COEXISTENCE OF GAP AND SEPTATE JUNCTIONS IN AN INVERTEBRATE EPITHELIUM

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ABSTRACT

The intercellular junctions of the epithelium lining the hepatic caecum of *Daphnia* were examined. Electron microscope investigations involved both conventionally fixed material and tissue exposed to a lanthanum tracer of the extracellular space. Both septate junctions and gap junctions occur between the cells studied. The septate junctions lie apically and resemble those commonly discerned between cells of other invertebrates. They are atypical in that the high electron opacity of the extracellular space obscures septa in routine preparations. The gap junctions are characterized by a uniform 30 A space between apposed cell membranes. Lanthanum treatment of gap junctions reveals an array of particles of 95 A diameter and 120 A separation lying in the plane of the junction. As this pattern closely resembles that described previously in vertebrates, it appears that the gap junction is phylogenetically widespread. In view of evidence that the gap junction mediates intercellular electrotonic coupling, the assignment of a coupling role to other junctions, notably the septate junction, must be questioned wherever these junctions coexist.

INTRODUCTION

In the past few years it has become clear that there are specialized pathways for the exchange of ions (and perhaps larger materials) between neighboring cells. In many instances there is good, if largely circumstantial, evidence that the gap junction found between both excitable and nonexcitable cells may provide a pathway for such intercellular exchange. Although gap junctions are found between most electrically coupled cells, there are several instances of cell coupling where this specialization appears to be absent. A case in point is the giant salivary gland of *Drosophila* larvae, in which cell-to-cell coupling has been extensively studied by Loewenstein and his coworkers. They suggest that the septate junction (septate desmosome), a type of contact often encountered between cells of invertebrates, may play a role in low-resistance coupling (16). Recently published work on freeze-etched preparation of septate junctions in the mussel has supported this view (6).

The present report documents the existence, side by side with septate junctions, of cell contacts which closely resemble the gap junctions of vertebrates. This finding again raises the question of the identity of the contact specialization(s) responsible for cell-to-cell, low-resistance coupling.

MATERIALS AND METHODS

Daphnia were obtained from the Connecticut Valley Biological Supply Co. (Hampton, Mass.) and identified as *Daphnia pulex* de Geer (Arthropoda: Crustacea). They were maintained as continuous cultures in distilled water at room temperature and fed with an infusion of wilted lettuce leaves.

Animals were fixed by immersion for 1–3 hr at room temperature in a modified Karnovsky's fixative (7) containing 2% formaldehyde and 5% glutaraldehyde buffered with 0.08 M sodium cacodylate to pH 7.4. They were then rinsed for ½ hr in 0.08 M sodium cacodylate buffer and postfixed for 1–2 hr at room temperature in 1–2% osmium tetroxide in

0.7 M collidine buffer, pH 7.4. A brief wash with 0.2 M sodium maleate buffer, pH 5.2, preceded en bloc staining with 1–2% uranyl acetate in sodium maleate buffer at pH 5.2. After another brief wash in maleate buffer, specimens were dehydrated in a graded series of ethanol and embedded in Araldite 6005 resin (Ciba Products Co., Summit, N.J.). En bloc staining with uranyl acetate was omitted in several experiments, as a control for possible alterations in structure due to staining.

In experiments employing lanthanum as a tracer of the extracellular space, one of two techniques was used. Animals were generally fixed in aldehydes as described above, and exposed to 1–2% lanthanum hydrosol (10) in the osmium tetroxide postfixative solution. Alternatively, the initial aldehyde fixation was omitted, and the animals were fixed only in the lanthanum-containing osmium tetroxide solution. Subsequent steps followed the outline above.

For electron microscopy, silver-to-grey sections were cut with glass or diamond knives on either a Porter-Blum Sorvall MT-2 or on an LKB Ultrotome III ultramicrotome. The sections were collected on carboned, celloidin-coated copper grids, stained with lead citrate (15), and examined with a Siemens IA electron microscope at 80 kv. For light microscopy, 0.5–2-μ sections were cut and stained with toludine blue in borax. Whole animals were examined as wet mounts immediately following immobilization with low concentrations of osmium tetroxide.

OBSERVATIONS

General Appearance of Caecal Cells

The hepatic caeca of *Daphnia pulex* are paired diverticula of the anterior midgut. These caeca are simple tubes, approximately 0.2 mm long and about 0.1 mm in diameter in adult animals of 2.5 mm body length. The wall of each caecum consists of a simple cuboidal epithelium 25 μ thick, with a prominent apical brush border and a thick basement lamina (Fig. 1). The epithelium is surrounded by several small groups of striated muscle fibers, and the whole structure lies within an open blood space (haemocoele). The cytoplasm of these cells is unremarkable except for the frequent occurrence of microtubules adjacent to the lateral cell boundaries.

