THE STRUCTURAL ORGANIZATION OF THE SEPTATE AND GAP JUNCTIONS OF *HYDRA*

ARTHUR R. HAND and STEPHEN GOBEL

From the Laboratory of Biological Structure and The Neural Mechanisms Section, National Institute of Dental Research, National Institutes of Health, Bethesda, Maryland 20014

ABSTRACT

The septate junctions and gap junctions of *Hydra* were studied utilizing the extracellular tracers lanthanum hydroxide and ruthenium red. Analysis of the septate junction from four perspectives has shown that each septum consists of a single row of hexagons sharing common sides of 50–60 A. Each hexagon is folded into *chair configuration*. Two sets of projections emanate from the corners of the hexagons. One set (*A* projections) attaches the hexagons to the cell membranes at 80–100-A intervals, while the other set (*V* projections) joins some adjacent septa to each other. The septate junctions generally contain a few large interseptal spaces and a few septa which do not extend the full length of the junction. Basal to the septate junctions the cells in each layer are joined by numerous gap junctions. Gap junctions also join the muscular processes in each layer as well as those which connect the layers across the mesoglea. The gap junctions of *Hydra* are composed of rounded plaques 0.15–0.5 μ in diameter which contain 85-A hexagonally packed subunits. Each plaque is delimited from the surrounding intercellular space by a single 40-A band. Large numbers of these plaques are tightly packed, often lying about 20 A apart. This *en plaque configuration* of the gap junctions of *Hydra* contrasts with their sparser, more widely separated distribution in many vertebrate tissues. These studies conclude that the septate junction may possess some barrier properties and that both junctions are important in intercellular adhesion. On a morphological basis, the gap junction appears to be more suitable for intercellular coupling than the septate junction.

INTRODUCTION

Recent physiological studies have demonstrated low-resistance intercellular pathways in many tissues (Loewenstein and Kanno, 1964; Furshpan, 1964; Barr et al., 1965; Penn, 1966; Bennett et al., 1967; Payton et al., 1969) and have stimulated intensive investigation of the morphology of intercellular junctions. Gap junctions have been designated as the morphological site of these intercellular pathways in several vertebrate tissues (Furshpan, 1964; Barr et al., 1965; Penn, 1966; Bennett et al., 1967) as well as in invertebrate tissues (Payton et al., 1969; Pappas et al., 1971).

In Loewenstein's initial studies in the *Drosophila* salivary gland, septate junctions were implicated in intercellular coupling (Wiener et al., 1964). However, later studies have shown that gap junctions are also found in this gland (Phillips and Rose, as cited by Hudspeth and Revel, 1971). Septate junctions are not confined to invertebrate epithelial tissues as originally thought. In a very few instances, they have also been described in vertebrate tissues (Barros and Franklin, 1968; Lasansky, 1969) including at least one site in the vertebrate central nervous system (Gobel, 1971). The function of the septate junction, some twelve

years after its discovery, is still poorly understood, and its possible role in intercellular communication is still being intensively investigated (Satir and Gilula, 1970; Gilula et al., 1970; Rose, 1971). In view of its distribution and the general lack of information about the functional properties of the septate junction, we have reexamined its structure utilizing extracellular tracers and uranyl acetate blockstaining techniques.

Hydra was selected as a model for this study for several reasons. The septate junctions, which join the outer and lumenal margins of the cells of the two layers (epidermis and gastrodermis), are readily accessible to extracellular tracers. The hydroid septate junction is well suited for a two-dimensional analysis since it is made up of regularly spaced, parallel septa (Wood, 1959) in contrast to the corrugated or honeycombed septa found in the septate junctions of higher species (Locke, 1965; Danilova et al., 1969; Satir and Gilula, 1970). In addition, a micrograph in Wood's study (1961) suggested to us that gap junctions also may occur in *Hydra*.

The present study describes a lattice-like organization of the septate junction in which individual septa consist of a chain of folded hexagons which are attached to the cell membranes and to each other by short projections. The cells of *Hydra* are also connected by gap junctions organized in closely packed, rounded plaques. The septate and gap junctions of *Hydra* are evaluated from a morphological standpoint with respect to their role in intercellular adhesion, as permeability barriers, and in intercellular coupling.

MATERIALS AND METHODS

Individual *Hydra* (*Pelmatohydra oligactis*, General Biological Supply House, Inc., Chicago, Illinois) were sucked up into a medicine dropper from the water in which they were shipped, allowed to relax, and ejected into a large volume of fixative. The animals were fixed for 30–60 min in a mixture of 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer, pH 7.4 (Karnovsky, 1965). After an overnight wash in 0.1 M sodium cacodylate buffer with 7.5% sucrose, pH 7.4, they were postfixed for 30–60 min in a solution of 1% OsO_4 in either the cacodylate buffer or Veronal acetate buffer (Zetterqvist, 1956), and stained in block with 0.5% uranyl acetate (Karnovsky, 1967). *Hydra* were also fixed in the above sodium cacodylate-buffered aldehyde solution which contained either 1–2% lanthanum hydroxide (Revel and Karnovsky, 1967) or 500 ppm ruthenium red (Luft, 1964). In these experiments, similar concentrations of these extracellular tracers were included in the buffer wash and in the OsO_4 solutions. In some experiments, *Hydra* were initially fixed in aldehydes for 5–10 min before being placed in fixatives with lanthanum hydroxide. There were no observable differences between the two lanthanum procedures. After dehydration in ethanol and embedding in epoxy resin (Spurr, 1969), thin sections of the mouth and body column were cut transversely and longitudinally, and stained with lead citrate (Venable and Coggeshall, 1965).

