# STUDIES ON AN EPITHELIAL (GLAND) CELL JUNCTION

## II. Surface Structure

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ABSTRACT

The surface structure of a gland epithelium (*Drosophila* salivary gland), particularly that at the junction between cells, was examined under the electron microscope. The junctional surface, which in the preceding paper was shown to be highly permeable to ions, has the following structural characteristics. About two-thirds of it are profusely infolded; the surface membranes of adjoining cells interdigitate and present desmosomes. The width of the intercellular space varies considerably. The remainder of the junctional surface, the third that abuts on the lumen, is rather straight. Here, the cell membranes are aligned parallel at a distance of 150 A, and interconnected at regular intervals of 100 A. The connecting material has a high electron opacity, and is about as thick as the cell membranes, but, unlike the latter, has no resolvable unit membrane structure. The surface at the cell base, which in the preceding paper was shown to be rather impermeable, is infolded and resembles the infolded junctional region. The luminal surface exhibits microvilli. Critical surface dimensions are given, and the implications of surface structure in intercellular permeability are discussed.

### INTRODUCTION

Evidence has been presented in the preceding paper that ions move rather freely from cell to cell in a gland epithelium (1). It was shown that there is no substantial diffusion barrier in the cell-to-cell direction, but that there is a strong barrier between the intercellular space and cell exterior. Two observations called for an explanation at the structural level: the lack of ion leakage along the intercellular space and the low diffusion resistance across the contact surfaces of adjacent cells. The former implies that the intercellular space is obstructed or very narrow; and the latter, that either the membrane area at the contact surface is large in relation to that of the rest of the cell surface, or that the permeability of the contact surface is high. One may expect to find information on at least two of these points at the fine structural level. The present study has been undertaken to provide this information and to correlate the structure with the electrophysiological measurements of the preceding article (1).

### MATERIALS AND METHODS

Salivary glands of third instar larvae of *Drosophila flavorepleta* were used in these studies. The glands were fixed for 3 hours in cold buffered 6.25 per cent

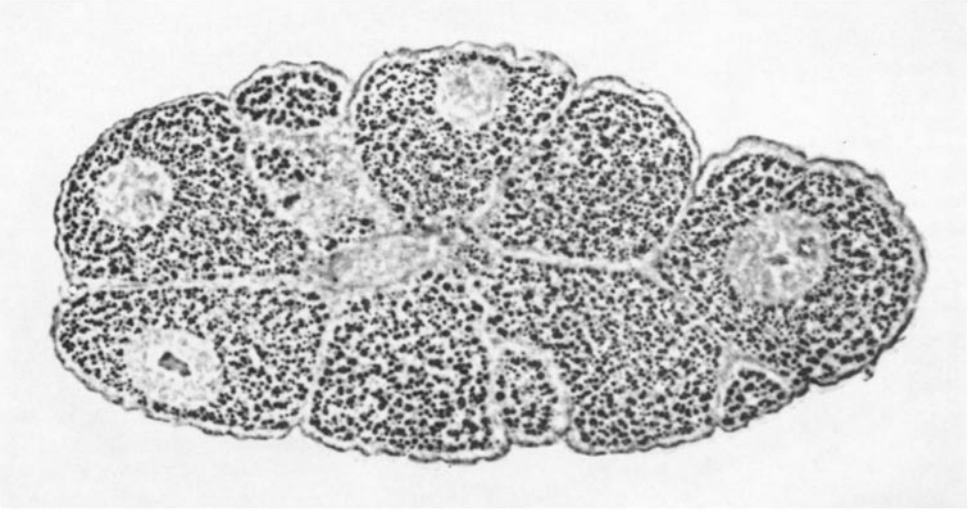


FIGURE 1 Phase contrast micrograph of an Araldite-embedded salivary gland. This tangential section shows the nuclei and numerous secretion granules in the cytoplasm. × 200.

