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Structure and Function of the Basal Lamina and of the Cell Junctions in the Midgut Epithelium (Stomach) of Female *Aedes aegypti* L. (Insecta, Diptera)

C. REINHARDT and H. HECKER

Introduction

Vertebrate and invertebrate epithelia are separated from connective tissue or from the haemocoel by a basal lamina (BL). The BL consists of a matrix within which filamentous structures may be embedded (FAWCETT 1966, ASHHURST 1968). Biochemical investigations of BL in various vertebrate tissues reveal filamentous tropocollagen-like macromolecules bound to antigenic matrix glycoproteins. The BL is composed of about 10% carbohydrate and 90% protein (KUPFER & GEYER 1968, RAMBOURG et al. 1969, PIERCE 1970, SPIRO 1970). In insect BL, fibrous collagen-like structure may be associated with the mucopolysaccharides of the matrix (ASHHURST 1968).

The following insect epithelia exhibit BL with regularly arrayed particles or grid-like structures in the amorphous matrix: ovarian follicular tubes (BERTRAM & BIRD 1961), salivary glands (ASHHURST 1968), ovotestis (COGGESHALL 1970), malpighian tubes (REINHARDT, unpublished observation on fleas). In addition, the following insect midguts contain grid-like structures in the BL matrix:

Heteroptera	<i>Ranatra linearis</i>	GOURANTON (1970)
	<i>Nepa cinerea</i>	GOURANTON (1970)
Coleoptera	6 different species	HOLTER (1970)
Siphonaptera	<i>Ctenophthalmus</i> spec.	RICHARDS & RICHARDS (1968)
	<i>Xenopsylla cheopis</i>	REINHARDT et al. (1972)
	<i>Echidnophaga gallinacea</i>	REINHARDT et al. (1972)
Nematocera, Culicidae	<i>Anopheles quadrimaculatus</i> ♀	VANDERBERG et al. (1967)
	<i>Aedes aegypti</i> ♀	BERTRAM & BIRD (1961)
		STÄUBLI et al. (1966)
		TERZAKIS (1967)
		HECKER et al. (1971)

Using light microscope histochemical techniques, HOLTER (1970) found protein structures in a carbohydrate containing matrix of the BL from coleopteran midguts. Ultrastructural, cytochemical and enzyme extraction studies on the cockroach midgut BL demonstrated a pattern of collagen-like particles embedded in a polysaccharide matrix (GOURANTON 1970).

Membrane junctions control cell-to-cell relations such as the movement of ions along the longitudinal and transverse axes of an epithelium. They also play an important role in the mechanical maintenance of cell contact (TONER & CARR 1968, SMITH et al. 1969).

Gap junctions (gj) or close junctions with a 20–40 Å gap, containing hexagonally arrayed particles with a center-to-center distance of 90–100 Å, have been

found in many vertebrate tissues where electrical coupling (small ion transport from cell to cell) has been demonstrated (REVEL & KARNOVSKY 1967, REVEL & GOODENOUGH 1970, GOODENOUGH & REVEL 1970, 1971, MCNUTT & WEINSTEIN 1970, CHALCROFT & BULLIVANT 1970, HUDSPETH & REVEL 1971, SPIRA 1971, 1971a).

In some invertebrates, *septate junctions* (sj) or septate desmosomes were originally thought to be responsible for ion transport from cell to cell (GILULA et al. 1970, SATIR & GILULA 1970, ROSE 1971). More recent investigations confirm the coexistence of sj and gj in *Chironomus* and lamellibranch electron coupled tissues (BERGER & UHRIK 1972, GILULA & SATIR 1971). Based on our knowledge of vertebrate gj, these results suggest that invertebrate gj may be primarily responsible for ion transport. In support of this hypothesis BERGER & UHRIK (1972) have shown that gj disappear in anisomotic (= uncoupling) fixatives. BULLIVANT & LOEWENSTEIN (1968) have found that sj do not change in uncoupling media. Coexistence of gj and sj appears to be quite common in epithelia of other invertebrates (cockroach midgut, HAGOPIAN 1970; sugar-mite proctodeum, NOIROT & NOIROT 1971, 1971a; mollusc egg capsule gland, FLOWER 1971; hydra gastrodermis, HAND & GOBEL 1972; *Periplaneta* midgut caeca, OSCHMAN & WALL 1972); however, these epithelia have not been tested for electrical coupling. It should be added that there are a few studies on invertebrate epithelia in which gj were not found in the junctional complex (SMITH 1966, LEIK & KELLY 1970, PRIESTER DE 1971).

Sj and gj both play an important role in cellular adhesion (WOOD 1959, GOODENOUGH & REVEL 1970, 1971, HUDSPETH & REVEL 1971, HAND & GOBEL 1972). While electronic coupling is thought to be correlated with gj, sealing function and barrier properties of the intercellular cleft (in the epithelial transverse axis) is correlated with sj (FLOWER 1971, BERGER & UHRIK 1972).

In the apical half of the midgut epithelium of cockroaches and termites (NOIROT & NOIROT 1967, 1972) and of collembolans (DALLAI 1970) an intercellular junction was found, the "*zonula continua*", filled with an electron dense, more or less homogeneous material. In addition, tight junctions have also been found in invertebrate epithelia (ANDERSON & HARVEY 1966, SATIR & GILULA 1970).

Maculae adhaerentes (ma) occur in a number of invertebrate tissues: the earthworm epidermis (COGGESHALL 1966); tracheal cells, follicular epithelia and rectal papillae of cockroaches (SMITH 1966); a sensillum of the termite leg (STUART & SATIR 1968); between the nerve cord and connective tissue (ASHHURST 1970), and midgut of a moth (SMITH et al. 1969); the hypodermis of a cockroach nymph (HAGOPIAN 1970); epithelia of cockroaches and termites (NOIROT & NOIROT 1972); and gut diverticula of leeches (HAMMERSEN & POKHAR 1972).

In invertebrate midgut epithelia *zonula adhaerentes* or "intermediary junctions" (ANDERSON & HARVEY 1966, SATIR & GILULA 1970, NOIROT & NOIROT 1972) and *hemidesmosomes* (SMITH et al. 1969, PRIESTER DE 1971) have been described.

The above three types of invertebrate desmosomes lack tonofilaments; their vertebrate counterparts (known to form attachment zones [FAWCETT 1966, ARMSTRONG 1970, HAGOPIAN 1970, SHEFFIELD 1970]) are larger and more complex.

In a study of imaginal midgut differentiation in *Aedes aegypti* by HECKER et al. (1971, 1971a), sj were found in both males and females whereas ma occurred only in females.

The present study was undertaken to obtain more detailed structural and cytochemical information on the BL and the various cell junction types found by HECKER et al. (1971) in the female *A. aegypti* stomach epithelium. During the course of this investigation further cell junctions were found; descriptions of these have been included in this paper.

Material and methods

Our strain of *Aedes aegypti* L. was originally derived from the Congo (Zaire) and has been maintained for many years in our laboratory. The preparation of the female midguts for electron microscopy has been described by HECKER et al. (1971).

In order to best preserve its original form, mechanical stretching of the "stomach" (posterior part of the midgut) was carefully avoided during dissection. In addition to double fixation in glutaraldehyde followed by OsO_4 , two other fixation procedures were used: a) a mixture of 3 or 5% glutaraldehyde and 2% acrolein in 0.1 M cacodylate buffer (pH 7.2–7.4) at 4°C/2 hrs, followed by OsO_4 ; b) the "triple fixation" technique (HECKER 1970). None of these three fixation procedures caused measurable differences in the preservation of junctional structures, such as the width of the intercellular cleft, and of the BL.

To determine if the first bloodmeal (BM) causes changes in the dimensions and structure of the stomach BL and cell junctions, 3-day-old females were examined immediately after BM and at the following other times: 12 hrs, 1, 2, 3, 5, and 7 days post BM. Control non-fed 3-day-old females were also studied. Measurements of investigated structures for the most important stages are shown in table 1.

