

# A DISTINCTIVE CELL CONTACT IN THE RAT ADRENAL CORTEX

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## ABSTRACT

Extensive cell contacts which resemble septate junctions occur between cells in the three major zones of the rat adrenal cortex. Characteristically, they extend between small intercellular canaliculi and the periendothelial space, frequently interrupted by gap junctions and rarely by desmosomes. Zonulae occludentes have not been identified in the adrenal cortex. Along this distinctive cell contact, the cell membranes of apposing cells are separated by 210–300 Å bisected by irregularly spaced 100–150-Å extracellular particles which are often circular in profile. In lanthanum preparations, these particles appear to form a continuous chain throughout the intercellular space and are visualized as an alveolate structure in sections parallel to the plane of the cell membrane. The cell membrane in the area of septate-like contact does not differ from nonjunctional areas of the cell membrane in freeze-fracture replicas. The cell contact retains its integrity after cell dispersion and after the separation of cell membranes from disrupted cells. The intercellular particles also persist after brief extraction in lipid solvents. Besides adherence, possible functions of this adrenal contact include maintenance of the width of the extracellular space, the provision of channels between intercellular canaliculi and the bloodstream, and utilization as cation depots. Similar structures are also present between adrenal cortical cells of several other species and between interstitial cells of the testis. This type of cell contact may, in fact, be a typical feature of steroid-hormone-secreting tissues in vertebrates.

## INTRODUCTION

The cells of the three distinctive zones of the rat adrenal cortex—the zona glomerulosa, zona fasciculata, and zona reticularis—all secrete steroid hormones and all possess the morphological features which generally distinguish steroid-hormone-secreting cells. That is, they are rich in mitochondria which usually have tubular or vesicular cristae and they are rich in smooth-surfaced endoplasmic reticulum (1, 2). A third feature, which has not received special attention in the past, is the form and distribution of their cell junctions, characteristics which may prove to be

common to many steroid-hormone-secreting cells in vertebrates.

In the order of increasing surface area occupied by contacts between adjacent cells, there are focal pentalaminar fusions, which are, at most, maculae rather than zonulae occludentes (3) in the rat; rudimentary desmosomes; extensive gap junctions (4, 5), and even more extensive septate-like zones of adhesion (3, 6). The intent of this paper is to describe in detail the distribution and fine structure of this zone of adhesion in the rat adrenal cortex, including observations on its appearance

after freeze-fracture; its permeability and staining with lanthanum, K-pyroantimonate, and horseradish peroxidase; its persistence after enzymatic cell dissociation, osmotic cell disruption, extraction with lipid solvents, and in hypophysectomized and in adrenocorticotroph-(ACTH)-stimulated rats. Several of its characteristics will be compared with those of gap and septate (7, 8) junctions. Finally, we will demonstrate the existence of this type of cell contact in the adrenal glands of other species and in another steroid-hormone-secreting tissue.

## MATERIALS AND METHODS

### Materials

The tissues used in the major portion of this investigation were the adrenal cortices of seven male and three female sexually mature Sprague-Dawley rats, two mature male guinea pigs, one sexually mature male miniature pig (The Hormel Institute, University of Minnesota, Minneapolis, Minn.), and two adult men. Adrenal glands were also obtained from two ACTH-stimulated and three hypophysectomized mature male Sprague-Dawley rats (see ref. 23 for details). The ovaries and testes of several of the rats and the testes of the minipig were also removed for electron microscope studies of corpora lutea and interstitial (Leydig) cells.

We employed the following reagents, enzymes, and substrates: horseradish peroxidase (type II), 3,3'-diaminobenzidine tetrahydrochloride, and hyaluronidase (type I) (Sigma Chemical Co., St. Louis, Mo.); protease (bovine pancreatic) and collagenase (type I) (Worthington Biochemical Corp., Freehold, N. J.); lanthanum nitrate and K-pyroantimonate (Fisher Scientific Co., Fair Lawn, N. J.).

### Methods

#### PREPARATION FOR ELECTRON MICROSCOPY

Fixation of tissues was initiated by local intravascular injection of 1-2 ml of Karnovsky's fixative (9), containing 1% formaldehyde and 3% distilled glutaraldehyde, and approximately 0.45 mM  $\text{CaCl}_2$  buffered to pH 7.4 with 0.1 M sodium cacodylate. After 2-3 min of perfusion, the tissue was removed, minced, and immersed in fixative for 4-6 hr at room temperature. In some experiments, 1% K-pyroantimonate (10) was added and appeared to improve the staining of cell junctions. Increased staining of cell membranes occurred whether or not  $\text{CaCl}_2$  was present in the fixative. 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%  $\text{OsO}_4$  (pH 7.4) with 5% sucrose. The tissues were then treated at room temperature for 1 hr 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. They were collected on carbon- and Formvar-coated grids, stained with alkaline lead (11) alone, or with 5% aqueous uranyl acetate followed by lead, and examined with a Siemens Ia electron microscope at 80 kv. For light microscopy, 1- $\mu$  sections were cut and stained with toluidine blue in borax.

### OTHER PROCEDURES

**LANTHANUM:** Tissue was infused with 1-2 ml of a solution of 3% lanthanum nitrate (5) in 0.05 M Tris-hydrochloride buffer, pH 7.2, after the addition of the lanthanum, with 4% sucrose added. After 1-3 min, the tissue was removed, finely minced, immersed in the lanthanum solution for a total of 5 min, and placed in fixative as described above.

**PYROANTIMONATE PRECIPITATION:** Regional adrenal blood vessels were injected with 1-2 ml of the formaldehyde-glutaraldehyde fixative, containing 5% K-pyroantimonate, pH 7.2, without calcium chloride. The pyroantimonate was dissolved for 30 min at 40°C in the buffer used to make up the fixative before addition of the aldehydes. Fixation was continued by immersing the minced tissue for 4-6 hr in the pyroantimonate-containing fixative. Subsequent processing was the same as above.

**HORSERADISH PEROXIDASE:** Physiological saline (1 ml) containing 60 mg of horseradish peroxidase and 5% sucrose was injected into the left ventricle of an adult male rat. 3 min later, the adrenals were removed and processed as described in ref. 12, using Karnovsky's diaminobenzidine (13) as the substrate.

**CELL ISOLATION:** Isolated adrenal cortical cells were prepared as described for liver cells (14), except that in this case, perfusion through the suprarenal vein was employed.

**CELL DISRUPTION:** Finely minced adrenal cortical tissue was shaken in a solution of 0.4 M sucrose in distilled water, pH 6.7, for 10 min. The sediment was then prepared for electron microscopy.

**FREEZE-FRACTURING:** For freeze-fracturing, small pieces of adrenal gland were fixed in formaldehyde-glutaraldehyde fixative for 30 min and then immersed in 20% glycerol for 4 hr. Pieces of tissue were mounted on cardboard discs, frozen rapidly in liquid Freon 22, and freeze-fractured in a Balzers apparatus (15, 16).

