# The use of hydra for studies of cellular ultrastructure and cell junctions

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# THE USE OF HYDRA FOR STUDIES OF CELLULAR ULTRASTRUCTURE AND CELL JUNCTIONS

BY

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## INTRODUCTION

Abraham Trembley is recognized as one of the pioneers of experimental biology. He was a highly inquisitive biologist yet a thoroughly disciplined investigator. He validated findings extensively by careful repetition and additional experimentation before committing them to definitive publication. Trembley was primarily an experimentalist, but he engaged in some descriptive morphology, and all of his work involved exceedingly careful observation. He was fully aware of the value of understanding structure as a basis for better understanding of function in living organisms. Modern cell biology perpetuates this principle by integrating information derived from a battery of sophisticated molecular, physiological and morphological techniques. The boundaries between these various investigative approaches have become increasingly indistinct and cell biology now transcends classical disciplines. Still, the value of thorough observation has not changed, and concentration on a single mode of investigation can reveal significant details that otherwise might be overlooked. The observations to be presented in this paper are based primarily on an ultrastructural approach. Actually, they are a combination of descriptive morphology and analysis of the effects of experimental manipulation. It is a visual approach not unlike that employed by Abraham Trembley, but utilizing more complex tools for visualization.

Hydra were first observed microscopically by van Leeuwenhoek some forty years prior to Trembley's classic experiments on regeneration and grafting. As readily available representatives of the phylum Cnidaria (Coelenterata), they still serve both investigative and instructional purposes. Nearly all of the great figures in biology during the 19th and early 20th centuries were well acquainted with hydra, having spent significant time in their early laboratory training examining them microscopically and

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repeating some of the Trembley experiments, often with their own innovative variations. Thus, it was natural that hydra were also utilized by several investigators during the early phases of the development of biological electron microscopy. The value of the higher resolution available through electron imagery was immediately obvious for elucidating nematocyst structure (Chapman and Tilney. 1958 a and b), but several important "firsts" in general cellular ultrastructure also were recorded during these early investigations. For example, those studies yielded the first unequivocal demonstration of true cytoplasmic bridges between synchronously developing somatic cells (nematoblasts) (Slautterback and Fawcett, 1959); the first description of the septate junction (Wood, 1959); and the first description of cytoplasmic microtubules (Slautterback, 1963). Those early studies also revealed close intercellular contacts that were suggested to play a role in direct cell-to-cell integration (Wood, 1961) (the configuration recognized in 1967 by Revel and Karnovsky as gap or communicating junctions), and the fact that cnidarian muscle processes contain two categories of contractile filaments, as do the muscle fibers of higher organisms (Slautterback, 1967).

Perhaps the most widely studied ultrastructural features of hydra have been the nerves and cell junctions. The junctions have aroused my special interest for several reasons. First, cnidarians have not evolved beyond the tissue level of organization and in hydra the only tissue represented is epithelium. Although not restricted to epithelia, cell junctions are developed in the greatest numbers and diversity in this tissue. Second, the animals are capable of great extensibility and rapid contraction, indicating that firm intercellular adhesion must occur to maintain consistent cellular topography. Third, hydra have a very primitive nervous system yet are capable of fairly intricate behavior, suggesting that direct cell-cell communication may be an important mediator of some of the activities. Fourth, extensive tissue reorganization must accompany regeneration phenomena and yet the animals are able to maintain their osmoregulatory abilities. Fifth, nematocytes develop in prodigious numbers in the body column, and most of them migrate to the tentacles for final positioning. In the tentacles, the four different types of nematocytes are firmly anchored to surrounding epidermal battery cells and to the underlying mesoglea. The sequence of events that results in nematocyte positioning in hydra occurs with extraordinary rapidity and specificity.

Hydra possess nearly the entire gamut of junctional arrangements found throughout the remainder of the animal kingdom. In view of the phylogenetic position of the Hydrozoa, this indicates that cellular junctions have a fundamental importance in the establishment and maintenance of the multicellular state, but it also indicates that there is a relatively limited spectrum of mechanisms that have evolved to a achieve cellular attachment. I will review here the current state of knowledge of three of the major junctional types of hydra: septate, gap and desmosome/hemidesmosome junctions.

## SEPTATE JUNCTIONS

Septate junctions are now recognized as the most characteristic intercellular junction of the invertebrates. Although variations in structural details occur in different phyla (Staehelin, 1974; Lane, 1978; Noirot-Timothee and Noirot, 1980; Green, 1981), the essential organizational features are identical from cnidarians to the arthropods and hemichordates. These junctions are arranged as zonular belts at apicolateral or basolateral surfaces of epithelial cells. In hydra the position is always apicolateral (Figure 1). At the ultrastructural level of resolution, the junction consists of a series of baffle-like partitions that span a 15-20 nm wide intercellular gap and adjoin apposed cell surfaces (Figure 2). The plasma membranes show periodic differences in density that correlates with septal location (Figure 3). Freeze-fracture replicas reveal linear arrays of intramembrane particles arranged in the same pattern as the extracellular septa (Figure 4). This image has a general resemblance to the freeze-fracture morphology of vertebrate occluding junctions where there are anastomosing intramembrane strands at the junctional site (Staehelin, 1974).

The initial suggestion that septate junctions probably perform the dual function of intercellular adhesion and restriction of paracellular permeability (Wood, 1959) has been amply supported by subsequent studies. Rudimentary vertebrate-type occluding junctions (tight junctions) have been found in specialized locations in a few arthropods (Lane, 1980), but even in those organisms tight junctions are limited in distribution, and the septate junction is by far the most prevalent junctional type.

