

# VARIATIONS IN TIGHT AND GAP JUNCTIONS IN MAMMALIAN TISSUES

DANIEL S. FRIEND and NORTON B. GILULA

From the Department of Pathology, University of California, San Francisco, California 94122, and the Department of Physiology-Anatomy, University of California, Berkeley, California 94720. Dr. Gilula's present address is the Department of Anatomy, Harvard Medical School, Boston, Massachusetts 02115.

## ABSTRACT

The fine structure and distribution of tight (zonula occludens) and gap junctions in epithelia of the rat pancreas, liver, adrenal cortex, epididymis, and duodenum, and in smooth muscle were examined in paraformaldehyde-glutaraldehyde-fixed, tracer-permeated (K-pyroantimonate and lanthanum), and freeze-fractured tissue preparations. While many pentalaminar and septilaminar foci seen in thin-section and tracer preparations can be recognized as corresponding to well-characterized freeze fracture images of tight and gap junction membrane modifications, many others cannot be unequivocally categorized—nor can all freeze-etched aggregates of membrane particles. Generally, epithelia of exocrine glands (pancreas and liver) have moderate-sized tight junctions and large gap junctions, with many of their gap junctions basal to the junctional complex. In contrast, the adrenal cortex, a ductless gland, may not have a tight junction but does possess large gap junctions. Mucosal epithelia (epididymis and intestine) have extensive tight junctions, but their gap junctions are not as well developed as those of glandular tissue. Smooth muscle contains numerous small gap junctions. The incidence, size, and configuration of the junctions we observed correlate well with the known functions of the junctions and of the tissues where they are found.

## INTRODUCTION

In a careful analysis of thin sections from various epithelia, Farquhar and Palade (10) first defined the junctional complex. They demonstrated that this epithelial complex is commonly composed of a zonula occludens (tight junction) which serves as a barrier to the diffusion of substances through the intercellular space, a zonula adherens (intermediate junction), and a macula adherens (desmosome). Subsequent technical innovations, particularly the introduction of a low molecular weight electron-opaque tracer (lanthanum) (8, 34) and the technique of freeze etching (3, 27, 28, 40), permitted a more thorough examination of the occludens portion of the junctional complex. These techniques also helped unmask another junctional

component, the gap junction (34) (nexus [6, 7]), now widely implicated in intercellular communication—that is, in cell-to-cell transfer of ions (ionic coupling) (9, 15, 20, 30, 31, 38) and in cell-to-cell transfer of cellular metabolites (metabolic coupling) (15).

With the use of lanthanum, this junction, heretofore considered to be of the occludens type, was conclusively demonstrated to have a 20–40 Å intercellular gap (34). Revel and Karnovsky further found that in lanthanum preparations, the intercellular gap contained a hexagonal array of subunits with a 90 Å center-to-center spacing, reminiscent of a similar subunit structure earlier observed by Robertson at the site of an electrical synapse

(37). The use of lanthanum and other tracers which permeate the gap junction (uranium salts, anionov, ruthenium) has demonstrated the wide distribution of the gap junction in invertebrate (19, 31, 38) and vertebrate (4, 16, 26, 34, 35) tissues and between cells in tissue culture (20, 33).

Recently the freeze-etch technique has also become a valuable asset to the identification and study of tight and gap junctions (5, 13, 16, 23, 26, 33, 39). The fracture process exposes the internal components of the cell membrane (3, 32), revealing specific membrane modifications related to these junctions, and permits assessment of the precise relationship of one cell junction to another as well as to unmodified regions of the cell membrane. Besides revealing the extent and distribution of cell junctions, the significance of this technique is that in some instances gap junctions cannot be positively identified in any other way.

In this study we examine tight and gap junctions in a variety of mammalian glandular (pancreas, liver, adrenal cortex) and mucosal (epididymis, small intestine) epithelia and in smooth muscle (epididymis, small intestine), with particular emphasis on their size, form, distribution, and interrelationships. We consider variations which could account for quantitative differences in ionic coupling between contiguous cells of various epithelia and raise several questions related to the inadequacy of present terminology and technology in the definition and identification of cell junctions.

## MATERIALS AND METHODS

### *Materials*

The tissues used in these studies were the adrenal cortex, liver, pancreas, duodenum, and epididymis (head and tail) of sexually mature male Sprague-Dawley rats. 15 rats and the following reagents and enzymes were employed. Hyaluronidase (Type I) (Sigma Chemical Co., St. Louis, Mo.), collagenase (Type I) (Worthington Biochemical Corp., Freehold, N. J.); lanthanum nitrate and K-pyroantimonate (Fisher Scientific Co., Pittsburgh, Pa.).

### *Methods*

#### PREPARATION FOR ELECTRON MICROSCOPY

Fixation of tissues was initiated by local injection of 1–2 ml Karnovsky's fixative (21), containing 1% formaldehyde and 3% distilled glutaraldehyde, and approximately 0.45 mM CaCl<sub>2</sub> buffered to pH 7.4 with 0.1 M sodium cacodylate. After 2–3 min of local

perfusion, the tissue was removed, minced, and immersed in fixative for 4–6 hr at room temperature. In some experiments, 1% K-pyroantimonate (22) was added and appeared to improve the staining of cell junctions. The tissues were washed overnight in 0.1 M sodium cacodylate buffer (pH 7.4) with 7% sucrose, followed by fixation for 2 hr at 4°C in acetate-Veronal-buffered 1% OsO<sub>4</sub> (pH 7.4) with 5% sucrose. The tissues were then treated for 1 hr at room temperature with buffered 0.5% uranyl acetate containing 4% sucrose, quickly dehydrated in graded ethanols, and embedded in Epon 812.

For electron microscopy, silver-to-grey sections were cut with diamond knives on a Porter-Blum Sorvall MT-2 microtome (Ivan Sorvall, Inc., Norwalk, Conn.). They were collected on carbon- and Formvar-coated grids, stained with alkaline lead alone or with 5% aqueous uranyl acetate followed by lead, and examined with a Siemens 1A electron microscope at 30 kv. For light microscopy, 1 μ sections were cut and stained with toluidine blue in borax.

#### OTHER PROCEDURES

**LANTHANUM:** Regional blood vessels or parenchymal tissues were injected with 1–2 ml of a solution of 3% lanthanum nitrate in 0.05 M Tris-hydrochloride buffer, pH 7.2, with 4% sucrose added. After vascular injection (1 min), the tissue was removed and placed in fixative as described above. After parenchymal tissue injection (3 min), the specimen was removed, finely minced, immersed in the lanthanum solution for a total of 5 min, and placed in fixative (see above).

**PYROANTIMONATE PRECIPITATION:** 1 ml of the saturated solution of pyroantimonate was injected into the lumen of the epididymis. K-pyroantimonate was dissolved for 30 min at 40°C in the buffer used to make up the fixative, before addition of the aldehydes. In all other instances, parenchymal tissue was injected with 1–2 ml of the formaldehyde-glutaraldehyde fixative containing 5% K-pyroantimonate, pH 7.2, without calcium chloride. Fixation was continued by immersing the minced tissue for 4–6 hr in the pyroantimonate-containing fixative. Isolated cells were exposed to pyroantimonate just by immersion in this mixture. Subsequent processing was the same as that specified above.

**CELL ISOLATION:** Isolated hepatic parenchymal cells were prepared as described by Berry and Friend (2).

**FREEZE FRACTURING:** For freeze fracturing, small pieces of tissue were placed in formaldehyde-glutaraldehyde fixative for 30 min and then immersed in 20% glycerol in 0.1 M cacodylate buffer for 3–4 hr. Pieces of tissue were mounted on cardboard discs, frozen rapidly in liquid Froon 22 (chlorodifluoromethane), and fractured in a Balzers ap-

paratus (Balzers AG, Balzers, Liechtenstein) with a stage temperature of  $-115^{\circ}\text{C}$  (3, 27). Electron micrographs of freeze-fracture replicas are mounted with the shadow from bottom to top, and all shadows are white.

### *Terminology Employed in this Paper*

**JUNCTIONAL COMPLEX:** The zonula occludens (tight junction), zonula adherens (intermediate junction), and macula adherens (desmosome), collectively. This term does not indicate whether or not a gap junction is present.

