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Comparative Ultrastructure of Arthropod Transporting Epithelia

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SYNOPSIS. The general organization of arthropod epithelia is compared to that of vertebrates. It is suggested that although ciliated epithelia, stratified epithelia and in some cases continuous muscle sheaths do not occur in arthropods, they have certain analogous structures which carry out the same functions. For example, the arthropod cuticle is compared to the squamous layer of vertebrate stratified epithelia, and complex arthropod basement membranes are compared to the muscle and connective tissue sheaths of certain vertebrate epithelia. The cellular organization of transporting epithelial cells is then discussed, with particular reference to elaboration of plasma membranes, and similarities and differences between vertebrates and arthropods, and between insects and crustaceans are pointed out. Specializations peculiar to insect cells are described, including the insertion of mitochondria into apical membrane microvilli, and the presence along this membrane of small particles called portasomes believed to be involved in active transport. Finally, it is shown that in the midgut of the insect *Manduca sexta*, distinct ultrastructural changes accompany loss of potassium transport activity during a larval molt and in the prepupal stage. The ultrastructural changes which occur include a proliferation of the basement membrane and muscle tissue underlying the epithelium, and a change in the morphology of the potassium transporting goblet cells. Possible correlations between ultrastructural changes and loss of transport activity are discussed.

INTRODUCTION

The comparative ultrastructure of arthropod transporting epithelia is an enormous subject, and there were several ways to approach it. In this paper I will use insect and crustacean examples to compare the structure of arthropod epithelia, as a group, to those of vertebrates. I will point out the distinctive features common to both insects and crustaceans, and also some of the more interesting differences between these two groups of arthropods. Finally, I will describe some of my more recent work on the midgut epithelium of the Tobacco Hornworn larva, *Manduca sexta*, and compare the structure of this epithelium in an actively transporting state with its structure when the transport is turned off.

GENERAL ORGANIZATION OF TRANSPORTING EPITHELIA

A generalized transporting epithelial cell is shown in Figure 1, together with some of the variations in epithelial organization which can occur in vertebrates (review by Berridge and Oschman, 1972). A simple epithelium (Fig. 1A) consists of a single layer of cells joined together to form a sheet or tube. The cells are asymmetric in both structure and function, with distinct apical and basal sides, and there may be elaboration of the apical, basal or lateral plasma membrane, often with some close mitochondrial association. The apical membrane forms microvilli or leaflets, and the basal membrane may be infolded to form narrow channels which penetrate the cell. The lateral membranes may also be quite convoluted, so that there is often extensive interdigitation of neighboring cells, and may also give rise to intercellular spaces or channels. The folding of the plasma membrane serves to increase the surface area of the cell, and to provide enclosed spaces where ion concentration gradients can build up. The epithelial cells are joined apically by a junctional complex, and rest on a basement membrane which provides support and stability for the cells, and may also act as a filter for large molecules.

In vertebrate epithelia, this basic structure can be modified in a number of ways. In some cases the apical surface of the cell may be covered with cilia in addition to microvilli (Fig. 1B). In addition to a basement membrane, some epithelia may have underlying mucosal and submucosal mus-

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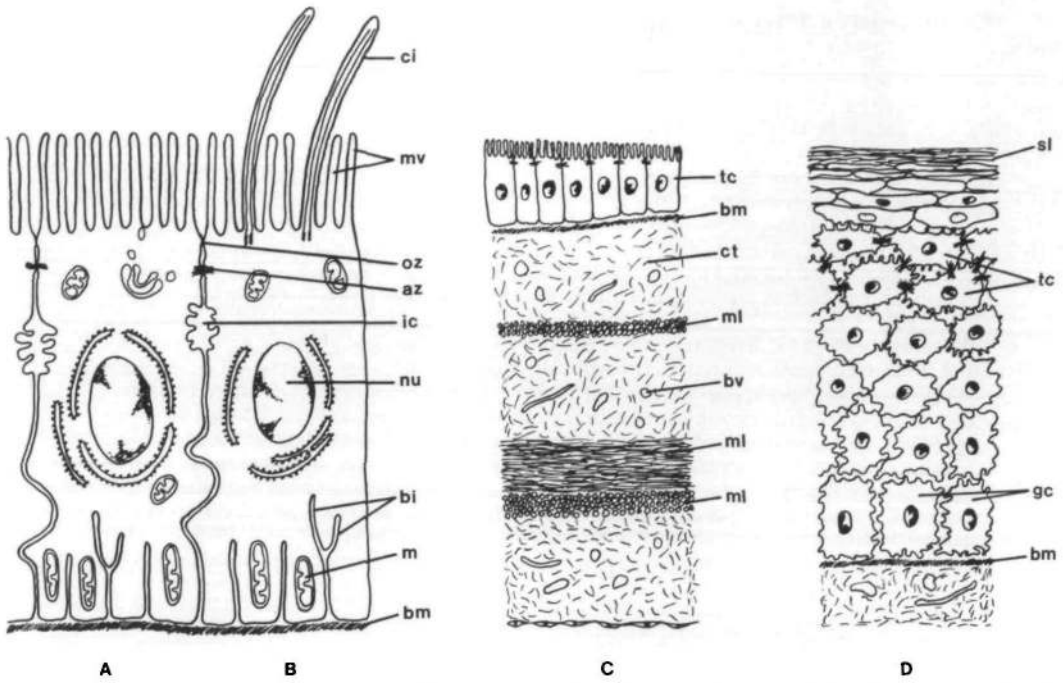


FIG. 1. Organization of vertebrate epithelia. A. A generalized transporting cell. B. A ciliated epithelial cell. C. A muscularized epithelium. D. A stratified epithelium. ci, cilium; mv, microvilli; oz, occluding zonule; az, adhering zonule; ic, intercellular canaliculus; nu, nucleus; bi, basal infolding; m, mitochondrion; bm, basement membrane; tc, transporting cell; ct, connective tissue; ml, muscle layer; bv, blood vessel; sl, squamous layer; gc, germinative cell.

cle sheaths and thick layers of connective tissue, through which blood vessels and capillaries pass to reach the epithelium (Fig. 1C). These layers not only produce muscular movements, but also provide additional support for the epithelium and prevent tearing or damage as a result of excessive distension. Some vertebrate epithelia may be stratified, consisting of several cell layers (Fig. 1D). Dividing cells are located basally, and differentiate into transporting cells as they move towards the apical surface of the epithelium. The very apical surface consists of a flattened or squamous layer of dead cells, which provide protection against mechanical damage or dessication. Depending on the tissue, this layer may be thick and keratinized, as in the frog skin, or thin and non-keratinized as in the cornea.

A careful search of the literature revealed no examples of ciliated epithelia, submucosal muscle sheaths or stratified epithelia in arthropods. However, I would suggest that where this type of organization would be required, arthropod epithelia have different but analogous structures which serve the same function. Comparing vertebrate and arthropod epithelia in this way proved to be extremely valuable, and led not only to some interesting analogies but also to some new insights into the functional organization of arthropod epithelia.

In the case of stratified epithelia, the protective function of the squamous layer in vertebrates has, in arthropods, been replaced by their very versatile cuticle. The cuticle, like the squamous layer, varies greatly in strength and thickness depending on its location. Where less protection is required, such as the gills (Fig. 2A) or the insect rectum, the cuticle is less than 2 μm thick. In contrast, the cuticle over the integument may become modified to serve a skeletal as well as a protective function, such as the heavily calcified cuticle of crabs