The wide separation of apposed plasma membranes at the septate junction has raised questions about the mechanism by which they could achieve an effective barrier function. It is clear that the mechanism must differ significantly from that of vertebrate occluding junctions where the intercellular space totally collapses in linear focal areas. It has been proposed that the septa introduce a marked increase in length and tortuosity to the intercellular pathway (Staehelin, 1974), and that special ion binding characteristics of the interseptal material probably also contribute to the establishment of a functional barrier (Wood and Kuda, 1980a). As would be expected for an occluding type junction, the size of septate junctions is noticeably greater in fresh water and terrestrial organisms than in closely related marine forms because of differences in requirements for osmoregulation by organisms living in the different environments. Studies with electron dense tracers indicate that septate junctions are not as effective in preventing paracellular flux as are vertebrate tight junctions (Staehelin, 1974; Lane, 1978). Nevertheless, in hydra the septate junctions are effective enough to maintain a favorable sodium gradient, which is the basis for osmoregulation in these fresh water organisms (Josephson and Macklin, 1967; Prusch et al., 1976).

Septate junctions are relatively stable, but they are still capable of rapid modulation. For example, successful regeneration of hydra polyps following cellular dissocia-

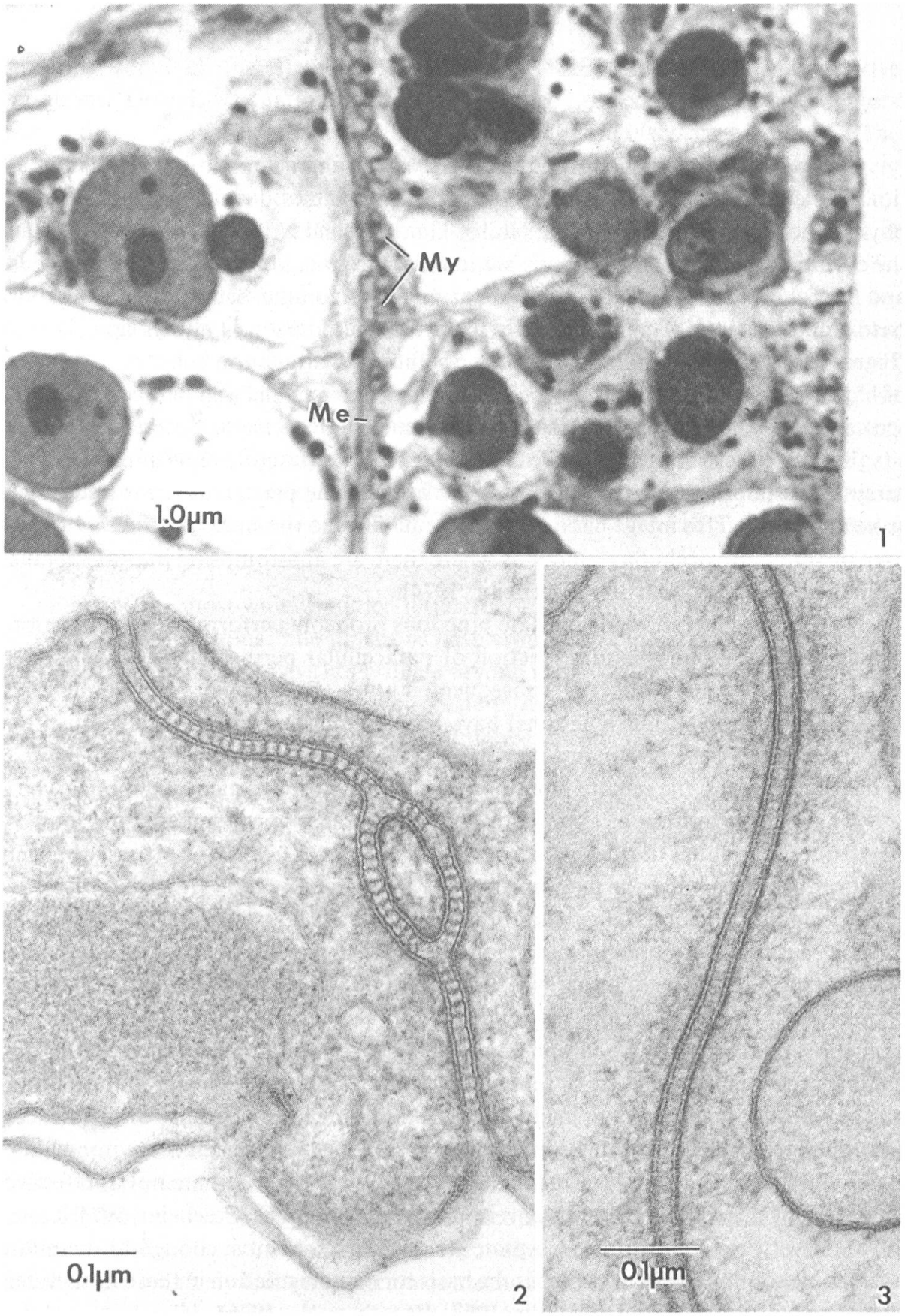



FIG. 1. — Light Micrograph of cross sectioned body wall of *Hydra oligactis.* The epidermis is at right. Apicolateral junctions appear at the arrows. My, myonemes, Me, mesoglea. X 3800.

FIG. 2. — Septate junction from regenerated *Hydra attenuata.* X 110,000.

FIG. 3. — Septate junction of adult *Hydra attenuata* showing intramembrane periodicity coincident with extracellular septa. X 133,400.

tion and reaggregation depends upon the ability to reestablish an epithelial configuration and osmoregulation. These essential phenomena occur in the first few hours of regeneration and are correlated with the rapid reassembly of septate junctions (Wood and Kuda, 1980a). The rapidity of this recovery strongly suggests that junctional subunits are dispersed within the membrane during cellular dissociation and can be reutilized for assembly of new junctions without the necessity of new synthetic activity.