**PROCEDURES REFERRED TO IN TABLE I:**  
(a) *Protease digestion.* Protease digestion of Epon thin

sections was executed for 45, 60, and 120 min as described by Monneron and Bernhard (17). (b) *Lipid extraction*. Finely sectioned adrenal cortex was fixed in the glutaraldehyde-formaldehyde fixative for 15 min and then extracted with two 10-min washes in 60% acetone at 4°C. In other experiments, small pieces of unfixed tissue were immersed in a chloroform-methanol mixture (2:1) for 1 hr. Subsequent processing was the same as above. (c) *Ruthenium red* was employed according to the method of Luft (18). (d) *Cytochemical precipitation* of lead-phosphate reaction product in an incubation medium containing adenosine triphosphate (ATP) and NaF or isoproterenol was performed according to the method of Reik et al. (19).

## OBSERVATIONS

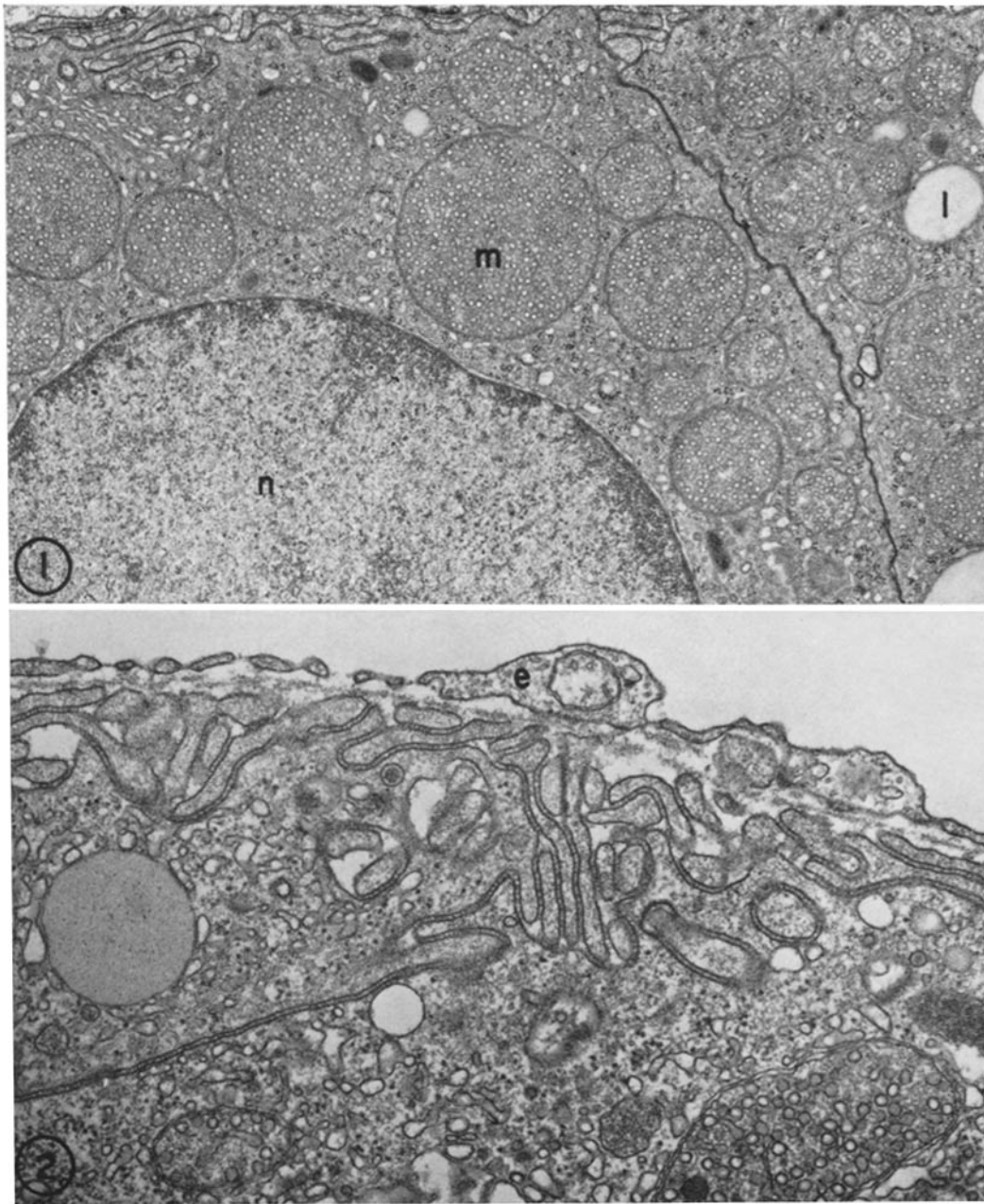
### *Distribution*

The zonation and fine structure of the rat adrenal cortex have been described in detail elsewhere (20–24). In optimally fixed preparations, the areas of contact between adjacent cells are extensive along the lateral cell surfaces and between interdigitating cell processes in the periendothelial spaces in all zones (Figs. 1, 2, 7, 9). At low magnification, the areas of contact have a greater inherent density than do simply apposed membranes. In the zona glomerulosa and zona reticularis, the density is largely attributable to extensive gap junctions (Fig. 13), but in the zona fasciculata a lesser portion of the increased density is due to gap junctions, being primarily due to the septate-like adhering zonule (Figs. 2, 5–7). In the zona fasciculata, where this cell contact is most extensive, it is present along uninterrupted lateral cell interfaces extending from one perisinusoidal space to the next; consistently surrounds small canaliculi between adjacent adrenal cortical cells (Fig. 3); and also envelops larger canaliculi formed by the abutment of four fasciculata cells (Fig. 4). Its point of greatest development is between the interdigitating cell extensions in the periendothelial space (Figs. 2 and 7), where it can be occasionally seen between basal infoldings of the same cell. Except for rare focal junctions, which appear tight in thin sections, small desmosomes, and canalicular-type separations between cells in the zona fasciculata, the septate-like adhering zonule is the interface between nearly all cells and constitutes a continuous structure extending between canaliculi formed by the parenchymal cells and the periendothelial space.

### *Fine Structure*

Higher magnifications of both the lateral and periendothelial regions reveal that the apposing cell membranes are separated by a constant space no less than 210 Å, punctuated by intercellular particles about 100–150 Å in diameter (Figs. 2, 3, 5). Some of the particulate intercellular structures almost touch, while others are several hundred angstroms apart (Fig. 5) so that the periodicity is irregular. Most, however, appear to truly bisect the intercellular space. Views which crisply reveal the trilaminar architecture of the 90-Å plasma membranes indicate that the intercellular structures are frequently circular (Fig. 5). In other areas, where the plane of section is slightly tangential to the plasma membranes, narrow cylindrical structures (Fig. 6), similar to those of the invertebrate septate junction, traverse the space at a fairly marked angle. In still other views, clear linear markings cross the intercellular space (Fig. 6, inset).