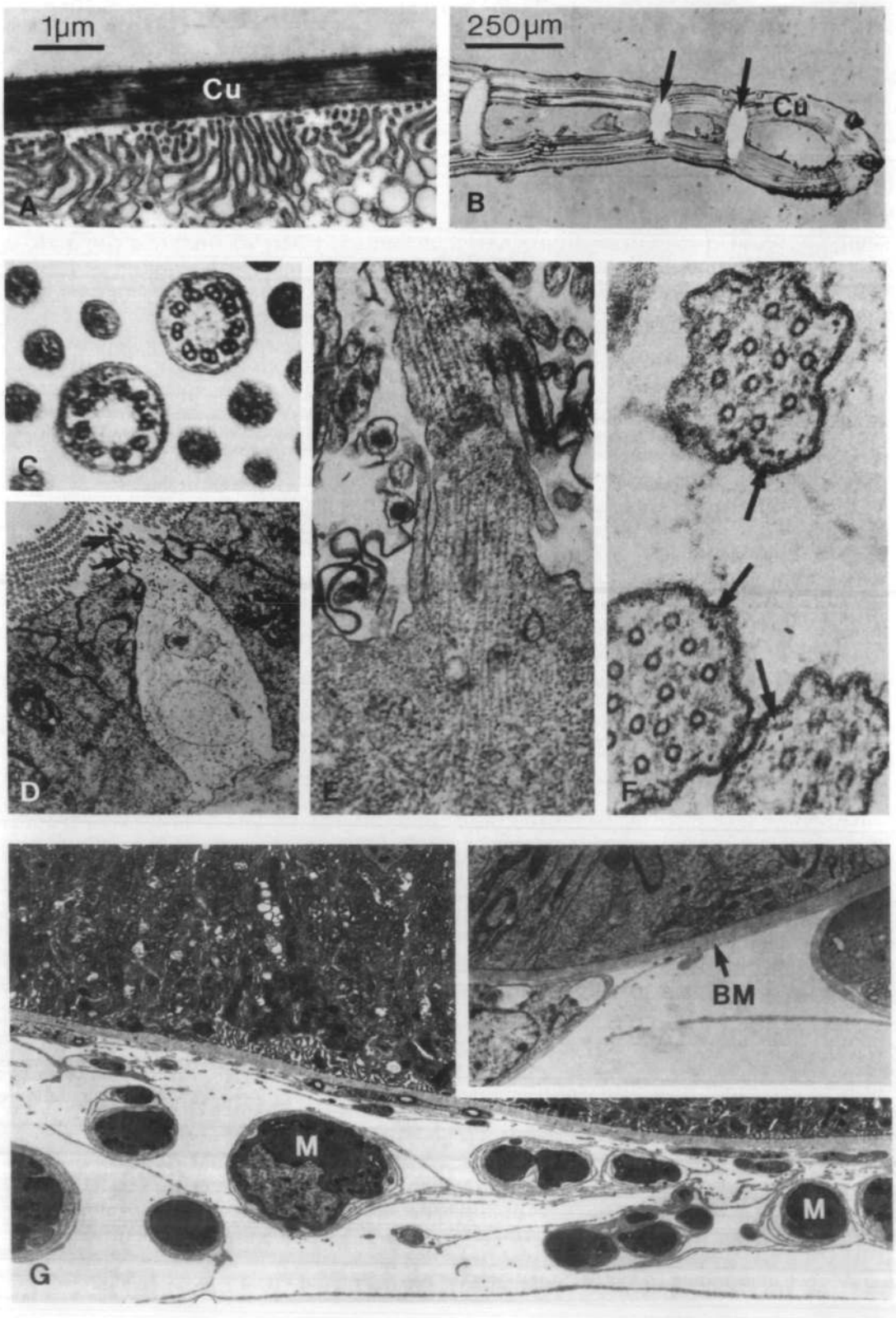
and lobsters, or the thick chitinized cuticle found in certain insects. Figure 2B shows a section through the leg of *Hydrocyrius colombiae,* a swimming water bug, in which the cuticle is over 100 μm thick, and has developed into skeletal struts which pass right through the leg (Neville, 1967).

In arthropods, cilia may occur in sperm cells or sensory cells, but ciliated epithelia similar to those found in vertebrates have never been reported for arthropods. However, some cilia-like structures have been found in arthropod epithelial cells. True cilia are characterized by the typical 9 plus 2 arrangement of microtubules, in which 9 pairs of microtubules surround 2 single ones, and have a basal body and rootlet. In *Leptestheria dahalacensis,* a primitive crustacean, biciliate cells have been found scattered along the midgut (Fig. 2D), but the cilia are 9 plus 0, and lack the two middle microtubules (Fig. 2C). The authors suggest that these cilia are sensory rather than motile, and that the ciliated cells may regulate the peristaltic movements of the gut. In the case of insects, Figure 2E shows axopods found in the Malpighian tubules of *Rhodnius prolixus* (Bradley and Satir, 1979). These axopods contain a varying number of microtubules (Fig. 2F); they have no basal bodies, but are apparently quite motile and sweep crystals of uric acid down the tubule lumen.

Associated with some vertebrate epithelia are underlying continuous muscle layers tightly bound to the epithelium by connective tissues, and therefore contraction of the muscle layers will produce an effect on the epithelium. However the mechanism for coupling muscular movements to movement of certain arthropod epithelia, which in many cases lack not only a submucosal muscle layer but also any continuous muscle sheath or connective tissue, presented an interesting problem which to my knowledge has not been considered before. Crustaceans have a limited capillary system, and connective tissue layers and thin muscle sheaths can develop. However insects have no capillary system at all, so that epithelia are bathed directly by the blood, and a continuous muscle sheath would constitute a considerable permeability barrier. Therefore in tissues such as the midgut, where muscular movements are required, the muscle layers are discontinuous. Now for contraction of a discontinuous muscle layer to have any effect on the epithelium, the muscles must somehow be connected to each other or to the epithelium, and preferably to both. To find what this connection might be, I examined the muscle layers surrounding the midgut of several species of lepidopteran larvae, including *M. sexta, Prodenia eridania,* and the gypsy moth *Lymantria dispar.* In all cases I found that the basement membrane serves to form a connecting network between the muscles and the epithelium (Cioffi, unpublished). Each of the muscle blocks is surrounded by a sheath of basement membrane, from which thin filaments extend to contact the basement membrane surrounding adjacent muscles (Fig. 2G) and the basement membrane underlying the epithelium (inset).

As I mentioned earlier, the muscle sheaths of vertebrate epithelia not only produce muscular movements, but together with layers of connective tissue provide support and stability for the epithelium. In the midgut of *M. sexta,* this function is served by an interesting arrangement of muscles and folds. The midgut is composed of six folded strips of tissue separated from each other by an unfolded strip along which runs a large longitudinal muscle (Cioffi, 1979). This organization allows the gut to increase its surface area by folding, while the unfolded strips with their continuous longitudinal muscle hold these folds in position and prevent the gut from becoming distended.

However in some cases, considerable distension must be permitted, as occurs in the midgut of the female *Aedes aegypti* mosquito following a large blood meal, to the extent that the midgut columnar cells become quite flattened. In this case the basement membrane underlying the epithelium seems to determine the limit of this distension (Reinhardt and Hecker, 1973). Before a blood meal, the basement membrane is very thick, consisting of several beaded layers (Fig. 3A), each of which forms a square grid in tangential section



(Fig. 3B). In each layer the grid lines may be at a different angle, as in Figure 3B, where the plane of section has passed through at least three different layers. When the gut is stretched by a blood meal, the beaded appearance of the basement membrane disappears (Fig. 3C), as the square grids are stretched to their maximum, or rhombic, configuration (Fig. 3D). Reinhardt and Hecker also suggest that since the holes in the grid increase in size when the gut is stretched, this could increase the permeability of the basement membrane and facilitate diffusion across the gut. However Houk *et al.* (1981) have found that the permeability of the basement membrane in fed and unfed *Culex tarsalis* mosquitoes is approximately the same, and also that particles less than 8 nm in diameter can pass right through the basement membrane. This implies that in mosquitoes at least, the basement membrane does not function as a permeability barrier, and its complex organization serves only as a mechanism to limit distension of the gut. Interestingly, in male mosquitoes, which do not suck blood, the midgut does not have this complex type of basement membrane (Reinhardt and Hecker, 1973).

In some crustaceans the midgut basement membrane seems to have a similar function to that of the female mosquito, of limiting distension of the gut epithelium. In fact, some of the most complex basement membranes ever described have been found in decapod crustaceans. In the intestine of the lobster, *Homarus americanus,* the basement membrane is extremely thick and dense, and is perforated by numerous pores (Factor, 1981), as shown in Figure 3E. Processes from the basement membrane extend into the connective tissue beneath (Fig. 3F), apparently providing additional strength and support for the epithelium. Factor also points out that in the fore and hind gut of the lobster, where the epithelium is covered apically by cuticle, this complex type of basement membrane does not occur, and he suggests that in this case the cuticle plays the same role as the basement membrane in the intestine.

In the gill, certainly, it is the cuticle which provides support for the gill epithelium. The gill of the blue crab, *Callinectes sapidus,* consists of a central stem which supports several flattened lamellae (Fig. 4). Each lamella consists of two sheets of epithelial cells covered externally by cuticle and separated by a blood space. At regular intervals across the lamella are pillar cells, which extend across the blood space and anchor the two epithelial sheets together (Fig. 3G). The pillar cells are packed with bundles of microfilaments which insert into the cuticle at each end of the cell (Fig. 3H; Cioffi, unpublished). This arrangement probably provides stability, and keeps the size of the blood space between the epithelial sheets constant.