To identify polysaccharides in junctions and in the BL the THIÉRY (1967) periodic acid-TCH-silver-protein stain technique as modified by JENNI (1971) was applied to ultrathin sections. To demonstrate alkaline amino acid rich proteins in cell coats and in the BL, midguts from 3-day-old-non-fed females were fixed in glutaraldehyde and then incubated in 1% phosphotungstic acid solution (e-PTA) (BLOOM & AGHAJANIAN 1968, MEYER 1969, PFENNINGER 1971).

Results

1. Stomach of unfed mosquitoes

1.1. Basal lamina (BL)

The heterogenous BL consists of 4–7 superimposed or stacked layers, which follow an undulating course along the base of the epithelial cell (TERZAKIS 1967). These layers are embedded in an amorphous matrix which is partly continuous with the BL of the muscle cells (HECKER et al. 1971). In cross sections of the BL (fig. 1) the layers exhibit regularly arranged structures ("beads"). A grid-like substructure can be seen in a tangent section parallel to the base of a cell (fig. 3). Diagram 1 is a schematic representation of the grid-like substructures in one layer (mesh width $300 \pm 5 \text{ \AA}$, table 1). Grid lines generally intersect at right angles. Two different electron lucent "holes" can be distinguished, "hole" I (diameter $\sim 200 \text{ \AA}$) between the grid lines and "hole" II (diameter $\sim 70 \text{ \AA}$) in the annular shaped intersections of the grid lines. Cross and tangential sections of one BL layer exhibit a continuous grid. The direction of grid lines may differ from one layer to the other. In fig. 3 several subsequent layers are hit by the section plane with each layer showing another grid line orientation.

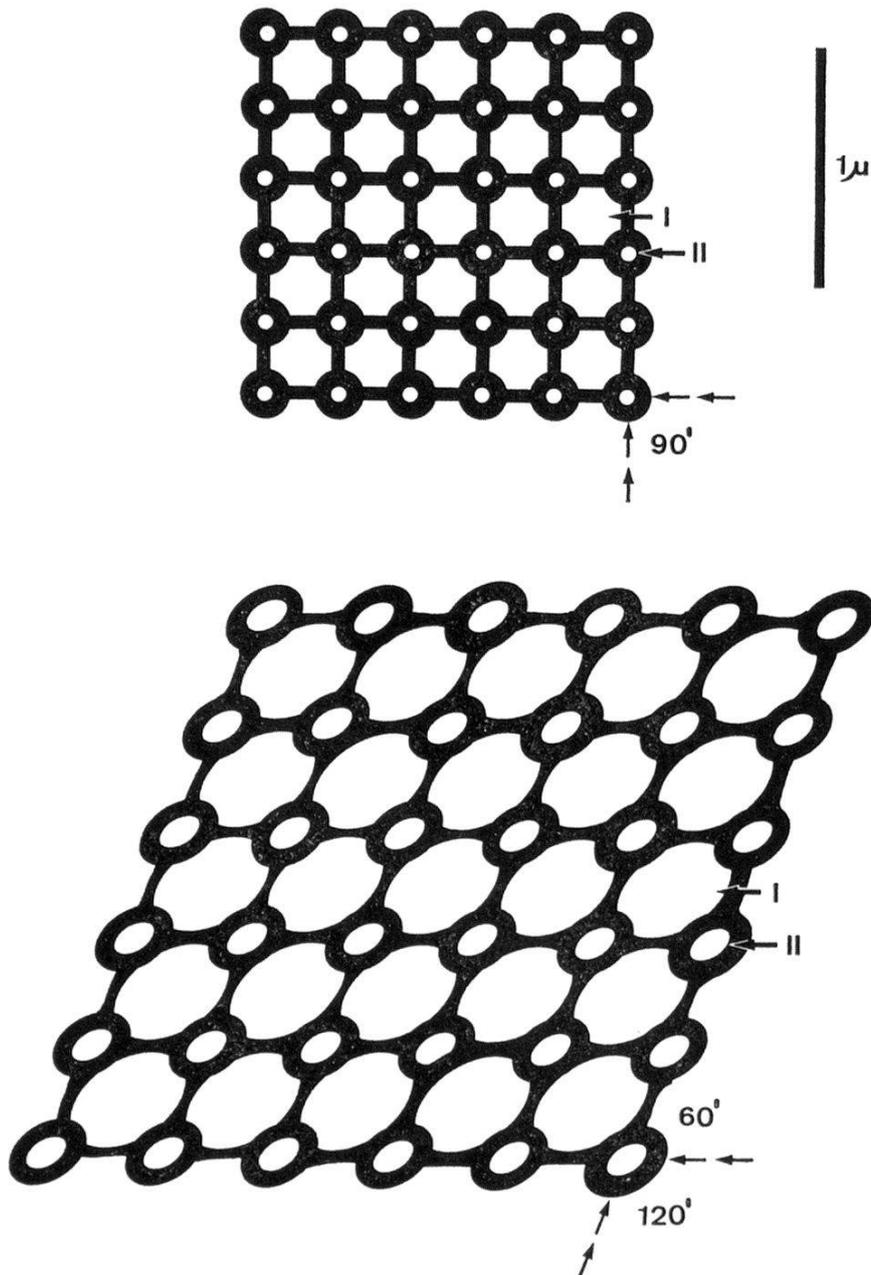
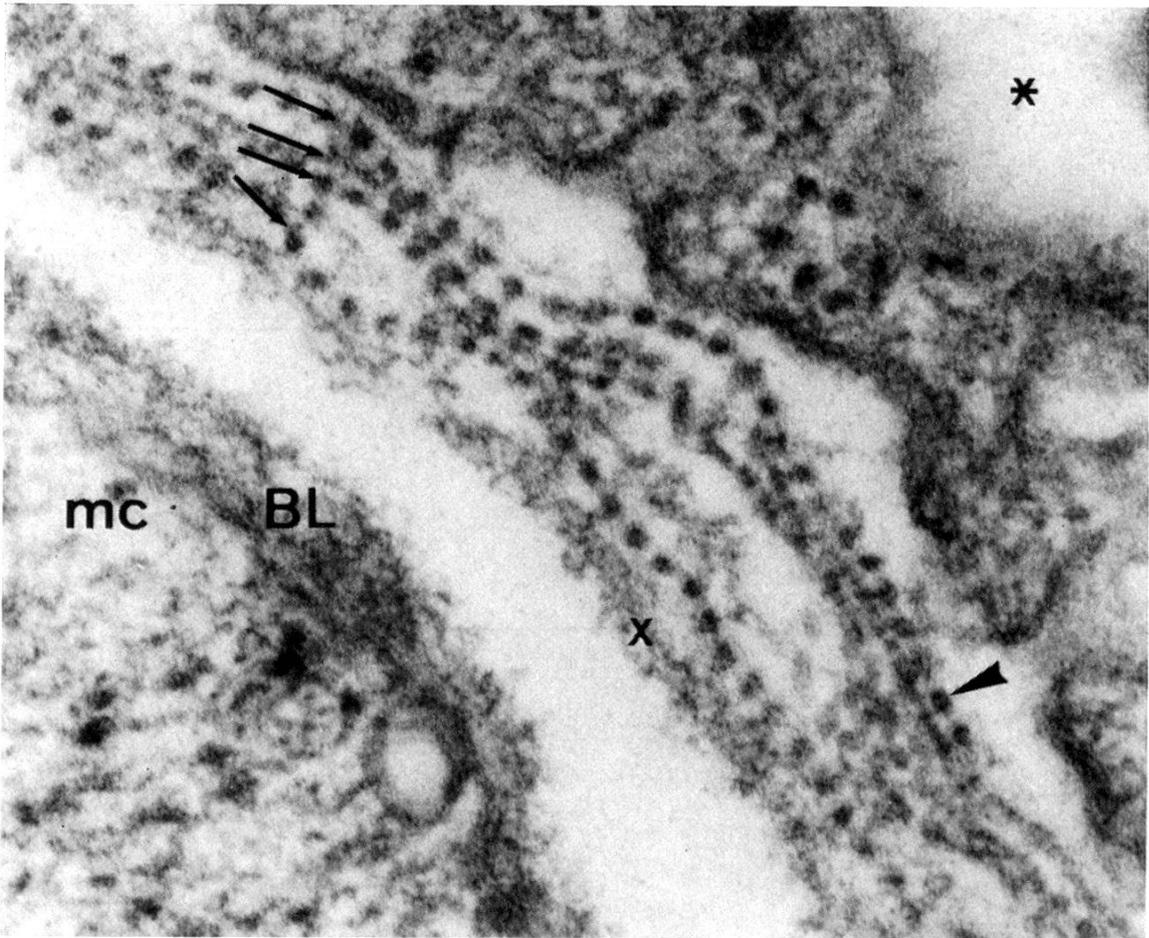


Diagram 1. Scheme of the regular grid in a BL-layer, unfed female mosquito (according to fig. 3). The grid lines ($\rightarrow \rightarrow$) intersect at right angles (90°). Two different electron lucent "holes" (I, II) can be distinguished.