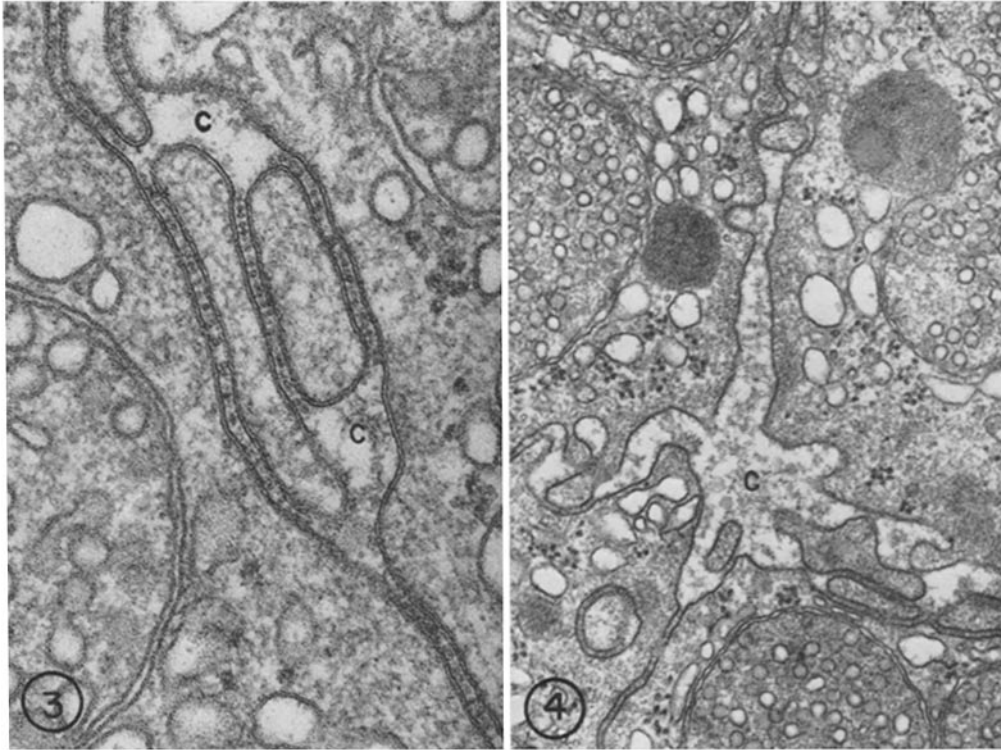
The permeability of the fenestrated sinusoidal endothelium (Figs. 7 and 8) to low molecular-weight tracers and the permeability and ubiquity of the zonulae in the periendothelial space permit visualization of several other structural features. Horseradish peroxidase (mol wt 40,000), generally excluded by gap junctions, permeates the extraparticulate portion of this distinctive cell contact (Fig. 9). Colloidal lanthanum, introduced either by perfusion or immersion as a 3% solution in Tris-hydrochloride buffer before fixation, outlines the extracellular particles (Figs. 10 and 11). As seen in Fig. 11, after lanthanum penetration, the intercellular particles seem to fill the length of the space more fully. Other views show that they are irregular in shape, some occasionally doughnut-like, with slender projections extending to the cell membranes (Fig. 11). Tangential views favor the impression that some traverse the space, and *en face* views reveal contiguous, thin-walled, alveolate structures (Fig. 16), the centers of which fill with lanthanum, demonstrating continuity with the extracellular space. Compare this view (Fig. 16) with a similar one of the gap junction (Fig. 15). The outside diameter of the intercellular particle of the zonule is larger than that of the gap junction particle. It is hollow, however, whereas the gap junction particle is almost solid.



**FIGURES 1-7** Electron micrographs of zona fasciculata cells of the rat adrenal cortex, fixed in buffered formaldehyde-glutaraldehyde. Figs. 2-7 contain 1% K-pyroantimonate and no  $\text{CaCl}_2$  and are postfixed in  $\text{OsO}_4$ . The pyroantimonate generally does not form a precipitate at this concentration but does aid visualization of cell junctions. Tissues were stained in block with uranyl acetate and stained after sectioning with uranyl acetate and lead.

**FIGURE 1** Low magnification of portions of two zona fasciculata cells. Note the marked density of the tangentially sectioned junction between the cells and the lesser density of the contact in the transversely sectioned periendothelial space (top). *l*, lipid; *m*, mitochondrion; *n*, nucleus.  $\times 16,000$ .

**FIGURE 2** The densities seen at low magnification are largely due to ubiquitous stippling of the extracellular space between the membranes of adjacent cells (both along their lateral surfaces and between their interdigitating cell processes in the periendothelial space). This type of membrane apposition is referred to as a septate-like zone of adhesion in the text. *e*, endothelial cell.  $\times 28,000$ .



FIGURES 3 and 4 This distinctive form of contact in the adrenal gland always surrounds small intercellular canalculi formed by the surfaces of two (Fig. 3) or more (Fig. 4) parenchymal cells. Other junctions are rarely found at these sites. *c*, canalculus. Fig. 3,  $\times 96,000$ ; Fig. 4,  $\times 29,000$ .

#### *Freeze-fractures*

Freeze-fractures of cells in all zones of the adrenal cortex readily reveal the gap junction's macular array of particles and hexagonal array of complementary depressions (Fig. 17, inset) as first described by Kreutziger (25). Freeze-fracture preparations of the zona fasciculata in areas where adhering zonules are nearly always seen in thin-sectioned material, that is, adjacent to gap junctions (Fig. 13), around cell indentations (Fig. 7), and between interdigitating cell processes (Figs. 2 and 7), have no distinctive identifying features (Fig. 17). Thus far, this septate-like cell contact has simply not presented a distinctive freeze-fracture appearance such as that which characterizes the gap junction or the septate junction of invertebrates. In fact, it is in no way distinguishable from the unmodified, nonjunctional portions of the cell membrane.

#### *Lipid Extraction, Cell Isolation, Cell Disruption*

With a mixture of chloroform and methanol, and with acetone, extraction of fresh adrenal cortical tissue and tissue briefly fixed in aldehydes does not remove the extracellular particles. Total osmotic disruption of cells results in separation of cell membranes in pairs, braced together by the particles of the septate-like cell contact (Fig. 18). The extracellular particles likewise persist on the surfaces of intact cells isolated by perfusion with hyaluronidase and collagenase, behaving structurally the same as tight and gap junctions, retaining the membrane of a formerly apposing but now disrupted cell on the surface of the intact cell (Fig. 19). We do not know, however, if this contact would resist dissolution after perfusion for longer periods. They do withstand immersion of the tissue in 2%  $\text{OsO}_4$  at 40°C for 2 days. In fact, the only method which disrupts the substructure of