**FREEZE-FRACTURE FACES:** When the fracture passes through the cell membranes in the region of the junctional complex, two internal complementary fracture faces are produced. Fracture face A, associated with the cytoplasmic portion of the membrane, contains the larger number of intramembranous random particles, the ridges of the zonula occludens, and the closely packed particles of the gap junction. The other fracture face, B, associated with the intercellular portion of the cell membrane, contains fewer particles and includes the furrows and depressions which complement the ridges and particles of face A. Chalcraft and Bullivant have demonstrated that the fracture faces are indeed complementary (5).

**TIGHT JUNCTION (ZONULA OCCLUDENS):** As seen in thin section, the sum of the pentalaminar fusions between contiguous cell membranes. This junction is impermeable to lanthanum. As seen in freeze fractures, the sum of the ridges and furrows on faces A and B, respectively. (Although a solitary continuous ridge may actually constitute an impermeable seal per se and therefore qualify as a tight junction, we do not refer to the single ridges observed among many as tight junctions. We refer to these as fasciae occludentes.)

**GAP JUNCTION (NEXUS):** As seen in thin section, the pentalaminar or septilaminar membrane appositions with a 20–40 Å gap, permeable to lanthanum, and possessing a periodic substructure. The total width of the gap junction (cytoplasm-to-cytoplasm) is greater than 150 Å, but less than 210 Å. As seen in freeze fractures, macular aggregates of polygonally arranged 80–90 Å membrane particles (face A) and complementary depressions (face B).

### OBSERVATIONS

#### *Cell Junctions in Glandular Epithelia*

**EXOCRINE PANCREAS:** The junctions between cells of the pancreatic acini will be consid-

ered first, since both tight and gap junctions are abundant in this tissue. Here gap junctions, especially, are present in various locations, configurations, and sizes. Examination of the pancreatic junctions introduces problems of identification and characterization which will be further explored in other tissues.

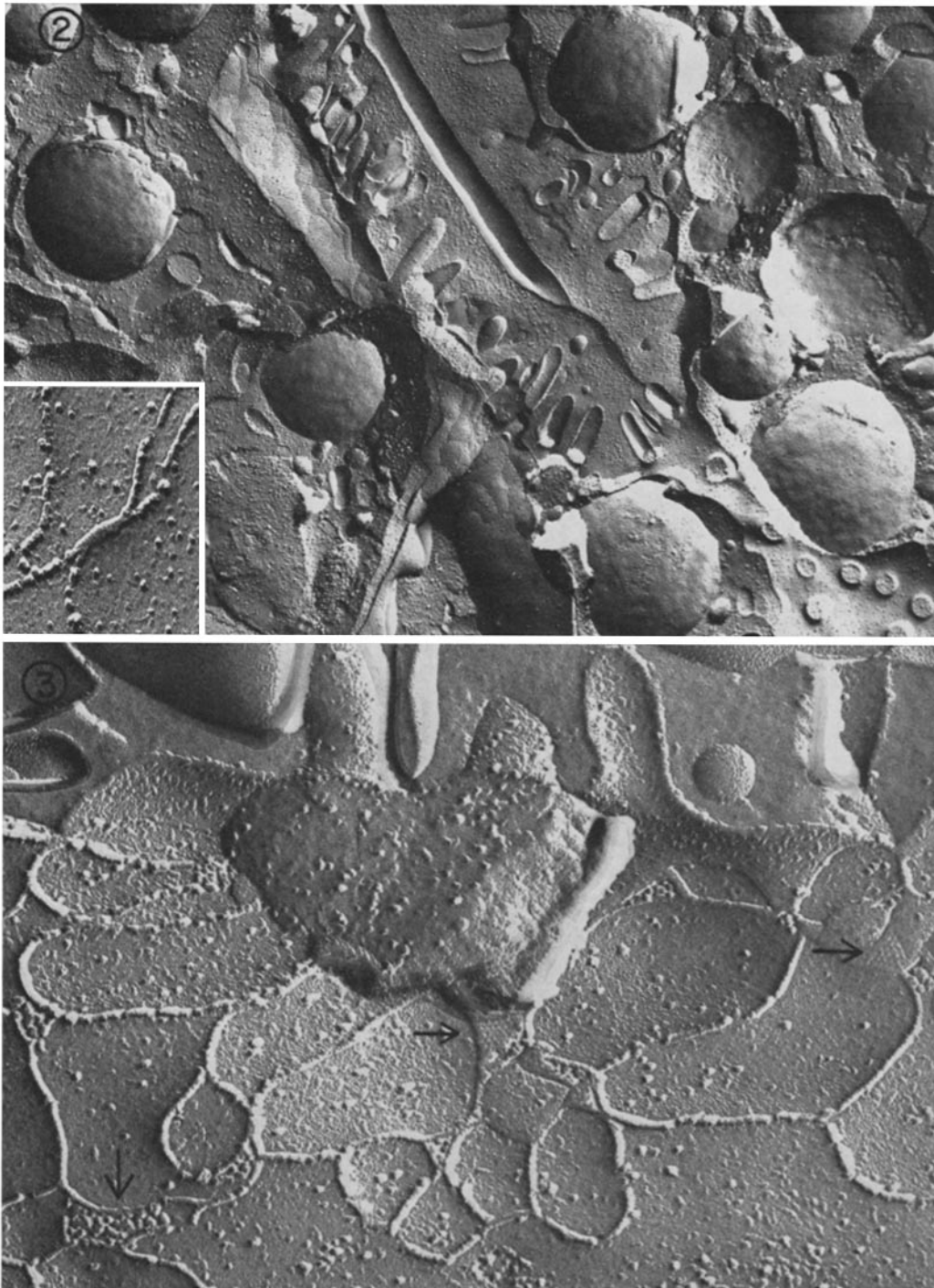
In thin sections of acini of the exocrine pancreas, junctional complexes are found at the luminal surfaces of all contiguous cells. Pentalaminar fusions (Fig. 1, inset *a*) of adjacent membranes are common along the interface between the luminal surface and the first macula adherens (desmosome). Morphologically, all points of membrane fusion appear alike, but lanthanum, introduced into the intercellular space from the basal, vascular pole of the cells, is excluded by some fusions (Fig. 1) but not by others (Fig. 1, inset *b*). The fusions which do not impede the flow of lanthanum are usually farthest from the luminal surface. The first fusion at the microvillar surface stops flow in either direction. The differences in the capacity of pentalaminar fusions to impede lanthanum indicate that some are continuous while others are discontinuous or focal, and that thin sections alone cannot distinguish between the two in all instances (Figs. 1 and 4). (Nor can we be certain that the thin-section image does not represent a punctate gap junction.) Freeze etching confirms the variations in components of the tight junction by showing short, focal ridges and furrows as well as extensive continuous ones (Figs. 2 and 3). Compared with the tight junctions of mucosal epithelia which we will examine shortly, this mixture of continuous and discontinuous ridges and furrows is more evident as well as looser in array. Freeze etching does not clarify the nature (tight *versus* gap) of all the punctate points of apparent fusion seen in thin section, since the minimum size of a puncta ( $\sim 80$  Å) is about the same as that of an ordinary membrane particle. Hence a junctional membrane comprised of a single membrane particle would be indistinguishable from the rest of the membrane.

Another aspect of pancreatic junctions elucidated by freeze etching is the complete enclosure of some gap junctions within the confines of the zonulae occludentes (Fig. 6), as noted in the liver by Kreutziger (23). Tracers permeate and help to reveal some gap junctions near the luminal surface (Fig. 5) and the more extensive ones abluminal to the junctional complex (Fig. 7), wherever the gap junctions are accessible to permeation by low molecular weight substances. Freeze etching readily



FIGURES 1-9 Figs. 1-9 are micrographs of pancreatic acinar cells.