CELLULAR ORGANIZATION

Cell junctions and lateral membranes

In vertebrate epithelia, the typical cell junctions are occluding and adhering zonules and maculae. Both the occluding and

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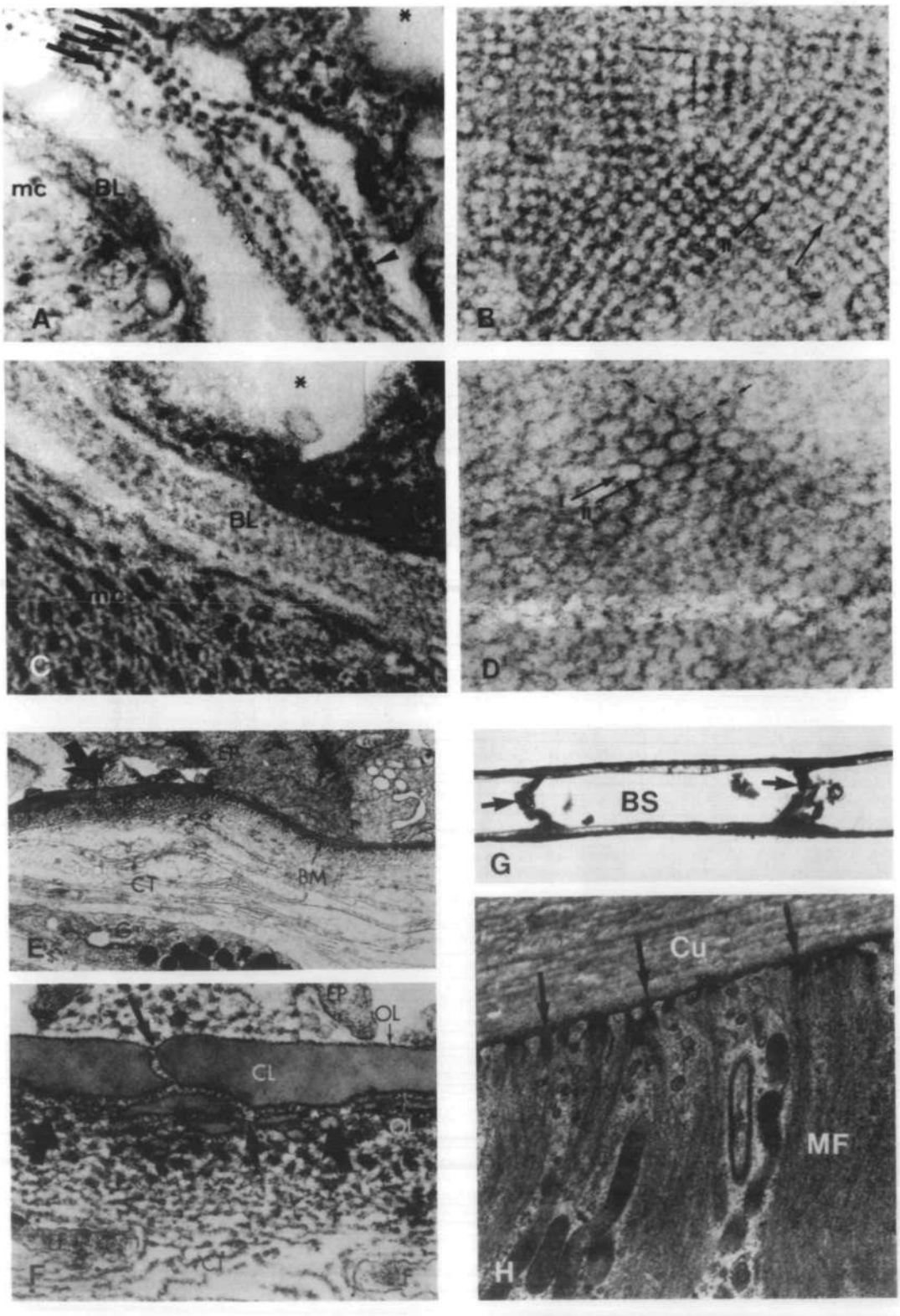
FIG. 2. Specializations of arthropod epithelia analogous to the vertebrate structures shown in Fig. 1B, C and D. A, B. Arthropod cuticle replaces the squamous layer of vertebrate stratified epithelia. A. The thin gill cuticle (Cu) of the crustacean *C. sapidus.* B. A section through the leg of the insect *H. colombiae* photographed under polarized light. The cuticle (Cu) is approximately 100 μm thick, and forms skeletal struts (arrows) which pass through the leg. C–F. Cilia-like structures in arthropod epithelia. D. Longitudinal section through a biciliate cell (arrows indicate cilia) in the midgut of the crustacean *L. dahalacensis.* ×6,300. C. Transverse section through the cilia shown in D, illustrating their 9 + 0 arrangement of microtubules. ×100,000. E. Longitudinal section through the base of an axopod in the Malpighian tubule of the insect *R. prolixus.* No basal body is present. ×21,000. F. Transverse section through the base of three axopods. They contain a varying number of microtubules, and in addition microfilaments are present (arrows) underlying the plasma membrane. ×97,000. G. The muscle and connective tissue sheaths of vertebrate epithelia are replaced in lepidopteran midgut by a network of basement membrane. The midgut epithelium of *Prodenia eridania* is shown. The muscle blocks (M) form a discontinuous muscle layer, with the blocks connected to each other and to the basement membrane underlying the epithelium (inset) by filaments of basement membrane. ×2,500; inset ×10,500. (B from Neville, 1967, with permission, from *Advances in Insect Physiology* 4, pp. 213–286. Academic Press, Inc. (London) Ltd.; C, D from Rieder and Schlect, 1978; E, F, from Bradley and Satir, 1979.)



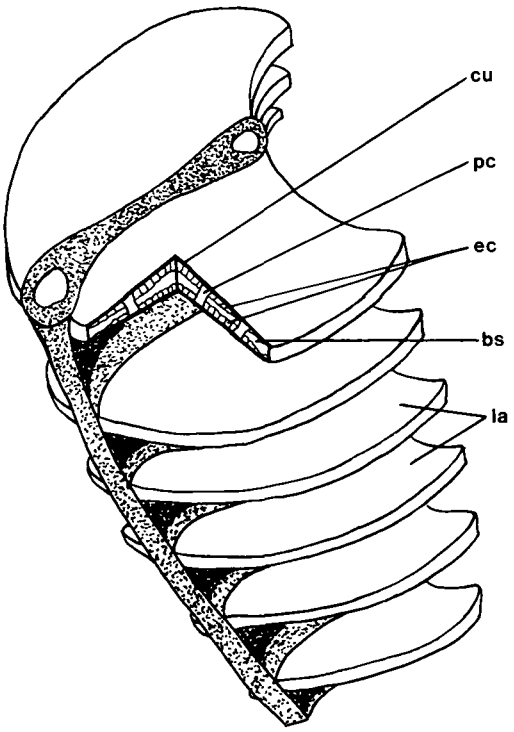


FIG. 4. The organization of *C. sapidus* gill. Cu, cuticle; pc, pillar cell; ec, epithelial cells; bs, blood space; la, lamella.

adhering zonules form a continuous band of adhesion around the cell, but are present at the very apical surface only. The occluding zonule appears to be tight to large ions and molecules (Farquhar and Palade, 1963), but the adhering zonule does not appear to be a diffusion barrier. Occluding and adhering maculae can occur at any site between adjacent cells, but since maculae are spot junctions they obviously do not constitute a permeability barrier. Both are sites of adhesion, but the occluding maculae may also function in cell to cell communication (Loewenstein and Kanno, 1964). All these junctions can be found in arthropods, but they have as their most prominent junction the septate desmosome, which does not occur in vertebrates. Unlike the occluding and adhering zonules, which form an apically located adhesive band less than 1 μm long, the septate junction forms a continuous area of adhesion over as much as the apical half to two thirds of adjacent cells, and may be found at the basal as well as the apical end of the cell. The need for such an extensive area of attachment between arthropod epithelial cells may be a reflection of the reduced or absent supporting layers of muscle and connective tissue which occur in vertebrates.

The organization of septate junctions will be described in detail by Dr. Hakim later in this symposium, so I will only briefly mention here their more general modifications related to their role in active transport. In arthropod epithelia, as in vertebrates, the lateral membranes of adjacent cells may separate to form intercellular spaces or canaliculi, particularly in epithelia where a large flow of water is coupled to active transport. The classical verte-

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FIG. 3. Support structures in arthropod epithelia. A–D. The midgut basement membrane of the female mosquito *A aegypti* A. Longitudinal section through the unstretched basement membrane before a blood meal. The basement membrane consists of several beaded layers (arrows) embedded in an amorphous matrix. A muscle cell (mc) and its basal lamina (BL) are also shown. B. Tangential section through the unstretched midgut basement membrane, showing its grid-like arrangement of holes. C. Longitudinal section through the stretched midgut basement membrane following a blood meal; the basement membrane is thinner than in A and the beaded appearance has disappeared. D. Tangential section through the stretched basement membrane; the square grid has changed to a rhombic one, and the holes are now larger. ×78,000. E, F The midgut basement membrane of the lobster *H. americanus* E. Longitudinal section through the midgut epithelium (EP) showing the dense basement membrane (BM) perforated by channels (arrow), and its network of processes into the underlying connective tissue (CT). ×5,600. F. The basement membrane at higher magnification, showing a branching channel, the central layer (CL) of the basement membrane and the fibrous outer layers (OL), processes of the basement membrane (large arrows) and strands (small arrows) which interconnect processes and bridge channels. ×27,500. G. Section through a gill lamella of *C. sapidus* showing pillar cells (arrows) bridging the blood space (BS) between the epithelial sheets. ×220. H. Portion of a pillar cell in contact with the cuticle. Arrows show bundles of microfilaments (MF) inserting into the cuticle (Cu). ×16,000. (A–D from Reinhardt and Hecker, 1973; E, F from Factor, 1981).

brate example is the gallbladder, in which the size of the large intercellular spaces changes with the transport rate (Tormey and Diamond, 1967). Transport related elaboration of lateral membranes does not occur to any great extent in crustacea, but perhaps the most highly developed lateral membranes ever described occur in the rectal papillae of the insect, *Calliphora*. The cells of the rectal papilla are joined by a septate desmosome at both their apical and basal surface (Berridge and Gupta, 1967). In addition there are several more short lengths of septate desmosome forming a series of bands around the cell. Where junctions are not present, the lateral membrane is infolded to form a series of flattened stacks which are closely associated with mitochondria, and which then open out to form larger intercellular spaces. Berridge and Gupta showed that when the cells were actively transporting ions, these spaces became large as they filled with fluid, but when the fly was starved the spaces collapsed as active transport processes were stopped.