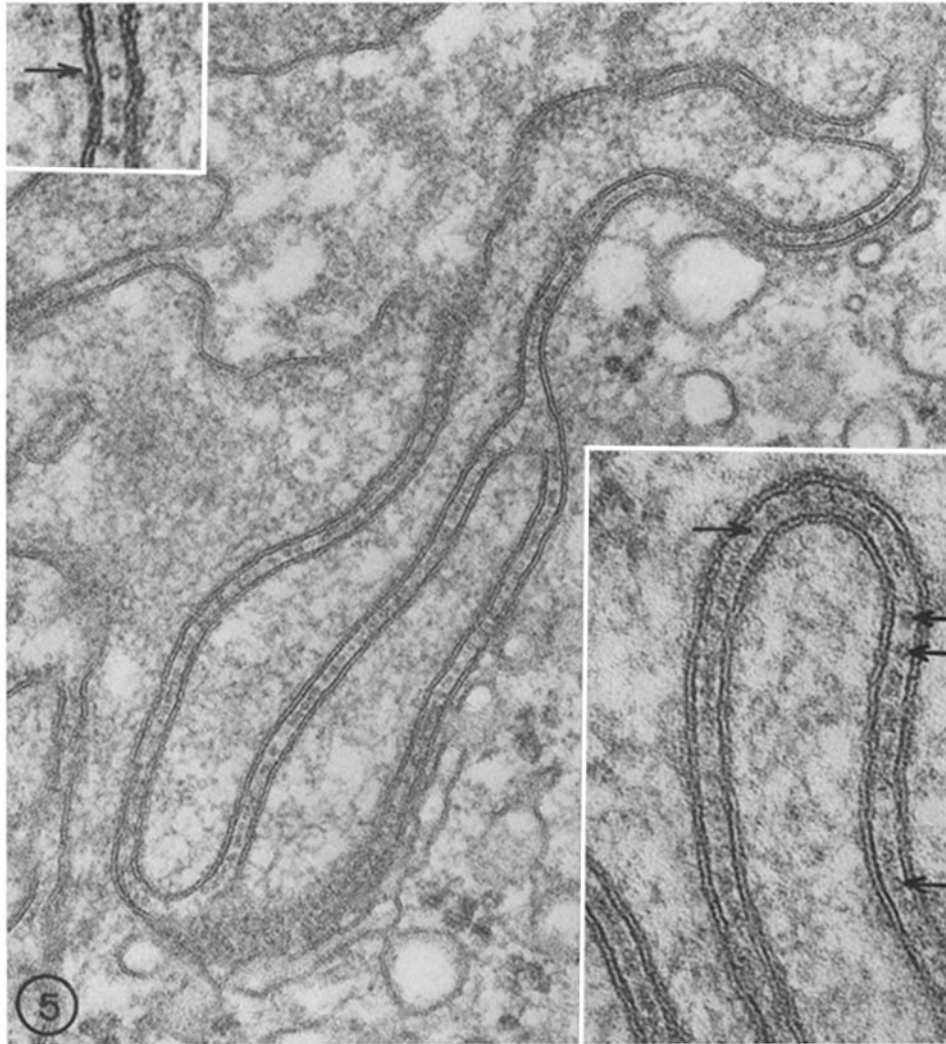


FIGURE 5 At higher magnifications, the circular shape of the intercellular particles (arrows) and their irregular spacing are evident. Note the absence of particles on the free surface of the cell membrane where cells are not closely apposed (upper area of micrograph). Observe also the close approximation of smooth-surfaced endoplasmic reticulum to the cell membrane in the region of this cell contact (lower area).  $\times 100,000$ . Upper inset,  $\times 200,000$ ; lower inset,  $\times 170,000$ .

the zonule is the digestion of thin sections with protease for at least 45 min.

Further observations on this cell contact in the rat adrenal cortex appear in the figure legends and in Table I.

#### *Other Species and Tissues*

Although they stain with less intensity, septate-like contacts are present in the same distribution in the adrenal cortex of the guinea pig, boar, mouse, and man (Fig. 20). As is the case in the

rat, they are accompanied by extensive gap junctions. Areas of contact identical to those of the adrenal cortex are also found between interstitial cells in the testes of the boar (Fig. 21) and the mouse, and similar membrane appositions are present between cells of the rat corpus luteum.

#### DISCUSSION

##### *Real Structure or Artifact?*

Various septate-type periodicities have been reported in vertebrate tissues. Those associated

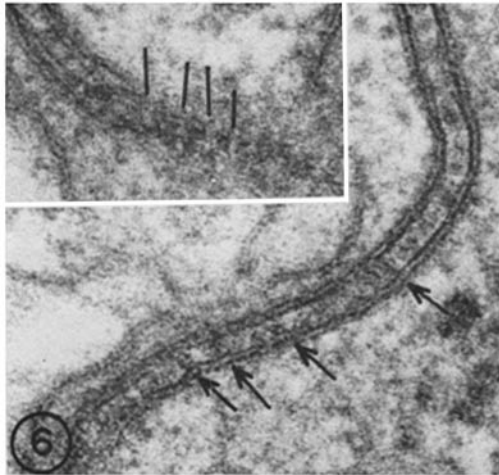


FIGURE 6 In some areas along the course of the cell contact, grazing sections reveal narrow cylindrical structures (arrows) or clear linear markings (bars) traversing the space. These types of images resemble the invertebrate septate junction. The close apposition of smooth-surfaced endoplasmic reticulum is again apparent.  $\times 180,000$ .

with synapses have been well documented as real structures (26, 27), but many others have been observed in pathological conditions (28) and considered artifactual (29). The cell contact described in this paper is present after fixation with aldehydes, with osmium tetroxide, and with a variety of fixative mixtures including phosphotungstic acid, glutaraldehyde, and osmium (6). The intercellular particles are also present after permeation of the intercellular space by tracers. They are, moreover, present in different physiologic states—under standard experimental conditions in rats of different sex and age, after hypophysectomy, and after ACTH stimulation—without appreciable alteration. The location of this cell contact is constant in the rat adrenal gland, in the adrenal glands of other species, and in at least one other steroid-hormone-secreting tissue. The septate-type periodicity does not appear between other types of cells in the same tissue nor between contiguous membranes of other organelles. The intercellular structures between apposing membranes remain after the cells are dispersed or their membranes isolated. On the basis of these persuasive findings, we conclude that this distinctive adrenal cell contact, like those found at synapses (26, 27), is also a real structure.