FIGURE 1 The tissue in *inset a* was fixed in buffered paraformaldehyde-glutaraldehyde containing 1% K-pyrosulfonate. The central linear density of the pentalammar fusion here does not stain well, due to its inaccessibility to pyrosulfonate at the point of fusion. The tissues in the principal figure and *inset b* were injected with lanthanum before fixation. Lanthanum penetrated the intercellular space from the vascular pole. Focal fusions (arrows) between contiguous cell membranes of the exocrine pancreas may correspond to one of several membrane modifications observed by freeze etching. Such thin-section pentalammar profiles may appear on fracture face A as continuous, smoothly contoured ridges, discontinuous ridges, beaded ridges, or small focal aggregates of gap-junction-like membrane particles. Consistent with this structural variation in thin-section pentalammar images which look alike, such structures may serve different biological functions. The structure in the main figure halts the lumenward flow of lanthanum at the distal limit of a zonula occludens, whereas one closer to the base of the cell (*inset b*) does not. Thus thin sections and tracer preparations alone do not permit positive classification of all junctional structures examined, and the impediment to the flow of tracers by tight junctions hinders visualization of gap junctions in the apical region of the junctional complex. Z, zymogen granule.  $\times 100,000$ ; *inset a*,  $\times 200,000$ ; *inset b*,  $\times 150,000$ .



**FIGURE 2** A low magnification view of a freeze-fracture replica through several pancreatic acinar cells. This micrograph reveals the distribution of the series of ridges and complementary linear depressions which constitute the tight junction. Subsequent micrographs will depict these at higher magnifications. The *inset* illustrates a feature common to all tight junctions examined—the fusion of two ridges on the A face.  $\times 28,000$ ; *inset*,  $\times 130,000$ .

**FIGURE 3** In this replica, both A and B fracture faces (arrows) of the gap junction are visible within compartments of the tight junction. This pattern of sequestration is also common in the liver. Note the relative amplitude of the enclosures formed by the ridges.  $\times 125,000$ .

confirms the configuration and size of gap junctions in these locations (Figs 6, 8, 9). Only freeze etching, however, reveals the gap junctions enclosed by continuous ridges of the zonulae occludentes. Completely surrounded, they are shielded from tracer permeation and consequently cannot be identified by the use of tracers in thin section. As illustrated in Figs 3 and 6, the gap junction particle aggregates of face A and the depressions of face B are encompassed by the zonula occludens's network of ridges and furrows, respectively.

**LIVER:** The junctional complex between adjacent hepatic parenchymal cells has been described in detail by other investigators (5, 10, 16, 18, 23, 25). In general, its tight and gap junctional components resemble those of the pancreas in all major respects. Again, the zonula occludens is only moderately extensive (Fig 12) and occasionally contains gap junctions, large gap junctions are also present below the junctional complex region of the parenchymal cells (Fig. 11). One novel observation is the fine delineation of the gap junction achieved with pyroantimonate as a tracer (Fig 10).

**ADRENAL CORTEX** Junctions of the adrenal cortex differ from those of other glandular epithelia such as the pancreas and liver. As visualized in thin section, the classic junctional complex is not present in the gland's three major cortical zones. Desmosomes are rudimentary, the extensive zones of adhesion have a distinctive appearance (12, 14), and the pentalaminar fusions characteristic of tight junctions are rare. Tracers readily pass between the cells and encompass areas of apparent fusion, indicating that such images (Fig 13) correspond to fracture-face profiles of discontinuous or beaded ridges or small gap junctions. In addition, tracers reveal that large gap junctions are common (Fig. 14), a fact which freeze etching confirms (Figs. 15 and 16). Actually, freeze etching demonstrates that gap junctions of various sizes and configurations are widely distributed throughout the cortex, but no definitive, smoothly contoured ridges or furrows are visible, implying that the pentalaminar profiles seen in thin sections of the adrenal cortex are probably gap junctions. Whether or not closely packed linear arrays of particles (inset, Fig. 16) represent portions of gap or tight junctions will be explored in the Discussion. In any case, pyroantimonate, lanthanum, and peroxidase all move freely around these structures, and pyroantimonate and lanthanum may move through them.

### *Cell Junctions in Mucosal Epithelium*

**EPIDIDYMIS:** Among the various epithelial cell contacts examined, the zonula occludens of the epididymis is the most highly developed. Epididymal columnar cells are joined at their microvillar surfaces by the characteristic junctional complex comprised of a zonula occludens up to several microns long, a zonula adherens, and multiple desmosomes. Although the zonula occludens occasionally has the pentalaminar image of membrane fusion along its entire length, such profiles are rare. Customarily the zonula occludens contains both regions of membrane fusion and regions where the intercellular space intervenes (Fig. 17, inset *b*), often varying in width from 20 A to more than 100 A. The occludens portion of the junctional complex excludes pyroantimonate from the intercellular space when the tracer is introduced via the lumen (Fig. 17, inset *b*) and excludes pyroantimonate and lanthanum introduced via the bloodstream. Fig. 17, inset *a* is a rare example where some lanthanum is seen near the microvillar surface. Tracers do patchily permeate small segments of the intercellular space in the basal area of the complex, but they are always checked in the apical region.

Below the junctional complex, lateral cell membranes generally maintain an intercellular space of 150 A or more. This vicinity fills completely with lanthanum.

With the freeze-etching technique, extensive membrane fracture faces of the lateral cell membranes are easily obtained. At low magnification, the ramifying ridges and complementary furrows of the zonula occludens throughout the apical region are particularly striking (Figs 17-19). Below the zonula occludens, in the region of the zonulae and maculae adherentes, no detectable modifications of the fracture face are evident. If anything, the membranes here have fewer particles than do other areas of nonjunctional membrane (Fig. 18).

In the epididymis, the ridges of the zonula occludens form a far-reaching horizontal network. Not always continuous individually, they may stretch abuminally for several microns. In the basal part of the zonula occludens, a variety of ridge deployments are observed. One is a ramifying, compartmentalizing, continuous band, another is a continuous but open network, and still another is a completely open array of isolated ridges (Figs. 17-20).

In most cases, the ridges are perpendicular to the axis of the cell. At the juncture of three cells,

however, as also noted by Staehelin et al. in the intestine (39), they are vertically disposed, coincident with the cellular axis. Usually one major vertical ridge is present at the cell juncture, giving rise to several short horizontal ones which merge with the major portion of the zonula occludens network (Fig. 20). (Compare this image with Fig. 12, which is a similar view of the hepatic tight junction.) All the ridges are about 80 Å wide, most are smoothly contoured, and some apparently consist of fused particles 80–85 Å in diameter (Fig. 18, inset *a*). Fused-particulate ridges frequently have missing portions, presumably representing the 80–85-Å particles associated with the complementary furrows of fracture face B (Fig. 19).

Since mistakenly reporting that macular gap junctions were not present in the epididymis (13), we have discovered some basal to the tight junctions in the rat (Fig. 18, inset *b*). Revel (personal communication and 36) has also found numerous ones intermingled with tight junctions, as well as basally, in the mouse.

**SMALL INTESTINE (DUODENUM):** In the mucosal epithelium of the duodenum, the freeze-fracture landscape of the tight junction is similar to that of the epididymis. But here the zonula occludens appears more compact (Fig. 21). The network of ridges and furrows is continuous at the microvillar surface, while some discontinuities are found in the basilar region. In the abluminal pole of the complex, we often observe isolated ridges. We have not found gap junctions concealed between the ridges of the small intestine's zonula

occludens, although small aggregates of particles, like those of the epididymis, are prevalent

### *Cell Junctions in Smooth Muscle*

**SMALL INTESTINE AND EPIDIDYMIS:** The cells of the smooth muscle of the small intestine and epididymis are joined by gap junctions (nexus) with the same permeability to intercellular tracers as those in epithelia. In fracture faces, cell membranes contain plaques of aggregated particles varying from 0.1  $\mu$  to 1  $\mu$  in diameter constituting the membrane differentiation associated with the gap junction (Fig. 22). While most of the particle arrays are macular, some are variegated in shape, including single-file distributions indistinguishable from the beaded ridges (inset, Fig. 22) associated with the zonulae occludentes in epithelia. Particularly in the small intestine, one smooth muscle cell makes numerous gap junctional contacts with either one or several neighboring muscle cells.