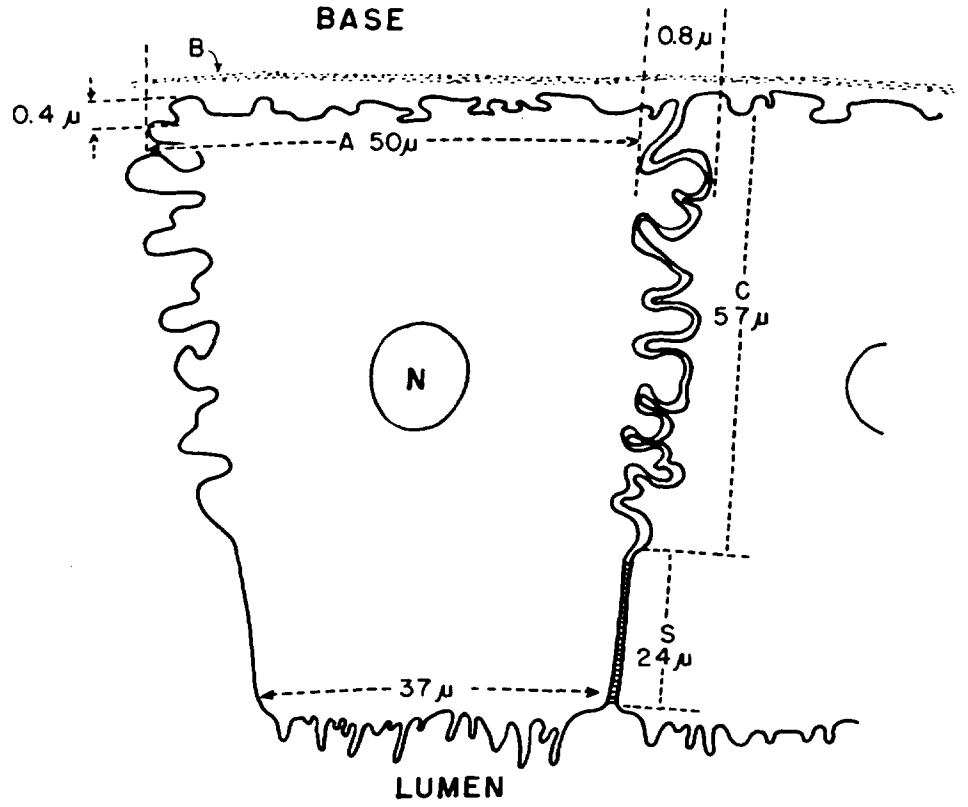



FIGURE 2 A schematic representation and average dimensions of the cell surfaces. *C*, convoluted segment; *S*, septate junction; *B*, basement membrane; *N*, nucleus. Dimensions given are average measures (see text).

glutaraldehyde (pH 7.6) *in situ*, following decapitation of the larvae; or after isolation from the larvae (2). Following rinsing and storage in cold phosphate buffer, the tissue was fixed in 2 per cent osmium tetroxide with sucrose (3) at pH 7.4 for 4 hours, dehydrated in acetone, and embedded in Araldite. Prefixation with glutaraldehyde resulted in better structural preservation, especially of cell membranes, than conventional osmium tetroxide fixation (4).

Two-micron thick sections of the Araldite-embedded tissues were studied in phase contrast. Thin sections were stained with lead hydroxide (5) and examined under a Siemens Elmiskop I.

## RESULTS

The salivary gland consists of a single layer of roughly cylindrical cells, radially disposed around a lumen (Fig. 1). The diameter of the lumen is about 45 $\mu$ in the caudal half and about 55 $\mu$ in the cephalic half of the gland. Average cell dimensions are: 80 $\mu$, lumen-to-base; 300 $\mu$, basal perimeter; 250 $\mu$, apical (luminal) perimeter (Fig. 2). Toward the cephalic end of the larva, and particularly at the duct, the lumen-to-base length becomes smaller, but the other dimensions remain essentially unchanged.

The cytoplasm of the gland cells, as seen under the electron microscope, contains abundant formations of rough-surfaced endoplasmic reticulum, as well as numerous dispersed ribosomes (Figs. 3 to 10). Numerous secretion droplets, vesicles, and vacuoles are present throughout the cytoplasm (Figs. 4 to 6, 8, and 10) (6). The electron opacity of the cytoplasm varies from cell to cell, which helps one to trace cytoplasmic extensions of individual cells (Figs. 4 to 8).

The cell surface presents three regions with distinctly different structural features. One region exhibits profusely infolded cell membranes and encompasses the basal surface and approximately two-thirds of the contact borders between adjacent cells (Figs. 3 to 7). A second region comprises the remainder of the contact surface (Figs. 4, 8, and 9). Here, the surface membranes of adjacent epithelial cells are relatively straight, and are separated by an intercellular space which contains a series of regularly spaced septa. The third structural variant is an apical brush border which is formed by numerous microvilli (Fig. 10). A more detailed description of the structural features of the surface membranes relevant to the electrical measurements follows below. A diagram summarizing these features is given in Fig. 2.

### *Basal Surface*

BASEMENT MEMBRANE: The basal portion of the epithelial cell rests upon a basement membrane, 0.2 to 0.4 $\mu$ thick, made of poorly defined filamentous material. In addition, a narrow dense layer is frequently observed within and parallel to the plane of the basement membrane. Occasional spur-like extensions of the basement membrane are present within the superficial portions of the extracellular phase of the basal infoldings (Fig. 3).

CELL MEMBRANE PROPER: The cell surfaces in this region present a complicated array of infoldings. These infoldings project in all directions, but, for the most part, seem to run parallel to the basement membrane (Fig. 3). The cytoplasmic processes bounded by these surface infoldings measure approximately 0.1 $\mu$ in their smaller dimension and involve the peripheral portion of the cell for an average depth of 0.4 $\mu$. The surface membranes of the cells exhibit a unit membrane structure (inset, Fig. 3) (7). The extracellular space between contiguous membranes shows no organized structure. It ranges in width from less than 100 to 450 A (average about 150 A).