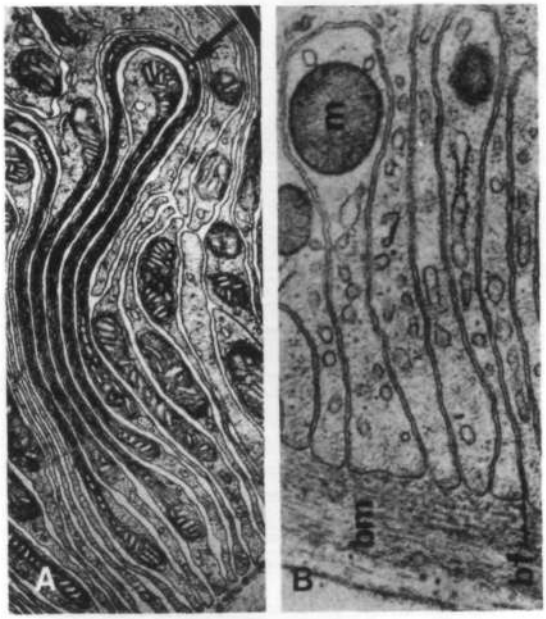


FIG. 5. Basal plasma membranes of arthropod transporting cells. A. Basal region of a salt transporting cell in the gill of *C. sapidus*. A cup-shaped mitochondrion (arrow) curves around one of the deep basal membrane infolds. ×10,500. B. Basal region of a Malpighian tubule cell from the cockroach *Periplaneta americana*. Mitochondria (m) are associated with the basal membrane infolds (bf) and there is a thick basement membrane (bm). ×30,000. (A from Copeland and Fitzjarrell, 1968; B from Berridge and Oschman, 1972).

Basal plasma membranes

Infolding of the basal plasma membrane and close mitochondrial association may occur in both vertebrate and arthropod epithelia, but is probably seen to its best advantage in the osmoregulatory organs of crustacea, the gills, antennal gland and bladder. In salt transporting cells from the blue crab gill, the basal plasma membrane is infolded almost to the level of the apical membrane. Between the basal infolds are numerous mitochondria which often have unusual shapes in that they may be cup-shaped or flattened, so that they curve around the basal membrane infolds (Copeland and Fitzjarrell, 1968; Fig. 5A). The antennal gland can be considered as the crustacean kidney, and consists of a coelomosac and labyrinth. The labyrinth cells resemble the salt cells in the gills, with deeply infolded basal membranes associated with mitochondria. However the cells of the coelomosac are podocytes, and have a similar structure and function to the podocytes of the vertebrate glomerulus (Schmidt-Nielsen *et al.*, 1968). Ultrafiltration occurs across the basal membrane of the coelomosac podocytes, as the hydrostatic pressure of the blood forces fluid between the foot processes. A diaphragm between the foot processes, and the basement membrane, probably both act as filters to exclude large molecules. No podocytes have ever been reported in insects, but then the insect blood does not have sufficient hydrostatic pressure for ultrafiltration to occur. In the Malpighian tubule (Fig. 5B), which can generally be considered as the secretory part of the insect kidney, fluid is probably moved across the basal membrane of the tubule cells by active transport (Berridge and Oschman, 1969), with the basement membrane acting as a filter to large molecules.

Apical plasma membranes

As a general rule, the apical membrane of cells involved in active transport tends

to form microvilli, and this is also true for arthropods, but in addition to this type of folding pattern some of the most elaborate apical membranes ever described occur in insects. For this reason, I will divide the apical membranes of arthropod transporting cells into three groups (Type I microvilli, Type II microvilli, and leaflets) according to their organization and structure. The term "microvilli" will be used to describe any finger-like apical membrane projections, while "leaflets" will be used to describe flattened or leaf-like apical membrane projections and infolds.

Type I microvilli

This term will be used to distinguish the brush border type of microvilli, typically present in the vertebrate intestine, in which all microvilli are the same size and length and are regularly arranged in a hexagonal pattern. Each microvillus is a long narrow cylinder and therefore circular in cross section, and a glycocalyx may be present. In arthropods, type I microvilli occur in the intestine of crustacea such as the nauplius of *Artemia salina* (Hootman and Conte, 1974; Fig. 6A), although in this case the glycocalyx is absent, and in the midgut columnar cells of insects such as the gypsy moth larva where there is a distinct glycocalyx (Fig. 6B). These microvilli are probably involved in absorption of nutrients, and in feeding *M. sexta* larvae occur only in the posterior region of the midgut (Cioffi, 1979). The anterior part of the midgut appears to be secretory rather than absorptive, and here the columnar cell apical membrane forms a dense branching network from which large vesicles break off. As will be shown later (see Fig. 7), when the larva stops feeding this secretory activity ceases, and the apical membrane forms microvilli similar to those of posterior midgut.

Leaflets

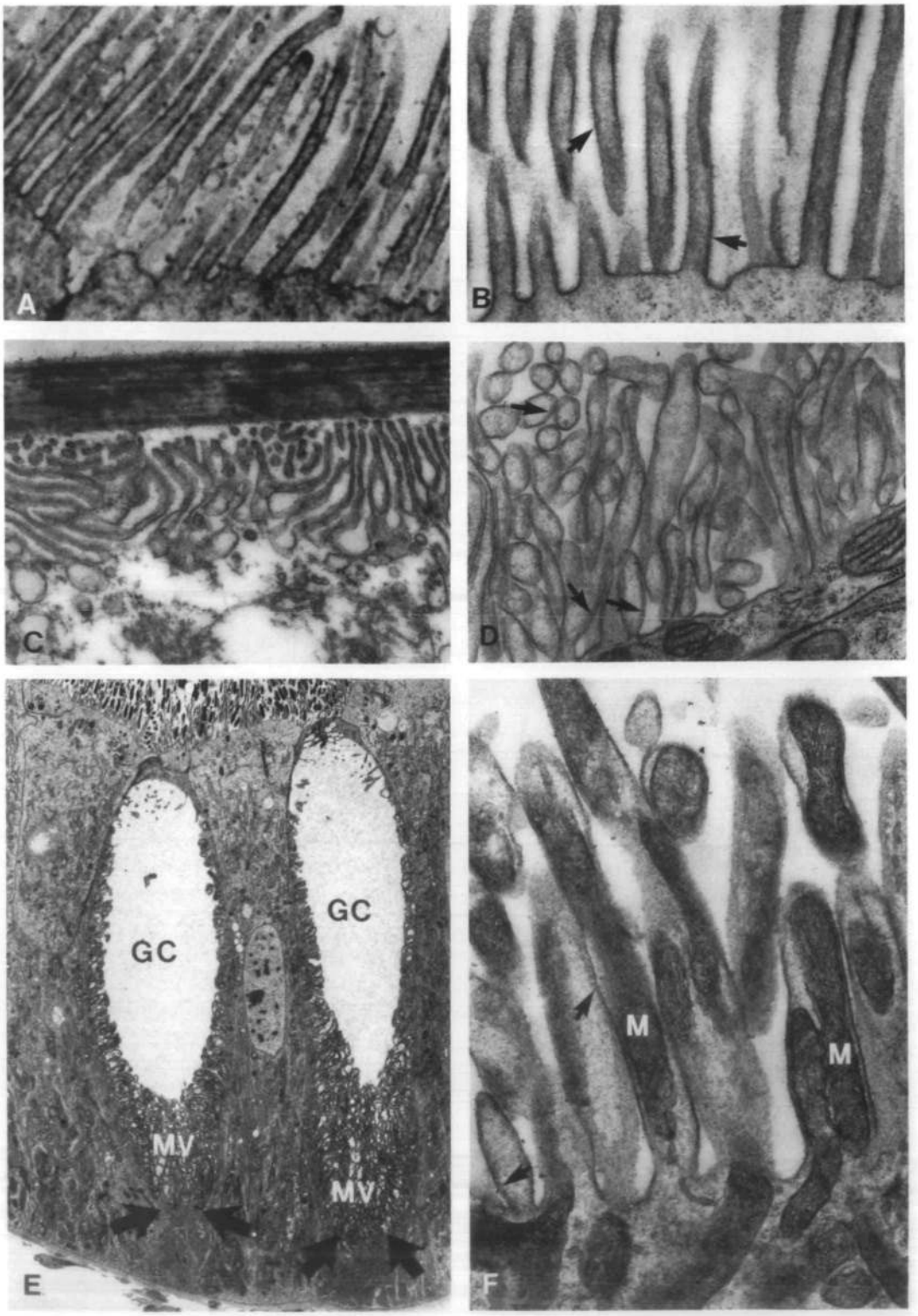
Apical membrane leaflets do not seem to occur in vertebrate epithelial cells, but are frequently found in arthropod epithelia. Some examples are the salt transporting cells of the blue crab gill (Fig. 6C), the salivary glands of *Calliphora* (Oschman and Berridge, 1970) and the rectal papillae cells of *Calliphora* (Berridge and Gupta, 1967).