RESULTS

Septate Junctions

The septate junctions of *Hydra* are found between the outer regions of adjacent epidermal cells and between the lumenal regions of adjacent gastrodermal cells where they follow a tortuous course, frequently uniting interdigitating processes of adjacent cells. An analysis of these septate junctions as seen in different section planes has led us to propose the following three-dimensional model for the structure of the septa (Fig. 1). Each septum is composed of a backbone which consists of a single row of hexagons sharing common sides of 50–60 A. Each hexagon is folded into *chair configuration*, a term used to describe a

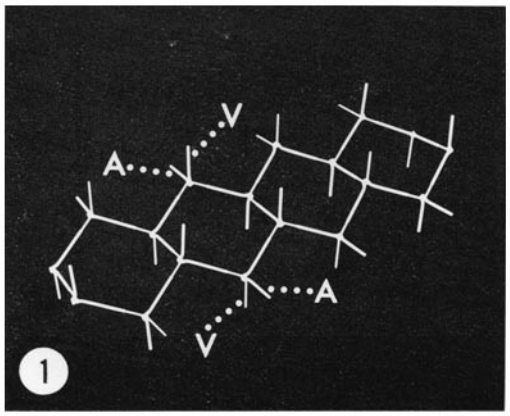


FIGURE 1 Photograph of a model of a portion of a single septum. Each septum consists of a backbone made up of a row of hexagons sharing common sides and folded into *chair configuration*. *V* projections extend vertically from each corner of the hexagons. In addition, each hexagon emits two *A* projections. In succeeding figures this model has been photographed on end (Fig. 6), from above (Fig. 9), and from its side (Figs. 13 and 16).

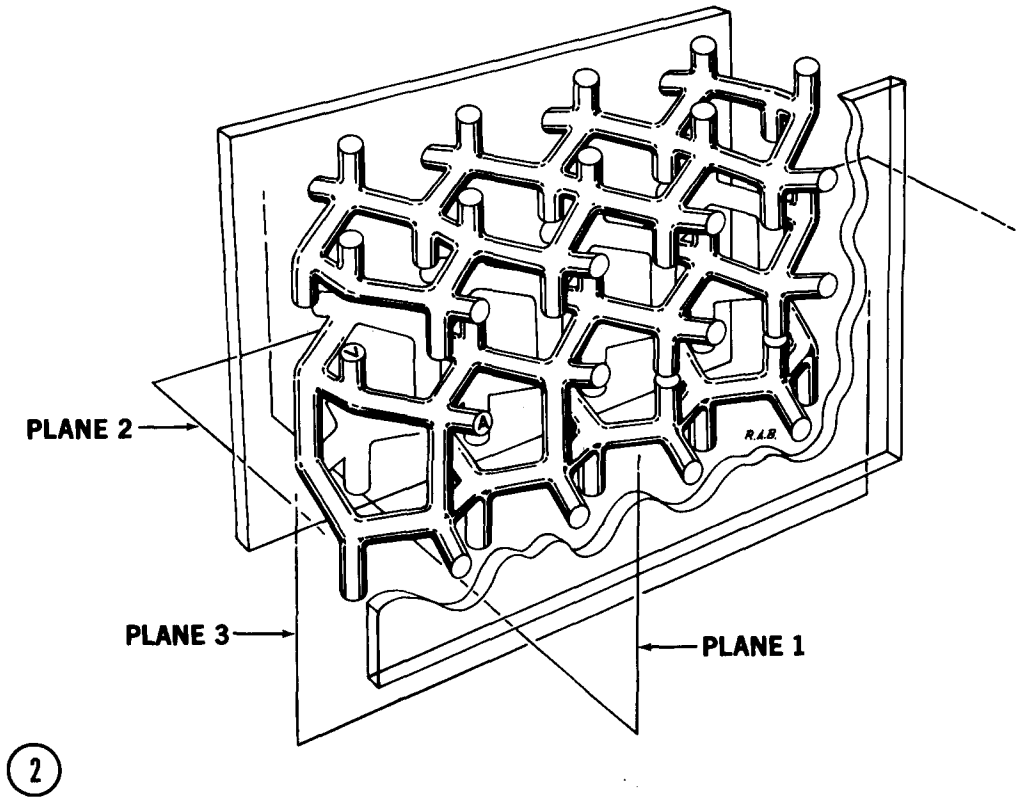


FIGURE 2 Schematic drawing illustrating the interseptal relationships between three septa and the orientation of the section planes used in the analysis. The folds of the upper and middle septa are oriented parallel to each other (see text) and their *V* projections are out of alignment. The folds of the middle and lower septa are inverted with respect to each other and show fusion of their aligned *V* projections at the left. The membrane in the foreground has been cut away in order to reveal the *A* projections which join the hexagons of the septa to the cell membranes.

specific configuration of the hexagonal structure of the cyclohexane molecule, in which opposite corners of the hexagon lie on different sides of an equatorial plane through the hexagon. Projections extend vertically from each of the corners of the hexagons either above or below the backbone, depending on the position of the corner with respect to the equatorial plane (Figs. 1 and 2). Such projections will be referred to as *V* projections. Another set of projections extend from each of the corners of the hexagons which face the cell membranes and attach the backbone to the membranes (Figs. 1 and 2). These projections will be referred to as *A* projections. Consequently, each corner of the hexagons is formed by four intersecting lines which meet in tetrahedral fashion.

When the model (Fig. 1) is viewed either on end (Fig. 6), from above (Fig. 9), or from its side (Figs. 13 and 16), one obtains different images with respect to the form of the backbone and the periodicity of the *A* and *V* projections. These different images result from superimposition of parts of the model and from foreshortening of some of the angles. The following description correlates electron microscope observations of septate junctions viewed from different planes with the model which has been photographed from corresponding angles. The structure of septa from junctions between gastrodermal cells and epidermal cells is similar and the model is based upon observations of junctions in both locations.

When a septate junction is viewed at right angles to the long axis of the septa, each septum extends across the intercellular space between adjacent cell membranes, producing the typical ladder-like image of septate junctions (Fig. 3).

Such views are defined as plane 1 (Fig. 2). Each septum as seen in plane 1 appears as a moderately electron-opaque bar, which emits *V* projections that extend toward the adjacent septa (Fig. 3). The periodicity of the septa varies between 130 and 170 A, although large interseptal spaces occasionally interrupt this pattern (Figs. 10 and 17). Lanthanum hydroxide and ruthenium red gain access to the septate junctions of both cell layers and fill the interseptal spaces. In the presence of these substances the septa span the intercellular space and are seen in negative contrast. They appear as folded electron-lucent bands 20–30–A thick, with *V* projections extending from the peaks of the folds (Figs. 4 and 5). The *V* projections of adjacent septa occasionally appear connected across the interseptal space (Figs. 3, 4, and 5). Fig. 6 shows the model (Fig. 1) viewed on end and illustrates the folds and peaks which result from the superimposition of parts of the backbone as well as the alignment of the *V* projections seen in plane 1.