## DISCUSSION

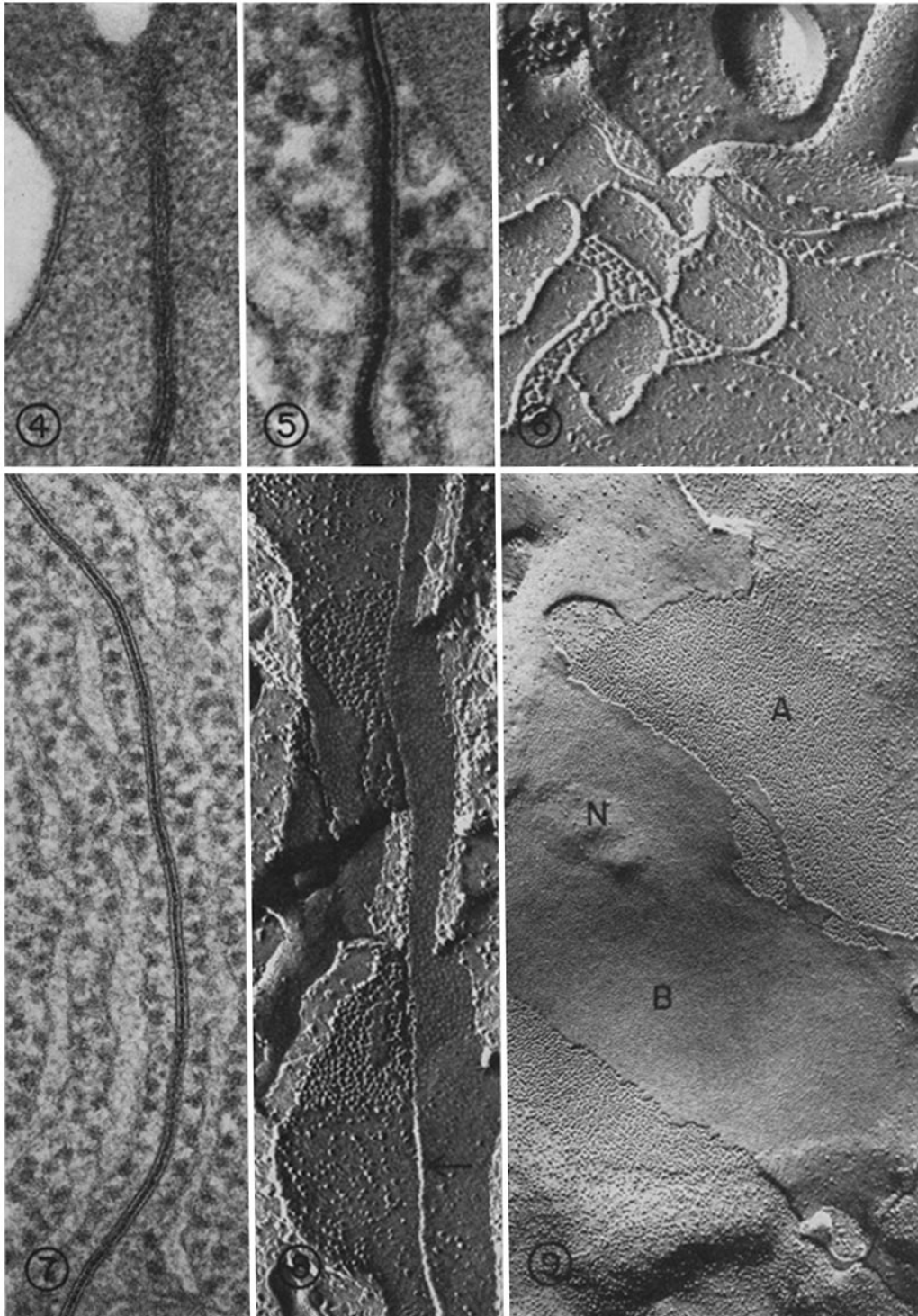
### *Components of the Junctional Complex*

The junctional complex is the most elaborate zone of contact between mammalian epithelial cells. It has long been established that the usual initial element of the complex, the zonula occludens, forms a barrier to the passage of substances from the lumen into the intercellular space lining cavitory organs and between cells which form the acini of exocrine glands (10). The second

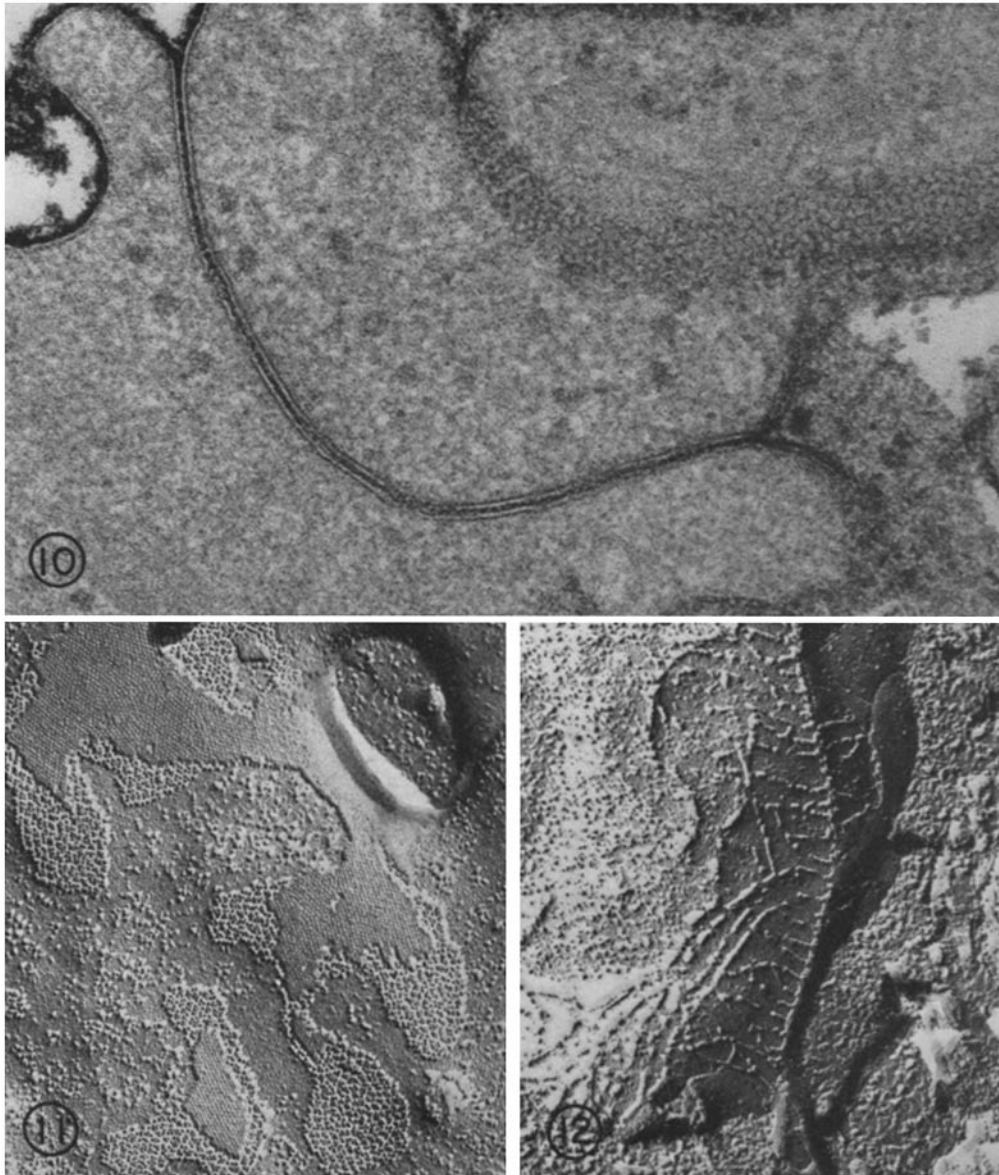
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FIGURES 4–6 Paraformaldehyde-glutaraldehyde (Fig. 4), lanthanum (Fig. 5), and freeze-fracture (Fig. 6) preparations through portions of the junctional complex. In the routine preparation, the nature of the apparent points of fusion is difficult to assess. In regions where ridges of the tight junction do not exclude the tracer, permeation with lanthanum reveals the periodicity and spacing suggestive of a gap junction. Freeze-fracturing definitively illuminates small gap junctions confined by ridges of the zonula occludens, which would not be detected in routine and lanthanum preparations. Fig. 4,  $\times 150,000$ ; Fig. 5,  $\times 190,000$ ; Fig. 6,  $\times 140,000$ .