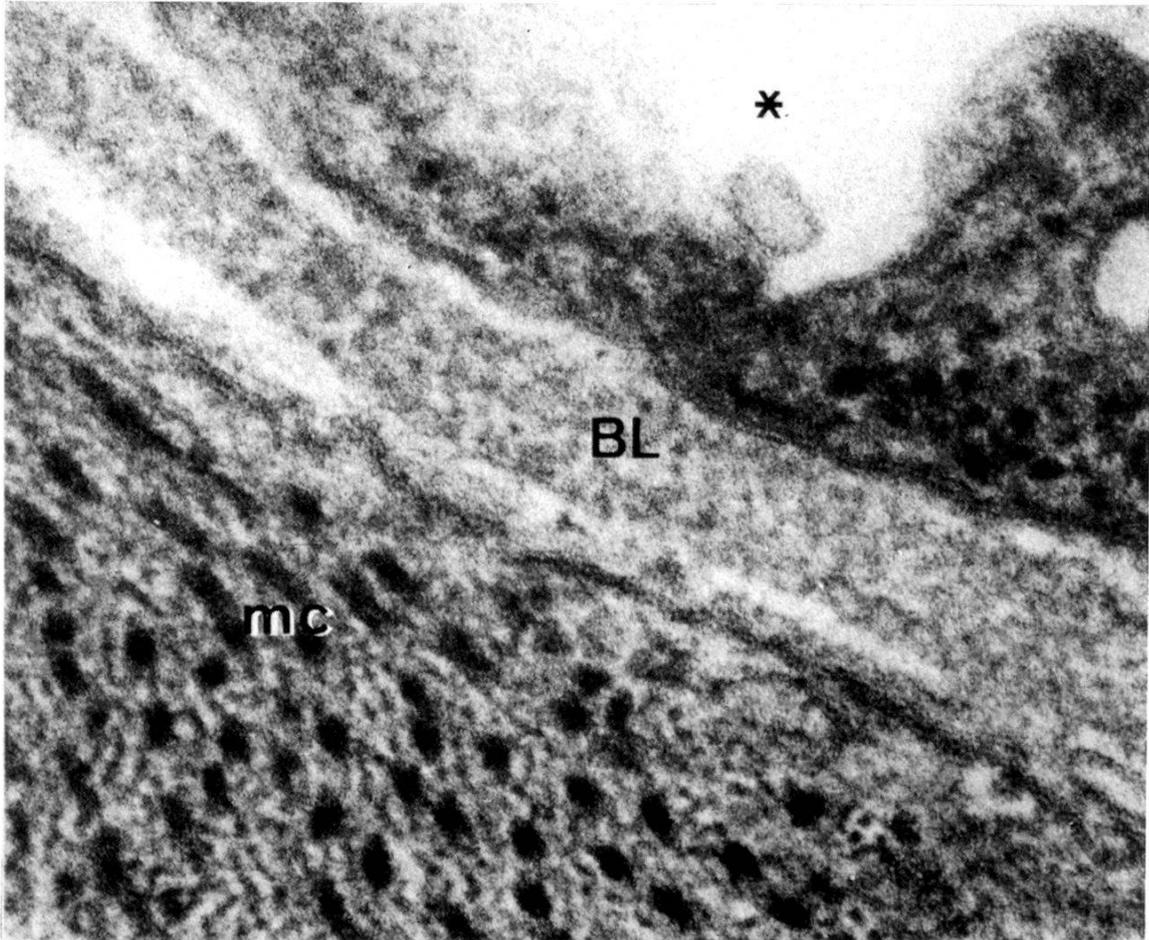
Diagram 2. Scheme of the expanded regular grid in a newly engorged mosquito (according to fig. 4). The square grid has changed to a rhombic one (angles 120° , 60° resp.). The longer diagonal axis of the rhombus coincides with the longitudinal axis of the stomach. The width of grid mesh and the "holes" (I, II) are distinctly enlarged. Same magnification as diagram 1.

Fig. 1. Stomach of female *A. aegypti*, cross section, 3 days after emergence. The basal lamina consists of several stacked layers ($\rightarrow \rightarrow$) each with a beaded appearance embedded in an amorphous matrix (x). The beads (\blacktriangleright) correspond to the annular intersections of gridlines (see fig. 3). Basal lamina (BL) of a muscle cell (mc) basal labyrinth (*). Fixation: 5% glutaraldehyde (G)/2% OsO_4 (O). 130,000 \times .

Fig. 2. Stomach, longitudinal section, immediately after bloodmeal (BM). The layers of the basal lamina (BL) cannot be distinguished, the BL is thinner and stretched. Muscle cell (mc), basal labyrinth (*). 3% G + 2% acrolein/2% O. 130,000 \times .



1



2

Table 1. Measurements (Å) of female *Aedes aegypti* stomach basal lamina and intercellular clefts before and after the first bloodmeal (BM)

	3 days after emergence without BM	immediately after BM	2 days after BM	5 days after BM
<i>Basal lamina</i>				
Thickness of the structured part	1050 ± 51 ¹	850 ± 41	1750 ± 115	1650 ± 47 ²
Width of mesh of the grid-like layers	300 ± 5	475 ± 17	330 ± 16	350 ± 5
<i>Width of the inter- cellular cleft</i>				
Gap junctions	36 ± 1.0	34 ± 1.2	35 ± 1.1	33 ± 1.1
Septate junctions	142 ± 5	147 ± 4	143 ± 2	143 ± 2.5
Maculae adhaerentes	172 ± 5	155 ± 5.5	172 ± 4	178 ± 4

¹ mean ± SE (n = 20)