### *Contact Surface*

CONVOLUTED REGION: The portion of the contact surface which extends from the base for about two-thirds of the radial length of the cell (57 $\mu$, average) exhibits numerous infoldings which resemble those of the basal surface. Here, cytoplasmic processes of adjacent cells interdigitate profusely. This is best seen in sections in which a dark cell lies next to a light one (Figs. 4 to 7). The dimensions of the cytoplasmic processes and their intercellular space are similar to those at the cell base. The fringe zone along the cell junction over which infoldings occur measures up to 0.8 $\mu$ in the cell-to-cell direction (Fig. 2). Occasional desmosomes (8–10) are situated along the convoluted surfaces of the contact margin (Fig. 7).

SEPTATE JUNCTION: The cell surface of the apical third of the contact border (24 $\mu$, average) differs markedly in structure from the convoluted segment. Its surface membrane is relatively straight and exhibits a unit membrane structure which has an electron opacity greater than that seen at the other cell surfaces (Figs. 4, 8, and 9). The membranes of adjoining cells are here separated by an intercellular space of rather constant width (150 A). The most striking feature of this junctional re-

gion is the presence of electron-opaque septa which bridge the intercellular space and make contact with the external aspects of both cell membranes (Figs. 8 and 9).

The septa are about 50 A thick, and are regularly spaced at intervals of 100 A perpendicular to the cell membrane. Septa are present in all planes of section. Tangential sections of the cell surfaces disclose polygonal arrays which probably represent the arrangement of septa in this plane (Fig. 9). Occasionally, interruptions in the cell surfaces, associated with apparent fusion of the inner and outer layers of the unit membranes, are noted (inset, Fig. 9). Whether such regions represent true discontinuities of the cell surface membranes is uncertain. Segmental thickenings which resemble a portion of the junctional complex described by Farquhar and Palade (11) are present along the contact margins near their junction with the apical cell border (Fig. 10).

### *Luminal Surface*

The apical cell surface has a brush border which is similar to that found in most glandular cells (Fig. 10). The microvilli measure from about 0.5 $\mu$ to 2.8 $\mu$ in length, and their surfaces display typical unit membrane structure (inset, Fig. 10).

## DISCUSSION

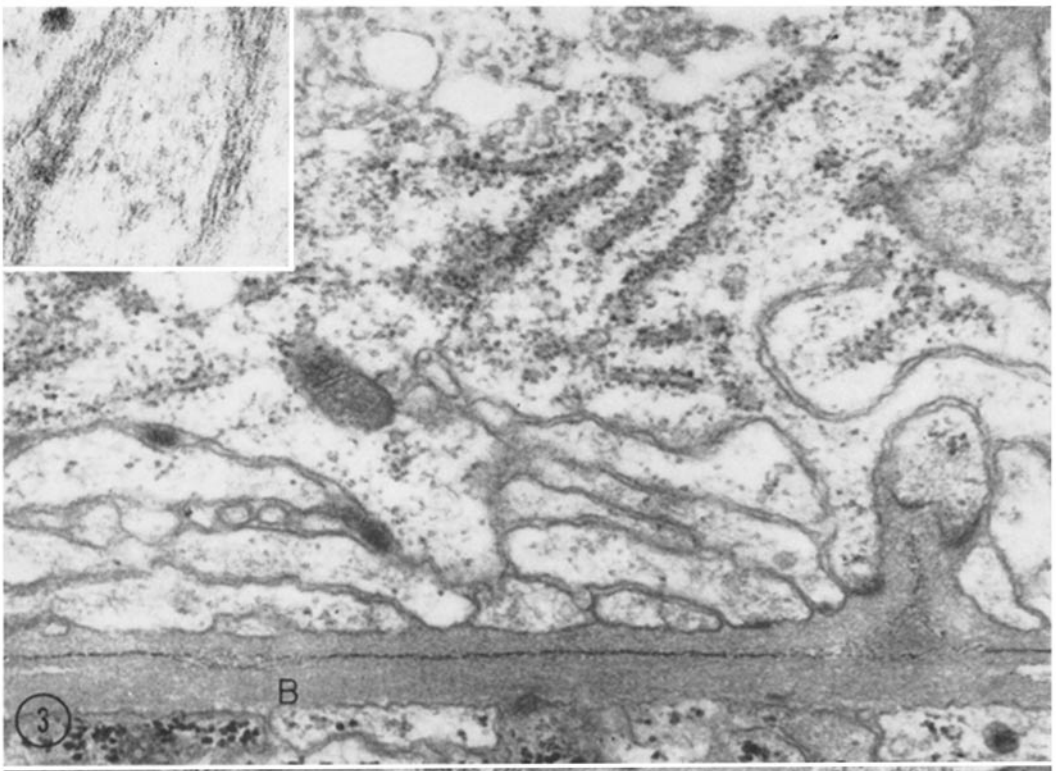
The present observations and measurements of cellular surface membranes provided the structural background for the determinations of membrane permeability described in the preceding paper (1). On the basis of these measurements, it was possible to estimate an upper limit for the relevant membrane area, and to show that ion permeability of the cell membrane is drastically altered at the contact surface (1).