FIGURES 7–9 Pyroantimonate (Fig. 7) and freeze-fracture (Figs. 8 and 9) preparations of extensive gap junctions below the junctional complex. Careful examination of Fig. 7 reveals the regular periodicity within this extensive gap junction permeated with pyroantimonate. Fig. 8 is a cross-fracture showing complementary A and B faces of a gap junction comparable to that in Fig. 7. In this instance, the fracture process exposed the B face of one cell membrane and crosses the intercellular space (gap) (arrow), where it then exposes the A face of the adjacent cell membrane. Fig. 9: The magnitude of some pancreatic gap junctions is more apparent here. Gap junctions of the liver and adrenal cortex are comparable in size. A region of nonjunctional membrane (*N*) is often found within the gap junctional plaque in both liver and pancreas. *A*, fracture face A; *B*, fracture face B. Fig. 7,  $\times 110,000$ ; Fig. 8,  $\times 105,000$ ; Fig. 9,  $\times 60,000$ .





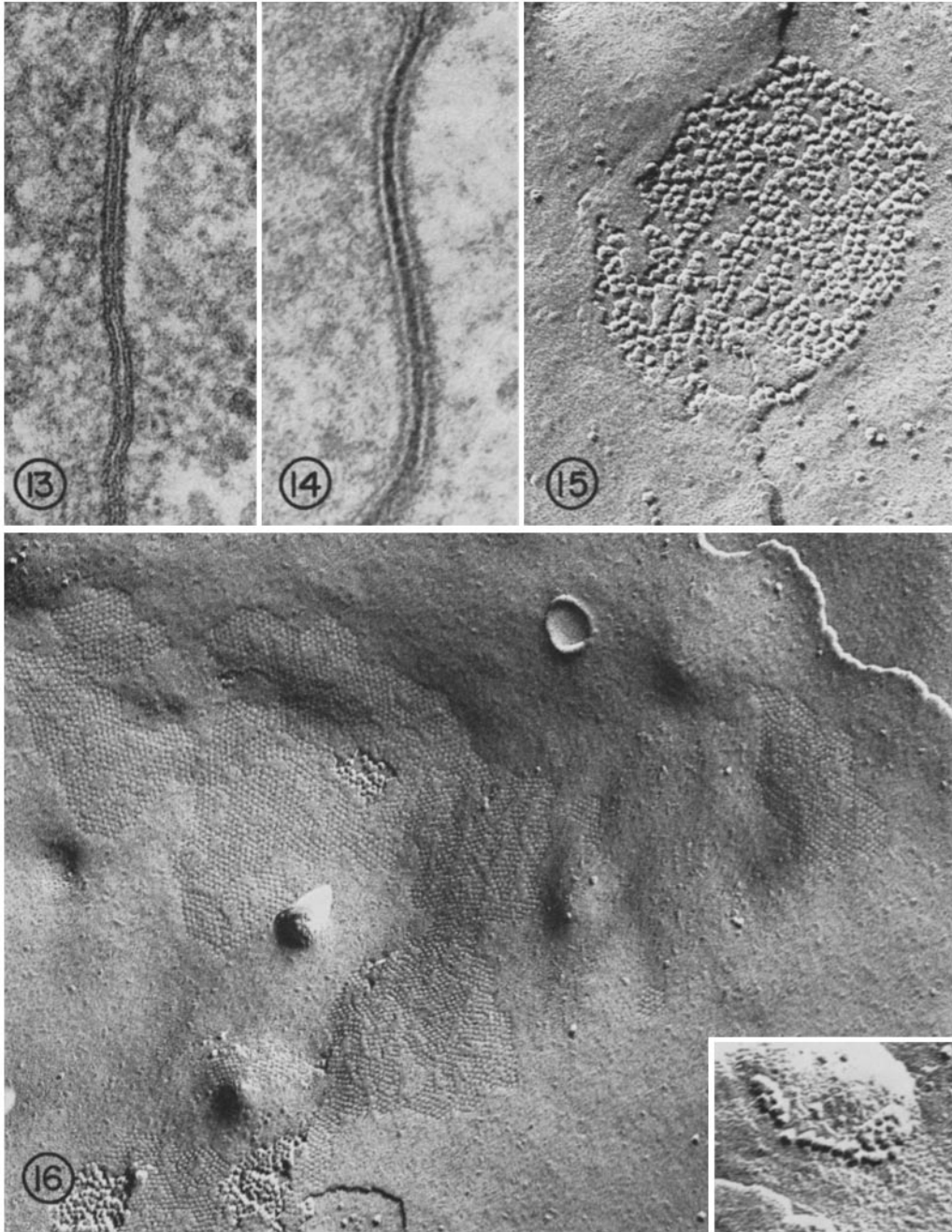


FIGURES 10-12 Rat liver.

FIGURE 10 Pyroantimonate preparation of a gap junction between two partially isolated hepatic parenchymal cells. Both the *en face* view of the intercellular particles (upper right) and a transverse view of the regular periodicity of a gap junction are visible and presumably represent two aspects of the same junction. The central density of the intercellular particles revealed by pyroantimonate is similar to that shown with lanthanum and negative staining.  $\times 160,000$ .

FIGURE 11 The magnitude of gap junctions in rat liver is confirmed by this freeze-fracture replica. The polygonal packing of the A and B face components is particularly striking.  $\times 70,000$ .

FIGURE 12 An A fracture face of part of the hepatic tight junction illustrates a recurrent pattern in the occluding zonules of all tissues examined. Numerous ridges extend perpendicularly from a thick continuous ridge commonly found at the juncture of two or more cells.  $\times 90,000$ .



FIGURES 13-16 Figs. 13-16 are routine (Fig. 13), lanthanum (Fig. 14), and freeze-etched (Figs. 15 and 16) preparations of adrenal cortex. Fig. 15 shows an A face to advantage; Fig. 16, a B face. Gap junctions are as extensive here as in those glandular epithelia which possess full junctional complexes such as the pancreas and liver. But in the adrenal gland, no readily identifiable elements of the tight junction are present. Isolated rows of particles on the A face (*inset*, Fig. 16) and depressions on the B face are commonly found adjacent to the usual macular aggregates of the gap junction. Similar rows of particles were observed in all other tissues, including those which have extensive tight junctions (epididymis) and those without (smooth muscle). Compare this *inset* with those in Figs. 18 and 22. Fig. 13,  $\times 130,000$ ; Fig. 14,  $\times 220,000$ ; Fig. 15,  $\times 150,000$ ; Fig. 16,  $\times 100,000$ ; *inset*,  $\times 150,000$ .