## GAP JUNCTIONS

The need for a mechanism to permit direct physiological exchanges between cells in multicellular organisms was recognized from the earliest elucidation of the cell theory (see Wilson, 1928; Baker, 1952). Cytoplasmic bridges were believed to be a common, if not universal, method to accomplish such intercellular communication until the increased resolution of electron microscopy demonstrated that such continuities were, in fact, relatively rare. Still, the physiological evidence is compelling that ions and small molecular weight metabolites are exchanged extensively between cells, and the morphological arrangement that is believed to mediate these exchanges is the gap or communicating junction (Figure 5). As already indicated, the existence in hydra of close cellular relationships that might be involved in direct cell-to-cell integration was reported in 1961 (Figures 6 and 7). Unfortunately, the potential importance of the observation was not initially recognized, and a definite description of the morphology of the gap or communicating junction did not appear until six years later (Revel and Karnovsky, 1967). Furthermore, the recognition that hydra possesses large numbers of gap junctions closely resembling those described by Revel and Karnovsky for vertebrate tissues did not come for another five years (Hand and Gobel, 1972). The latter study also revealed a distinct plaque-like organization of hydra gap junctions, but the basis for that morphology was not appreciated until these junctions were later examined by the freeze-fracture technique (Filshie and Flower, 1977; Wood, 1977).

Freeze-fracture images of hydra gap junctions showed that the aggregated intramembrane junctional particles were primarily partitioned to the E-fracture face (the half of the membrane lying closest to the extracellular space) rather than the P-fracture face as is the case for vertebrate gap junctions (Figure 4). Until that time the arthropods were the only group of organisms known to possess this reversed orientation of gap junctional particles. The freeze-fracture replicas also showed that the gap junctions of hydra contained an amorphous interparticle component and that intercellular adhesion was stronger in these junctions than for the vertebrate gap junctional counterparts (Figures 4 and 8). Evidence for strong adhesion resides in the high frequency of junctional profiles in which the fracture plane has deviated out of the membrane into the subjacent cytoplasm (Figure 8) a configuration only rarely observed with vertebrate

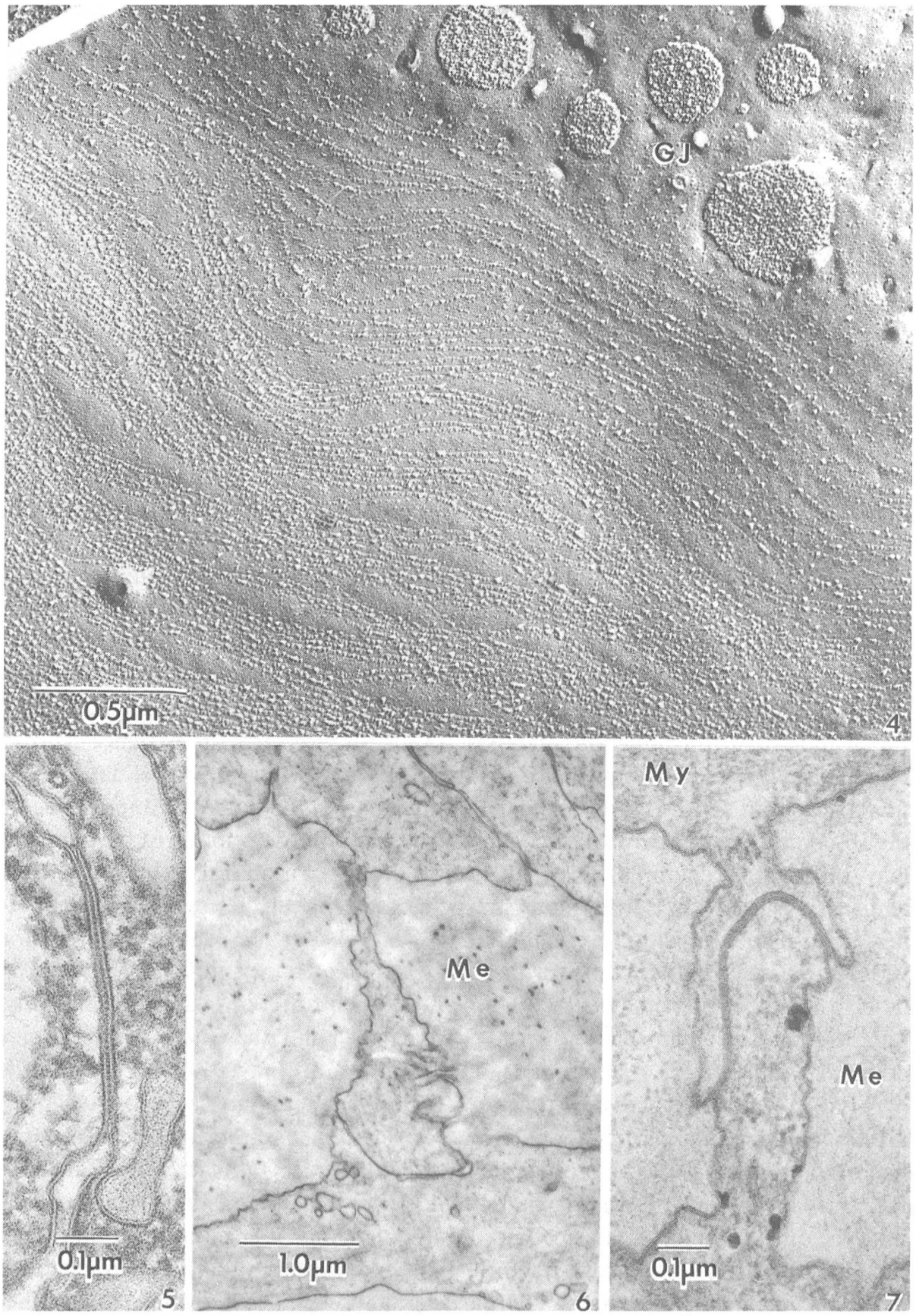



FIG. 4. — Freeze-fracture image (E-fracture face) of septate junction region (bottom) and several gap junctions (GJ). 42,600.