The lateral surfaces of a cell are not extensively convoluted, and their most striking specializations are intercellular junctions. Approximately the apical third of the cell membrane is involved in a septate junction, which forms a belt or zonule surrounding the whole cell. The numerous gap junctions have a more basal location. A third structure, possibly also an intercellular junction, occurs as small plaques of regularly arranged intercellular material.

A description of these specializations is outside the scope of this paper, especially as there is good evidence to indicate that the structures do not really represent a cell-to-cell junction.

Septate Junction

The septate junction forms a belt-like structure (zonula) around the cells. It reaches the luminal border of every caecal cell, and from there extends basally for up to 10 μ (Fig. 1). The junction runs uninterruptedly in the apical-basal direction save for the occasional appearance of structures resembling interfacial canals. In the areas of junctional contact the apposed plasma membranes are separated by an intercellular space 175 A wide (Figs. 2 and 3). In routinely prepared specimens the septa which extend between cells are best seen when they are cut transversely. Because the intercellular space has a relatively high inherent density, the septa are not well defined in other planes of section. The septa may appear to be continuous with the central, clear zone of the adjacent unit membrane, while in other instances a dense line, corresponding to the outer leaflet of the plasma membrane, can be seen at the root of the septum.

Introduction of lanthanum hydrosol into the preparation reveals additional details of the caecal septate junction. Transverse sections of the junction stained with lanthanum still show an extracellular space 175 A wide (Fig. 4). Now, however, density of this space has increased considerably and the septa appear in striking relief. As shown by *en face* views of lanthanum-treated junctions, contiguous septa in some regions are parallel to each other and separated by as little as 35 A, while in other areas the septa are separated from one another by variable distances, and are inclined at various angles to one another (Figs. 5–7). Large numbers (20 or more) of mutually parallel septa are observed only rarely. The septa do not always follow courses parallel to the apical surfaces of the cells; the obliquity of many septa with respect to the apical cell surface explains the paucity of septa observed in some transverse sections of septate junctions in normally prepared material.