= difference not significant

≠ difference significant

² compared to the first column

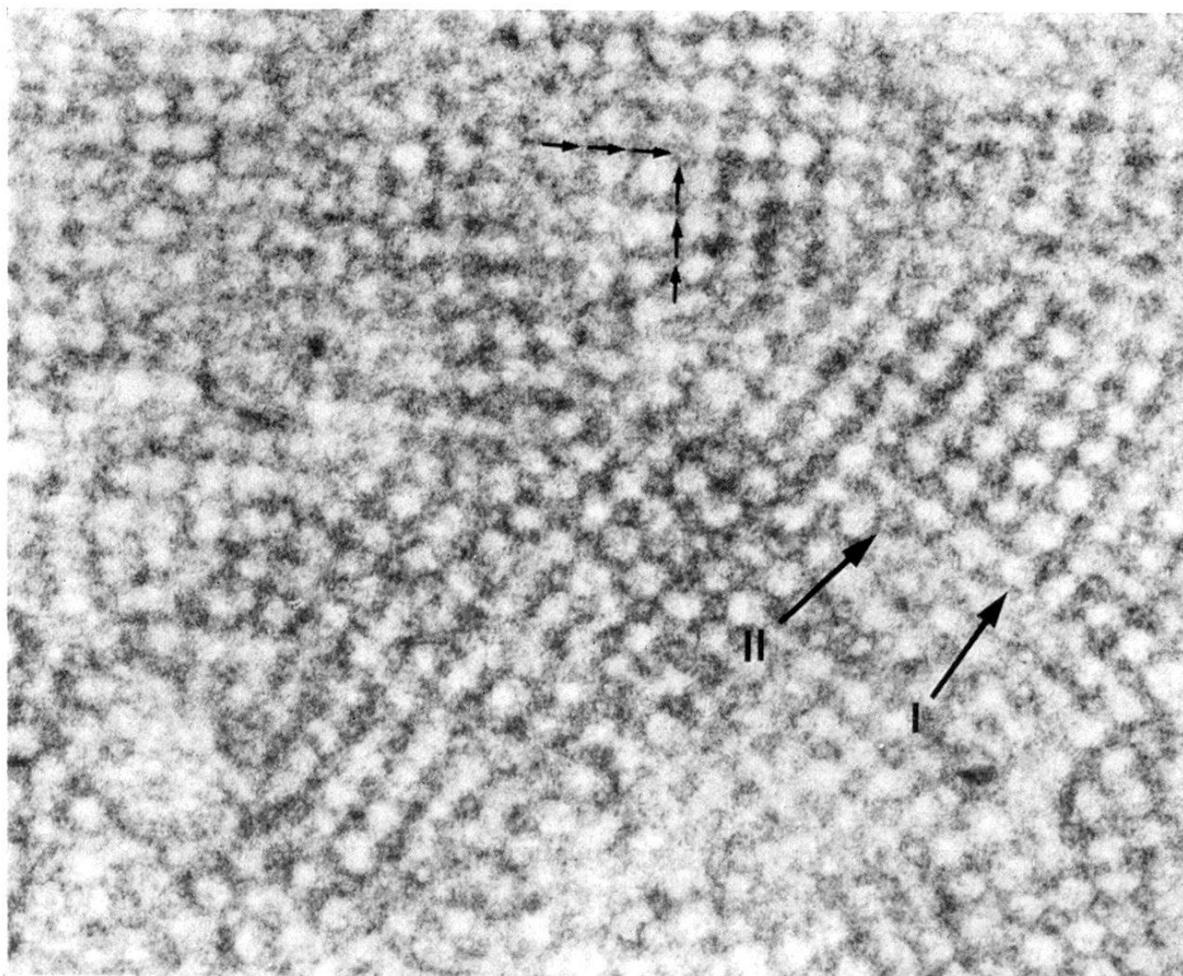
} confidence limit 95% according
to Student's t-test

Annular intersections appear as dense “beads” in cross section (fig. 1). When different layers of the BL are closely packed and parallel to one another, the stacked dense beads may give a “striated aspect” (HECKER et al. 1971) or may even simulate short dense “rods” (BERTRAM & BIRD 1961).

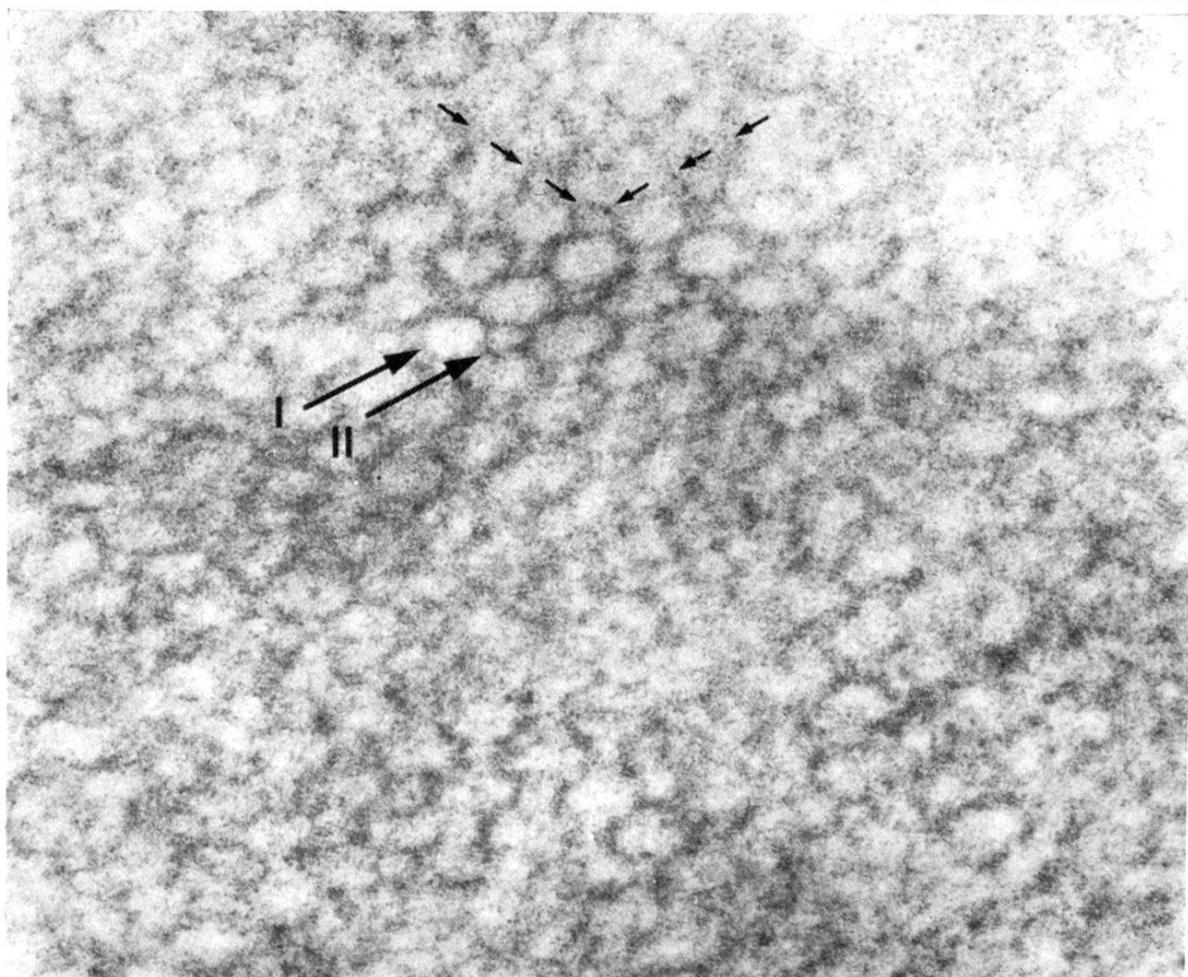
Polysaccharides could not be demonstrated in the BL matrix nor in the structured layers, however, tracheal chitin was marked with silver

Fig. 3. Stomach, 3 days after emergence, tangent section plane through the basal lamina. Grid-like structure of the BL layers (→ → →). Two types of “holes”: I between the gridlines and II in the annular intersections of the gridlines (see diagram 1). 3 layers are hit in this section plane showing 3 different gridline orientations. 5% G/2% O. 130,000 ×.

Fig. 4. Stomach, immediately after BM, tangent plane through the BL. Expanded grid-like structure with greater “holes” I and II. The gridlines (→ → →) intersect at angles of about 60° and 120°, respectively (see diagram 2). 3% G + 2% acrolein/2% O. 130,000 ×.



3



4

precipitations (fig. 11). Incubation with e-PTA did not stain for proteins in the BL (fig. 9).

1.2. Junctions

In the apical half of the epithelium, membranes of neighbouring cells run in a more or less straight manner (fig. 5). Intercellular clefts in this region are often darkly contrasted, resembling the “zonula continua” (NOIROT & NOIROT 1967, 1972; DALLAI 1970]. Several gj are always found between the sj (fig. 5, 12, 13).

In the region of the basal labyrinth, the intercellular cleft is electron lucent as well as folded and irregular in width (fig. 6). Here, ma are usually present near the cell base. Hemidesmosome-like patches line the basal cell boundaries (fig. 9).

Zonulae adhaerentes, tight junctions and “jonctions scalariformes” (FAIN-MAUREL & CASSIER 1972) were not observed in the stomach of female *A. aegypti*.

1.2.1. Gap junctions (gj)

The average width of the intercellular cleft of gj measures $36 \pm 1 \text{ \AA}$ (table 1). In the apical part of the cell gj alternate with sj (fig. 12). Sometimes, gj occur in the basal part of the cell, where their gaps open into wide intercellular spaces. The diameter of a single gj, presumably macular in shape (HAND & GOBEL 1972), may measure up to 1μ (fig. 5, 12). Very thin cross sections of gj not only exhibit an electron lucent 36 \AA gap, they also show small intercellular gap constrictions (fig. 13). These constrictions probably represent the hexagonal subunits of the gj membranes (REVEL & KARNOVSKY 1967, HAND & GOBEL 1972).