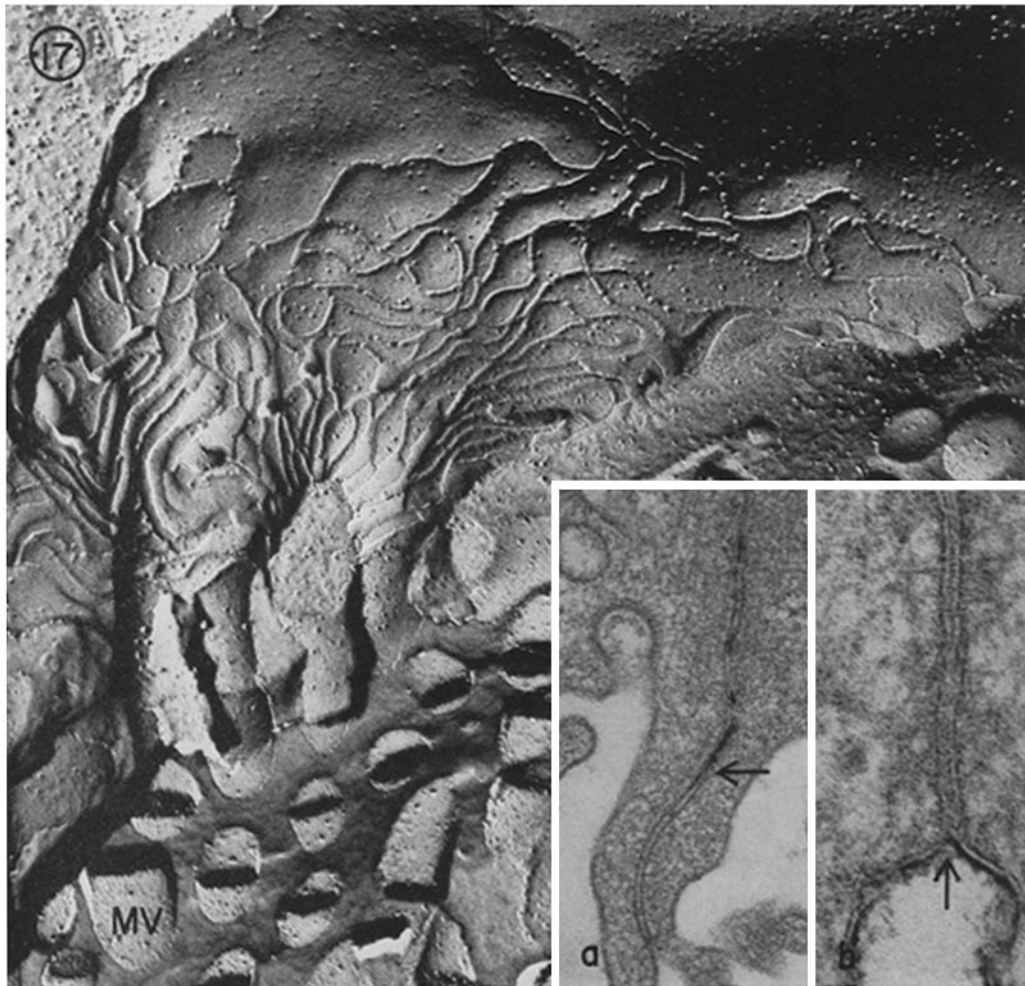


FIGURE 17 The lower half of this freeze-fracture image through the ridges and furrows of the zonula occludens corresponds to the area shown in thin section in *inset a*. The *inset* demonstrates that small amounts of tracer can occasionally percolate from the basal pole of the cell up toward the microvillar surface (arrow), although tracer flow is usually impeded several microns from this surface. In *inset b*, pyroantimonate is totally excluded from the intercellular space (arrow) after luminal introduction of the tracer. *MV*, microvilli.  $\times 80,000$ .

element, the zonula adherens, generally serves as the insertion site for microfilaments of the terminal web. And the final element, the macula adherens (desmosome), provides structural resistance to lateral shearing forces. The epithelial-type junctional complex is not found in muscle (smooth, skeletal, and cardiac), neural tissue, or most endocrine glands (the thyroid gland is an exception). The junctional complex as a whole, as well as the

zonula occludens, is confined to epithelia which permit no interchange of large molecules between the luminal contents and the intercellular space. Many epithelia have both tight and gap junctions, possibly serving as seals and means of intercellular communication and reflecting the diverse requirements of the tissue in its normal functioning. Other epithelia apparently need primarily one or the other type of junction. Careful identification of

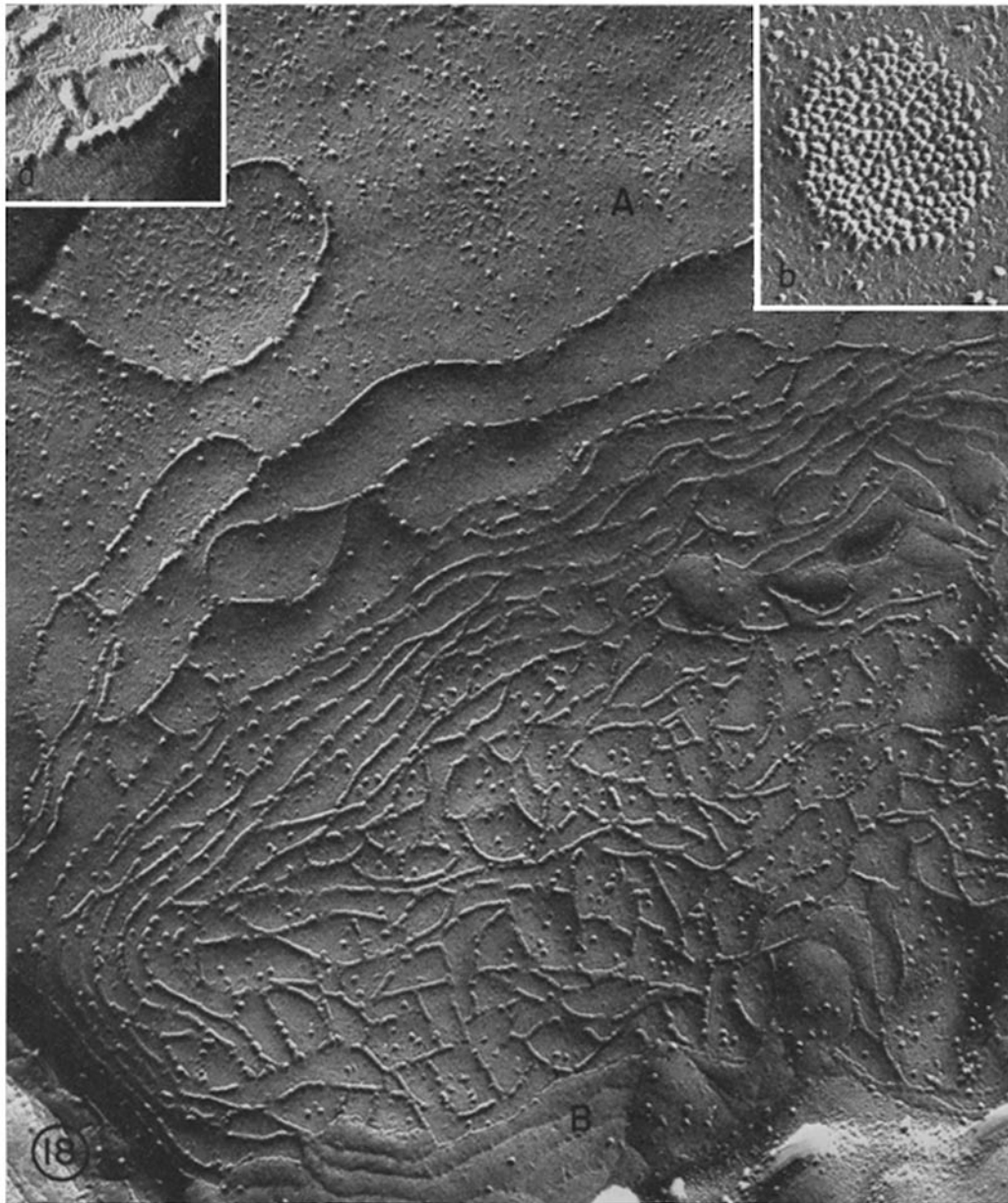


FIGURE 18 This fracture face is characteristic of the extensive, ramifying, compartmentalized tight junction of the epididymis. The basal, larger compartments (upper portion) are like those found in the pancreas and liver, while the apical, smaller ones (lower portion) resemble those of the intestine. Single, doublet, and triplet arrays of membrane particles are more common in the smaller compartments. All types of ridges—smoothly contoured continuous, discontinuous, fascial, and beaded—are apparent in this field, and the width of ridges and membrane particles is similar. *Inset a* depicts a beaded ridge which forms part of the epididymal zonula occludens. As illustrated in Figs. 16 and 22, such beaded ridges are indistinguishable from linear particle arrays associated with gap junctions. Their classification and function are undetermined. *Inset b* is a gap junction found near the base of an epididymal epithelial cell.  $\times 90,000$ ; *inset a*,  $\times 175,000$ ; *inset b*,  $\times 105,000$ .