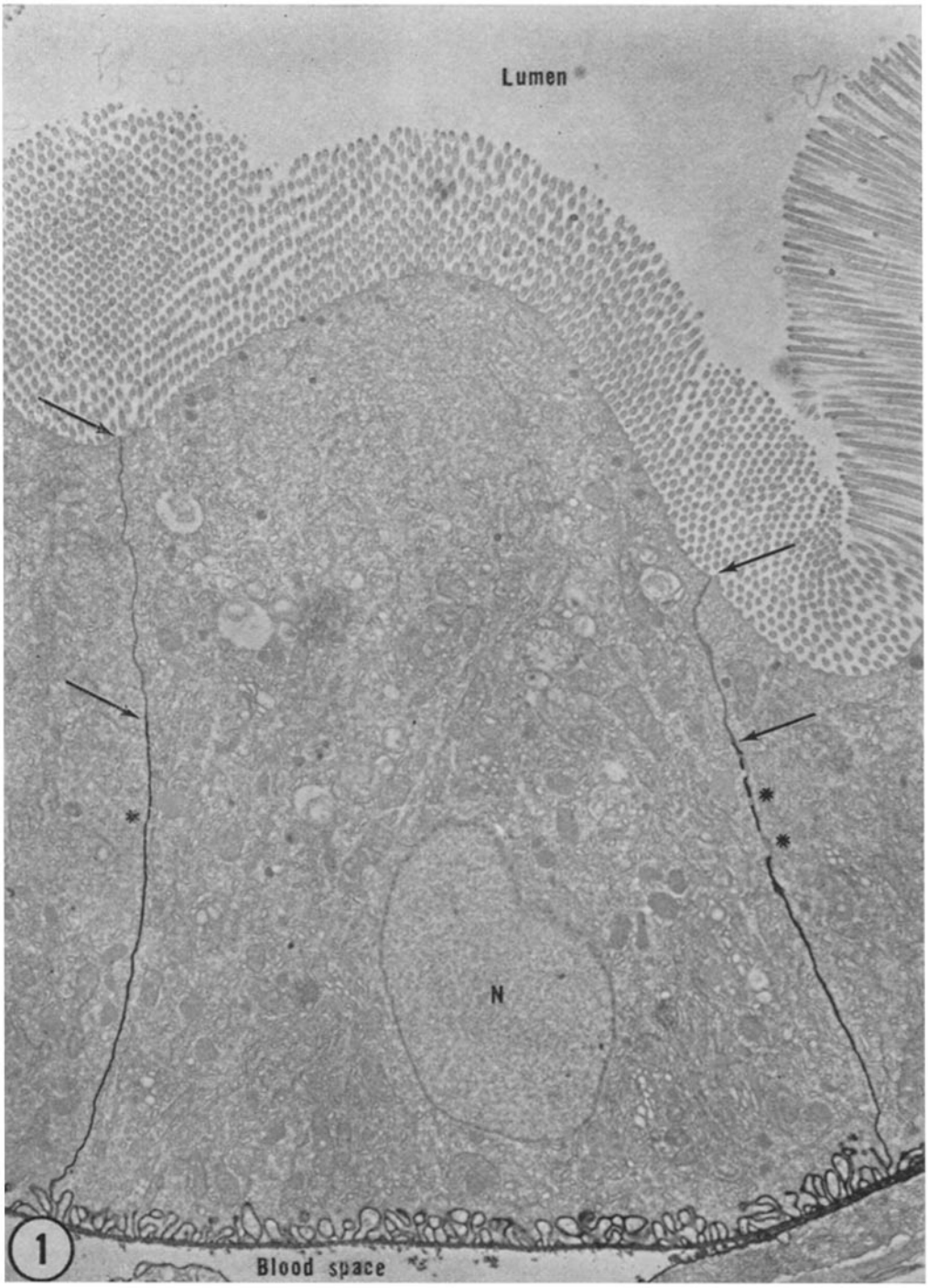
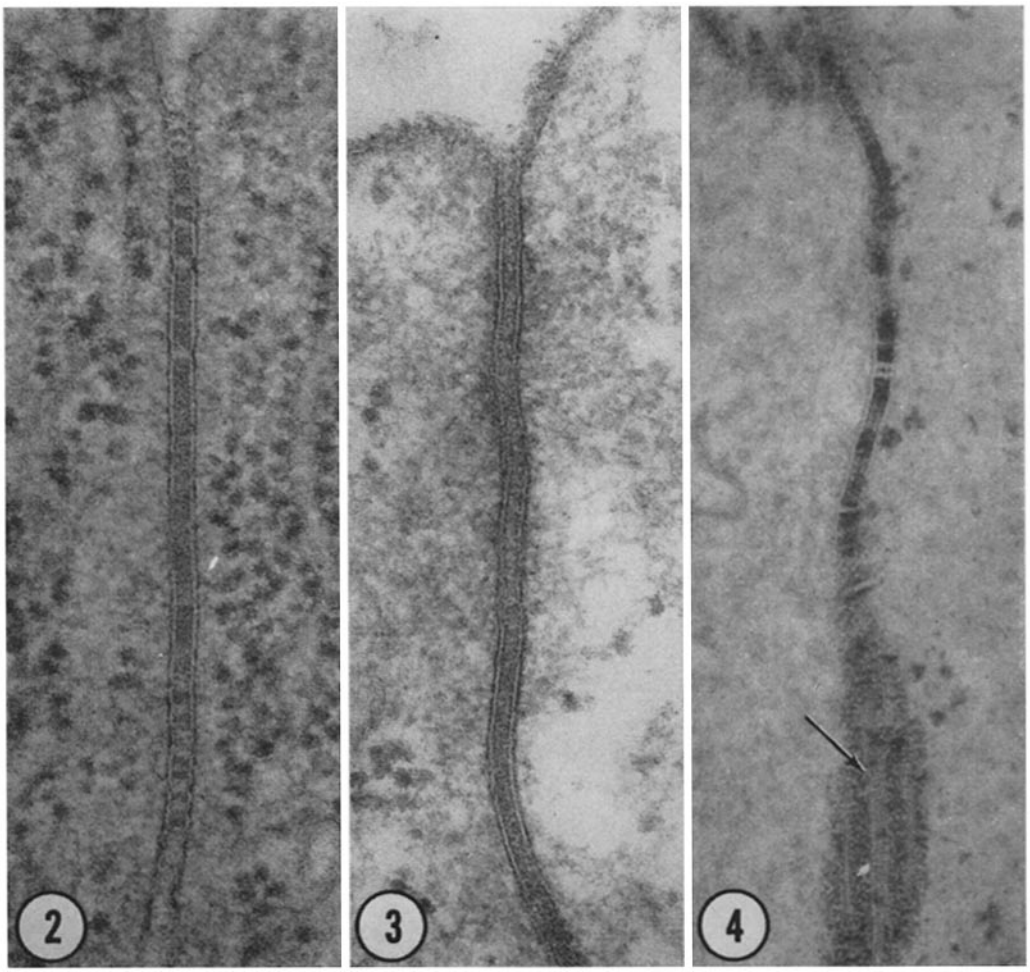