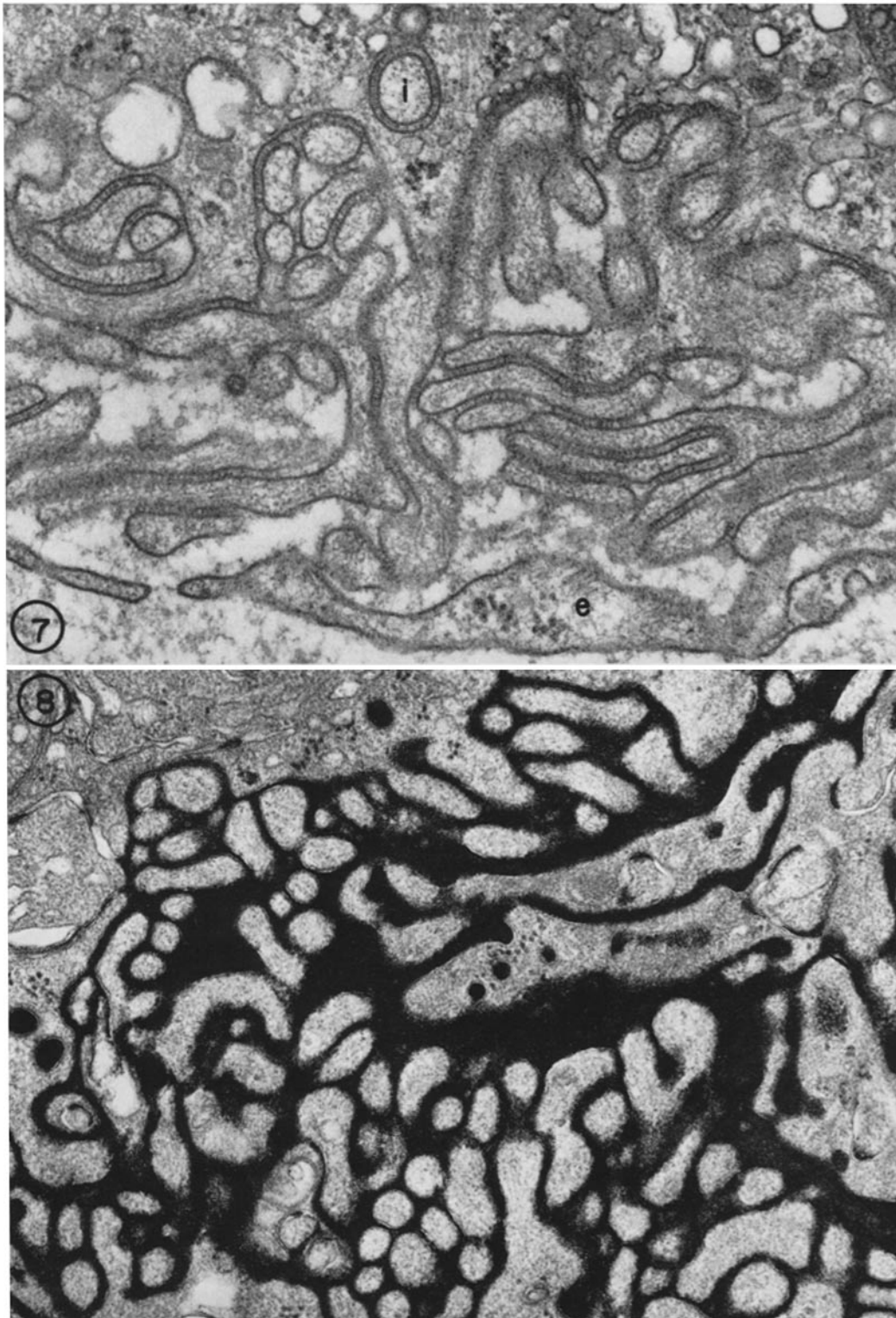
### Significance

Specialized regions of apposing cell membranes which perform a function different from that of unmodified cell interfaces are broadly referred to as cell junctions. Thus if we use the term “junction” loosely, those commonly recognized in vertebrates include tight junctions (3) (zonulae occludentes), which act as impermeable seals between cells; gap junctions (4, 5, 30), permeable to lanthanum, widely considered to be the sites of low-resistance coupling between cells (31–33); intermediate junctions (zonulae adherentes), permeable to horseradish peroxidase, which function as an anchoring site for intracellular filaments in some tissues but for which a general function has not yet been ascribed; and desmosomes (maculae adherentes), which offer resistance to lateral shearing forces. As a modified interface between apposing cells, possessing at least one specialized function, this adrenal zonule fulfills the criteria for a junction in respect to general usage of the term. We prefer, however, to use the more inclusive “cell contact” in reference to membrane appositions which do not reveal distinguishing modifications in freeze-fracture preparations. Doing so separates tight, gap, and septate junctions from desmosomes, zonulae adherentes (intermediate junctions), and septate-like cell contacts.

The significance of this particular cell contact found in the adrenal cortex lies in its restricted distribution outside of the nervous system, its occurrence in mammals other than rodents, and its presence in steroid-hormone-secreting tissues besides the adrenal cortex. That is, its distribution in epithelia is much more limited than the components of the junctional constellation designated above. Second, it occurs, along with extensive gap junctions, in the adrenal cortices of species other than the rat: it is present in identical distribution in the mouse (illustrated in a recent paper by Shelton and Jones [34]), in the guinea pig<sup>1</sup>, the hog<sup>1</sup>, and in man. Third, it is definitely present in less distinct form in the rat corpus luteum and between other cells of the rat ovary.<sup>1</sup> These three factors (*a*) the limited distribution of this type of cell contact in vertebrates, (*b*) the fact that it is not species-specific, and (*c*) its presence in steroid-hormone-secreting tissues other than the adrenal cortex, may result in the presence of this type of contact becoming a morphological criterion

<sup>1</sup> D. S. Friend, unpublished observations.





FIGURES 7 and 8 Lanthanum freely permeates between the interdigitating cell processes of the periendothelial space (Fig. 8) in an area comparable to that shown in Fig. 7. There are no zonulae occludentes to bar the passage of tracers in this region. *i*, cell indentation; *e*, endothelial cell. Fig. 7,  $\times 56,000$ ; Fig. 8,  $\times 45,000$ .