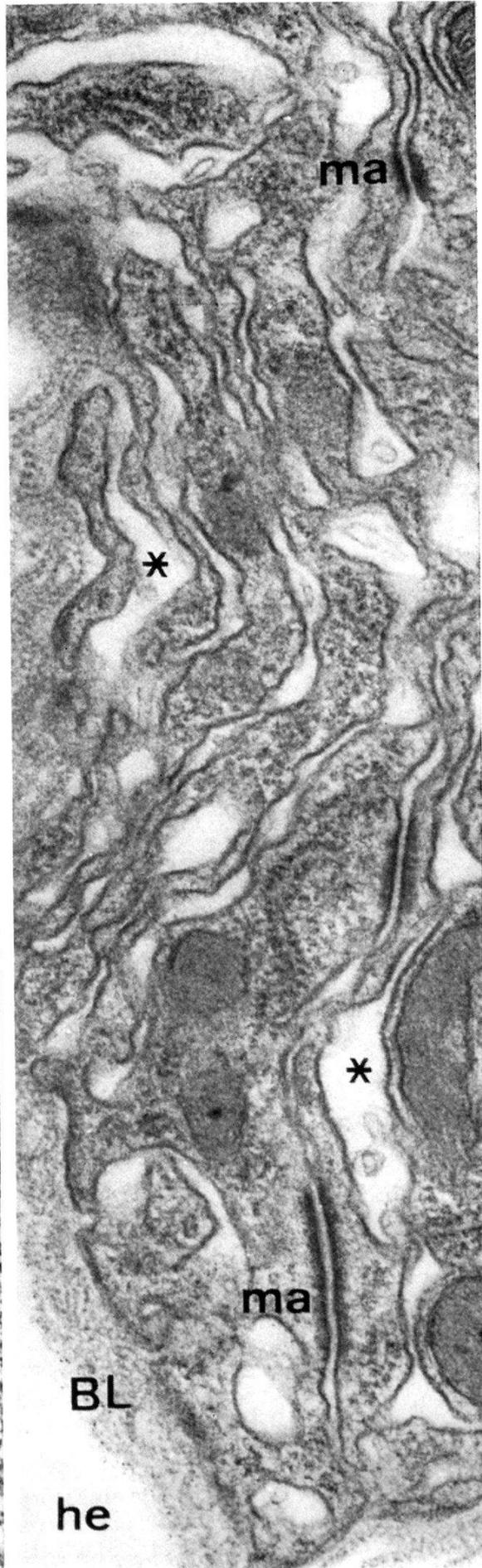
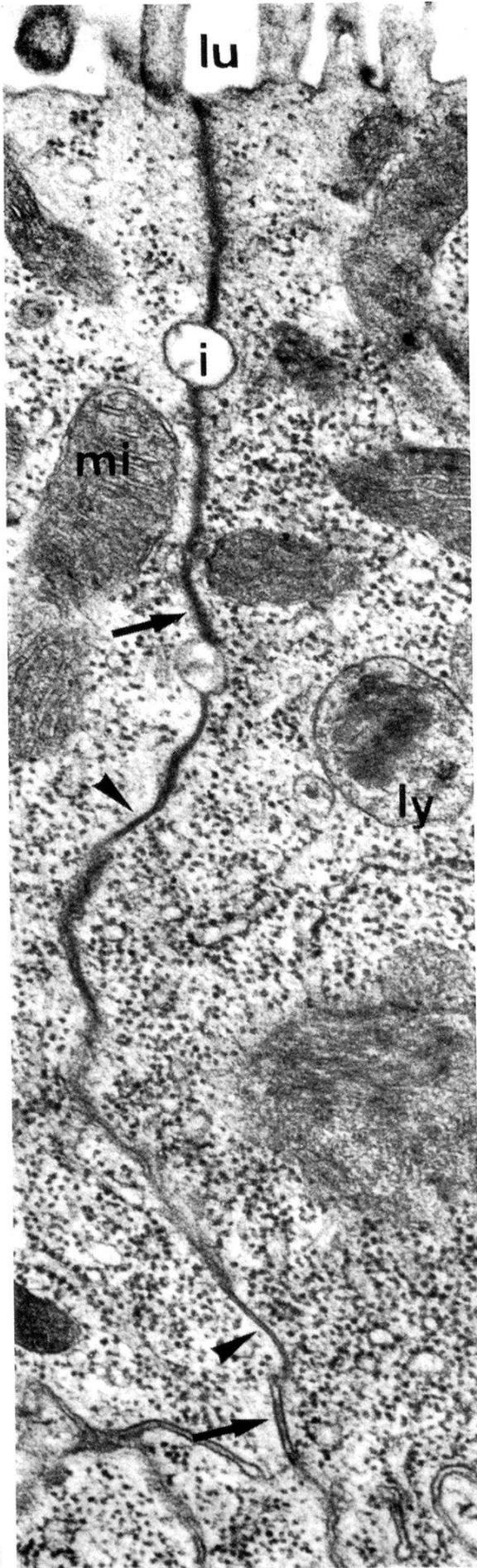
Polysaccharides can be demonstrated in gj where they fill the whole intercellular cleft (fig. 10).

1.2.2. Septate junctions (sj)

The width of the intercellular cleft of sj measures $142 \pm 5 \text{ \AA}$ (fig. 12, table 1). Depending upon the section plane, septa are rarely sharply outlined by the fixations used, and seem not to be as regular as those found in other invertebrate epithelia (BULLIVANT & LOEWENSTEIN 1968, GILULA et al. 1970, LEIK & KELLY 1970, FLOWER 1971, HAND & GOBEL

Fig. 5. Stomach, 3 days after emergence, cross section. The apical part of the intercellular cleft reveals the coexistence of gap (▶) and septate junctions (—→). Lumen (lu), mitochondria (mi), intercellular space (i), lysosome (ly). 3% G/2% O. 40,000 ×.

Fig. 6. Stomach, 5 days after BM, longitudinal section. Maculae adhaerentes (ma) of different length are present in the zone of the basal labyrinth (*). Basal lamina (BL), hemocoel (he). Triple-fixation. 45,000 ×.



1972). For this reason electron micrographs showing intercellular clefts lacking septa and resembling “zonulae continuae” cannot be interpreted with certainty as septate-free intercellular spaces. Moreover, TCH-silver and e-PTA staining of sj reveal many tangentially sectioned sj thereby supporting the idea of a sj “zonula” encircling the apical cell half (fig. 10, 14). Septate-like structures are sometimes found in the basal part of the epithelium.

TCH-silver staining revealed polysaccharides in the microvilli glycocalyx and on cell membranes (“coat”) in the apical zone of the intercellular cleft (gj, sj and vesicular loose contacts) (fig. 10, 16). In the region of the sj, a positive stain sometimes occurs between the leaflets of the cell membrane (fig. 16). The septa do not appear stained. More or less parallel rows of septa are seen in tangential sections; these rows are not visible in control sections (fig. 10, 15). Apical intercellular spaces become darkly contrasted while septa in these spaces remain electron lucent after e-PTA treatment (fig. 14). Despite the septa, DALLAI (1970) described similar cytochemical reactions for “zonulae continuae”.