FIGURE 1 A low-power electron micrograph of a typical caecal cell, with lanthanum hydrosol filling the lateral and basal extracellular spaces and staining the basement membrane. Various organelles, including the nucleus (*N*), mitochondria, apical microvilli, and endoplasmic reticulum can be recognized. Septate junctions are situated apically (between paired arrows); lanthanum has been largely excluded from these junctions in this preparation. Several gap junctions (*), seen as regions less densely stained by the lanthanum tracer, occur along the cell boundaries. × 4700.



FIGURES 2–4 Electron micrographs of transverse sections of apical septate junctions.

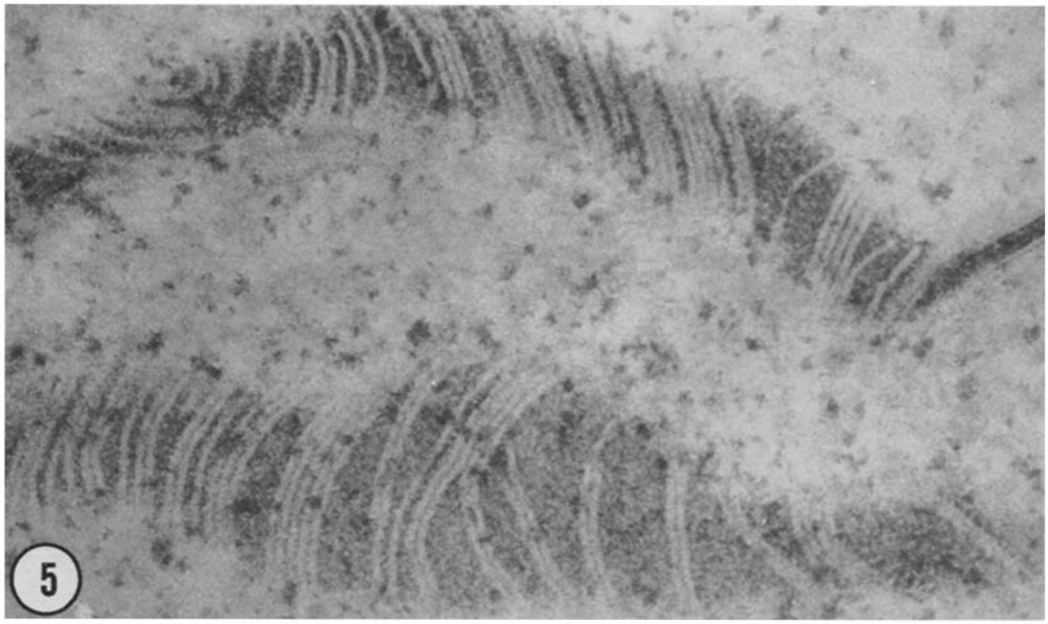
FIGURE 2 A septate junction in which the transversely sectioned septa are seen as interruptions of the dense extracellular space. Although some septa appear thicker than others, tangential sections of similar material reveal that this results from varying obliquities of septa of identical thickness. × 125,000.

FIGURE 3 A similar junction situated immediately adjacent to the caecal lumen. Here the septa are so oblique with respect to the plane of section that they are not evident. × 125,000.

FIGURE 4 A septate junction treated with lanthanum hydrosol; the tracer increases the contrast in densities of septa and extracellular space. Note the regular substructure in some septa (arrow). It is unclear whether this periodicity lies within the septum itself, or at the point of septal insertion into the membrane. × 125,000.