The remaining points that emerge from the studies are speculative. They concern the location and the structural basis of the increased permeability. The electrical measurements and fluorescein experiments of the preceding paper show that an increase in permeability occurs at the contact membranes, but give no information on where it occurs. At the structural level, the question is then whether there are discernible modifications in surface membranes at the contact surface. It is, of course, too much to expect that changes in permeability to relatively small ions would show up under the electron microscope; but the permeability change here may well be a much coarser one and also include particles of larger size. Electron micrographs of the contact membranes show a number of suggestive structural specializations at the level of the septate junction. There, the unit membranes of adjoining cells are closely aligned and interconnected by electron-opaque bridges. The nature of the bridges and the way they connect with the unit membranes are not clear. Similar bridges have previously been described in *Hydra* by Wood (12) and in hydroids by Overton (13). The bridges in *Hydra* epithelia are interpreted by Wood (12) to consist of unit membrane material. In the present case, it appears unlikely that the bridges are unit membranes; a double leaflet is not discernible, and the electron opacity of the bridges is lower than that of the outer leaflets of the contact membranes, where a unit membrane structure is clearly visible (Fig. 9). As to the type of interconnection between contact membranes, a number of possibilities present themselves; three are depicted in the diagram of Fig. 11. Possibilities *A* and *B* (Fig. 11) differ only with respect to the continuity of the outer layer of the unit membrane. It is not possible to distinguish between them due to the close apposition and similar electron opacity of septa and unit membranes. We are at present quite unable to distinguish between these three possibilities.

The bridges are also obvious candidates for the diffusion barrier found in the radial direction of ion flow. They divide the intercellular space into many compartments (about 2,000 per junction) which lie in series in the direction intercellular space–cell exterior of current flow. Thus, if the

FIGURE 3 Electron micrograph of basal aspect of an epithelial cell. The basement membrane (*B*) and surface infoldings of the cell membrane are noted. × 50,000. The unit membrane structure of the infolded surface membranes is seen in the inset. × 200,000.