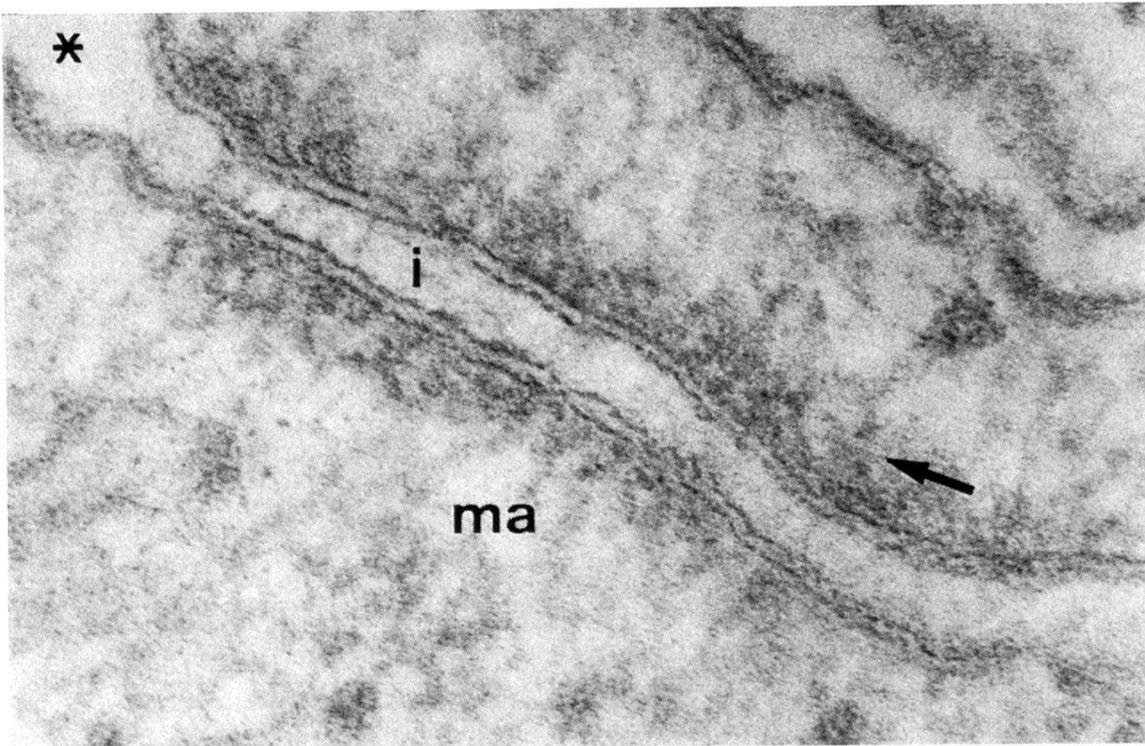
1.2.3. Maculae adhaerentes (ma)

The intercellular cleft of ma measures $172 \pm 5 \text{ \AA}$ (table 1). Usually, ma are found in the region of the basal labyrinth, bordering on the wide intercellular spaces (fig. 6, 7). Ma do not occur between basal labyrinth infoldings of a single cell, rather they are found between adjacent cells. Occasionally, they occur between sj (HECKER et al. 1971). The electron-dense “maculae” of this desmosome vary in length from 0.1–0.5 μ and has a thickness of 150–300 \AA . These values are comparable to those reported by SMITH (1966), HAGOPIAN (1970) and ASH-HURST (1970). Polysaccharides could not be demonstrated in the ma by the TCH-silver staining method. Incubation in e-PTA solution stains the “maculae” very intensively; the intercellular cleft, however, usually is not stained (fig. 8).

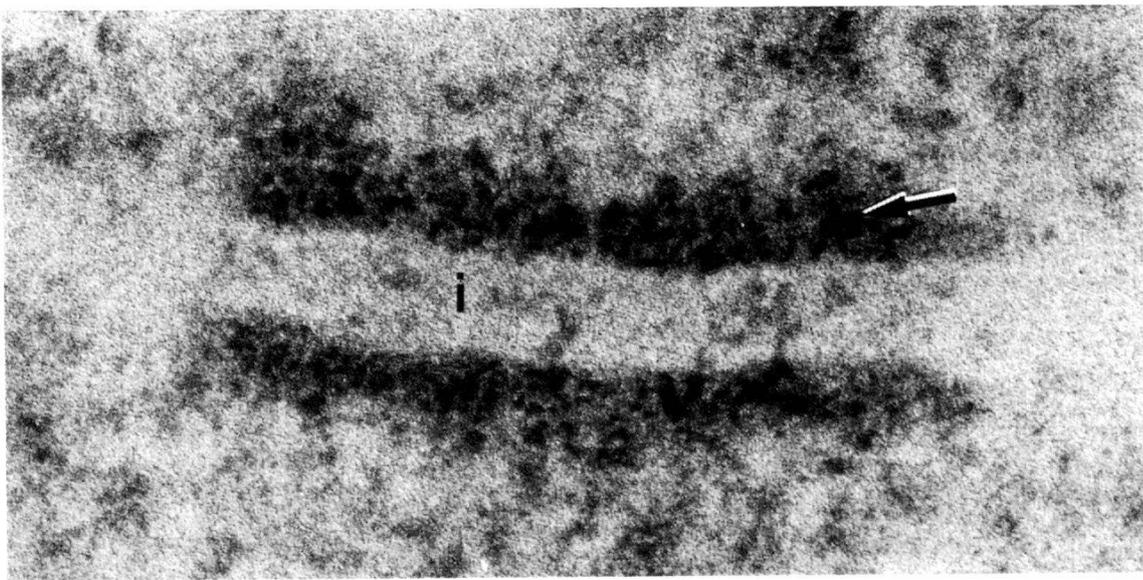
Fig. 7. Stomach, 7 days after BM. A macula adhaerens (ma) in the zone of the basal labyrinth (*) exhibits electron dense “maculae” (—→). The intercellular cleft (i) of the ma leads directly into the wide spaces of the basal labyrinth. 5% G/2% O. 260,000 \times .

Fig. 8. Stomach, 3 days after emergence. Incubation with e-PTA solution intensively contrasts the “maculae” (—→) of a ma. Intercellular cleft (i). 5% G. 260,000 \times .

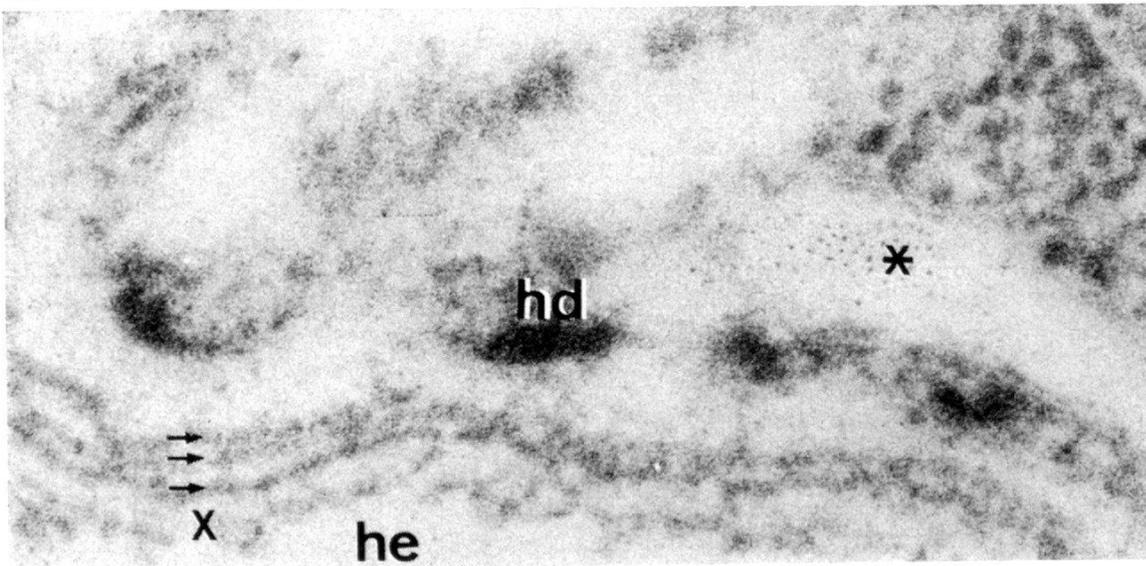
Fig. 9. Stomach, 3 days after emergence. Incubation with e-PTA solution contrasts the hemidesmosomal plaques (hd). The layers (↔) and the matrix (x) of the basal lamina are not stained. Basal labyrinth (*), hemocoel (he). 5% G. 130,000 \times .



