bridge is indicated by arrow. × 15,000.

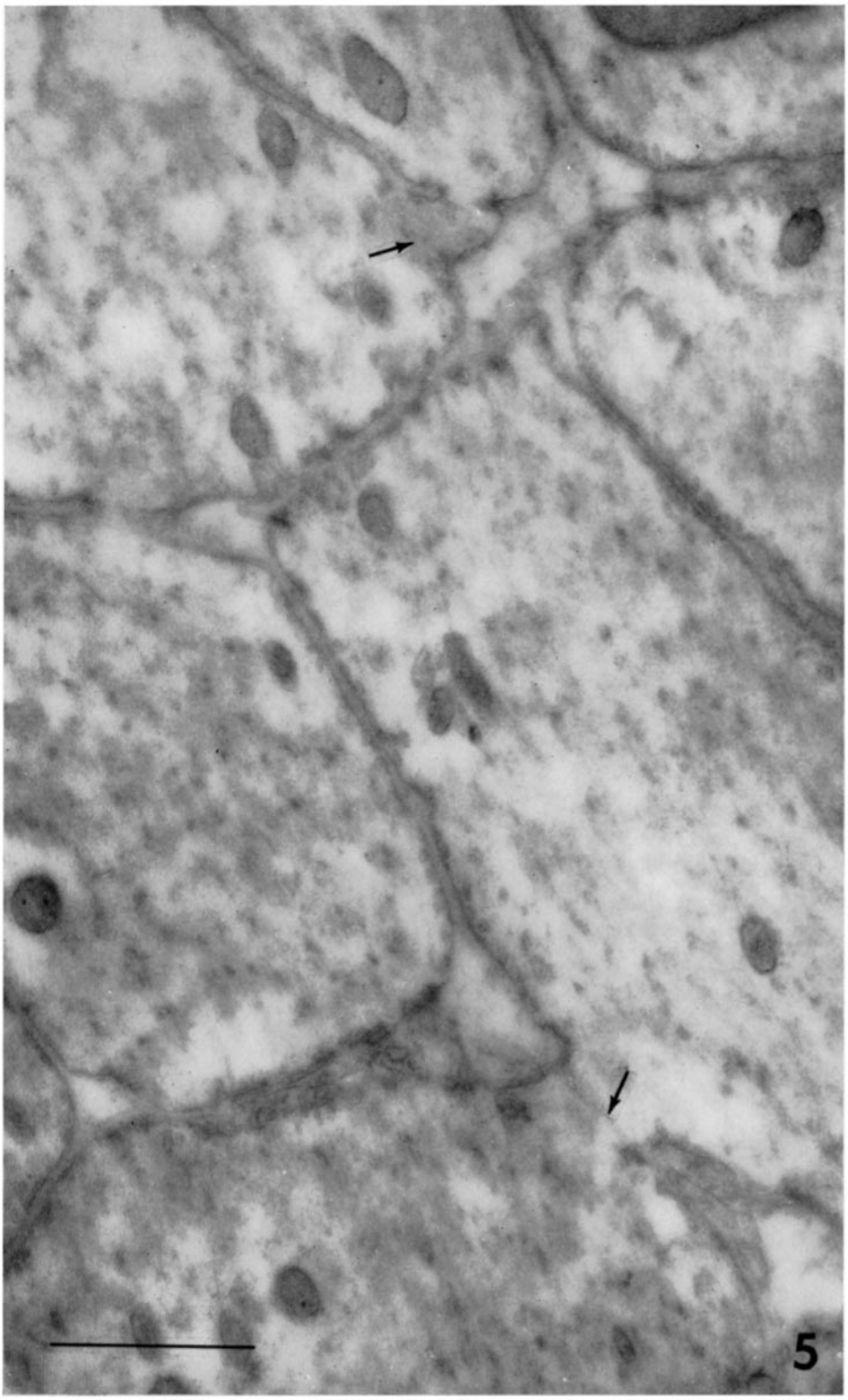




(Thaemert: Anastomotic intercellular bridges)

PLATE 40

FIG. 5. Portions of transversely sectioned smooth muscle cells from the wall of the pyloric stomach of a rat. Transverse, diffuse lines are indicated by arrows. × 30,000.



(Thaemert: Anastomotic intercellular bridges)