Septate junctions oriented so that the section plane passes between the septa provide opportunities to view the septa from above. Such views are defined as plane 2 (Fig. 2). The junction, as seen in plane 2, contains a rather amorphous band of

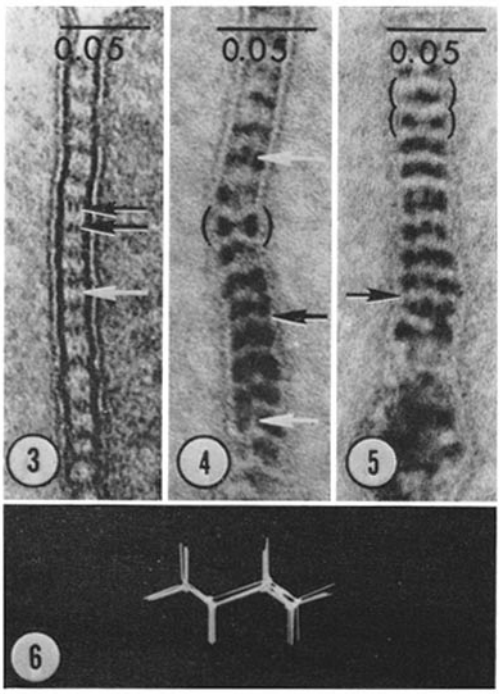


FIGURE 3 Plane 1 view of a septate junction. The septa extend across the intercellular space, and *V* projections (black arrows) extend toward adjacent septa. Some septa appear to be joined by their *V* projections (white arrow). In some instances the outer leaflets of the cell membranes are thickened following uranyl acetate block staining. Gastrodermis. × 243,000.

FIGURES 4 and 5 In plane 1 views of septate junctions filled with extracellular tracers, the septa appear as folded electron-lucent bands (black arrows). *V* projections (white arrows) emanate from the peaks of the folded septa. The pairs of septa within the brackets are inverted with respect to their folds. At the top of Fig. 4 the septa become progressively thickened and blurred as they come out of plane 1 alignment. Epidermis. Ruthenium red. × 258,000.

FIGURE 6 A photograph of the model viewed on end which corresponds to a plane 1 view of a septum. This shows a folded band with projections emanating from each peak.

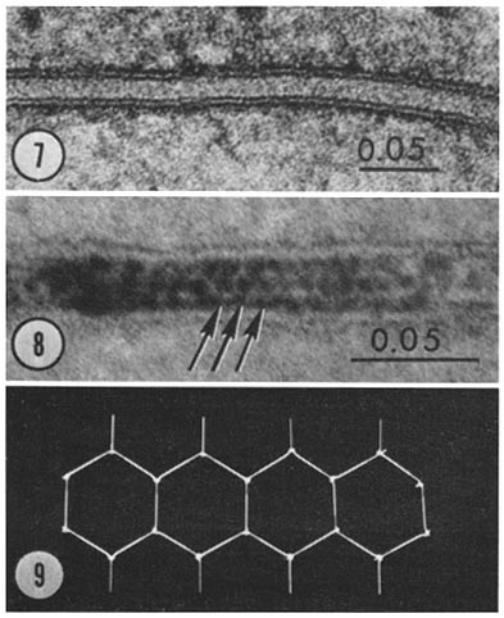


FIGURE 7 A plane 2 view reveals moderately electron-opaque material within the junction. Epidermis. × 210,000.

FIGURE 8 In plane 2, tracers outline a row of hexagons (arrows) with a periodicity of 80–100 A. *A* projections attach the hexagons to the cell membranes at similar intervals. Epidermis. Ruthenium red. × 340,000.

FIGURE 9 A photograph of the model viewed from above which corresponds to a plane 2 view of a septum. This shows a row of hexagons with their *A* projections. The *V* projections are not visible because of superimposition, and the folded nature of the hexagons is not evident because of foreshortening.

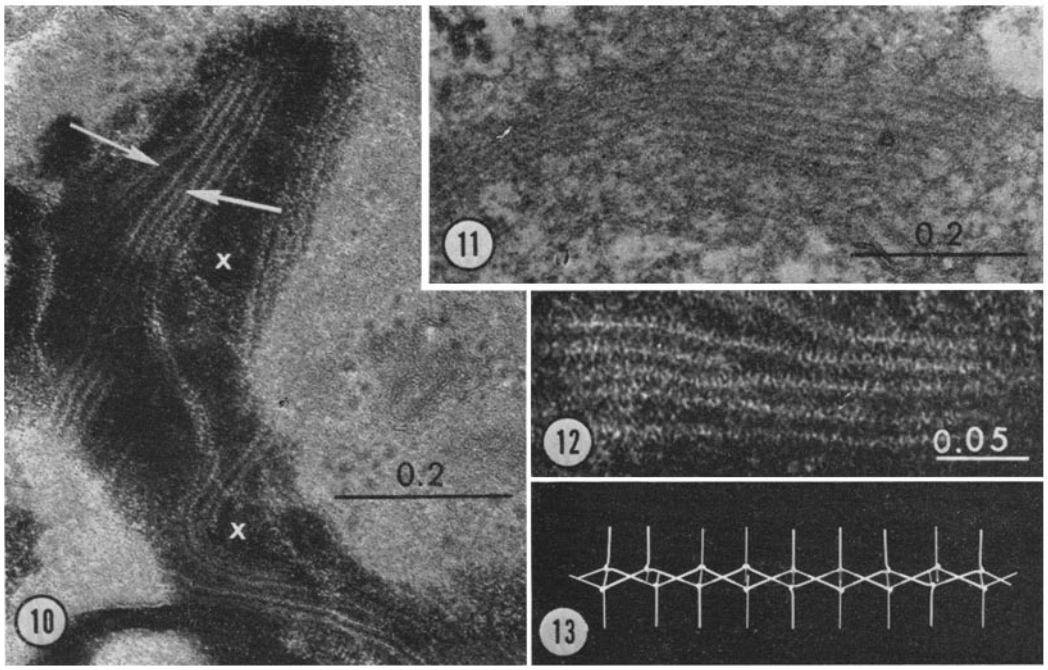


FIGURE 10 In plane 3, the septa show gentle curvatures along their length and often enclose wide interseptal spaces (*X*). A few septa terminate without extending the full length of the junction (arrows). Epidermis. Lanthanum hydroxide. × 117,000.