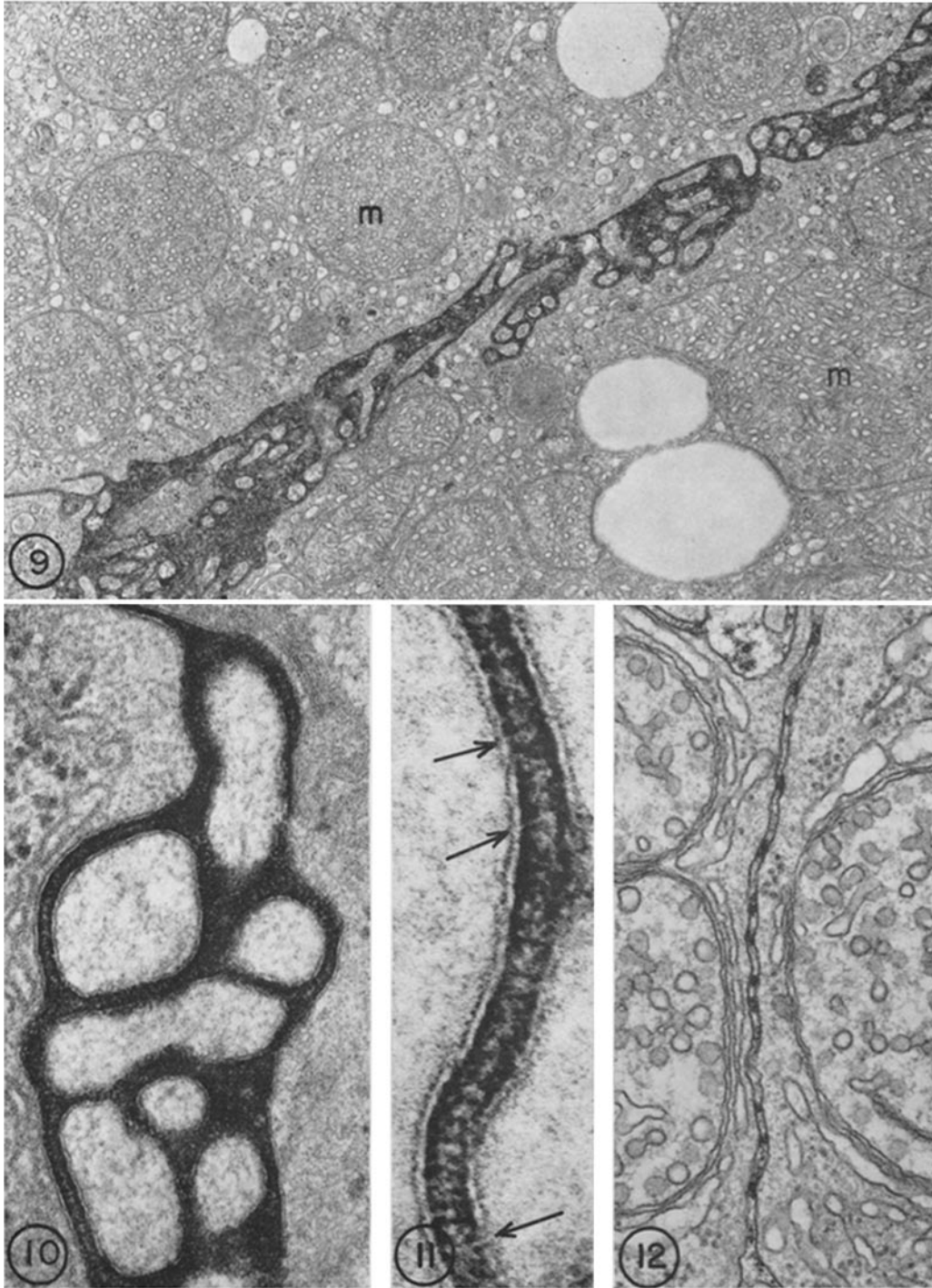
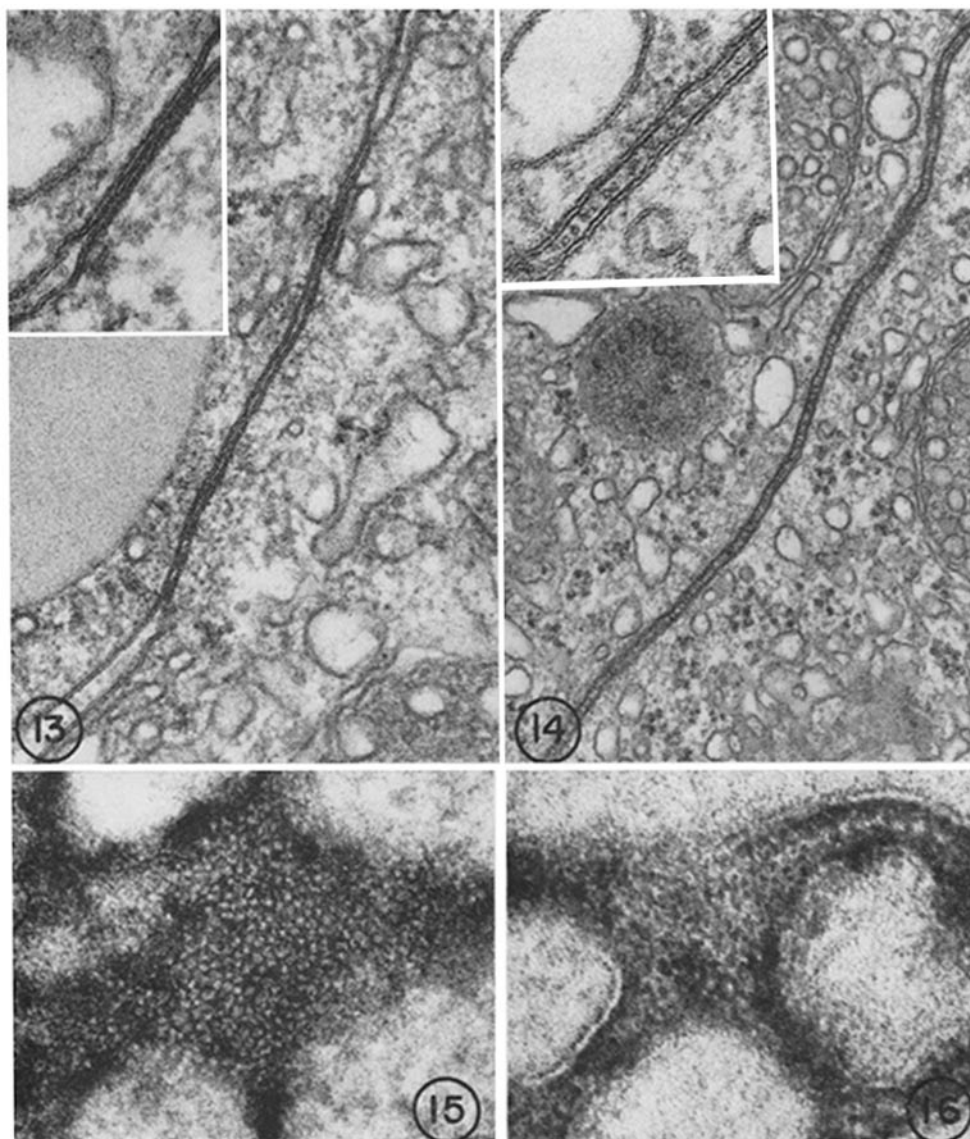


FIGURE 9 Horseradish peroxidase, retarded by gap junctions, flows through the septate-like zonulae. The different configurations of mitochondrial cristae in this micrograph indicate that this interface is between the zona fasciculata (upper cell) and another zone of the adrenal cortex (lower cell). *m*, mitochondrion.  $\times 18,000$ .

FIGURES 10 and 11 Lanthanum fully permeates the septate-like zone of adhesion. In such preparations, the particles of the intercellular space are seen to actually form a continuous chain. Arrows indicate where projections of the irregularly-shaped particles touch the cell membranes. Fig. 10,  $\times 90,000$ ; Fig. 11,  $\times 320,000$ .

FIGURE 12 K-pyrosulfonate, used as a 5% solution in the aldehyde fixative, encrusts some of the extracellular particles (perhaps indicative of high concentrations of cations [e.g., calcium] at these sites.)  $\times 52,000$ .



FIGURES 13-17 Micrographs comparing the gap junction and the septate-like cell contacts of the adrenal cortex in routine preparations, after lanthanum permeation, and in freeze-fracture replicas.

FIGURE 13 Gap junctions are large and frequent between cortical cells, commonly adjacent to septate-like adhering zones (inset). A space of  $\sim 20$  A separates the contiguous cell membranes, but the regular periodicity of its extracellular particles is difficult to resolve.  $\times 60,000$ . Inset,  $\times 130,000$ .

FIGURE 14 The adhering zonule is comparatively more extensive and more common than the gap junction. The space between cells is more than 200 A and its irregularly-spaced extracellular particles are readily visualized.  $\times 50,000$ . Inset,  $\times 130,000$ .

FIGURE 15 Viewed *en face*, lanthanum-treated gap junctions reveal a polygonal packing of subunits, some of which have central dense spots.  $\times 200,000$ .

FIGURE 16 A similar view of a lanthanum-treated septate-like zone of adhesion, demonstrating its alveolate (honeycomb) structure composed of continuous thin septa. Lanthanum permeates the alveolate structures.  $\times 260,000$ .