FIG. 5. — Gap junction between epidermal cells. Note periodicity in gap region. X 82,800.

FIG. 6. — Close contact between epidermal (top) and gastrodermal (bottom) cells across mesoglea (Me) (micrograph taken in 1959). X 15,800.

FIG. 7. — Gap junction at region similar to Fig. 6 (micrograph taken in 1974) Me, mesoglea; My, epidermal myoneme. X 67,500.

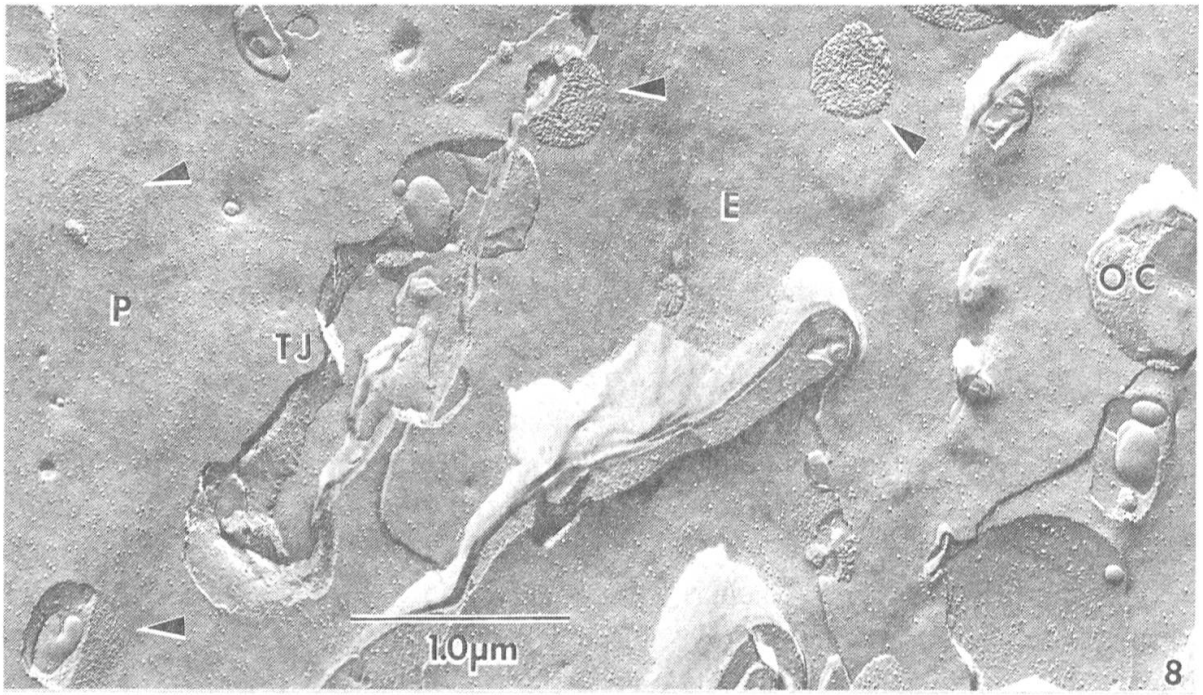
gap junctions (Wood, 1977). It is the presence of an amorphous material surrounding the junctional particles that constitutes the principal structural difference between the gap junctions of hydra and those of most other organisms. Therefore, it is natural to suspect that the amorphous material contributes in some way to the other unusual morphological features of these junctions. The nature of the amorphous material is not known. Subsequent studies in my laboratory on other hydrozoans and representatives of the Anthozoa and Scyphozoa have demonstrated that the same type of gap junction is present throughout the phylum Cnidaria. Junctions with a similar morphology have been reported for several other invertebrate groups, but in all other groups other types of gap junctions with a more conventional morphology are also found. In the Cnidaria, this is the only type of gap junction for which there is unequivocal evidence at the present time. There is physiological evidence that hydra epithelial cells are dye coupled (Fraser and Bode, 1981); it is presumed that gap junctions are responsible for the coupling.