FIGURE 11 The septa appear as thin, flattened sheets in plane 3 views of routinely fixed junctions. The curving nature of the septa is also evident. Gastrodermis. × 116,000.

FIGURE 12 Plane 3. A portion of Fig. 10 at higher magnification. In the presence of tracers the septa appear as a central band 20–30-A thick with *V* projections extending from each side at 40–50-A intervals. × 246,000.

FIGURE 13 When the model is viewed from the side, the backbone partially superimposes to form a central band, with *V* projections extending from both sides of the backbone.

moderately electron-opaque material between the membranes (Fig. 7). When the interseptal spaces are filled with lanthanum or ruthenium red, a row of small hexagons is outlined. These hexagons share common sides 50–60-A–long, and have a center-to-center spacing of 80–100 A (Fig. 8). *A* projections, about 30-A–long, leave the corners of the hexagons which face the cell membranes and appear to contact the latter perpendicularly, in effect joining the hexagonal backbone to the cell membranes (Fig. 8). The *A* projections are also spaced 80–100-A apart. Fig. 9 shows the appearance of the model when viewed from above and illustrates the hexagons and the *A* projections. The *V* projections are not evident in this plane because of superimposition, and the folded nature of the hexagons cannot be discerned because of foreshortening.

Section planes which pass through septate junctions parallel to the cell membranes are defined as plane 3 (Fig. 2), and provide side views of the long axis of the septa. In plane 3, the septa appear as thin, flattened sheets which commonly exhibit gentle curvatures (Fig. 11). Groups of septa parallel each other for varying distances but may occasionally diverge to encompass fairly large interseptal spaces (Fig. 10). In most junctions, a few septa can be found which do not extend the full length of the junction (Fig. 10). In the presence of lanthanum or ruthenium red each septum consists of a linear backbone 20–30-A –thick. The *V* projections on each side of the back-

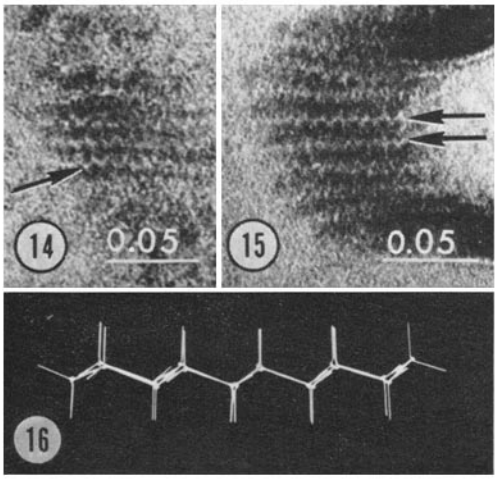
bone are spaced about 40–50 A apart (Fig. 12). The *V* projections from adjacent septa occasionally appear in register and probably fuse in the interseptal space. Fig. 13 is a photograph of the long axis of the model viewed from its side, illustrating the partial superimposition of the backbone and the alignment of the *V* projections.

If one end of the model, oriented as in Fig. 13, is raised to a point where the model forms an angle of 30° with the plane of the page, the backbone and the *V* projections assume a configuration which is shown in Fig. 16. The form of the backbone seen in septate junctions viewed in this plane (Figs. 14 and 15) is a result of the superimposition of the sides of the hexagons and the *A* projections. The periodicity of the *V* projections on either side of the backbone is ~100 A, or about twice that of the *V* projections as they appear in plane 3 views.

Gap Junctions

The epidermal and gastrodermal cells are also joined extensively by gap junctions. These junctions span the intercellular space at numerous points basal to the septate junction (Fig. 17). They are also found between muscular processes which lie on the mesoglea (Fig. 18) and between processes of each layer which meet within or cross the mesoglea (Fig. 19). A gap junction may also lie quite close to a septate junction (Figs. 17 and 22). When sectioned transversely, the gap junctions are short (usually 0.5 μ or less), occur in series, and are separated by short irregular dilations of the intercellular space (Figs. 18 and 20). On rare occasions one finds what appears to be an exceptionally long gap junction (Figs. 21 and 22). The cell membranes lie 30–40 A apart within the junction (Fig. 18).

When *Hydra* are fixed in the presence of lanthanum hydroxide, the lanthanum permeates the gap junctions and appears as a 75-A-thick line within transversely sectioned gap junctions (Fig. 22). Lanthanum is always retained in the gap junctions, although it is usually lost from the irregular intercellular spaces between adjacent gap junctions during processing of the specimens. In a few instances, however, the lanthanum is also retained within these spaces as well as within the gap junctions. When such areas are sectioned approximately parallel to the cell membranes, i.e., *en face*, the topographical and structural organization of the gap junctions is clearly revealed (Fig. 22). Each junctional region consists of numerous, tightly packed, rounded plaques surrounded by irregular pools of intercellular space. The plaques range from 0.15 to 0.5 μ in diameter. 65 individual plaques have been counted in an 8 μ^2 area. In this *en plaque* configuration, some plaques lie within 20 A of each other (Fig. 22). Each plaque is encircled by a thin electron-lucent band ~40-A-thick. The interior of each plaque consists of hexagonally packed subunits. Each subunit is clearly outlined by the lanthanum and is ~85 A in diameter. A central electron-opaque particle, ~20 A in diameter, is visible in most of the subunits. As in vertebrate gap junctions, the subunits have a center-to-center spacing of 95–110 A. The subunits are separated from the electron-lucent band by a relatively amorphous, lanthanum-filled zone 50–150-A-thick.



FIGURES 14 and 15 A folded band (arrows) with *V* projections from each peak is observed when septate junctions are sectioned obliquely. Epidermis. Fig. 14, Ruthenium red. Fig. 15, Lanthanum hydroxide. ×246,000.

FIGURE 16 When one end of the model is raised from its position in Fig. 13 to form an angle of 30° with the page, the sides of the hexagons and the *A* projections superimpose to form a folded band, and the *V* projections extend from each peak.