Gap Junction

Beneath the septate junction of a caecal cell, the intercellular space expands to an irregular width of 150–500 A. This basic conformation persists to the point at which basal infoldings begin, where the intercellular space characteristically enlarges. Macular structures of two varieties interrupt this pattern over small areas of membrane apposition (Fig. 1). Of these only one, the gap junction, will be discussed. The other, consisting of an array of regularly spaced electron densities in the intercellular space, will be described elsewhere. The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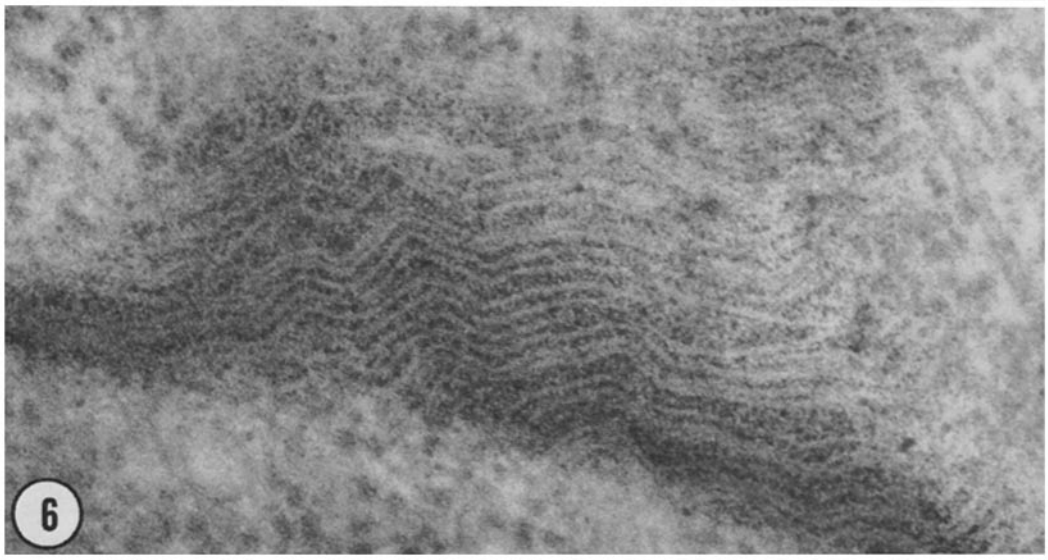
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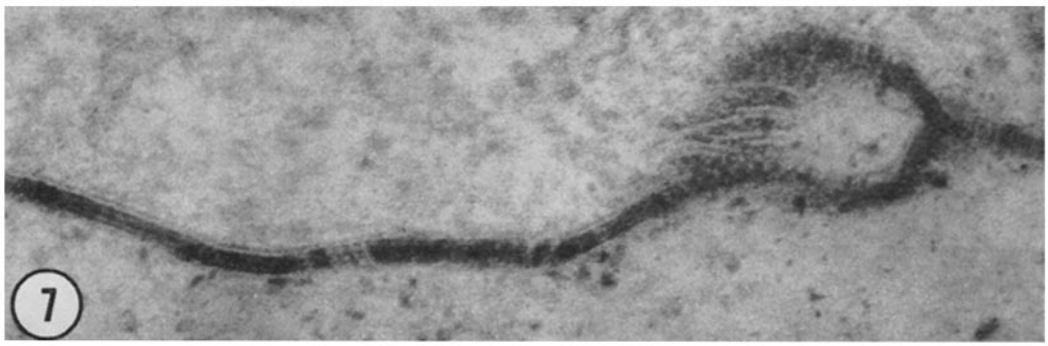
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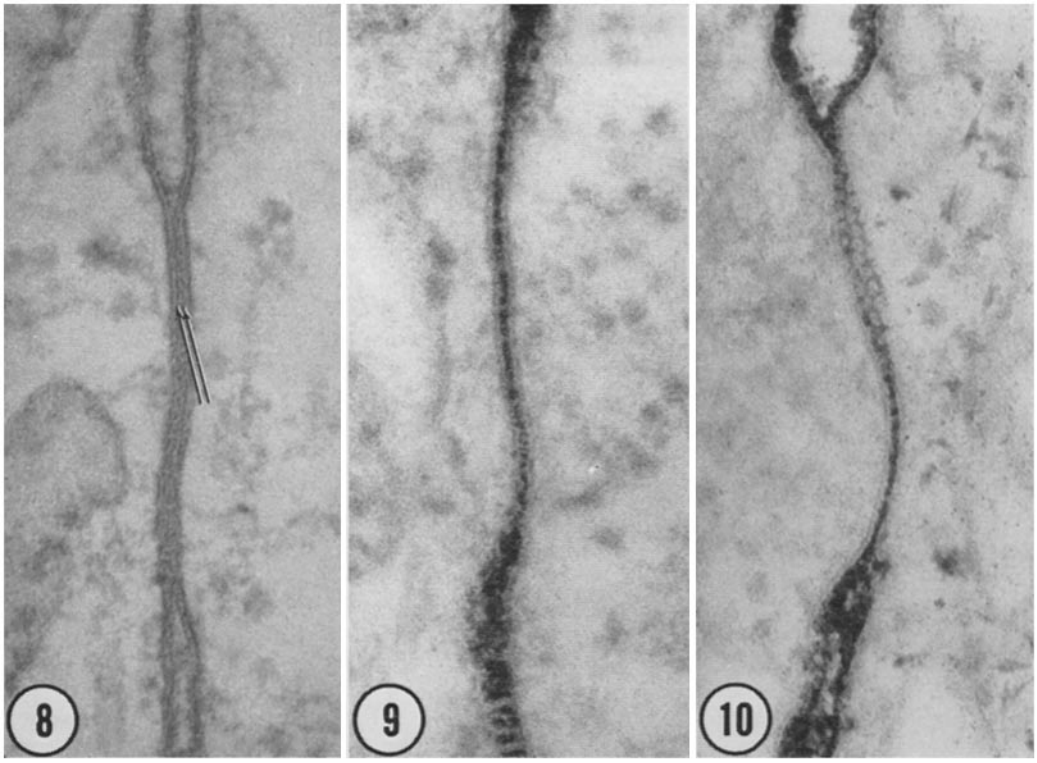