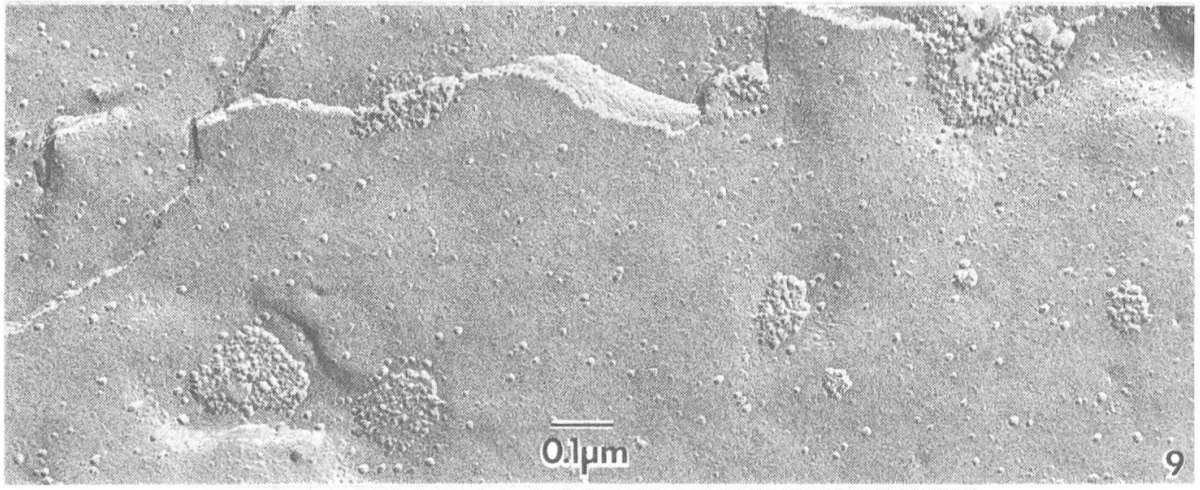
Gap junctions are present in both epithelial layers of hydra, and they also adjoin layers across the thin mesoglea (Figure 7), but only the principal epitheliomuscular cells are involved; interstitial cells, nematocytes and nerve cells do not possess gap junctions. As with septate junctions, gap junctions are disrupted by cellular dissociation, but they are rapidly reassembled during the early stages of regeneration (Wood and Kuda, 1980b). The amorphous interparticle component is present at all stages of junctional reassembly (Figure 9). The rapidity of reappearance of gap junctions under these conditions is strongly suggestive that the junctional subunits are dispersed in the membrane by the dissociation process and that the subunit can be reutilized for new junction formation. This mechanism of gap junction assembly is known to occur in other experimental systems as well (Yancy, et al., 1979).

## DESMOSOME/HEMIDESMOSOME JUNCTIONS

The integrity of any epithelial layer depends on cellular adhesion, both between apposed cells and between the layer of cells and underlying supportive tissue. All cellular junctions are involved to some degree in adhesion, but desmosomes and hemidesmosomes appear to be designed exclusively for an adhesive function. These junctions typically display both intracellular and extracellular densities that are associated with focal areas of insertion at the cell membrane of 10-12 nm cytoplasmic filaments (intermediate filaments) (Lazarides, 1982).

In hydra, a tandem arrangment of desmosomes and hemidesmosomes anchors each nematocyte simultaneously to its surrounding epithelial battery cell and to the subjacent mesoglea (Figures 10 and 11) (Wood and Novak, 1983). This specialized attachment complex (the NBM complex) has three remarkable features (Figures 12 and 13). 1) The two junctions are fascial in organization, that is, they are much larger



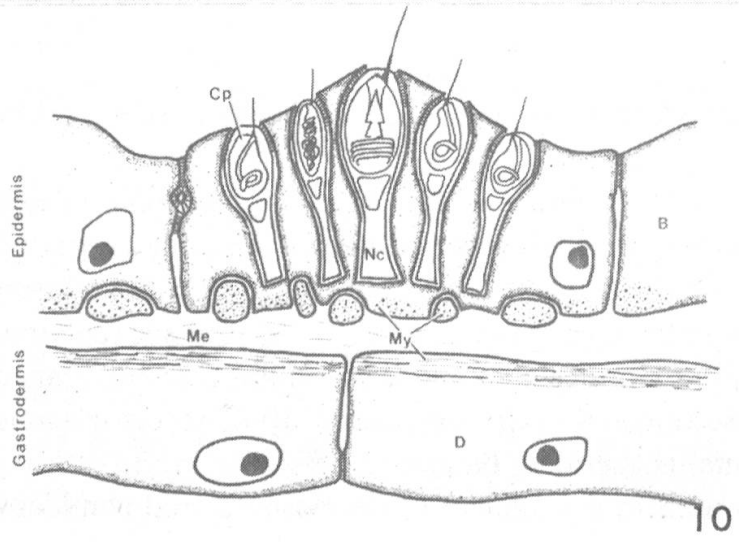
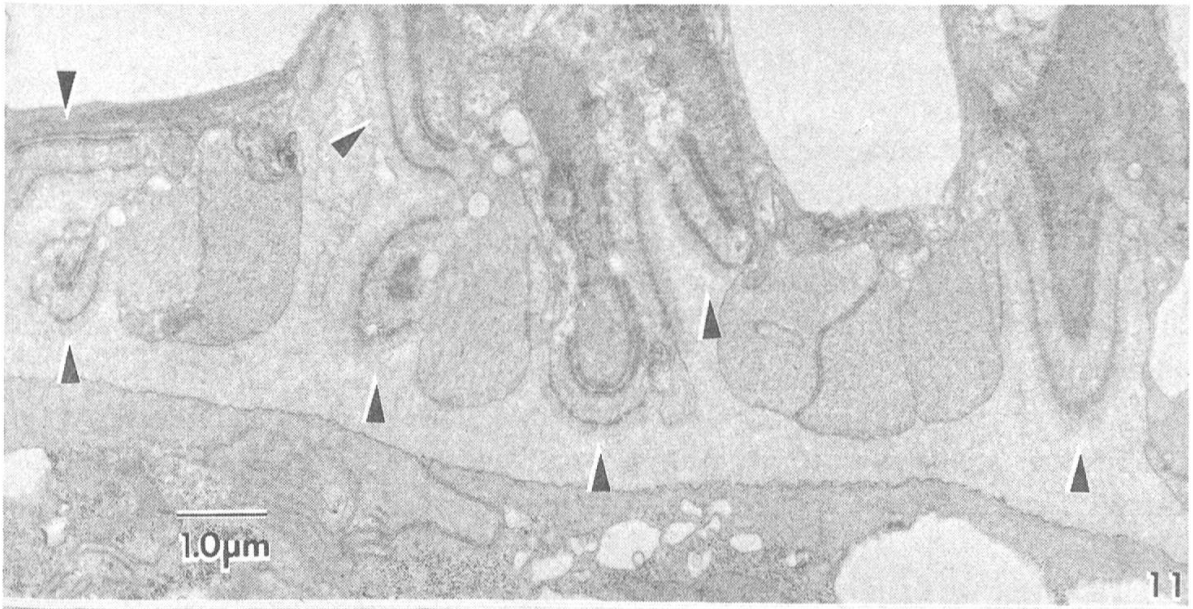
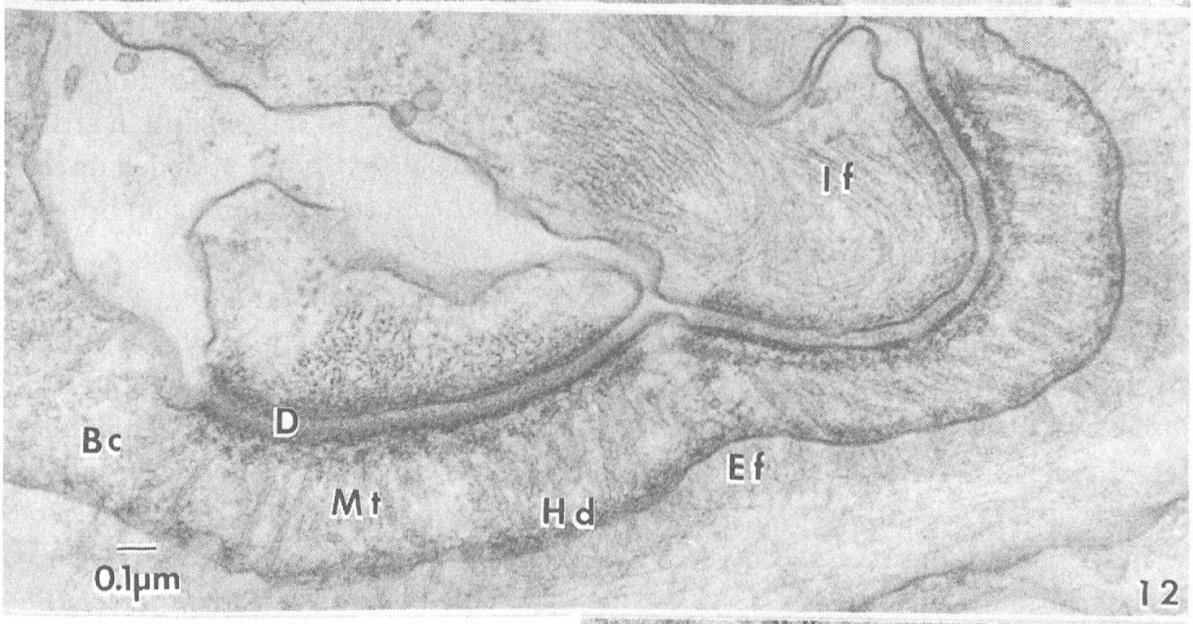