Type II microvilli

In contrast to type I, type II microvilli have no regular pattern or organization. Neighboring microvilli may vary in size and length, and also show a variety of profiles in cross section. Microvilli of this type occur in a number of vertebrate epithelial cells (see Berridge and Oschman, 1972). In arthropods, type II microvilli occur, for example in the labyrinth cells of the crab, *Uca mordax* (Schmidt-Nielsen *et al.*, 1968), in the goblet cells of larval lepidopteran midgut (Fig. 6D) and in insect Malpighian tubules (see Fig. 6F).

Where type II microvilli or leaflets are present, the apical membrane may be additionally infolded to form a large intracellular cavity or canaliculus. This organization is seen in the parietal cells of mammalian stomach (Ito and Winchester, 1963). I was unable to find any examples of this type of infolding in crustacea, but in insects it occurs in the salivary glands of *Calliphora* (Oschman and Berridge, 1970) and in the midgut goblet cells of lepidopteran larvae (Anderson and Harvey, 1966; Smith *et al.*, 1969; Cioffi, 1979). In goblet cells, the invaginated apical membrane may reach almost to the basal membrane and the cavity, packed with apical membrane microvilli, may occupy as much as 80% of the cell volume. The midgut goblet cells of *Prodenia eridania* are shown in Figure 6E.

In insects, two additional types of apical membrane elaboration have been described, neither of which occur in crustacea or, to my knowledge, in vertebrates. Firstly, in many insect transporting cells with type II microvilli or leaflets, small 10–12 nm particles are present on the cytoplasmic side of the apical membrane. These particles are believed to contain the cellular mechanism for active ion transport, and have been named portasomes to reflect that function (Harvey, 1980; review by Harvey *et al.*, 1981). Secondly, although mitochondria are generally associated with transporting membranes, in some insect cells where portasomes are present, mitochondria are actually inserted into the apical



membrane microvilli or leaflets, bringing them very close to the portasomes present on the apical membrane. This organization occurs in Malpighian tubule cells (Berridge and Oschman, 1969; Bradley and Satir, 1981) and is illustrated for *M. sexta* Malpighian tubule in Figure 6F, in the midgut goblet cells of lepidopteran larvae (Anderson and Harvey, 1966; Smith *et al.*, 1969; Cioffi, 1979), in the rectal pad cells of the cockroach, *Periplaneta americana* (Oschman and Wall, 1969), and in the anal sac of Thysanurans (Noirot and Noirot-Timothée, 1971). The close association between mitochondria and portasomes may provide a readily available supply of energy for active transport, but is apparently not essential. In *M. sexta,* potassium transporting goblet cells are present along the entire length of the midgut. However in anterior midgut the goblet cavity reaches almost to the base of the cell (see Fig. 8A) and mitochondria are inserted into the portasome covered microvilli which line the cavity, whereas in posterior midgut the cavity occupies only the apical half of the cell, and while mitochondria are present in the goblet cell cytoplasm they are not intimately associated with the transporting apical membrane and its portasomes (Cioffi, 1979). However both regions of the midgut are able to transport potassium, with the posterior region working slightly more efficiently than the anterior (Cioffi and Harvey, 1981). Therefore the structural differences between anterior and posterior goblet cells could indicate that the two regions have a different mechanism of potassium transport, or that the close association between mitochondria and portasomes in anterior midgut is necessary for another process in addition to potassium transport. This interesting association between mitochondria and portasomes will be discussed for Malpighian tubules by Dr. Bradley later in this symposium.

ULTRASTRUCTURAL CHANGES ASSOCIATED WITH LOSS OF TRANSPORT ACTIVITY

While simply examining the fine structure of a transporting cell can provide a lot of information about transport processes in that cell, it is sometimes possible to show that in a single epithelium there are definite changes in cell structure when the transport is turned on and off.

There are several ways of inhibiting transport with poisons or anoxia, but in arthropods, molting occurs several times during the animal's life, and many of its transport processes are temporarily and naturally shut down. I was able to make use of this fact to examine ultrastructural changes associated with loss of transport activity in the midgut of *M. sexta.* In this study I compared the structure of the midgut from feeding fourth and fifth instar larvae with that of larvae molting from fourth to fifth instar, and also with prepupae.

Both the molting larva and the prepupa are quite inactive physically; they stop feeding and the gut empties out completely. To check that the midgut potassium transport system was also turned off at these times, I carried out some open circuit flux measurements using Rb-86 as

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FIG. 6. Apical plasma membranes from arthropod transporting cells. A. Type I microvilli forming the brush border of the intestine of the nauplius of *A. salina.* Neighboring microvilli are similar in size and shape, but lack a glycocalyx. ×30,000. (From Hootman and Conte, 1974.) B. Type I columnar cell microvilli forming the brush border of the midgut of the gypsy moth *L. dispar.* Neighboring microvilli are similar in size and shape, and a glycocalyx (arrows) is present. ×30,000. C. Apical membrane leaflets from a salt transporting cell from the gill of *C. sapidus* ×15,000. D. Type II microvilli from a posterior midgut goblet cell of *M. sexta.* Microvilli are irregularly arranged and vary in size and shape. Portasomes (arrows) are present on the cytoplasmic side of the apical membrane. ×25,000. E. Goblet cells from the middle region of the midgut of the lepidopteran larva *P. eridania.* The apical membrane is infolded almost to the base of the cell (arrows), and type II microvilli (MV) project into the goblet cavity (GC). ×3,000. F. Type II microvilli from the Malpighian tubule of *M. sexta.* Individual microvilli vary in size and shape, and most of them contain an elongated mitochondrion (M). Portasomes (arrows) are present on the cytoplasmic side of the apical membrane. ×35,000.

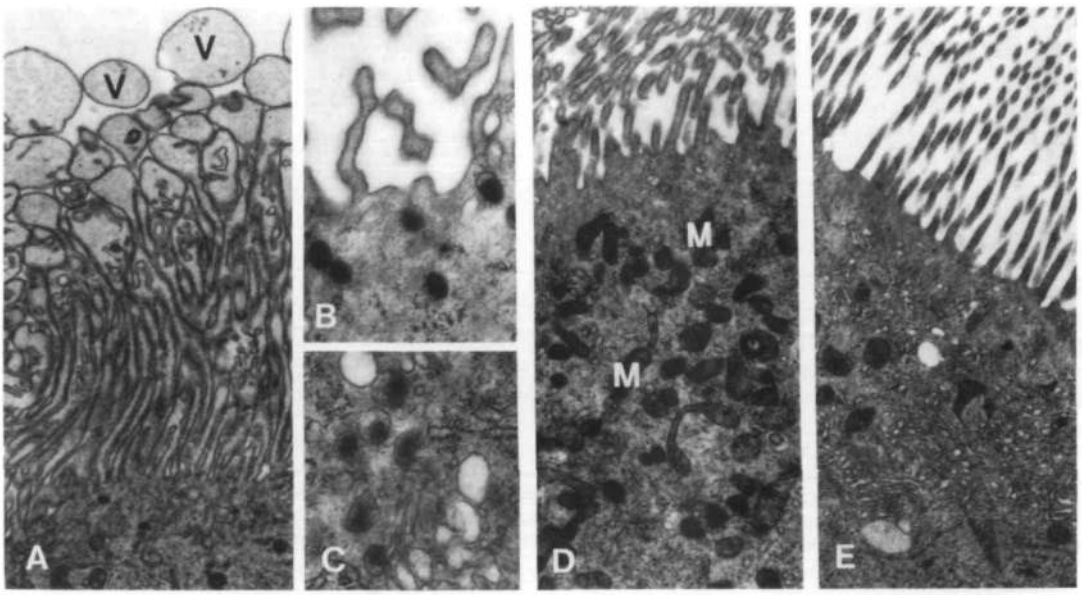


FIG. 7. The apical region of columnar cells from the anterior midgut of *M. sexta*. A–C. A feeding fifth instar larva. D. A larva molting from fourth to fifth instar. E. A prepupal larva. A. In the feeding larva the apical membrane forms a branching network from which large vesicles (V) break off. Arrows indicate small Golgi vesicles with dense contents just below the apical membrane. ×7,000. B. Golgi vesicles from A shown at higher magnification. ×19,000. C. Golgi complex producing vesicles. ×25,000. D. In a molting larva the secretory activity of the apical membrane ceases, and type I microvilli form. Golgi vesicles are no longer present below the apical membrane, but the apical cytoplasm becomes packed with mitochondria (M). ×7,000. E. In the prepupa, secretory activity of the apical membrane also ceases. but there is no accumulation of mitochondria. ×7,000.

a tracer for potassium, and confirmed that there is no net flux across the midgut in either the molting larva or the prepupa (Cioffi, unpublished). However, this comparison becomes potentially even more interesting when one considers that the midgut potassium transport is only temporarily turned off in the molting larva, and when the molt is over the midgut will recover its transport activity; but when transport is shut down in the prepupa, the gut will never recover because it is shed completely and replaced by a new pupal epithelium. Therefore this study provided the opportunity to look for ultrastructural changes associated with both a reversible and an irreversible loss of transport activity. Of course with this type of study it is always possible that there will be no visible changes at all, but the midgut proved to be a very fortunate choice of epithelium. I was able to identify several changes at both a gross and fine structural level, and I would now like to describe some of these results, none of which have been previously published.