FIGURES 8–10 Electron micrographs of gap injunctions of caecal cells in transverse section.

FIGURE 8 The gap junction in routinely fixed material, with an extracellular space of approximately 30 A clearly visible between the arrows. × 173,000.

FIGURE 9 A gap junction after infiltration of the extracellular space with lanthanum hydrosol. Part of a septate junction is also seen in the lower part of the field. × 173,000.

FIGURE 10 Another lanthanum-treated gap junction. As the junction twists, one can compare the transverse appearance of the gap junction with the tangential view which is characterized by an array of electron-lucent particles. × 173,000.

two types of intercellular specializations occur with no apparent relationship to one another; they may lie contiguously, or may be separated widely. Either structure may occur as an isolated macula, or as a cluster of several maculae separated by small regions of intercellular space of typical nonjunctional dimensions.

At a caecal gap junction, the irregular inter-

FIGURES 5–7 Electron micrographs of lanthanum hydrosol–treated septate junctions.

FIGURE 5 A section tangential to the cell membrane, showing the septa as elongated structures forming a loose, irregular pattern. This is the most frequently encountered configuration of the septate junction of *Daphnia*. × 145,000.

FIGURE 6 Another tangentially sectioned septate junction displaying a compact, moderately regular array of septa. × 145,000.

FIGURE 7 A septate junction, demonstrating the continuity of the structure giving rise to the transverse and tangential appearances shown in preceding figures. × 145,000.

cellular space abruptly narrows to a 30 A "gap," slightly wider than that generally reported for mammalian gap junctions (11, 14) (Fig. 8). Observations of tangential sections and of serial cross-sections of these junctions indicate that they have the shape of a macula approximately 0.5 μ in diameter (Figs. 11 and 13). Throughout the extent of a junction, the membranes involved maintain a uniform separation; regions of actual contact of the outer leaflets of adjacent membranes are not observed.

Treatment of the hepatic caecum with lanthanum tracers during or after fixation reveals additional features in the gap junctions. In sections transverse to the plane of a junctional macula, lanthanum is observed to fill the attenuated intercellular region (Figs. 9 and 10). In some transverse sections, the density of the tracer-filled junction shows periodic interruptions by less dense regions. Sections oblique to the macular plane, and those cutting the macula *en face*, reveal that the intercellular space of the caecal gap junction contains rounded, electron-lucent particles of approximately 95 A diameter (Figs. 11-13). These particles are variously disposed in different regions of a junction; some particles are tightly packed in hexagonal array, while others lie more widely separated and less regularly arranged. In regions of close hexagonal packing, center-to-center distances approximate 120 A. The majority of the particles have at their centers punctate densities 15 A or less in diameter.

Some of the material examined was fixed after exposure to hypertonic saline solutions. Under these conditions the cells decreased in volume, greatly enlarging the extracellular space. As in previously studied cases, the apposed cell membranes at the sites of gap junctions retained their normal spacing.