FIGURE 4 Contact surfaces. The arrow indicates transition between *C*, the convoluted segment, and *S*, the straight segment (septate junction). In addition, secretion granules, vacuoles and rough-surfaced endoplasmic reticulum are present in the cytoplasm of the light (*L*) and dark (*D*) cells. × 32,000.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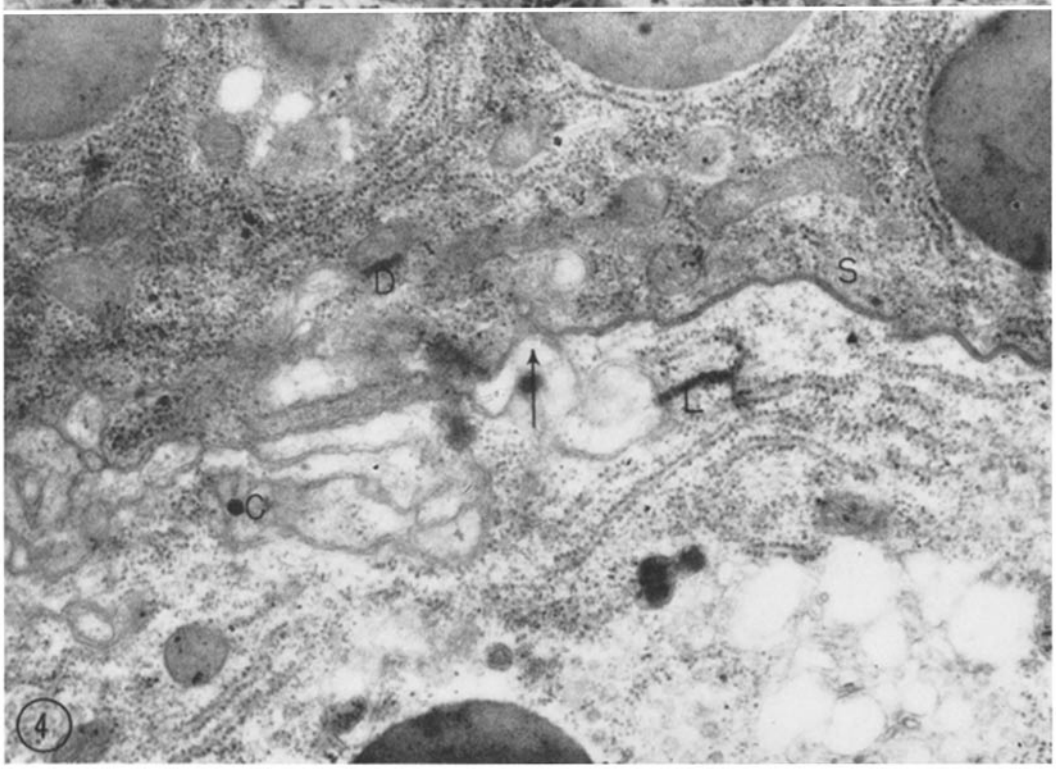
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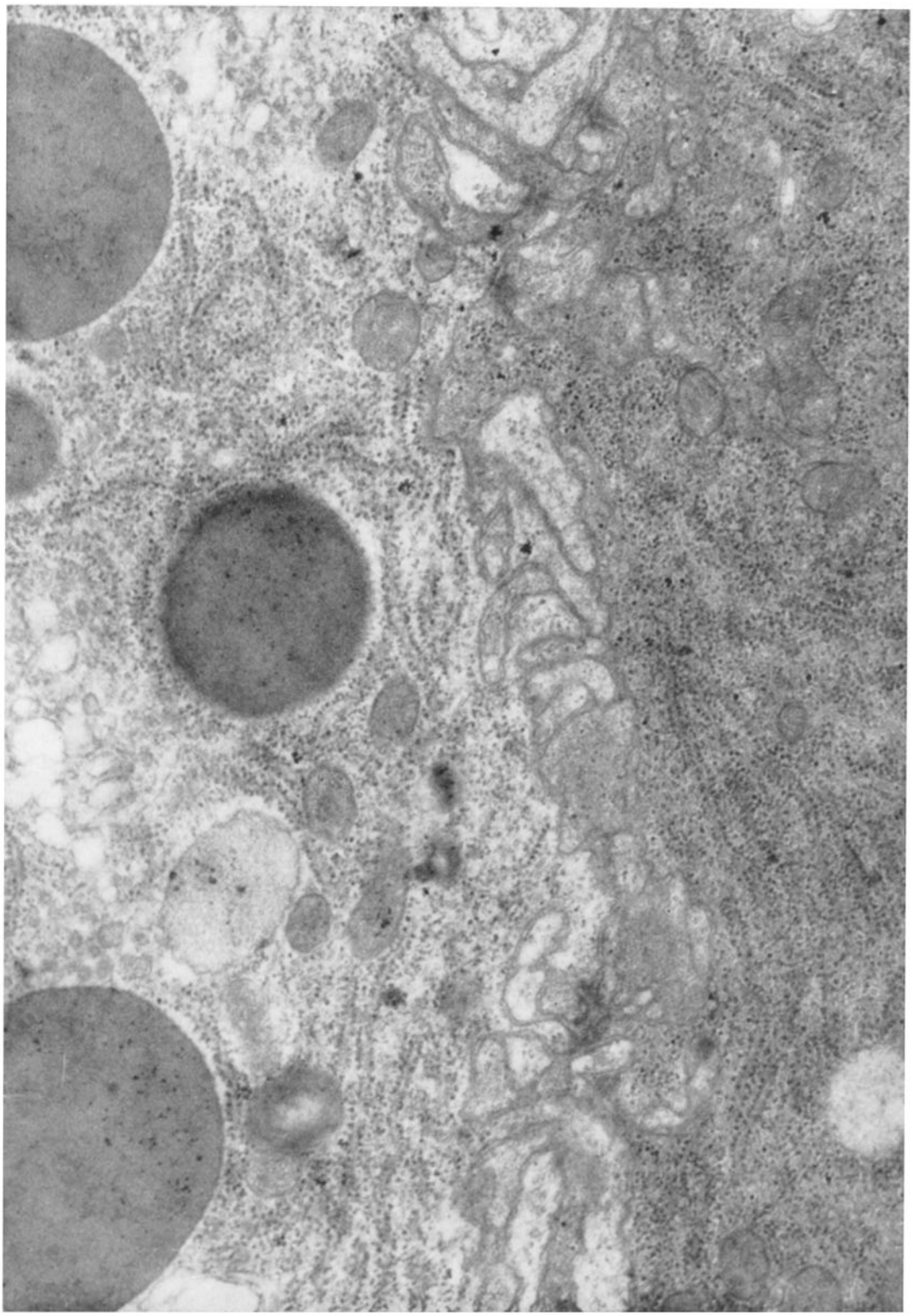
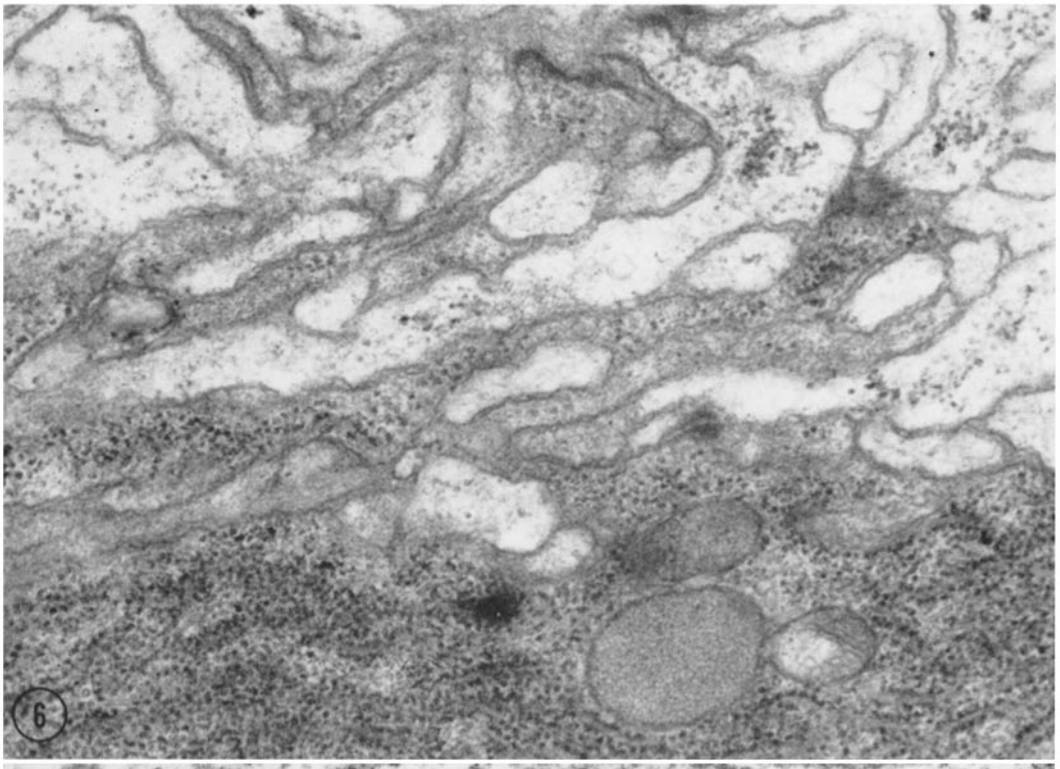