DISCUSSION

From our observations we have drawn the following conclusions about the distribution of the intercellular junctions of *Hydra*. Septate junctions join the outer and lumenal surfaces, respectively, of

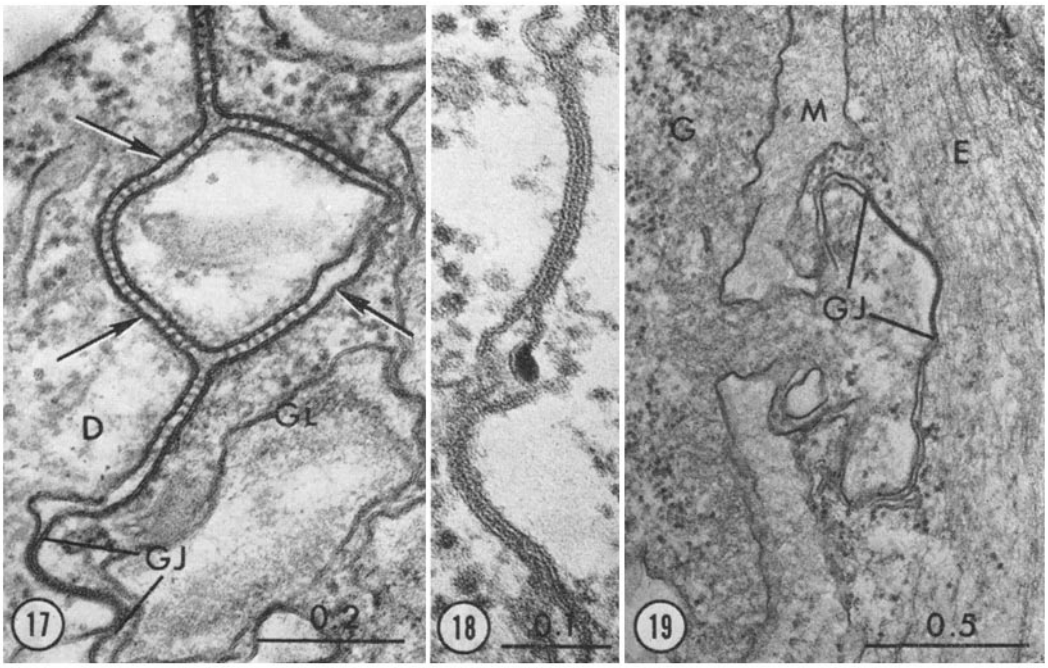


FIGURE 17 A gap junction (*GJ*) is located basal to a septate junction that contains several wide interseptal spaces (arrows). *D*, digestive cell; *GL*, gland cell. Gastrodermis. × 97,000.

FIGURE 18 Two short gap junctions join adjacent muscular processes. The cell membranes lie ~ 30 A apart in the junctions. A dilation of the intercellular space separates the two junctions. Epidermis. × 144,000.

FIGURE 19 Part of the muscular process of a gastrodermal cell (*G*) crosses the mesoglea (*M*) and lies within a recess of the muscular process of an epidermal cell (*E*). A 0.5 μ long gap junction (*GJ*) joins these processes. × 43,000.

the epidermal and gastrodermal cells. While they completely encircle the apical regions of adjacent cells, their extent with respect to the short axis of the animal varies considerably. In some instances they are quite long and tortuous, while in others they are short and consist of only a few septa. Some junctions may run parallel to the outer or lumenal cell membranes for short distances.

Basal to the septate junctions, adjacent cells of each layer are joined extensively by numerous gap junctions in *en plaque* configuration. Gap junctional plaques also join the muscular processes in each layer as well as those which connect the layers across the mesoglea. While desmosome-like structures occur between muscular processes (Haynes et al., 1968), they are conspicuously absent from other areas of the cells. Tight junctions (*zonulae occludentes*) have not been found in *Hydra*.

Morphology of the Septate Junction

Since the initial description of the septate junction (Wood, 1959), several models of the septa have been suggested. Generally these models were similar, and, like Wood's original one, each proposed that the septa consisted of separate barlike plates. These plates were, depending upon the species, either parallel to each other (Wood, 1959; Wiener et al., 1964; Danilova et al., 1969), somewhat corrugated (Gilula et al., 1970), or organized into a honeycomb (Locke, 1965; Bullivant and Loewenstein, 1968; Danilova et al., 1969). Recent advances in preparative techniques such as uranyl acetate block staining (Karnovsky, 1967) and the use of electron-opaque intercellular

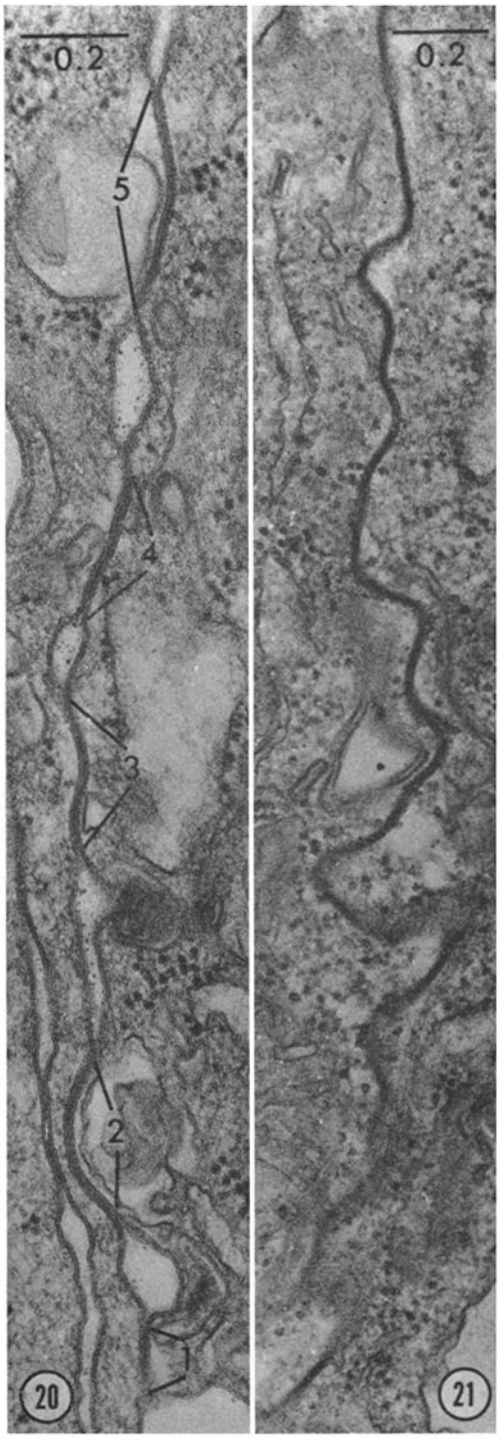


FIGURE 20 A series of five short gap junctions (1–5), each less than 0.5 μ long, join two epidermal cells. The gap junctions are separated by irregular dilations of the intercellular space. Epidermis. × 70,000.