7



8



9

1.2.4. Hemidesmosomes

Electron-opaque patches of hemidesmosomes occur along the basal cell membrane next to the BL. These patches are smaller in dimension than the “maculae”. As was true for the ma “maculae”, hemidesmosomes exhibit a negative TCH-silver reaction and a positive e-PTA reaction (fig. 9).

2. Newly engorged mosquitoes (stomach immediately after bloodmeal)

2.1. Basal lamina

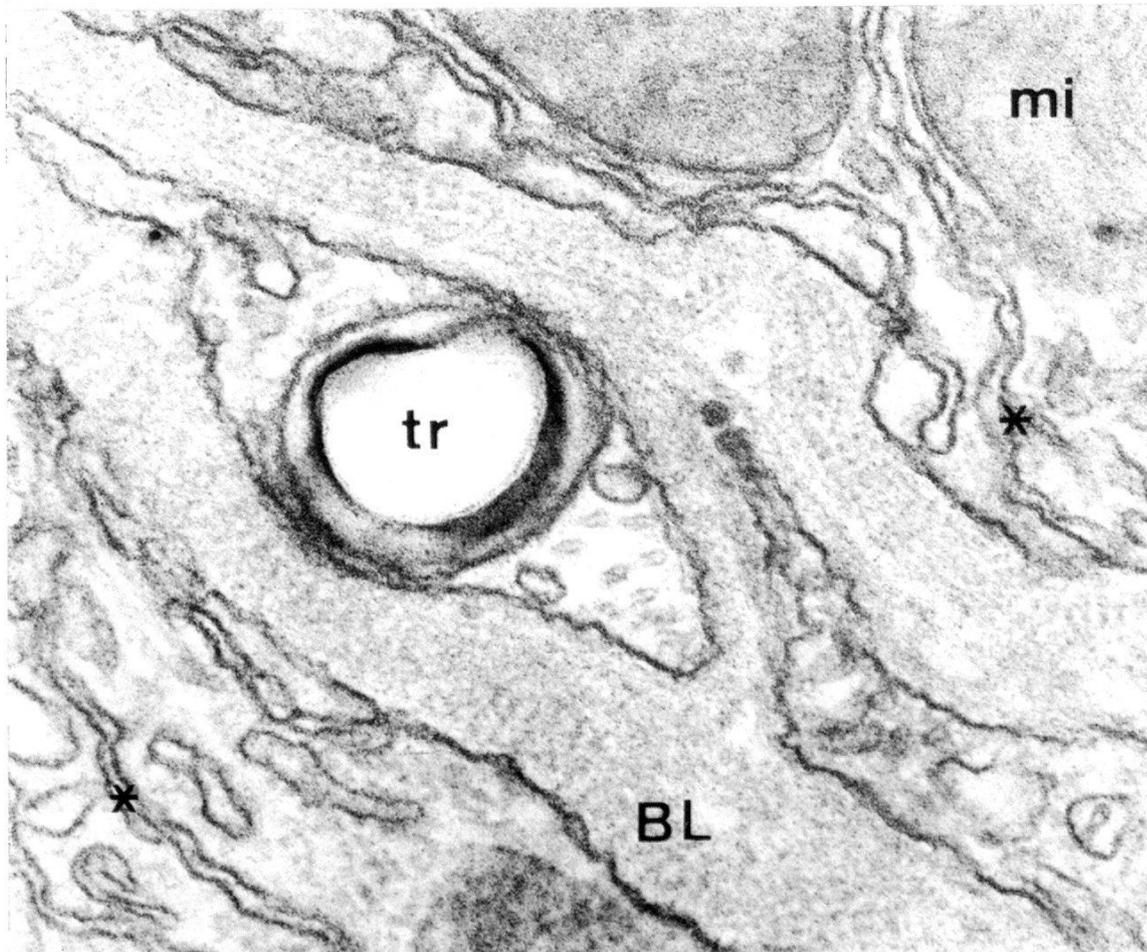
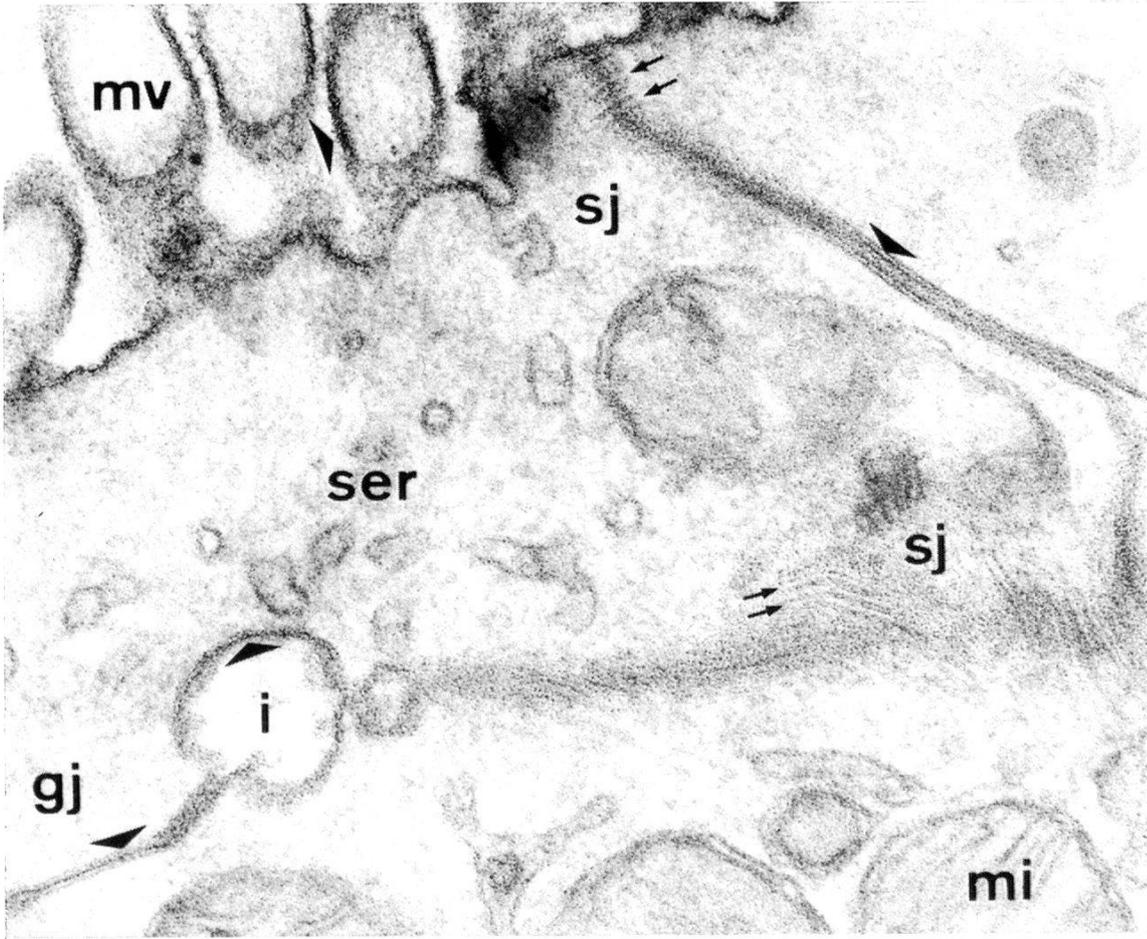
When the female mosquito takes up its bloodmeal, the blood passes through the fore part of the midgut to the stomach (CHRISTOPHERS 1960, GOODING 1972). The resulting mechanical stretching of the stomach epithelium and the flattening of the cells distinctly changes the fine structure of the BL. Electron micrographs show that the folded and previously clearly distinguishable BL layers become stretched, lying so closely together that they are no longer visually separable (fig. 2). In addition, the thickness of the BL is reduced. Tangential sections readily reveal the expansion of the grid structure of the layers (fig. 4). There is an expansion of mesh width ($475 \pm 17 \text{ \AA}$) and of “hole” diameter ($\sim 400 \text{ \AA}$ for “hole” I and $\sim 200 \text{ \AA}$ for “hole” II) (table 1). Gridlines often intersect at 60° angles in the longer diagonal axis of the grid (diagram 2). This corresponds to the greatest expansion of the BL coordinating with the longitudinal axis of the midgut.