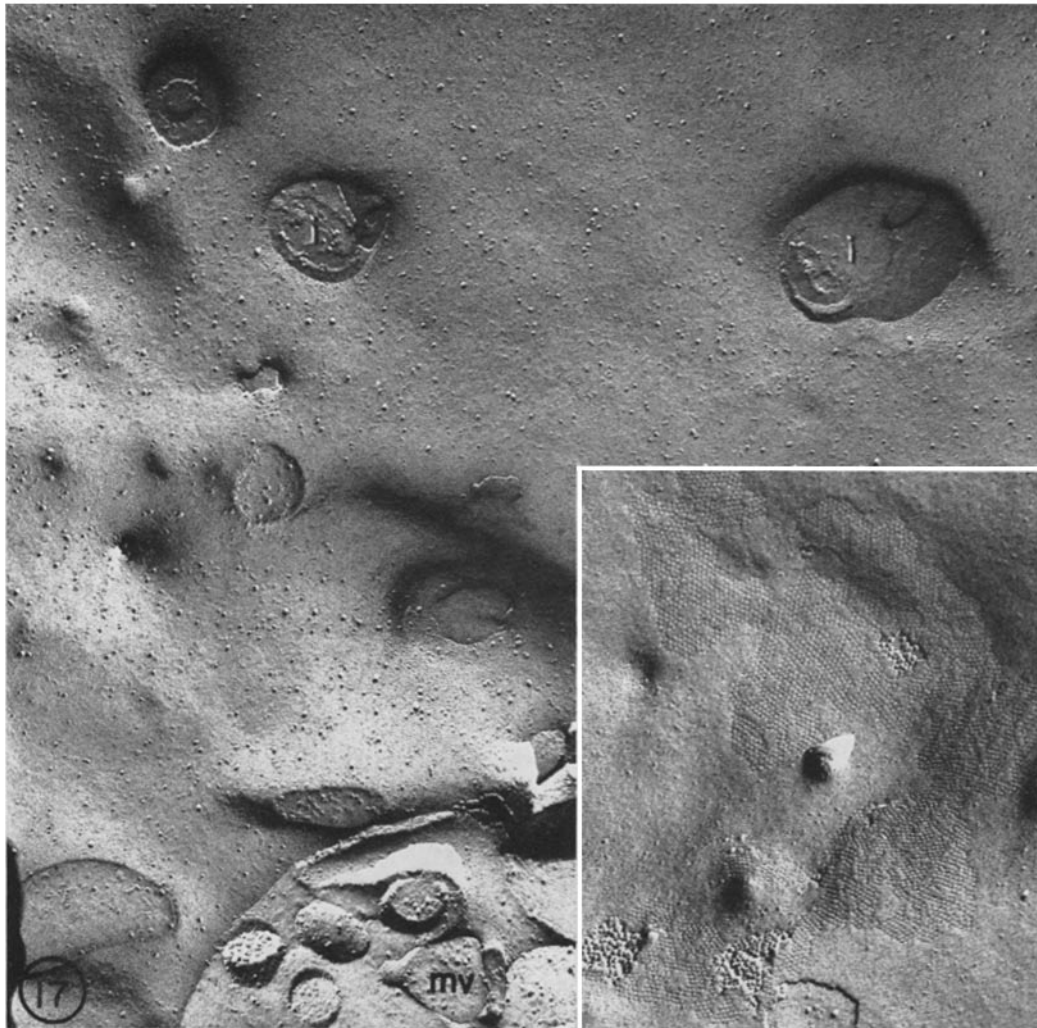


FIGURE 17 Unlike the arrays of particles and depressions observed in freeze-fracture replicas of the cell membrane in the region of the gap junction (inset), no noteworthy array of particles or depressions is revealed in a comparable replica in a region where zones of adhesion are commonly present. This area does not differ from nonjunctional portions of the cell membrane. *i*, cell indentation; *mv*, microvilli.  $\times 62,000$ . Inset,  $\times 90,000$ .

for the tentative identification of steroid-hormone-secreting cells.

#### *Structure and Functions*

The septate-like zone of adhesion, although superficially resembling the septate junction of invertebrate species (both have a honeycomb appearance when viewed *en face* and cylindrical-appearing structures traversing the extracellular

space in other views), differs in many of its fundamental properties. The adrenal contact zone has no substantial septa, does not stain intensely with ruthenium red, lacks a regular periodicity, is not present between all mucosal epithelial cells (as is the case with septate junctions in invertebrates), and lacks a freeze-fracture image of regular linear arrays of particles and depressions. The last point, whether or not membrane modifications are dis-

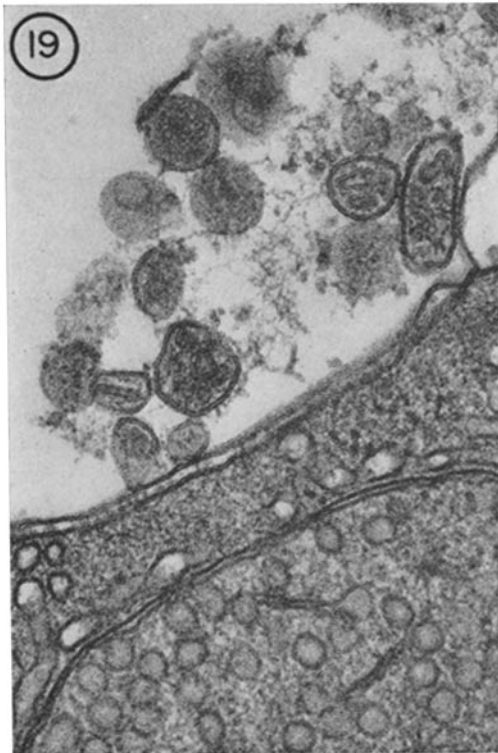
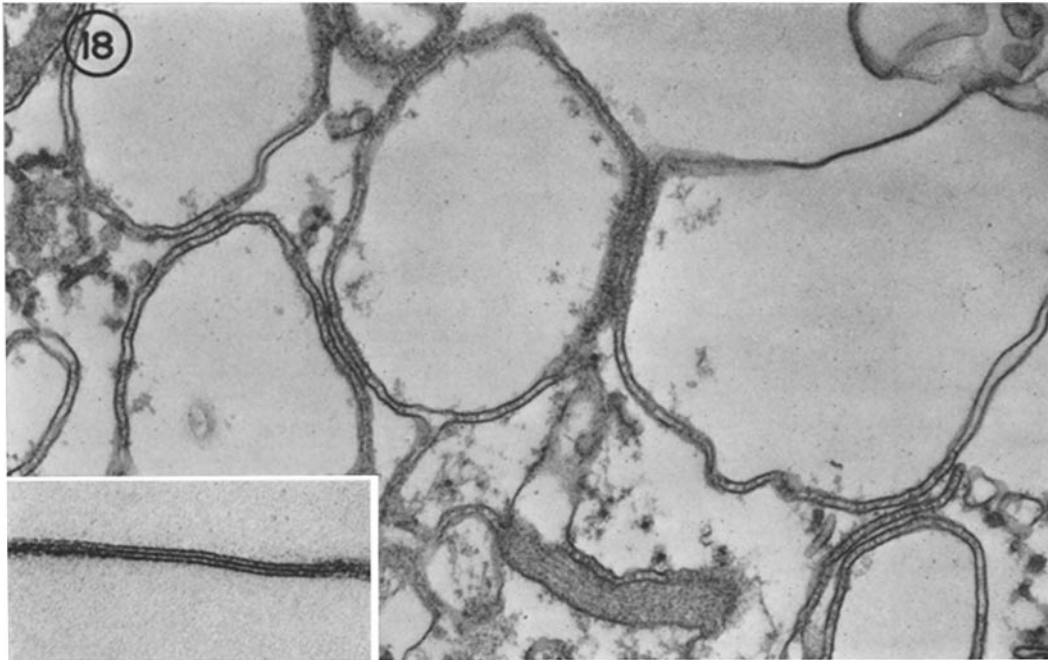