FIGURE 21 Rarely, one encounters a long gap junction; this one is about 4 μ. Gastrodermis. × 63,000.

tracers such as lanthanum hydroxide (Revel and Karnovsky, 1967) and ruthenium red (Luft, 1964) have provided an opportunity to examine the structure of the septate junction in greater detail. It has been possible to depict the three-dimensional structure of a septum as well as some interseptal relationships by utilizing the side length of the hexagon and by comparing the periodicity of the projections and the form of the backbone in the four different views. The four views used in the analysis were selected because they provide critical alignments of the projections and the backbone of the septa. Small deviations from these alignments produce thickened, blurred images of the septa. Essentially, each septum consists of a chain of hexagons, each of which is folded in chair configuration. *V* projections emanate from each of the corners of the hexagons and extend alternatively above or below each hexagon toward the adjacent septa. Two *A* projections join each hexagon to the cell membranes.

Considerable variation in interseptal relationships exists within a septate junction. Plane 1 views show that adjacent septa are inserted within the junction either inverted or parallel to each other with respect to the folds of their backbones (Figs. 4 and 5). Of 104 pairs of septa counted, 57 were in the inverted configuration. In addition, groups of three or more septa may be either parallel or inverted.

V projections often appear to connect adjacent septa (Figs. 3 and 4). The opportunity for such interseptal connections involves two factors: inverted versus parallel pairing of adjacent septa; and superimposition of the hexagons of adjacent septa (Fig. 2). Maximum opportunity for interseptal connections exists in two situations: (*a*) adjacent septa are inverted and their hexagons are superimposed (middle and lower septa of Fig. 2); and (*b*) adjacent septa are parallel and their hexagons are out of phase. Of course, such considerations are based on a rigid model that cannot encompass possible elastic properties or small irregularities in a septum. They nevertheless suggest considerable variation in interseptal connections and in the rigidity of the junction itself.

Morphology of the Gap Junction

The gap junctions of *Hydra* consist of rounded plaques, generally 0.5 μ or less in diameter. The term *en plaque* configuration has been chosen to denote the tight packing of the individual plaques which make up a gap junctional area. The *en plaque* configuration contrasts with the distribution of gap junctions in vertebrate tissues where individual junctions are more widely separated from each other. The shape of individual vertebrate gap junctions has not been determined, although there are suggestions that some may also be rounded plaques (Robertson, 1963; Kreutziger, 1968; McNutt and Weinstein, 1970).

FUNCTIONAL IMPLICATIONS OF THE INTERCELLULAR JUNCTIONS OF HYDRA

Intercellular Adhesion

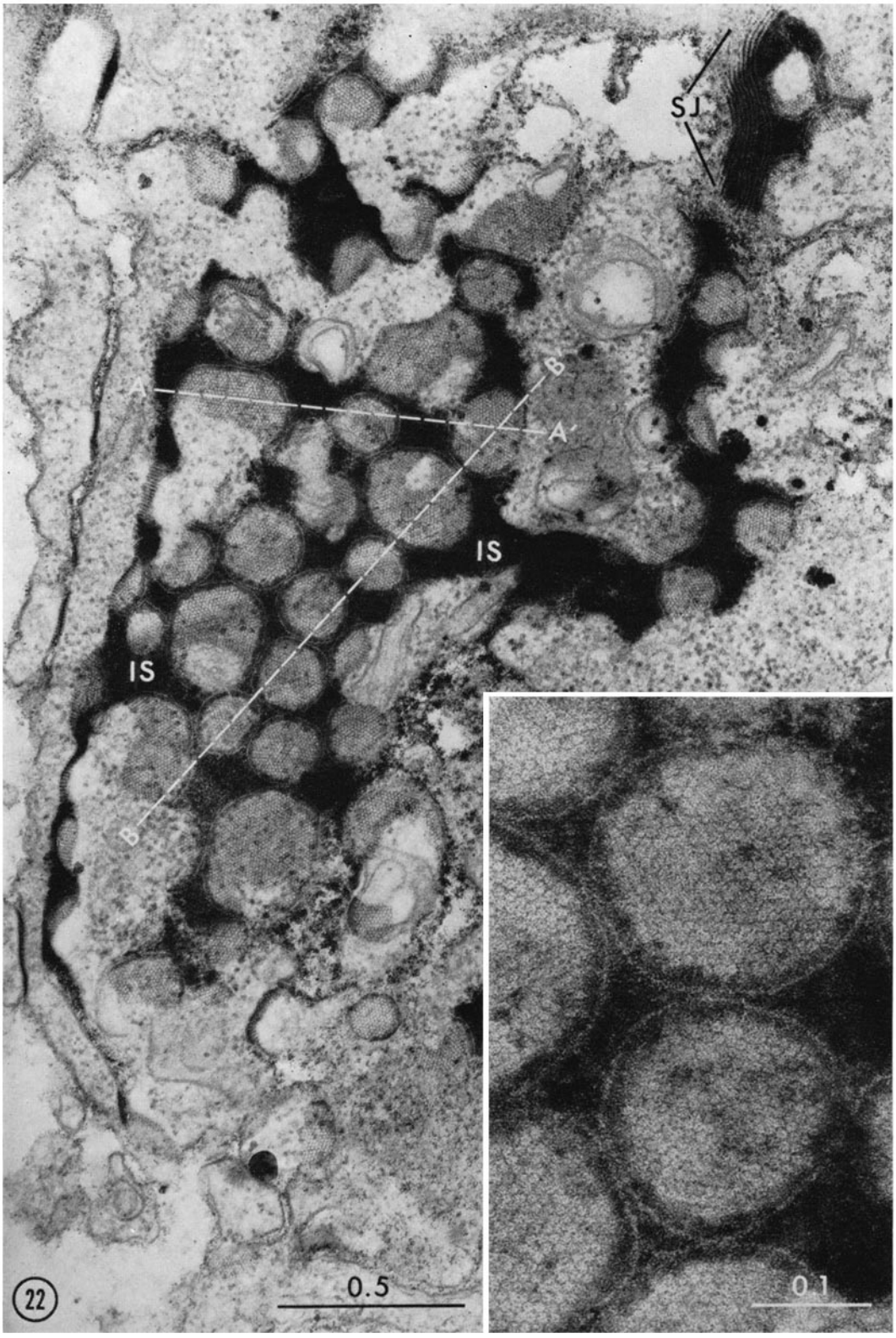
The septate junctions as well as the gap junctions are undoubtedly of prime importance in maintaining topographical relationships within the two epithelial sheets of *Hydra* as the individual cells undergo drastic changes in shape during contraction and relaxation. The structure of the septa, their interseptal connections, and the manner in which they are attached to the cell membranes suggest that the septate junction is a site of firm intercellular adhesion. Gap junctions are also known to be sites of firm intercellular contact which resist osmotic (Brightman and Reese, 1969) and mechanical disruption (Goodenough and Revel, 1970). The *en plaque* configuration of the gap junctions suggests a firm adhesion of the more basal parts of the cells of *Hydra*.