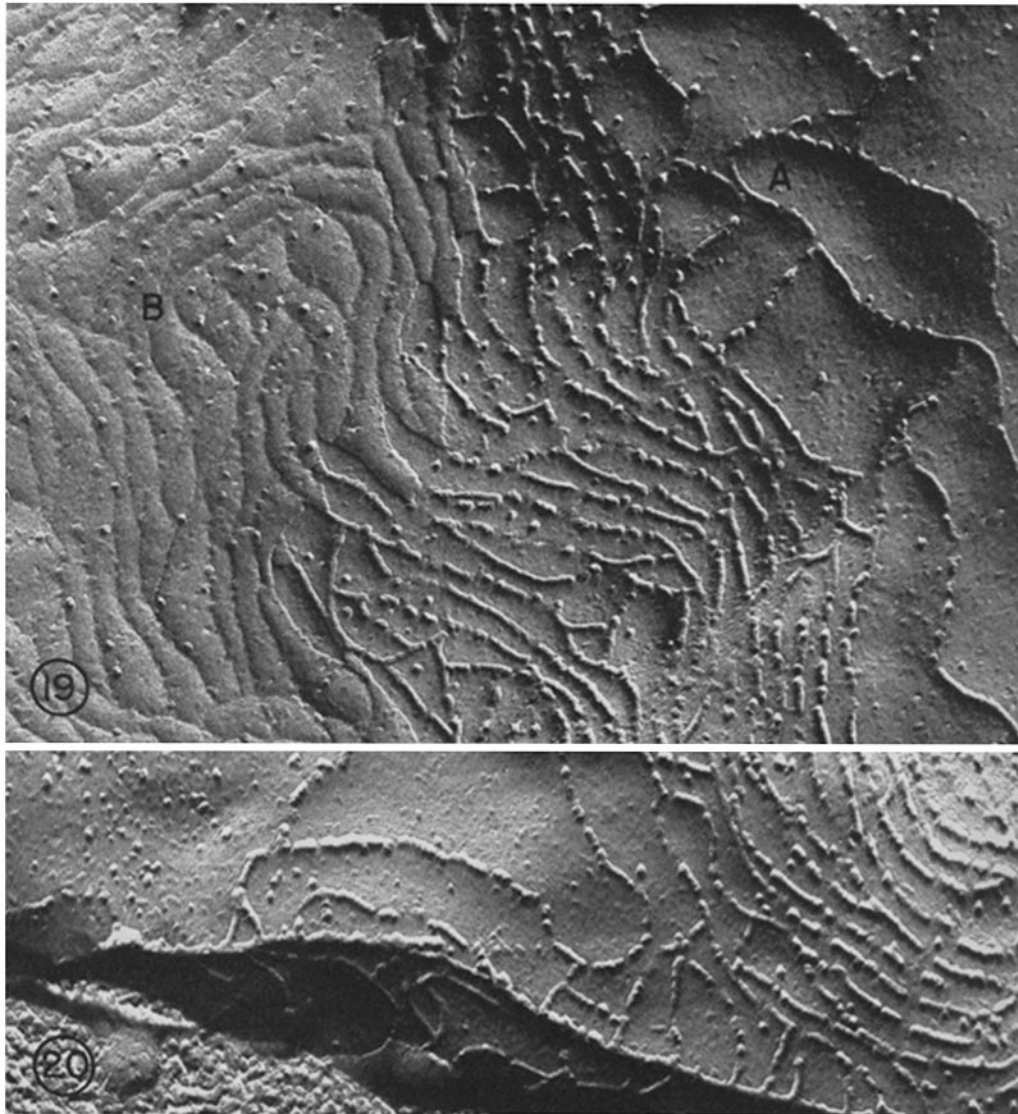


FIGURE 19 Precise complementarity between ridges (face A) and furrows (face B) of the tight junction is customary in the epididymis. Some particles are associated with furrows of the B face; presumably these have been dislodged from the ridges, perhaps accounting for some of their apparent discontinuities. Other particles on both the A and B face (Fig. 18) are not directly associated with either the ridges or the furrows. Their relationship to junctional structure is not known.  $\times 115,000$ .

FIGURE 20 Like the horizontal continuous ridge of the tight junctional element which seals the lumen, the lateral vertical ridge where several cells abut is also continuous. Its multiple branches extend horizontally in opposite directions. Compare this figure with Fig. 19, a similar image of liver tissue.  $\times 115,000$ .

the junctions present enhances understanding of the tissue's function.

The observations in this study indicate that (a) the tight junction (zonula occludens) varies considerably in size and configuration, (b) the gap

junction is both a frequent component of the junctional complex and an independent structure distal to the complex, (c) the variations present in the constituents of cell junctions correlate well with the specialized functions of the cells with which



FIGURE 21 Duodenal mucosa. The tight junction of small intestinal epithelium is similar to that of the epididymis in all major respects, although it is less extensive. Some isolated furrows (fasciae occludentes), like those frequently found in the epididymis, are visible some distance from the microvillar surface.  $\times 52,000$ .

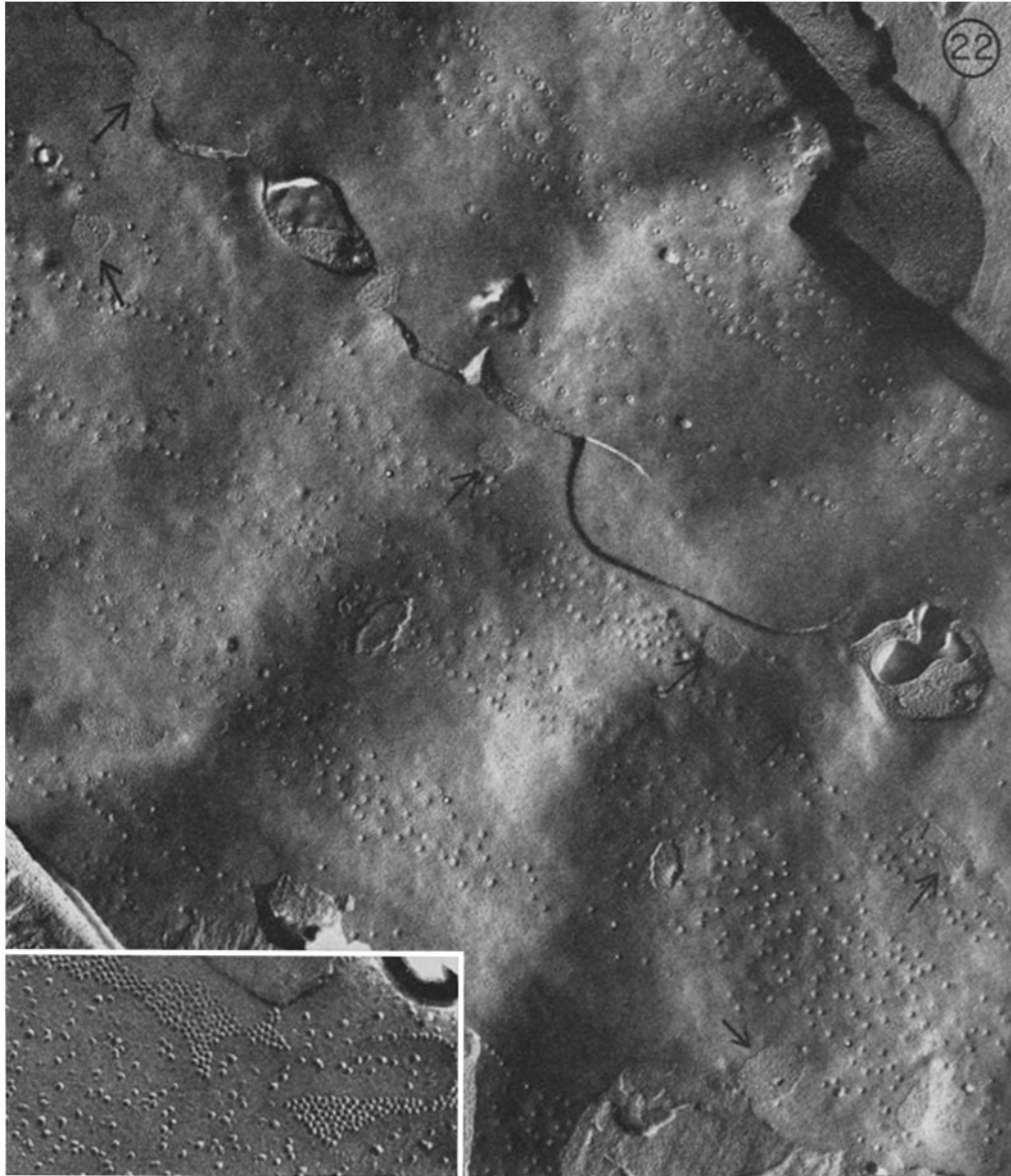
the junctional elements are associated, and (d) existing criteria for classifying junctional elements are inadequate for all the cell contacts seen in thin-sectioned and freeze-etched preparations.