Columnar cell ultrastructure

The columnar cells are probably involved in digestive processes rather than potassium transport, but it was interesting to find that in anterior midgut the unusual secretory microvilli, described earlier and shown in Figure 7A, cease this secretory activity in the molting larva (Fig. 7D) and the prepupa (Fig. 7E). The production of Golgi vesicles for secretion at the apical membrane (Fig. 7B, C) is also stopped. Neither of these changes is surprising, since all feeding has also stopped at these times. However, there is another change for which I have no explanation at this time; during the molt from fourth to fifth instar, the apical cytoplasm of the columnar cells

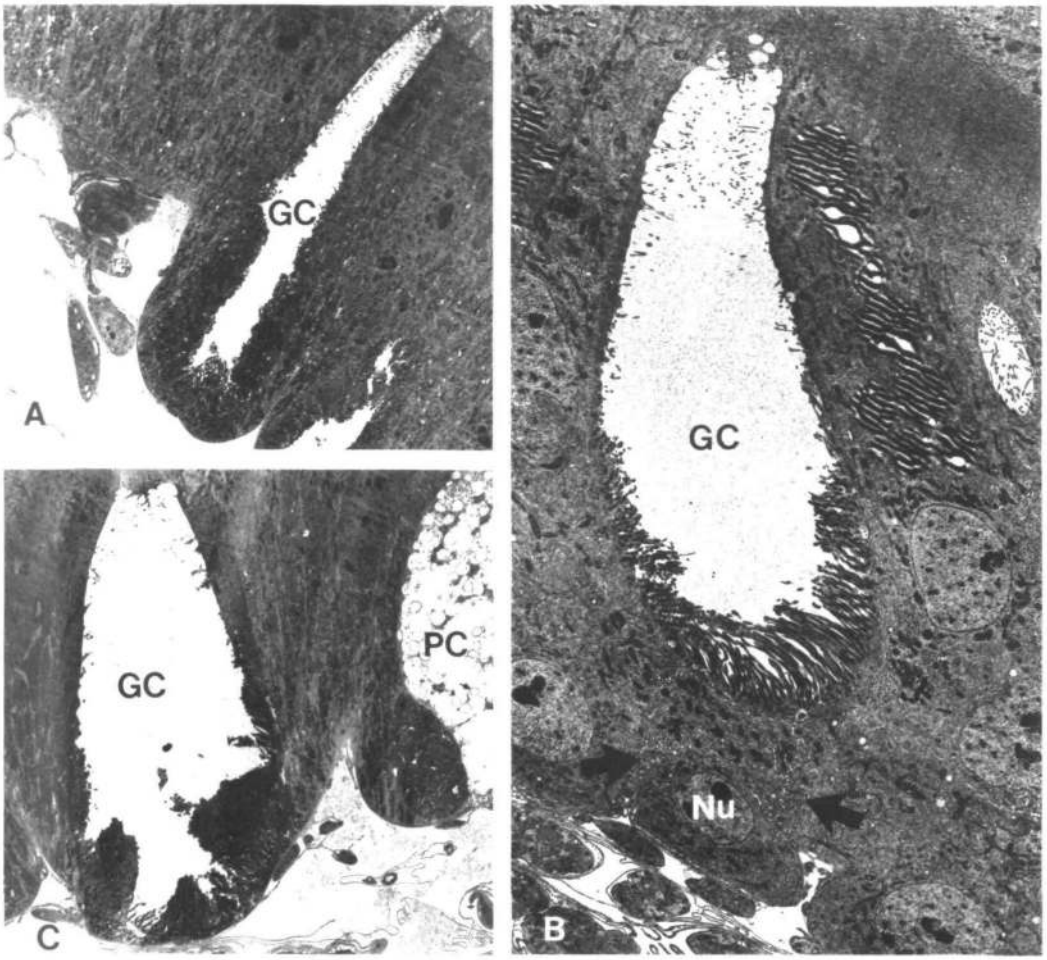


FIG. 8. Goblet cells from the anterior region of the midgut of *M. sexta*. A. A feeding fifth instar larva. B. A larva molting from fourth to fifth instar. C. A prepupal larva. In the feeding larva, A, and the prepupa, C, the base of the goblet cell projects below the level of the epithelium and the apical membrane is infolded to form a cavity (GC) reaching almost to the basal membrane. A developing pupal epithelial cell (PC) can be seen in C. In the molting larva, B, the base of the goblet cell is retracted into the epithelium, and a stalk of cytoplasm (arrows) containing the goblet cell nucleus (Nu) separates the infolded apical membrane from the basal membrane. A, C, ×1,000; B, ×2,200.

becomes filled with mitochondria (Fig. 7D). This does not occur in the prepupa (Fig. 7E).

Goblet cell ultrastructure

The goblet cells showed several interesting changes which may be associated with loss of potassium transport activity in molting and prepupal larvae. During the molt from fourth to fifth instar, there is a dramatic change in the shape of the goblet cells. In both the fourth and fifth instar larvae, the goblet cells in the anterior region of the midgut have a large rounded base which characteristically dips down below the level of the columnar cells and projects into the haemolymph (Fig. 8A). The cavity formed by invagination of the apical membrane reaches almost to the base of the cell, and the nucleus occupies a position lateral to the cavity. This arrangement would facilitate diffusion of potassium from

the blood into the cell, not only by exposing a large surface area of basal membrane but also by pushing the basal membrane through the unstirred layer close to the epithelium and into the blood. In the molting larva, however, the goblet cells are retracted back into the epithelium (Fig. 8B). The cytoplasm flows down to form a stalk separating the basal membrane from the invaginated apical membrane, and the nucleus moves to a position ventral to the cavity. Interestingly, this change in goblet cell shape does not occur in the prepupal stage (Fig. 8C), even though the potassium pump is lost at this time. I did, however, get the opportunity to examine the midgut of a larva which was spontaneously molting from a fifth to a sixth instar larva instead of entering the prepupal stage, and I was pleased to see that in this case the goblet cells were undergoing the same morphological changes as in the molt from fourth to fifth instar.

The goblet cavity opens into the midgut lumen through a valve-like arrangement shown in Figure 9A. In the fourth or fifth instar larvae, there are small spaces between the microvilli forming the valve, so that there is a tortuous pathway leading from the cavity to the lumen. However in both the molting larva and the prepupa, the valve appears to become sealed, and some dense material collects between the microvilli (Fig. 9B, C).

In posterior midgut, the goblet cells showed no change in shape during molting, but even in a feeding larva the cavity occupies only the apical half of the cell and the base of the goblet cell is more or less level with that of adjacent columnar cells. However in both molting and prepupal larvae, the goblet cell valve becomes sealed in the same way as the anterior midgut goblet cells. In addition, the cavity of posterior midgut goblet cells becomes filled with an electron dense, granular material (Fig. 10).

In both anterior and posterior midgut, the cellular site of active potassium transport is believed to be the apical membrane of the goblet cell. I was therefore disappointed to find that in molting and prepupal larvae there were no changes in the

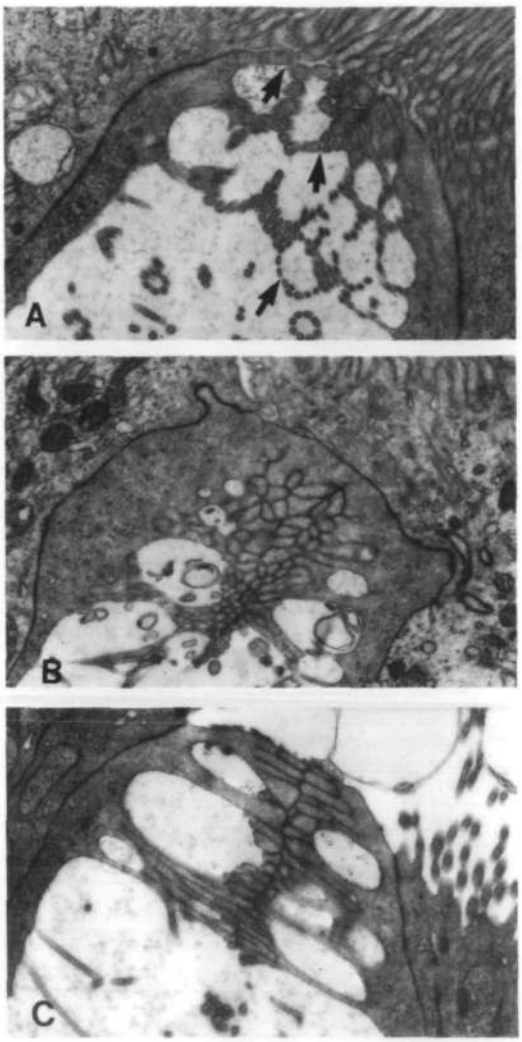
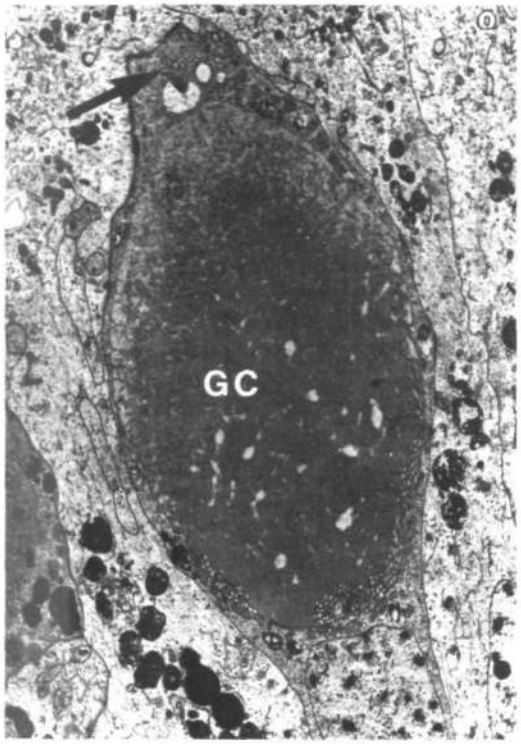