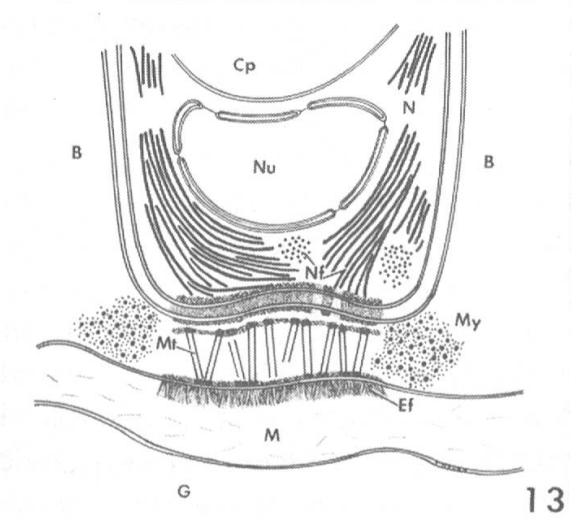

FIG. 8. — Freeze-fracture of lateral surfaces of two epidermal cells. Gap junctions appear on both E- and P-fracture faces (arrows). Images of torn out junctional areas (TJ) and overlying cytoplasm (OC) also occur. X 23,000.

FIG. 9. — Freeze-fracture image (mostly E-fracture face) of gap junctions during reassembly after tissue dissociation and reaggregation. X 63,000.

FIG. 10. — Diagram of battery cell-nematocyte arrangement in tentacles (drawing by P. L. Novak). B, battery cell; Cp, capsule; D, gastrodermal cell; Me, mesoglea; My, myonemes; Nc, nematocyte.



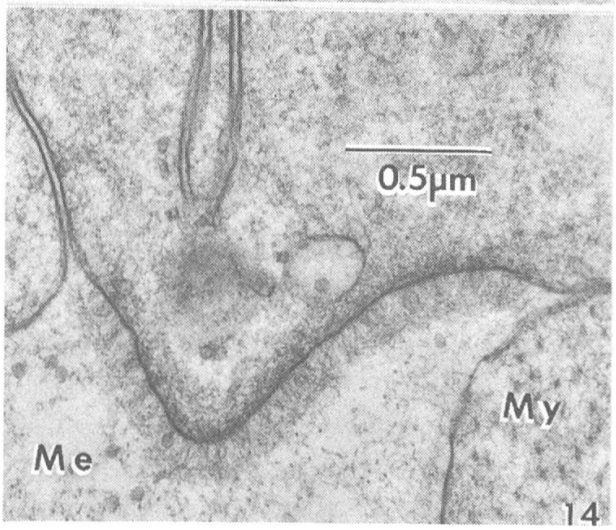




FIG. 11. — BNM junctions (arrows) at the base of a battery cell. Several nematocytes are being anchored simultaneously. X 9100.