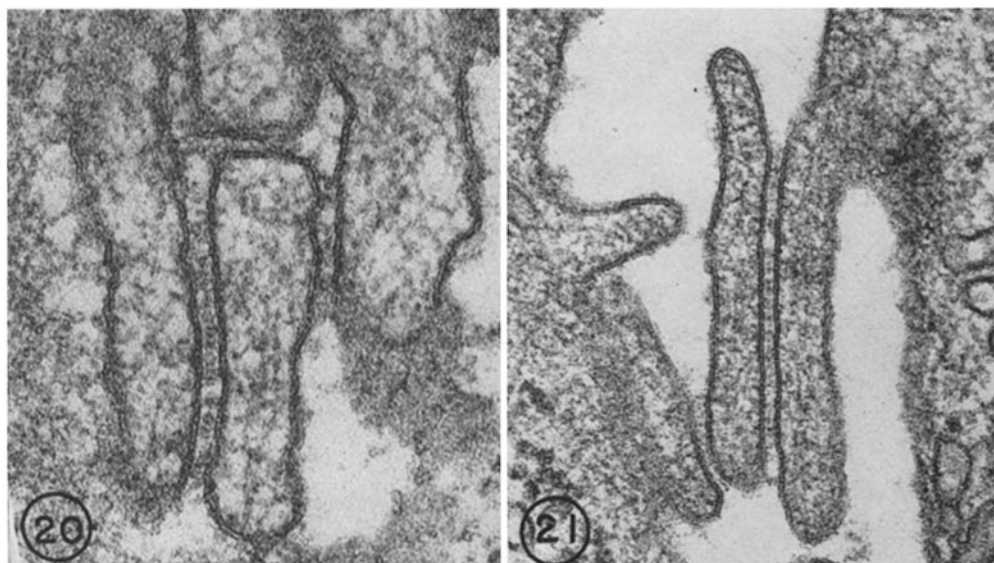
FIGURE 18 Cell membranes isolated from adrenal cortical cells appear in pairs, evidently held together by the particles of the septate-like zones of adhesion as well as gap junctions (inset). Inset is an enlargement of the junction in the upper right portion of the micrograph.  $\times 36,000$ . Inset,  $\times 100,000$ .

FIGURE 19 The same cell contacts fasten the cell membrane of a disrupted cell to the surface of an isolated intact cell.  $\times 80,000$ .

TABLE I  
*Adrenal Septate-like Zone of Adhesion*

Procedure	Result
Freeze-fracture	No distinguishing modification
Cell membrane isolation	Contact remains intact
Pyroantimonate, lanthanum, HRPase tracers	Penetrate
Protease digestion (sections), 45 min	Extracellular particles digested
Lipid solvents	Extracellular particles persist
Hyaluronidase, collagenase perfusion, 10 min	Contacts remain intact
Thiocarbohydrazide, silver-proteinase staining (43)	Same as nonjunctional membrane
Ruthenium red staining	Less than endothelial cell membrane
Wachstein-Meisel ATPase procedure	Less than erythrocyte membrane
Reik (19) medium with NaF or isoproterenol	No enhancement
ACTH-stimulation and hyophysectomy	No appreciable change

cerned by freeze-fracturing, seems particularly important to us, since such a major difference in the structure of septate junctions and septate-like cell contacts might also reflect a major difference in function. So different in structure, it is unlikely that the adrenal cell contact serves the same function in vertebrates as that ascribed to the septate junction in invertebrates (8, 35). The absence of membrane modification of the adrenal zone of adhesion in freeze-fractures, particularly, seems to eliminate the question of ionic or molecular communication between cells through this structure. In view of the absence of membranous structures to align with the intercellular particles, it is reasonable to assume that passageways are not present between the cytoplasmic matrices of contiguous cells. This theoretical point is of particular concern when we consider similar if not identical structures found in the vertebrate nervous system at synaptic endings of visual cells (26) and at axo-axonic connections in the basket formation of the cerebellum (27). These are designated by different names (basal junctions in the visual cells and septate junctions in the cerebellum) but they require further investigation (freeze-fracturing) to determine whether or not they are actually different structures. At the moment, we believe both



FIGURES 20 and 21 The septate-like zone of adhesion is a common form of membrane apposition in the human adrenal cortex (Fig. 20), as well as in the rat, boar, guinea pig, and mouse. It is less extensive, but consistently present, between interstitial cells of the boar (Fig. 21) and mouse testis. Fig. 20,  $\times 130,000$ ; Fig. 21,  $\times 70,000$ .

these cell contacts and the one in the adrenal cortex to be the same.

The septate-like adrenal contact's lack of freeze-fracture characteristics actually groups it with the desmosome and the zonula adherens of the typical junctional complex. Desmosomes also exist in adrenal tissue, although they are sparse and poorly developed, so that this form of adhering zonule is an unlikely substitute for the desmosome. It could be considered, however, as a major modification of the zonula adherens as initially described by Farquhar and Palade (3). It has a fairly wide extracellular space, it is a zonule, and it adheres, but there the resemblance ends. It lacks a dense filamentous network on its cytoplasmic surface, and it is not a usual component of the junctional complex (3).

The fine structure of this adrenal contact suggests other functions besides adherence. Considered as a series of doughnut-shaped structures with narrow cylindrical struts buttressing the cell membranes, the intercellular particles are well suited to hold the membranes apart, preventing the collapse of the intercellular space. Indeed, the minimal separation of 210 Å between apposing cell membranes seems to confirm this concept. In addition to holding the cells apart, the particles divide the extracellular space into at least two compartments. One, freely permeable to lanthanum and horseradish peroxidase, is the space surrounding the particle, between its narrow extensions. The other, a space permeable to lanthanum but probably not to peroxidase, is in the center of the doughnut-like structure. (A third, theoretical compartment would be a channel within the walls of the central particle and its extensions, similar to the channels postulated for gap and septate junctions.) The extracellular channels which the particles partition, taken together with their deployment between canaliculi and the periendothelial spaces, attract the speculation that they provide a microcanalicular system for the flow of secretory product from the parenchymal cells to the bloodstream. The extracellular space, specially structured for the flow of substances, would also explain the lack of zonulae occludentes in the adrenal cortex. Although the biochemical dynamics initiating steroid-hormone secretion are akin to those initiating exocrine secretion (36), the final egress of secretory product from the cell is probably different (1, 37), with the lipid-soluble hormones weeping through the

cell membranes over a wide area. A channel for conveying the hormones to the bloodstream would then seem useful, and the adrenal contact zone could well serve that purpose. At present, however, no evidence for this hypothesis exists.

Another possible function, implied by the encrustation of the extracellular particles with pyroantimonate (Fig. 12), is that they may be a site of high concentration of cations. According to the most recent analyses (38, 39), the pyroantimonate precipitate could well contain calcium. The binding of the precipitate is probably not due to polysaccharides, since this zonule does not stain intensely with ruthenium red or silver proteinate methods for glycoprotein and polysaccharides in the same preparations where deep staining of endothelial cell membranes and glycogen is achieved. The possibility, then, that calcium is present in high concentration in the particles may be significant, since calcium plays a critical role in corticosteroid secretion (36). ACTH may shift calcium from a rapidly exchanging to a more slowly exchanging pool (39), and the particles, contiguous with the cell membranes, could provide one of the depots for the cation. Theoretically, this site could also serve for binding trophic hormone (ACTH) or carrier protein (transcortin).

Insight concerning the organization of steroidogenic epithelia in vertebrates may also be gained from further exploration of invertebrate epithelia (41, 42), since closely associated gap and septate junctions without zonulae occludentes are common to both.

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