FIG. 9. Goblet cell valves from the anterior region of the midgut of *M. sexta*. A. A feeding fifth instar larva. B. A larva molting from fourth to fifth instar. C. A prepupal larva. In the feeding larva, A, small spaces (arrows) between the microvilli forming the valve provide a tortuous pathway from the goblet cavity to the midgut lumen. In the molting, B, and prepupal, C, larvae, the spaces between the valve microvilli are sealed. ×10,200.

portasomes covering this membrane, or, in the case of anterior midgut, in its association with mitochondria. In Malpighian tubules, where mitochondria are also inserted into the microvilli of the transporting apical membrane, the mitochondria move in and out of the microvilli as



transport is stimulated or turned off respectively (Ryerse, 1977; Bradley and Satir, 1981; also see Bradley, 1984). A similar movement of mitochondria also occurs in the Malpighian tubules of *M. sexta* (Cioffi, unpublished), with mitochondria present in the tubule microvilli in feeding fourth and fifth instar larvae (Fig. 11A) but not in the molting and prepupal stages (Fig. 11B, C). It is possible that this mitochondrial movement does not occur in anterior midgut goblet cells because, as suggested earlier, the mitochondria associated with the goblet cell apical membrane are involved in other processes in addition to potassium transport.

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FIG. 10. The apical portion of a goblet cell from the posterior region of the midgut in a prepupal *M. sexta* larva. In posterior midgut the goblet cavity occupies only the apical half of the cell, but in molting and prepupal larvae the cavity (GC) becomes filled with an electron dense material. The valve (arrow) also becomes sealed. ×3,300.

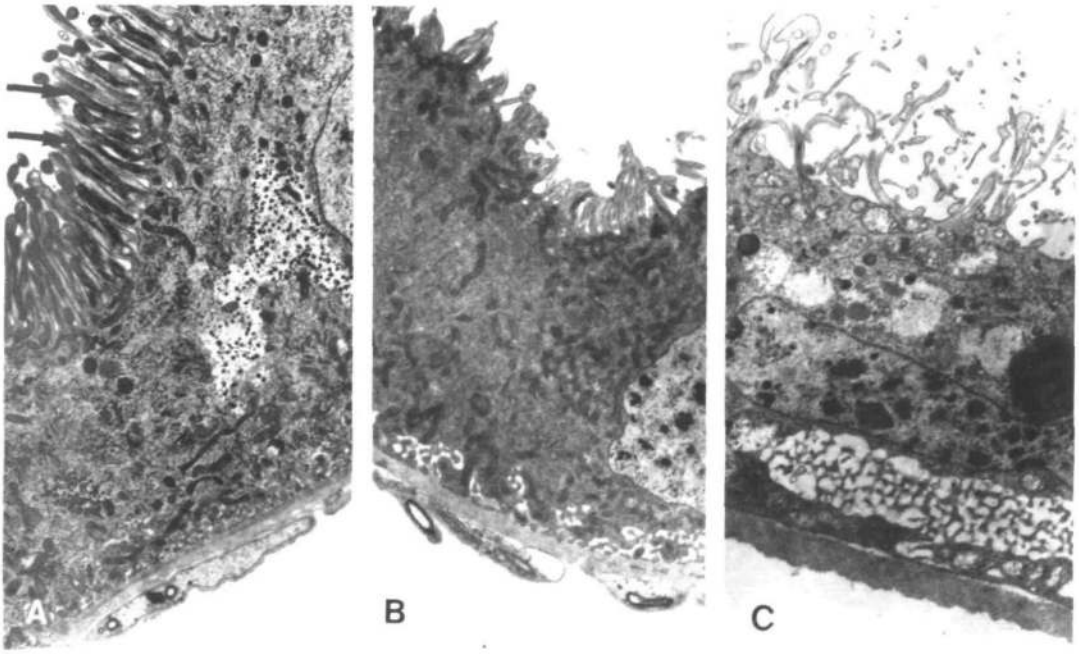

FIG. 11. Malpighian tubule cells from *M. sexta*. A. A feeding fifth instar larva. B. A larva molting from fourth to fifth instar. C. A prepupal larva. In the feeding larva, A, mitochondria (arrows) are present inside the apical membrane microvilli, but in the molting, B, and prepupal, C, larvae the microvilli are smaller and do not contain mitochondria. ×5,500.

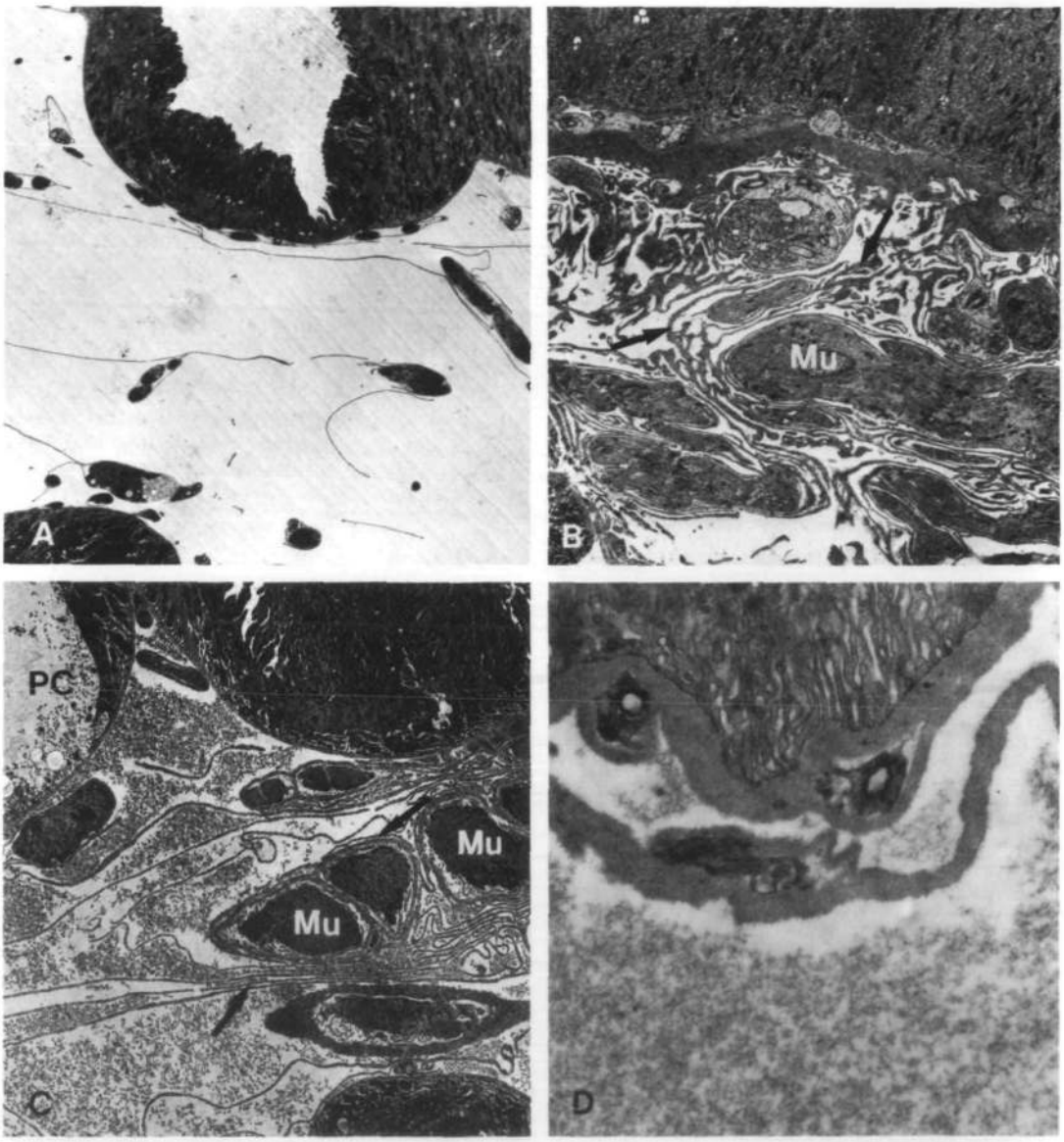

FIG. 12. The basement membrane and muscle layers underlying the midgut epithelium of *M. sexta*. A. A feeding fifth instar larva, B. a larva molting from fourth to fifth instar. C, D. A prepupal larva. In the molting larva, B, and the prepupa, C, there is proliferation of basement membrane material (arrows) and muscle (Mu). A developing pupal epithelial cell (PC) can be seen in C. In the prepupa there is also accumulation of granular material between the muscle blocks and layers of basement membrane. This granular material is shown at higher magnification in D. A, ×1,300; B, ×2,000; C, ×2,400; D, ×10,300.