FIGURE 5 The convoluted contact surfaces of a dark and light cell are shown. × 40,000.



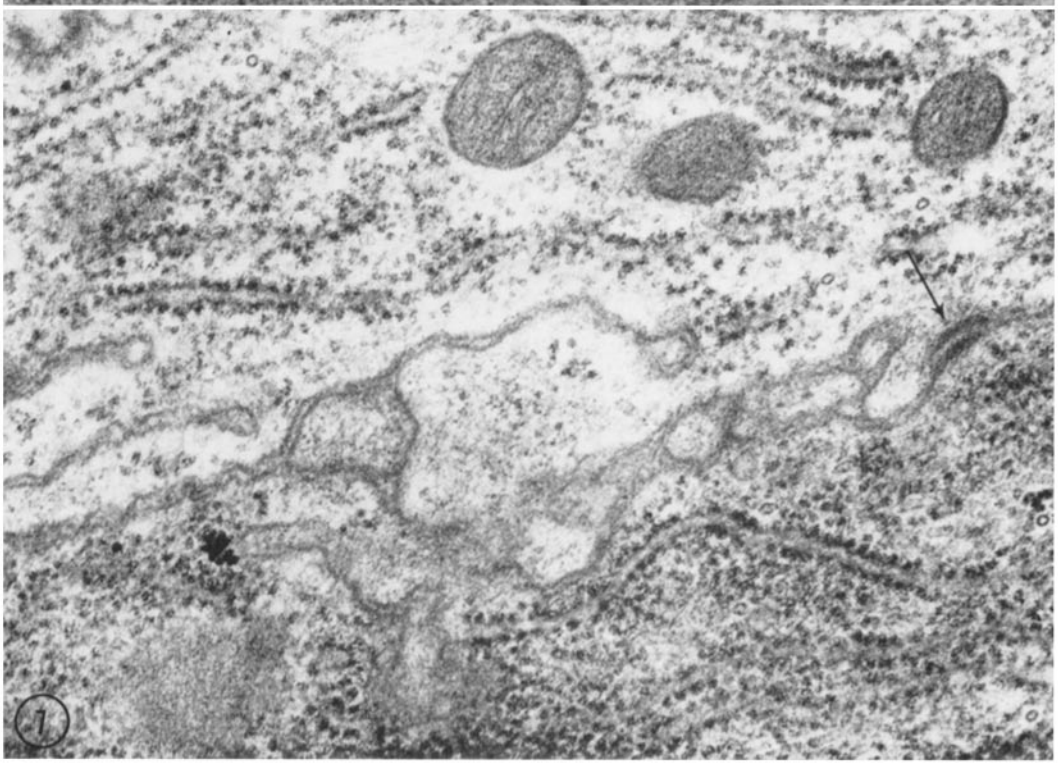




FIGURE 6 This electron micrograph shows the interdigitation of cytoplasmic processes of a dark and light cell. × 45,000.

FIGURE 7 A desmosome (arrow) is present along the convoluted contact surface. × 63,000.

septa are continuous around the cell perimeter, they would add up to an adequate diffusion barrier in this direction, even if their resistivity were only two orders of magnitude higher than that of the intercellular fluid. (For a complete discussion of this point, see pages 578 and 580 of the preceding paper (1).)