FIG. 12. — BNM junction showing tandom arrangement of a desmosome (D) and a hemidesmosome (Hd). Intermediate filaments (If) converge on the desmosome, microtubules (Mt) span the battery cell process (BC) and the underlying mesoglea has oriented fine filaments (Ef). X 46,200.

FIG. 13. — Diagram of NBM complex (drawing by P. L. Novak). B, battery cell cytoplasm; Cp, nematocyst capsule; Ef, extracellular filaments; G, gastrodermis; M, mesoglea; Mt, microtubules; My, myoneme; Nf, nematocyte filaments; Nu, nucleus.

FIG. 14. — Part of NBM complex at the base of a battery cell of an epithelial animal. Me, mesoglea; My, myoneme. X 30,500.

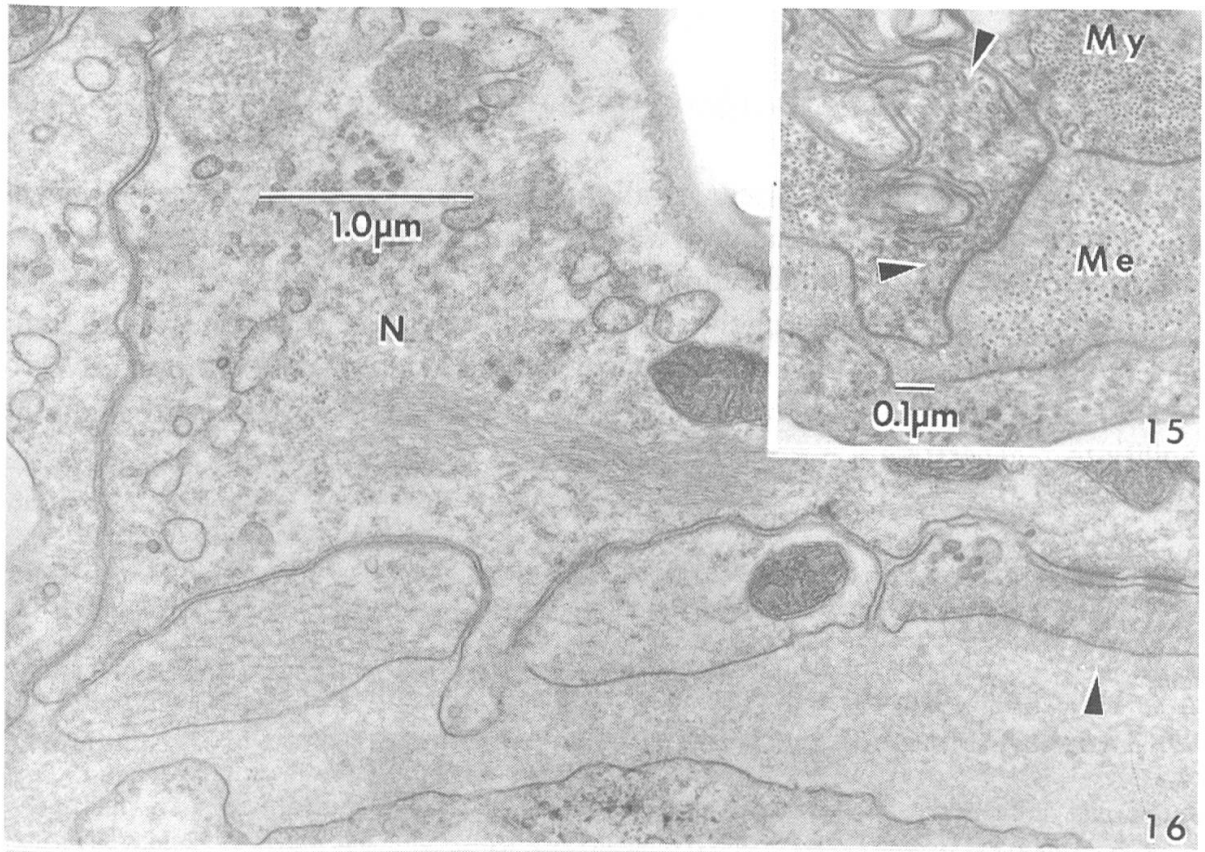
than the typical macular forms of desmosomes and hemidesmosomes found in most organisms. 2) The hemidesmosomal portion of the complex has microtubules rather than intermediate filaments for cytoskeletal reinforcement. 3) The underlying mesoglea shows a localized differential organization. This differential organization is detectable even after a long term absence of nematocytes, as in cultured "epithelial" animals (Figures 14 and 15).

Although the NBM junctional complex is fascial in general appearance, desmosomal portions are heterogeneous in morphology. Formation of this junction appears to result from a sequential lateral recruitment of smaller macular units (Novak and Wood, 1983).

The presence of microtubules oriented in the direction of greatest mechanical stress as part of a hemidesmosomal attachment is not unique to the NBM junctional complex of hydra. A similar arrangement occurs in other cnidarians (Westfall, 1971), and is known to occur at sites of skeletal muscle attachment to exoskeleton in arthropods (Caveney, 1969). In the latter situation the muscle fiber is not attached directly to the exoskeleton but an intervening epidermal epithelial cell containing an oriented bundle of microtubules serves as an intermediary.