DISCUSSION

It has been postulated that both gap and septate junctions mediate low-resistance electrical coupling between cells. A correlation between the presence of gap (then termed "tight") junctions and electrical coupling was established as early as 1963 by Robertson, who studied an electrical synapse of the goldfish brain (13). In the cases of cardiac muscle and smooth muscle, two other excitable tissues in which electrotonic coupling has been demonstrated (1, 3), gap junctions can again be found (4, 11, 12). They have also been observed

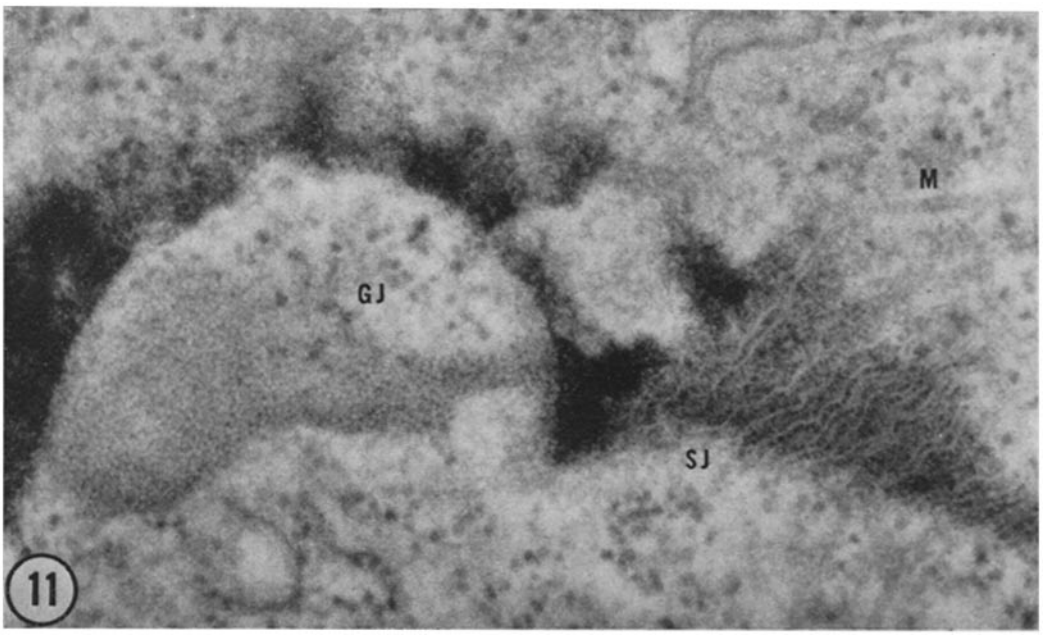
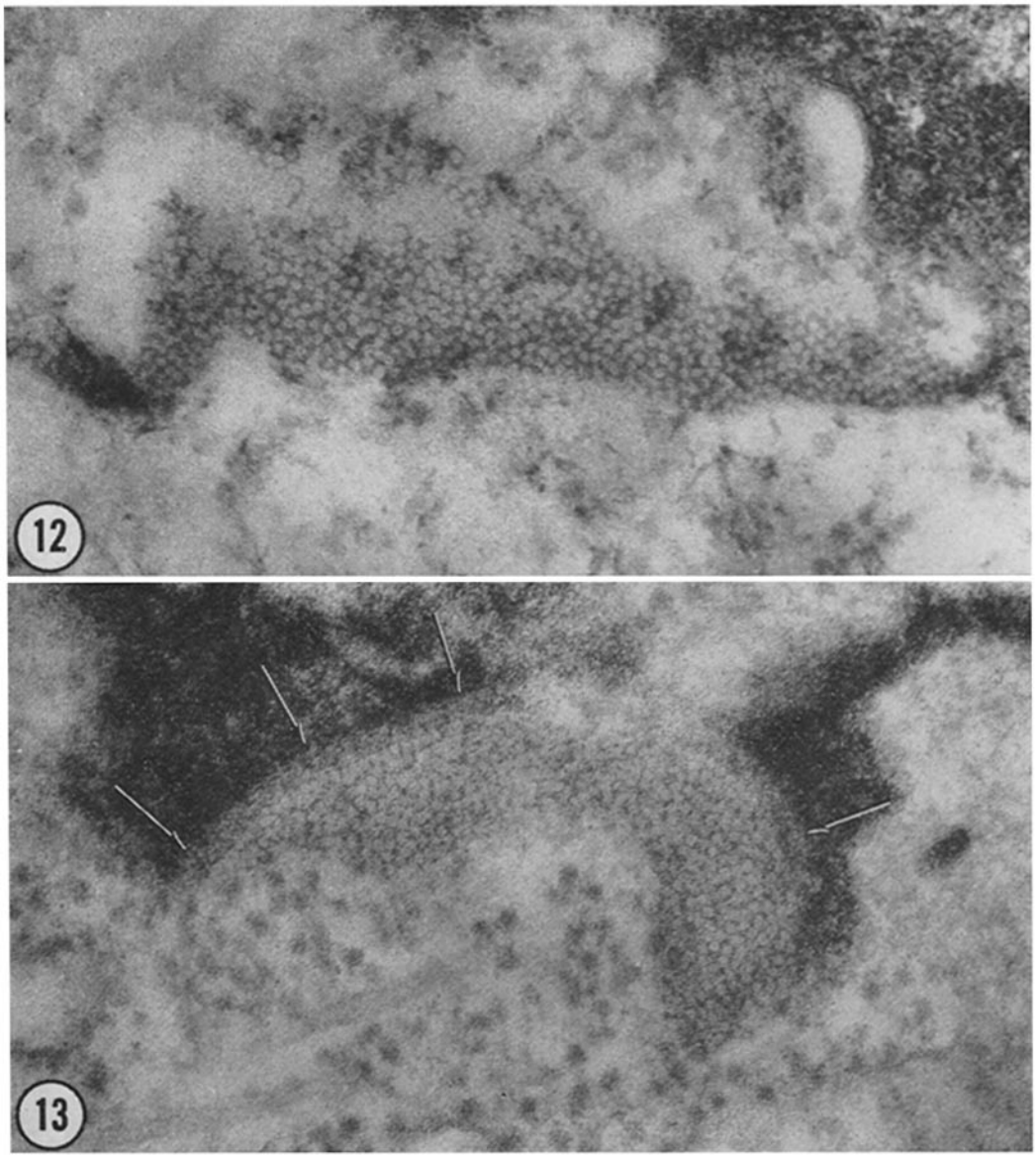