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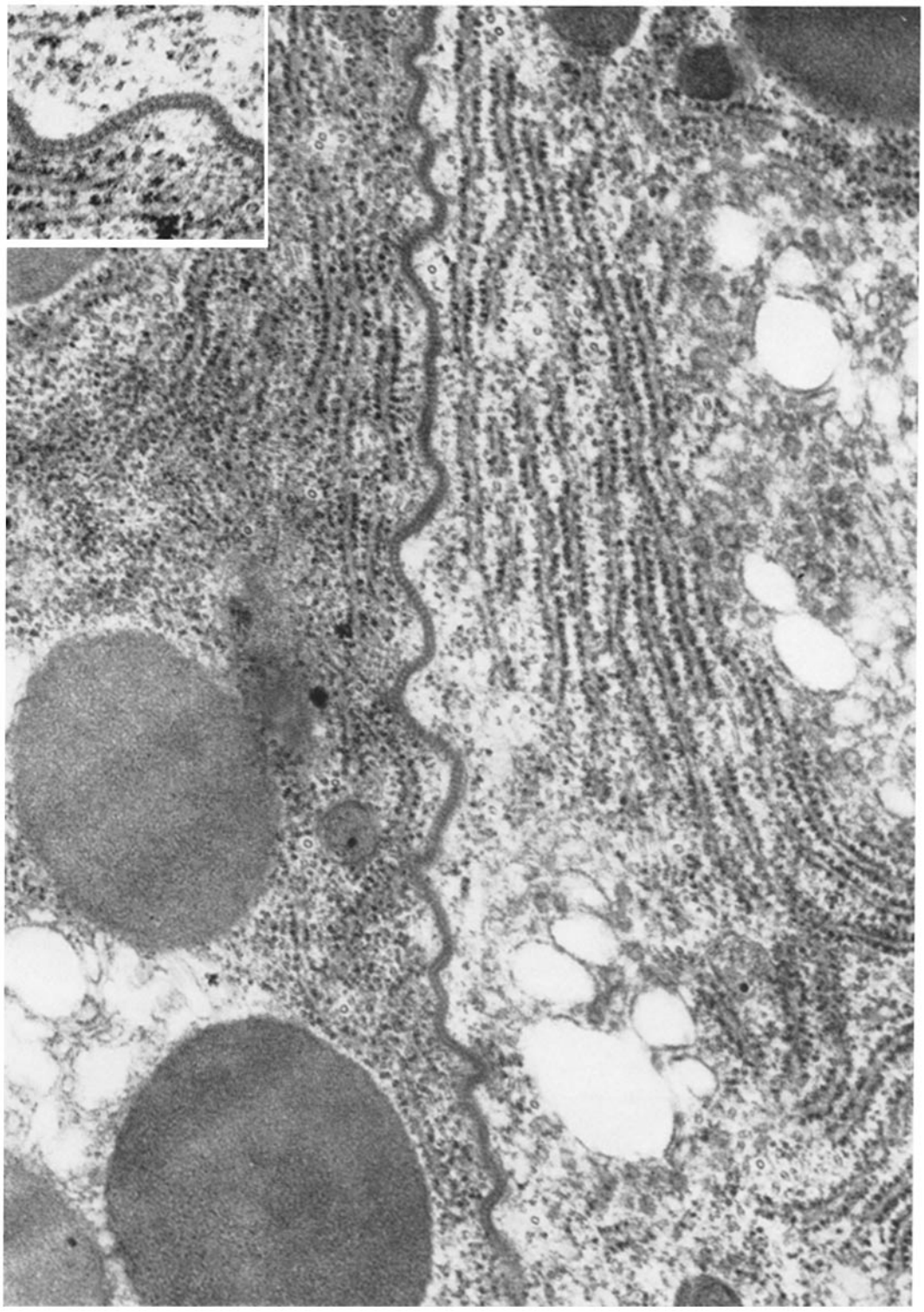
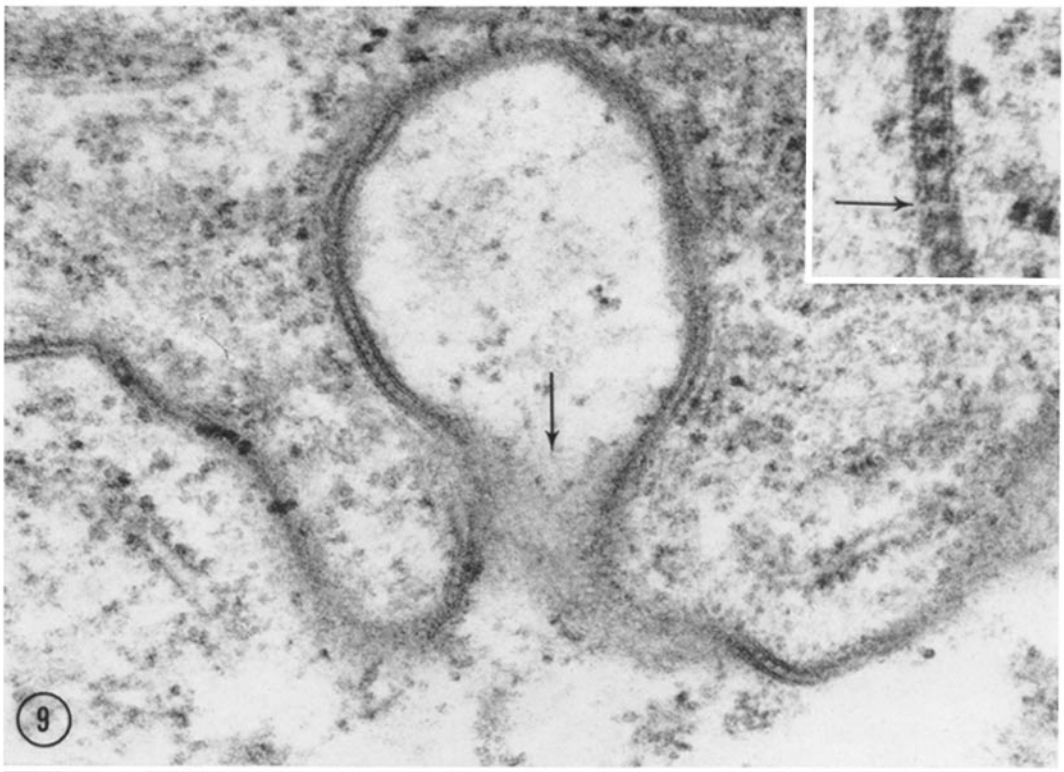