A morphological specialization of the extracellular material where the nematocyte attachment complex transmits its mechanical pull to the mesoglea is entirely predictable. However, the existence of a modified form of this specialization in the absence of nematocytes is surprising. In experiments using grafts of proximal halves of normal hydra to distal halves of epithelial animals, migrating nematocytes appear in the tentacles within four to six hours. The migration pathway is along the outer surface of interdigitated epidermal cell myonemes, which lie adjacent to the mesoglea (Campbell and Marcum, 1980). Migrating nematocytes extend short processes between the myonemes to make contact with the underlying mesoglea (Figure 16). It seems reasonable to suggest that the hemidesmosomal site, normally already partially differentiated prior to the arrival of a nematocyte, may serve as one type of signal to the migrating nematocyte that a mounting site is available. Once a mounting site is "recognized" the nematocyte reorients with its cnidocil facing outward and rapidly forms the complete NBM junctional complex (Figure 17). Although a direct role in these events of the hemidesmosomal site, with its local difference in the nature of the mesoglea, is at this time purely speculative, it should be noted that a specialized role of extracellular matrix components in directing cellular positioning is well documented for other experimental systems. For example, the external lamina of vertebrate skeletal muscle contains molecular clues that direct regenerating nerve fibers to prior synaptic sites (Sanes, et al., 1978).

To Abraham Trembley hydra were truly remarkable organisms. From a modern perspective hydra are no less remarkable. They are a somewhat aberrant fresh water representative of a largely marine phylum. In one sense their cellular junctions could be regarded as highly specialized. In another aspect, however, the junctions of hydra



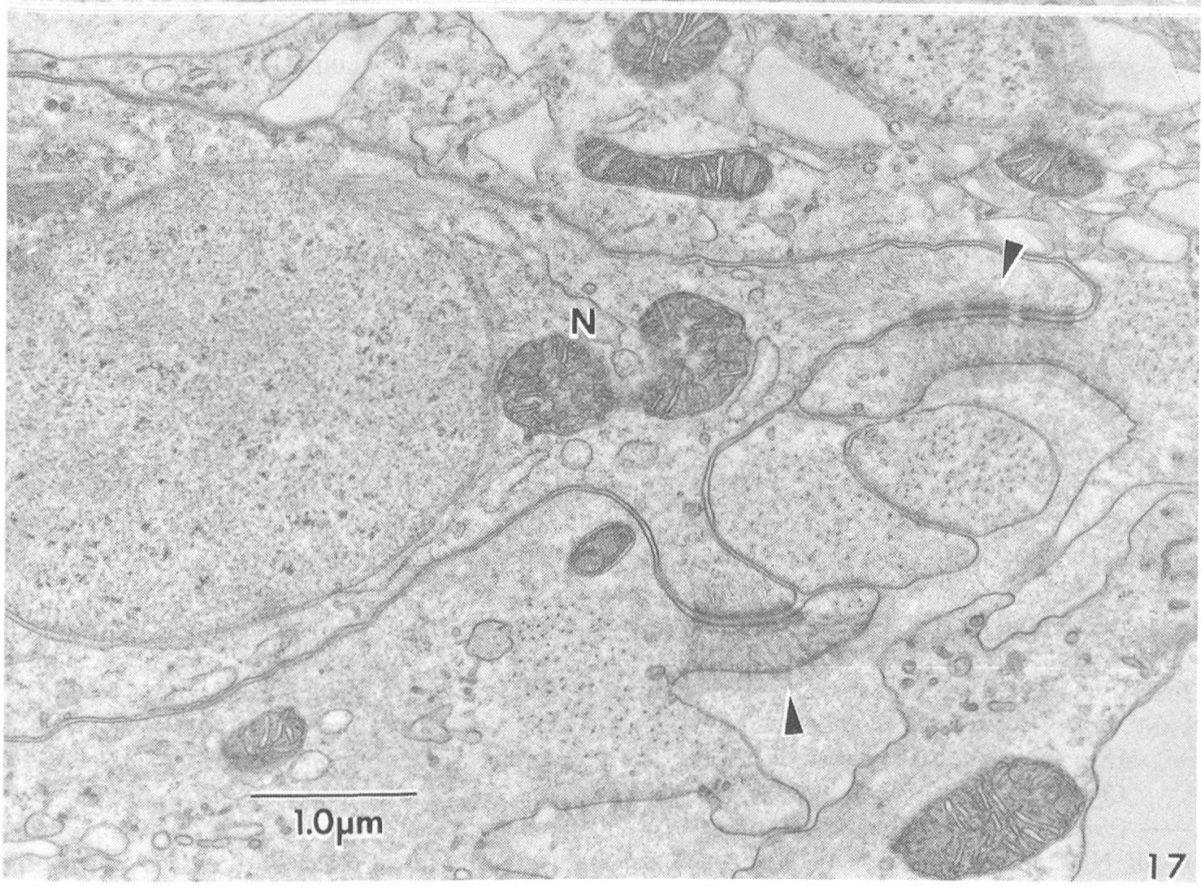




FIG. 15. — Part of NBM complex at the base of a battery cell of an epithelial animal showing associated microtubules (arrows). Me, mesoglea; My, myoneme. X 43,700.

FIG. 16. — Nematocyte (N) in the process of mounting in a battery cell six hours after grafting part of a normal animal to an epithelial animal. Processes of the nematocyte contact the mesoglea (left) and a BNM junction is forming (arrow). X 22,750.

FIG. 17. — Mounting nematocyte (N) showing forming BNM junctions (arrows) the junction at right shows three separate desmosomal junctions, whereas the one at left is single. X 17,500.

are reflective of the general needs of all multicellular organisms to develop means for cellular attachment, to maintain a controlled internal environment and to have effective intercellular communication. Thus, Trembley's intriguing little animals still serve a useful role in elucidating the structure and function of cellular junctions some two hundred forty years after their momentous contributions as a prototype experimental animal for the study of regeneration and grafting. Asexual budding and the ability to regenerate are not the only indications of the immortality of hydra!

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