Permeability Barriers

In several different systems (Revel and Karnovsky, 1967; Brightman and Reese, 1969) electron-opaque tracers such as lanthanum hydroxide have been demonstrated within gap junctions. These observations have suggested the existence of extracellular channels within the junction (Pappas et al., 1971) which are presumably permeable to water and small ions. The present study demonstrates that lanthanum hydroxide and ruthenium red readily penetrate the spaces in the lattice-like structure of the septate junction and gain access to the more basal intercellular spaces. However, in spite of these observations and the "open" appearance of the septa, several factors suggest that the septate junction may possess some barrier properties. (*a*) In preliminary studies, we have tested septate junctions in vivo by soaking living *Hydra* in horseradish peroxidase (mol. wt. 40,000, equivalent radius 25–30 A [Karnovsky, 1967]) or cytochrome *c* (mol. wt. 12,000, equivalent radius 15 A [Karnovsky and Rice, 1969]). With each of these tracers, the reaction product was found at a maximum depth of the fourth interseptal space in the epidermal septate junction. Neither of these tracers penetrates the complete depth of the septate junction in spite of the relatively large spaces ($\sim$50–60 A) in the hexagonal lattice. (*b*) The spaces in the septal lattice may actually be filled by substances which are not revealed by our preparative methods. Ruthenium red, which stains acid mucopolysaccharides (Luft, 1964), may be staining components of the intercellular space rather than inertly filling a potential space in the lattice. There is some evidence to suggest that lanthanum hydroxide also stains or binds to some extracellular substances (Doggenweiler and Frenk, 1965; Lesseps, 1967; Overton, 1968). (*c*) If the hexagonal backbone and projections of a septa bear even a weak electrical charge, a septate junction consisting of a stack of 30 or more septa would present a considerable barrier to the movement of many substances through the junction. (*d*) Independent electrophysiological studies of *Hydra* (Josephson and Macklin, 1967, 1969) have demonstrated a high resistance across the body wall and suggest that a barrier to the flow of ions exists at some point in the path from the digestive cavity to the exterior.

Intercellular Coupling

To date, intercellular coupling has not been demonstrated in *Hydra*. However, the paucity of neural elements, especially in the body wall, suggests that such mechanisms may be important in contraction and relaxation of the animal. There are at least two factors which, from a morphological standpoint, suggest that the gap junction is better suited for intercellular coupling than the septate junction. The minimum intercellular distance between cells at the gap junction is $\sim$30 A compared to $\sim$200 A through the septal backbone. A 10–20-A intercytoplasmic channel (McNutt and Weinstein, 1970; Pappas et al., 1971)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could be more readily accommodated within the 85–A subunit of the gap junction than in the 20-A–thick lattice of the septate junction.

Each junctional plaque in *Hydra* is sharply demarcated from the surrounding intercellular space by a thin 40-A band. While lanthanum hydroxide is usually washed out of the intercellular spaces between the plaques during processing, it is retained between the subunits within the plaque. This suggests that the lanthanum may be bound to the subunits or that its movement between the subunits may be relatively small compared to movement through the intercellular spaces which bathe the plaques. Loewenstein and Kanno (1964) pointed out, in their initial discussions of low-resistance intercellular pathways, the need for a barrier or seal to effectively separate the intercytoplasmic channels from the surrounding intercellular space. While barrier properties may be inherent within the design of the individual subunits (see Pappas et al., 1971), it is quite likely that the 40-A band surrounding each plaque isolates the subunits of the gap junction from the intercellular space and forms a barrier to the movement of some substances between the intercellular space and the gap junction.

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REFERENCES

BARR, L., M. M. DEWEY, and W. BERGER. 1965. Propagation of action potentials and the structure of the nexus in cardiac muscle. *J. Gen. Physiol.* **48**:797.

BARROS, C., and L. E. FRANKLIN. 1968. Behavior of the gamete membranes during sperm entry into the mammalian egg. *J. Cell Biol.* **37**:C13.

BENNETT, M. V. L., G. D. PAPPAS, M. GIMÉNEZ, and Y. NAKAJIMA. 1967. Physiology and ultrastructure of electrotonic junctions. IV. Medullary electromotor nuclei in Gymnotid fish. *J. Neurophysiol.* **30**:236.

BRIGHTMAN, M. W., and T. S. REESE. 1969. Junctions between intimately apposed cell membranes in the vertebrate brain. *J. Cell Biol.* **40**:648.

BULLIVANT, S., and W. R. LOEWENSTEIN. 1968. Structure of coupled and uncoupled cell junctions. *J. Cell Biol.* **37**:621.

DANILOVA, L. V., K. D. ROKHLENKO, and A. V. BODRYAGINA. 1969. Electron microscopic study on the structure of septate and comb desmosomes. *Z. Zellforsch. Mikrosk. Anat.* **68**:852.

DOGGENWEILER, C. F., and S. FRENK. 1965. Staining properties of lanthanum on cell membranes. *Proc. Nat. Acad. Sci. U.S.A.* **53**:425.

FURSHPAN, E. J. 1964. "Electrical transmission" at an excitatory synapse in a vertebrate brain. *Science (Washington).* **144**:878.