FIGURE 11 An electron micrograph of lanthanum-treated caecal tissue, demonstrating the coexistence of gap (*GJ*) and septate (*SJ*) junctions between two cells. Both junctions have been cut tangentially. It is apparent that the gap junction is macular in shape and lies largely within the section. Several microtubules (*M*) lie in the cytoplasm adjacent to the junctions. × 91,000.



FIGURES 12 and 13 Electron micrographs of tangential sections of lanthanum hydrosol–treated gap junctions in caecal cells.

FIGURE 12 A gap junction consisting of an array of electron-lucent particles, some with small central densities. Particle diameters here are approximately 95 A, and the closest center-to-center spacings are roughly 120 A. This structure closely resembles the gap junction of mammalian tissues except that the particle dimensions of caecal junctions are slightly larger than those found in mammalian tissues. × 144,000.

FIGURE 13 A large portion of a gap junctional macula. Note that this macula is sharply circumscribed, and that it is enclosed by a belt of relatively electron-lucent material (arrows). Such boundaries are frequently encountered in the present material. × 144,000.

between nonexcitable cells such as liver cells (11), which are linked to one another by low-resistance electrical pathways (10). The most direct evidence for the involvement of gap junctions in electrical communication is the persistence of coupling between cardiac muscle cells after disruption of the desmosomal components of the intercalated discs (5).

The involvement of septate junctions in electrical coupling has been inferred by Loewenstein and his collaborators after study of the salivary glands of *Drosophila* and of *Chironomus* (8, 9). They have examined the intercellular contacts of these tissues and have concluded that the junctions most suitable for mediating electrotonic coupling are septate junctions (14). This idea has recently been amplified by studies of freeze-etched junctions in the mussel gill filaments (6).

The relationship between any particular junction and the physiological phenomenon of low-resistance intercellular coupling is at present only circumstantial. However, the evidence supporting a coupling function for the gap junction is now compelling, and the widespread occurrence of this structure argues for its universal application in this role. It seems unlikely that, if the gap and the septate junctions carry out the same physiological functions, both would occur in the same tissues. However, there have recently been several unpublished reports of the presence of gap junctions, along with septate junctions, in the *Drosophila* salivary gland.[1] Preliminary investigations on *Mya* and on *Hydra* by Revel, Hay, and Rosenblith,[2] and results described orally by Gilula and Satir,[3] as well as the observations reported in the present communication, indicate that gap and septate junctions can defintely coexist. Accordingly, it may be unnecessary to postulate a coupling function for the septate junction; those coupled invertebrate systems which have been considered to contain only septate junctions merit reinvestigation with the more refined methods that are now available. Such study may well provide a simpler and more coherent view of the relationship between structure and function in cell-to-cell communication.

[1] Unpublished observations; D. M. Phillips and B. Rose, personal communications; R. S. Reese, personal communication in Bennett and Trinkaus, 1970 (2).

[2] Presented as a demonstration at the meeting of the American Association of Anatomists, Kansas City, Mo., April, 1968.

[3] Additional data on the occurrence of gap junctions along with septates was presented by Gilula and Satir at the meeting of the American Society for Cell Biology, San Diego, Calif., November, 1970.

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