FIGURE 8 This electron micrograph presents the septate junction. Regularly spaced septa, which bridge the intercellular space, are present. × 60,000. The inset is similar. × 78,000.

FIGURE 9 Views of surface membranes at the septate junction. The plane of section appears to be tangential with respect to the contact surface in one area. Polygonal arrays (arrow), which probably represent the septa, are present in this plane. × 90,000.

Unit cell membranes and septa at high magnification are seen in the inset. There appear to be discontinuities in the cell surface membranes (arrow) in one region. × 200,000.

FIGURE 10 Luminal surface. Microvilli and the luminal end of a septate junction with segmental thickenings of the contact margins can be seen (arrows). × 30,000. The surfaces of the microvilli exhibit a unit membrane structure in the inset. × 200,000.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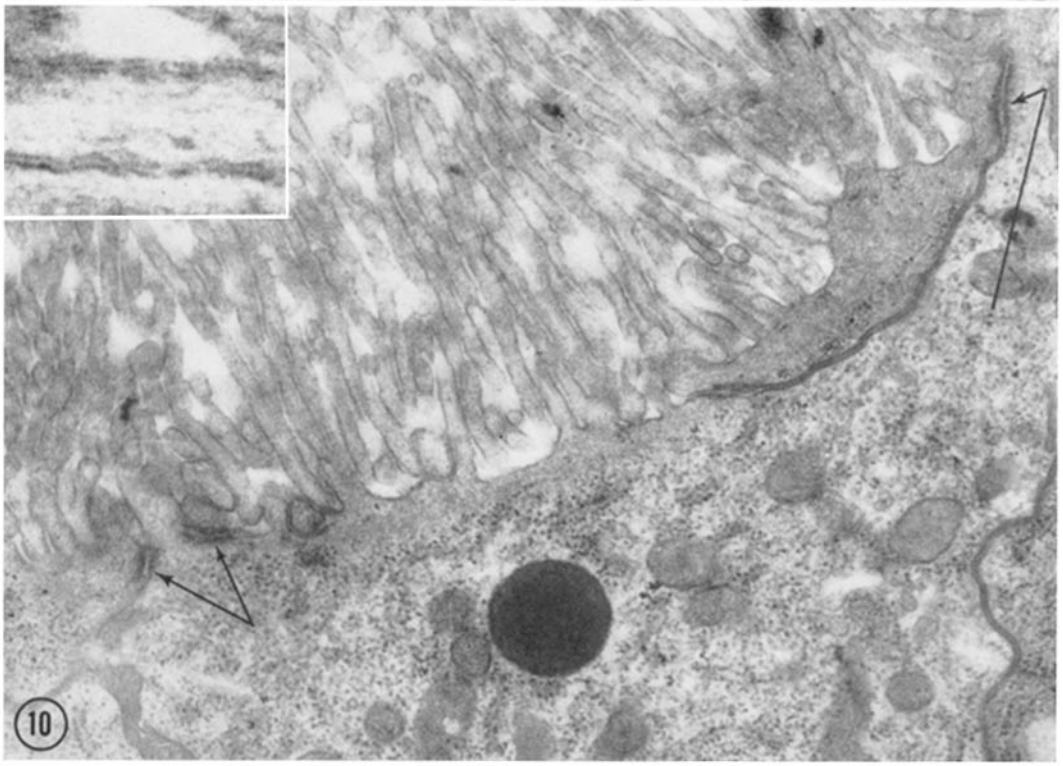
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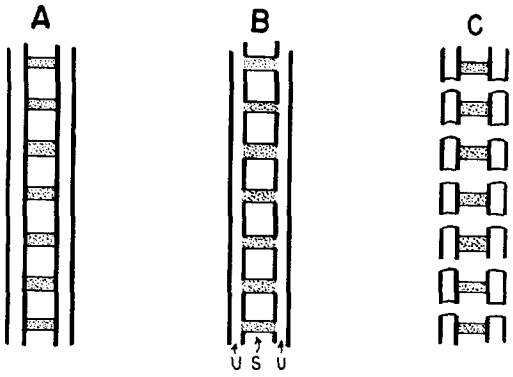


FIGURE 11 Diagram of three possible types of interconnections between septa (*S*) and unit membranes (*U*). In *A*, the septa connect uninterrupted surface membranes. In *B*, the outer layer of the surface membrane is modified and merges with the septum material. In *C*, the surface membranes are discontinuous between septa (see text).

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