Basement membrane and muscle layers

In addition to changes in goblet and columnar cell ultrastructure, when compared to the fourth and fifth instar larvae both the molting and prepupal stages show an enormous proliferation of basement membrane and muscle tissue (Fig. 12A–C). Each of the muscle blocks becomes wrapped in whorls of basement membrane, and the spaces between the muscle layers and

between the muscle layers and the epithelium are packed with basement membrane. In the prepupa (Fig. 12C), particularly at the base of the epithelial folds, there is an accumulation of granular material which resembles loosely packed basement membrane material (Fig. 12D), and which may later condense to make additional layers of basement membrane.

Ultrastructural changes in relation to potassium transport

Under conditions of active transport it is believed that potassium entering the goblet cells either actively or passively is then actively transported across the goblet cell apical membrane into the cavity, and from there passes through the valve and into the midgut lumen. In midguts of molting and prepupal larvae, the sealed goblet cell valve, and in the case of posterior midgut, the formation of a "plug" in the goblet cavity both seem to form a physical barrier between the apical membrane of the goblet cell and the midgut lumen. Similarly, the proliferated basement membrane and muscle layers may also form a permeability barrier between the blood and the basal membrane of the goblet cell. The retraction of the goblet cell, which occurs in the molting larva, is a further impedence to ion movement. All these changes are reversible, since they all occur in the molting larva and then the cells return to normal after the molt. An interesting question is why do the goblet cells retract only in the molting larva and not in the prepupa, and the answer may be found in a consideration of the Rb-86 fluxes at each stage. Recall that there is no net flux across the midgut in either the molting or prepupal stage. However these preliminary studies also showed that during the molt there is a considerable but equal leak of Rb in both directions across the gut, whereas in the prepupal stage the gut is extremely tight to Rb, and I was unable to obtain any counts above background in either direction. More detailed flux measurements are in progress, but the results obtained so far suggest that in the prepupa the basal membrane of the goblet cell may undergo an irreversible change which makes it extremely impermeable to potassium, so that retraction of the cell is unnecessary.

Many of these ideas can be tested. For example, I have been able to digest away the cells from a midgut epithelium mounted on a chamber, leaving only the basement membrane and muscle layer, so that it should be possible to test the permeability of this preparation at different larval stages. I also hope to examine the permeability of the basal plasma membrane in subcellular preparations, using the techniques of Cioffi and Wolfersberger (1983) to isolate the membrane fractions. The aim of these experiments is to show a direct correlation between a specific ultrastructural event and a specific biochemical or physiological one. The midgut of *M. sexta* seems to be an excellent system for studying such correlations; distinct ultrastructural changes accompany loss of transport activity, and in addition the tissue can be conveniently manipulated *in vitro* to obtain short circuit current and ion flux measurements. Hopefully, experiments of this type will lead not only to a better understanding of potassium transport in the midgut, but also of the factors which control transport in other epithelia.

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REFERENCES

Anderson, E. and W. R. Harvey. 1966. Active transport by the *Cecropia* midgut. II. Fine structure of the midgut epithelium. J. Cell Biol. 31:107–134.

Berridge, M. J. and B. L. Gupta. 1967. Fine structural changes in relation to ion and water transport in the rectal papillae of the blowfly, *Calliphora*. J. Cell Sci. 2:89–112.

Berridge, M. J. and J. L. Oschman. 1969. A structural basis for fluid secretion by Malpighian tubules. Tissue and Cell 1:247–272.

Berridge, M. J. and J. L. Oschman. 1972. *Transporting epithelia.* Academic Press, New York.

Bradley, T. J. 1984. Mitochondrial placement and function in insect ion-transporting cells. Amer. Zool. 24:157–167.

Bradley, T. J. and P. Satir. 1979. Insect axopods. J. Cell Sci. 35:165–175.

Bradley, T. J. and P. Satir. 1981. 5-Hydroxytryptamine-stimulated mitochondrial movement and microvillar growth in the Malpighian tubules of the insect *Rhodnius prolixus.* J. Cell Sci. 49:139–161.

Cioffi, M. 1979. The morphology and fine structure of the larval midgut of a moth (*Manduca sexta*) in relation to active ion transport. Tissue and Cell 11:467–479.

Cioffi, M. and W. R. Harvey. 1981. Comparison of potassium transport in three structurally distinct regions of the insect midgut. J. Exp. Biol. 91:103–116.

Cioffi, M. and M. G. Wolfersberger. 1983. Isolation of separate apical, lateral and basal plasma membrane from cells of an insect epithelium. A procedure based on tissue organization and ultrastructure. Tissue and Cell 15:781–803.

Copeland, D. E. and A. T. Fitzjarrell. 1968. The salt absorbing cells in the gills of the blue crab (*Callinectes sapidus* Rathbun) with notes on modified mitochondria. Z. Zellforsch. Mikrosk. Anat. 92: 1–22.

Factor, J. R. 1981. Unusually complex basement membranes in the midgut of two decapod crustaceans, the stone crab (*Menippe mercenaria*) and the lobster (*Homarus americanus*). Anat. Rec. 200: 253–258.

Farquhar, M. G. and G. E. Palade. 1963. Junctional complexes in various epithelia. J. Cell. Biol. 17: 375–412.

Harvey, W. R. 1980. Water and ions in the gut. *In* M. Locke and D. S. Smith (eds.), *Insect biology in the future "VBW 80,"* pp. 105–119. Academic Press, New York.

Harvey, W. R., M. Cioffi, and M. G. Wolfersberger. 1981. Portasomes as coupling factors in active ion transport and oxidative phosphorylation. Amer. Zool. 21:775–791.

Hootman, S. R. and F. P. Conte. 1974. Fine structure and function of the alimentary epithelium in *Artemia salina* nauplii. Cell Tissue Res. 155:423–436.

Houk, E. J., J. L. Hardy, and R. E. Chiles. 1981. Permeability of the midgut basal lamina in the mosquito, *Culex tarsalis* Coquillett (Insecta, Diptera). Acta Trop. 38:163–171.

Ito, S. and R. J. Winchester. 1963. The fine structure of the gastric mucosa in the bat. J. Cell Biol. 16: 541–577.

Lowenstein, W. R. and Y. Kanno. 1964. Studies on an epithelial (gland) cell junction. I. Modifications of surface membrane permeability. J. Cell Biol. 22:565–586.

Neville, A. C. 1967. Chitin orientation in cuticle and its control. *In* J. W. L. Beament, J. E. Treherne, and V. B. Wigglesworth (eds.), *Advances in insect physiology 4,* pp. 213–286. Academic Press, New York, London.

Noirot, C. and C. Noirot-Timothée. 1971. Ultrastructure du proctodeum chez le Thysanoure *Lepismodes inquillinus* Newman (=*Thermobia domestica* Packard). J. Ultra. Res. 37:335–350.

Oschman, J. L. and M. J. Berridge. 1970. Structural and functional aspects of salivary fluid secretion in *Calliphora.* Tissue and Cell 2:281–310.

Oschman, J. L. and B. J. Wall. 1969. The structure of the rectal pads of *Periplaneta americana* L. with regard to fluid transport. J. Morphol. 127: 475–509.

Reinhardt, C. and H. Hecker. 1973. Structure and function of the basal lamina and of the cell junctions in the midgut epithelium (stomach) of female *Aedes aegypti* L. (Insecta, Diptera). Acta Trop. 30: 213–236.

Rieder, N. and F. Schlect. 1978. Erster nachweis von freien cilien im mitteldarm von arthropoden. Z. Naturforsch. 33C:598–599.

Ryerse, J. S. 1977. Control of mitochondrial movement during development of insect Malpighian tubules. Proc. Microscop. Soc. Can. 4:48–49.

Schmidt-Nielsen, B. K., H. Gertz, and L. E. Davis. 1968. Excretion and ultrastructure of the antennal gland of the fiddler crab *Uca mordax.* J. Morphol. 125:473–495.

Smith, D. S., K. Compher, M. Janners, C. Lipton, and L. W. Wittle. 1969. Cellular organization and ferritin uptake in the midgut epithelium of a moth, *Ephestia kuhniella.* J. Morphol. 127:41–72.

Tormey, J. McD. and J. M. Diamond. 1967. The ultrastructural route of fluid transport in rabbit gallbladder. J. Gen. Physiol. 50:2031–2060.