#### *Variations in Distribution and Configuration of Tight Junctions*

Tracers introduced via the bloodstream often permeate the intercellular space of the liver and exocrine pancreas from the basal pole all the way

to the luminal surface before they are completely halted. In the epididymis, however, tracers are usually stopped several microns from the luminal surface, at the far end of the zonula occludens. Freeze fracturing clarifies these observations, confirming the general continuity of the barrier at the microvillar surface of all tissues examined, while revealing variations in tight junction structure at the opposite end.

Short, isolated ridges (fasciae) often accompany the major, continuous components of the tight



**FIGURE 22** Smooth muscle, duodenum. Many gap junctions (arrows) are present in configurations varying from regularly concentric maculae to geographic clusters and single strands (*inset*). The image in the *inset* demonstrates the difficulty in interpreting the true "junctional" nature of particle aggregates. Tight junctions have not been seen in this tissue. The replica also reveals the linear deployment of the annuli of numerous endocytic vesicles.  $\times 20,000$ ; *inset*,  $\times 100,000$ .

junction. Commonly, the isolated segments are basal to the main occluding structure. Their close association suggests a developmental relationship—one, perhaps the progeny of the other, caught in transition during membrane differentiation,

whether fusing or dissolving, we do not know. In the intestine and epididymis, the ridges basal to the continuous luminal one are composed of short, straight segments which join at acute angles, forming an extensive, closed, ramifying network. In

the liver and pancreas, the ridges consist of longer, curved segments which join at obtuse angles to form a more open network. The lengthy ridges in the liver and pancreas, more discontinuous than their counterparts in the intestine and epididymis, permit the tracing of potential passageways from the base to the apex of the zonula occludens. This is not the case in the epididymis and intestine, where imperforate ridge configurations stretch up to several microns from the apical surface. Variations in the tightness of the zonula occludens, therefore, have a structural basis which is revealed by freeze etching.

### *Correlation in Tight Junction Morphology and Tissue Function*

The epididymis modulates the composition of its luminal content by selective secretion and absorption along its span (24)—a process similar to the varying regional transport system within renal tubules. Fluctuations in fluid volume, electrolytes, and amphiphilic proteins in the head, body, and tail of the epididymis are presumably critical to the normal maturation of sperm. Whatever the mechanisms for modulating the composition of epididymal fluid, seals preventing the exchange of large molecules between the lumen and the extracellular space, and zonulae, perhaps functioning directly in transepithelial ion permeation as well (1), are important adjuncts in the over-all regulatory function of the tissue. In addition, shielding sperm from the host's immune system and preventing the escape of sperm or its degradation products also obviate the production of auto-antibodies to sperm, a situation which could cause sterility. The extensive tight junction thus protects against sterility in at least two ways: by helping to maintain the epididymal luminal environment and by providing a barrier between sperm and the host's immunologically competent cells. The significance of tight junctions in other epithelia may be considered in a similar light, functioning in the separation of two disparate environments (10, 11, 25). In the epididymis, this function is actually a matter of species survival, and the tight junction is perhaps the most elaborate tight junction yet found.

What of epithelia without tight junctions? The zona fasciculata of the adrenal cortex seems to exemplify an epithelial tissue which requires the free flow of large molecules between cells and periendothelial space, presumably for the sake of

an unhindered flux of steroid hormones from parenchymal cells to the bloodstream. This tissue appears to lack occluding zonules—in fact, a distinctive cell contact is present, perhaps to assist in keeping the intercellular spaces patent (12, 14). A very high degree of synchrony is desirable in this epithelium, and gap junctions which are extensive probably provide for the ionic coupling observed (Jensen and Friend, unpublished observations).

### *Variations in Incidence and Location of Gap Junctions*

Whether or not tight junctions or the complete junctional complex are present, gap junctions have the same variations in location and configuration in all tissues where they appear. The macular gap junction is a prominent element in the junctional complexes of the pancreas and liver. In these glandular epithelia, they are seen bound by ridges of the zonula occludens, adjacent to open ridges at the base of the zonula, and as independent elements toward the basal surfaces of cells. In tissues where they are part of the junctional complex, gap junctions are generally more extensive toward the basal lamina. In tissues which lack junctional complexes such as the adrenal cortex, independent gap junctions are found in the same variety of locations with respect to the polarity of the cell and in the same variety of sizes and configurations. Obviously, tight junctions are not a requisite for the functioning of gap junctions or that of the tissue in general. The intestine (duodenum) and epididymis, both of which have larger tight junctions than are found in glandular tissue, have fewer macular gap junctions in the apical regions of the cells. Diminutive gap junctional plaques have been recently identified in fracture faces of the basal cellular region of the small intestine (D. Goodenough, personal communication).

### *Problems in Identifying and Classifying Junctional Elements*

While the continuous ridge-type tight junction and the large macular-type gap junction are generally identified with ease, certain configurations are indistinguishable by currently available techniques. For as well as observing components of the tight junction in the continuous ridge conformation, we also see them as interrupted belts, beaded ridges, or mere puncta (short or single points of



apparent fusion) which lanthanum can encircle, thereby affording the impression that they are gap junctions in thin sections. We do not know if beaded ridges, particularly, should be considered as portions of tight junctions or as linear gap junctions. For gap junctions (in our opinion) may also exist in a variety of arrays besides the familiar hexagonal packing of uniform particles in a macular configuration. Two or three strands of particles may extend from the macular array, eventually tapering to an isolated, beaded ridge, as is the case in the adrenal cortex and in smooth muscle. And even more difficult to interpret are the triplets, doublets, and single isolated particles with complementary depressions. Do any or all of these particulate configurations function as gap junctions? At present, morphological criteria and experimental approaches do not resolve the problem of distinguishing whether or not the varieties of "puncta" function as gap junctions, focal tight junctions, or neither. Nor is it known whether large zonulae occludentes can act as electrotonic junctions.

Resolution of these problems awaits the discovery of a tissue truly devoid of macular gap junctions. Thus far, none has been found.

#### THE NEED FOR A MORE EXPLICIT DEFINITION OF A CELL JUNCTION

The term *cell junction* generally refers to a variety of contacts between two similar cells or between a cell and a closely apposed extracellular structure, wherever modifications of the cell membrane or intercellular space are sufficient to impart to it a distinctive appearance in thin section. With the application of this concept, numerous "junctions" are commonly recognized—tight junctions, gap junctions, intermediate junctions, desmosomes, septate junctions, and others. With the advent of new techniques, we are finding major differences among these contact areas—differences which perhaps should be exploited to narrow our definitions.

Several means for classifying cell contacts exist. One is to separate those which have recognizable membrane modifications in freeze-fracture replicas from those which do not. Thus in the case of the junctional complex, gap and tight junctions could be separated from intermediate junctions and desmosomes. Further discrimination can be made on the basis of the susceptibility of a cell contact to divalent cation-chelators and proteolytic enzymes, to which tight and gap junctions are resist-

ant while zonulae and maculae adherentes are not—they usually separate upon exposure to these agents (2, 9, 17, 29). In addition, proteases digest the plaques of desmosomes but do not affect the associated modifications of many other junctions. Discrimination may also be made on the basis of permeability, such as the exclusion of lanthanum by tight junctions but not by others. And more important than the differences in fine structure, freeze fracturing, dissociation, digestion, and permeability are the differences in the unique functions of the specific junctions, such as gap junction mediation of ionic and metabolic coupling. Ultimately, any classification of cell junctions devised will have to take all these factors into account. In anticipation of that development, we suggest that reports of new or incompletely described contacts include descriptions of all the foregoing parameters whenever possible. Naming the type of junction by fine structural impression alone is frequently insufficient.

Presently, the known cell contacts would seem to fall into two general categories which could suitably encompass new ones: those wherein apposing membranes seem to share a structural component as revealed by freeze fracturing (gap, tight, and septate) and those with no such mutual bond. Theoretically, those sharing a membrane component are most likely to participate in intercellular interactions such as ionic and metabolic coupling and absolute adhesion, while those without a mutual component probably fulfill another type of function. On this basis, we have elected to use the term *cell junction* for those which apparently share a structural component, and the term *cell contact* for all others. For us, the term *cell junction* then has a definite operational connotation in addition to its present general structural connotation.

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