GILULA, N. B., D. BRANTON, and P. SATIR. 1970. The septate junction: A structural basis for intercellular coupling. *Proc. Nat. Acad. Sci. U.S.A.* **67**:213.

GOBEL, S. 1971. Axo-axonic septate junctions in the basket formations of the cat cerebellar cortex. *J. Cell Biol.* **51**:328.

GOODENOUGH, D. A., and J. P. REVEL. 1970. A fine structural analysis of intercellular junctions in the mouse liver. *J. Cell Biol.* **45**:272.

HAYNES, J. F., A. L. BURNETT, and L. E. DAVIS. 1968. Histological and ultrastructural study of the muscular and nervous systems in *Hydra*. I. The muscular system and the mesoglea. *J. Exp. Zool.* **167**:283.

HUDSPETH, A. J., and J. P. REVEL. 1971. Coexistence of gap and septate junctions in an invertebrate epithelium. *J. Cell Biol.* **50**:92.

JOSEPHSON, R. K., and M. MACKLIN. 1967. Transepithelial potentials in *Hydra*. *Science (Washington).* **156**:1629.

FIGURE 22 In the presence of extracellular tracers, face views reveal the organization of the gap junctions of *Hydra*. The gap junctions are organized in rounded plaques ranging from 0.15 to 0.5 μ in diameter. Each plaque is surrounded by irregular pools of intercellular space (*IS*). Transverse sections through this junctional area would show either a series of short gap junctions as in plane *AA'* (see Fig. 20), or one long junction as in plane *BB'* (see Fig. 21). At the upper right, several plaques lie against a septate junction (*SJ*) which has been sectioned in plane 3. A few transversely sectioned gap junctions are seen at the left. Gastrodermis. Lanthanum hydroxide. × 59,000. Inset: The gap junctional plaques at higher magnification. Each plaque is sharply delimited from the intercellular space by a thin electron-lucent band ~40-A–thick. The interior of the plaques contains the typical hexagonally packed 85-A subunits with their 20-A central electron-opaque particle. In each plaque, a relatively amorphous zone ~100-A–wide separates the subunits from the surrounding band. × 185,000.

JOSEPHSON, R. K., and MACKLIN, M. 1969. Electrical properties of the body wall of *Hydra*. *J. Gen. Physiol.* **53**:638.

KARNOVSKY, M. J. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *J. Cell Biol.* **27**:137A.

KARNOVSKY, M. J. 1967. The ultrastructural basis of capillary permeability studied with peroxidase as a tracer. *J. Cell Biol.* **35**:213.

KARNOVSKY, M. J., and D. F. RICE. 1969. Exogenous cytochrome c as an ultrastructural tracer. *J. Histochem. Cytochem.* **17**:751.

KREUTZIGER, G. O. 1968. Freeze-etching of intercellular junctions of mouse liver. Proceedings, 26th Annual Meeting, EMSA. C. J. Arceneaux, editor. Claitor's Publishing Division, Baton Rouge, La. 234.

LASANSKY, A. 1969. Basal junctions at synaptic endings of turtle visual cells. *J. Cell Biol.* **40**:577.

LESSEPS, R. J. 1967. The removal by phospholipase C of a layer of lanthanum-staining material external to the cell membrane in embryonic chick cells. *J. Cell Biol.* **34**:173.

LOCKE, M. 1965. The structure of septate desmosomes. *J. Cell Biol.* **25**:166.

LOEWENSTEIN, W. R., and Y. KANNO. 1964. Studies on an epithelial (gland) cell junction. I. Modifications of surface membrane permeability. *J. Cell Biol.* **22**:565.

LUFT, J. H. 1964. Electron microscopy of cell extraneous coats as revealed by ruthenium red staining. *J. Cell Biol.* **23**:54A.

MCNUTT, N. S., and R. S. WEINSTEIN. 1970. The ultrastructure of the nexus. A correlated thin-section and freeze-cleave study. *J. Cell Biol.* **47**:666.

OVERTON, J. 1968. Localized lanthanum staining of the intestinal brush border. *J. Cell Biol.* **38**:447.

PAPPAS, G. D., Y. ASADA, and M. V. L. BENNETT. 1971. Morphological correlates of increased coupling resistance at an electrotonic synapse. *J. Cell Biol.* **49**:173.

PAYTON, B. W., M. V. L. BENNETT, and G. D. PAPPAS. 1969. Permeability and structure of junctional membranes at an electrotonic synapse. *Science (Washington).* **166**:1641.

PENN, R. D. 1966. Ionic communication between liver cells. *J. Cell Biol.* **29**:171.

REVEL, J. P., and M. J. KARNOVSKY. 1967. Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *J. Cell Biol.* **33**:C7.

ROBERTSON, J. D. 1963. The occurrence of a subunit pattern in the unit membranes of club endings in Mauthner cell synapses in goldfish brains. *J. Cell Biol.* **19**:201.

ROSE, B. 1971. Intercellular communication and some structural aspects of membrane junctions in a simple cell system. *J. Membrane Biol.* **5**:1.

SATIR, P., and N. B. GILULA. 1970. The cell junction in a lamellibranch gill ciliated epithelium. Localization of pyroantimonate precipitate. *J. Cell Biol.* **47**:468.

SPURR, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastruct. Res.* **26**:31.

VENABLE, J. H., and R. COGGESHALL. 1965. A simplified lead citrate stain for use in electron microscopy. *J. Cell Biol.* **25**:407.

WIENER, J., D. SPIRO, and W. R. LOEWENSTEIN. 1964. Studies on an epithelial (gland) cell junction. II. Surface structure. *J. Cell Biol.* **22**:587.

WOOD, R. L. 1959. Intercellular attachment in the epithelium of *Hydra* as revealed by electron microscopy. *J. Biophys. Biochem. Cytol.* **6**:343.

WOOD, R. L. 1961. The fine structure of intercellular and mesogleal attachments of epithelial cells in *Hydra*. *In* The Biology of *Hydra* and of some other Coelenterates. W. F. Loomis and H. M. Lenhoff, editors. University of Miami Press, Coral Gables, Fla. 51.

ZETTERQVIST, H. 1956. The ultrastructural organization of the columnar absorbing cells of the mouse jejunum. Thesis. Karolinska Institutet, Stockholm, Sweden.