2.2. Junctions

Epithelial stretch does not markedly alter junction ultrastructure; intercellular cleft width values for gj and sj are not significantly different before and after the bloodmeal. The ma width, however, is significantly smaller ($155 \pm 5.5 \text{ \AA}$) after blood intake (table 1).

Fig. 10. Apical part of a stomach cell, 7 days after BM. Demonstration of polysaccharides (PA, 18 hours TCH). The intensive contrast of the glycocalyx (►) and of the intercellular membrane “coats” of the gap junction (gj), septate junction (sj) and of the region of vesicular loose contact (i) indicate polysaccharides (►). A tangential sectioned sj exhibits parallel rows of polysaccharide positive lines (⇔). Mitochondria (mi), microvilli (mv), vesicles of the smooth endoplasmic reticulum (ser.). 5% G/2% O. 69,000 ×.

Fig. 11. Basal part of a stomach cell, 7 days after BM. Demonstration of polysaccharides (PA, 24 hours TCH). Precipitations only in tracheal chitin (tr) and not in the basal lamina (BL). Mitochondria (mi), basal labyrinth (*). 5% G/2% O. 69,000 ×.



3. Stomach of mosquitoes during blood digestion

3.1. Basal lamina (BL)

Two days after a bloodmeal most of the blood is digested. The BL becomes irregularly thickened and now consists of 5–10 stacked layers. Grid dimension values between those for unfed and for engorged females are now apparent, suggesting that stretching is reversible (table 1).

After *five days*, blood digestion is completed. BL thickness remains constant and the grid structure becomes more regular. There is a further reduction in mean mesh width, however, it is still significantly larger than the pre-bloodmeal mesh width (table 1).

3.2. Junctions

No further alterations were observed regarding the ultrastructure of the gj and sj and the intercellular ma cleft width returns to the pre-bloodmeal value (table 1).

Discussion

1. Stomach of female mosquito

1.1. Basal lamina (BL)

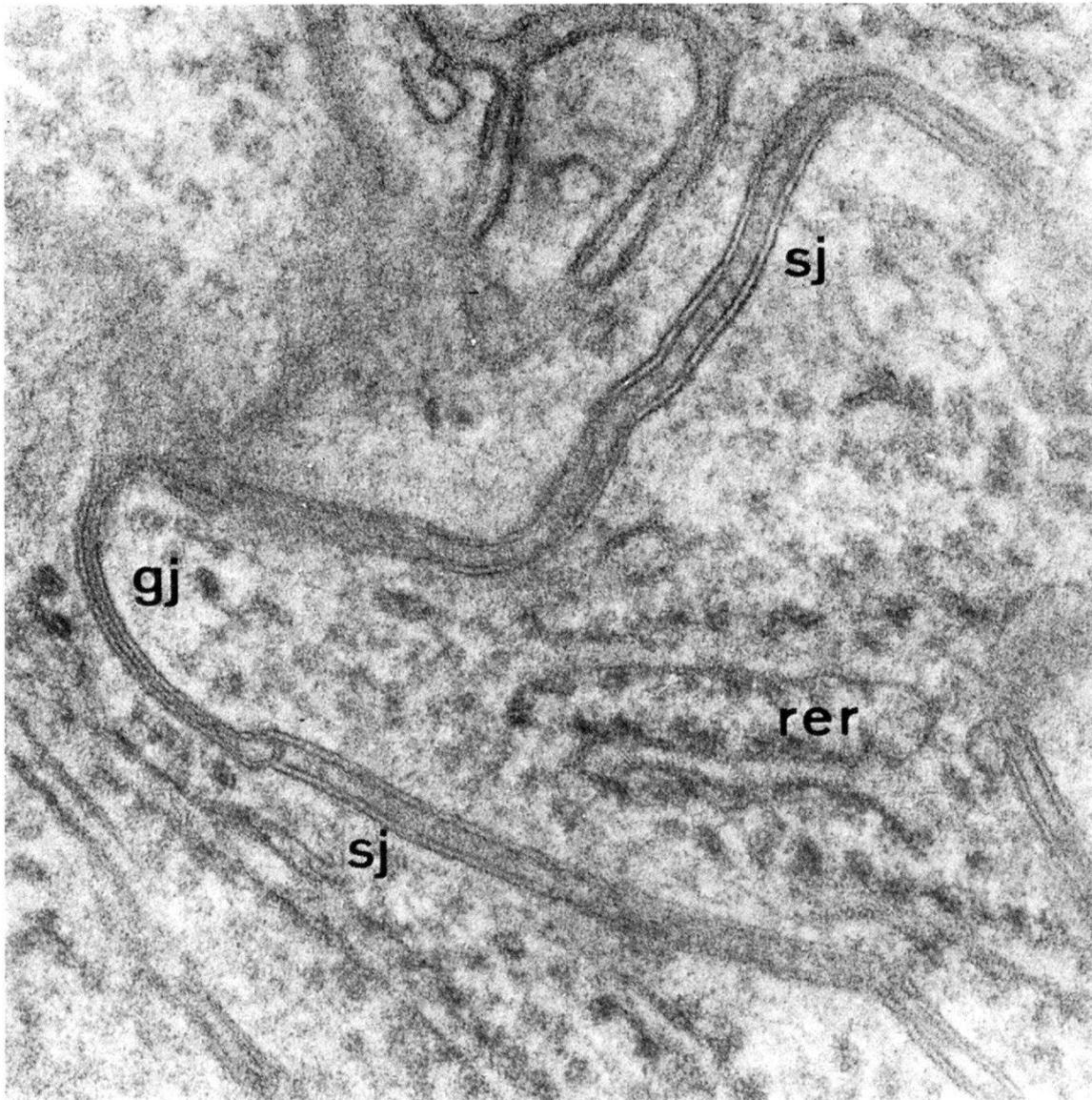
1.1.1. Function of the BL during bloodmeal and digestion

Influenced by the bloodfilled and flattened stomach epithelium, the BL expands in all directions. The structured layers stretch mainly in the longitudinal axis of the stomach (i.e. the longer diagonal axis of the grid in diagram 2). Expansion capacity of the structured part of the BL, as shown in diagram 2, suggests that it may be a limiting factor in midgut expansion during engorgement. This would indicate a mechanical function for the BL (ASHHURST 1968, SPIRO 1970). BL

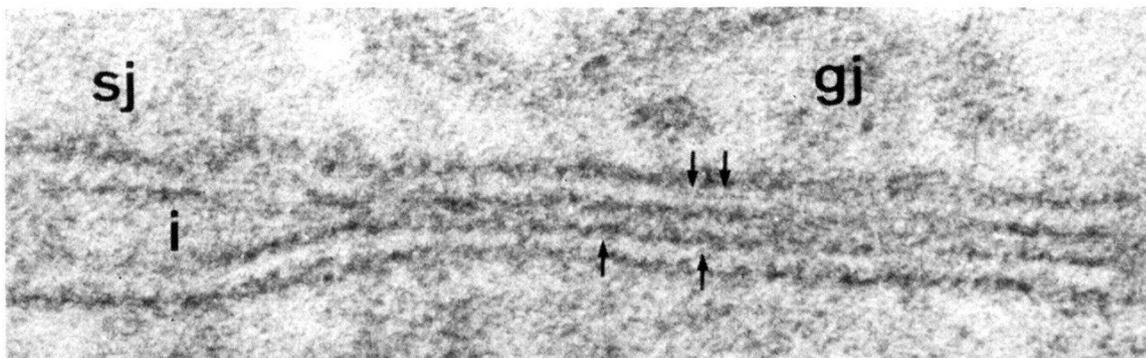
Fig. 12. Stomach, 7 days after BM. In the apical half of a cell a gap junction (gj) is placed between septate junctions (sj). Rough endoplasmatic reticulum (rer). 5% G/2% O. 130,000 ×.

Fig. 13. Stomach, 2 days after BM. The gap of a gap junction (gj) shows constrictions (→) which may be in correlation with the hexagonal 90 Å subunits. Intercellular cleft (i) of a septate junctions (sj). 5% G/2% O. 500,000 ×.

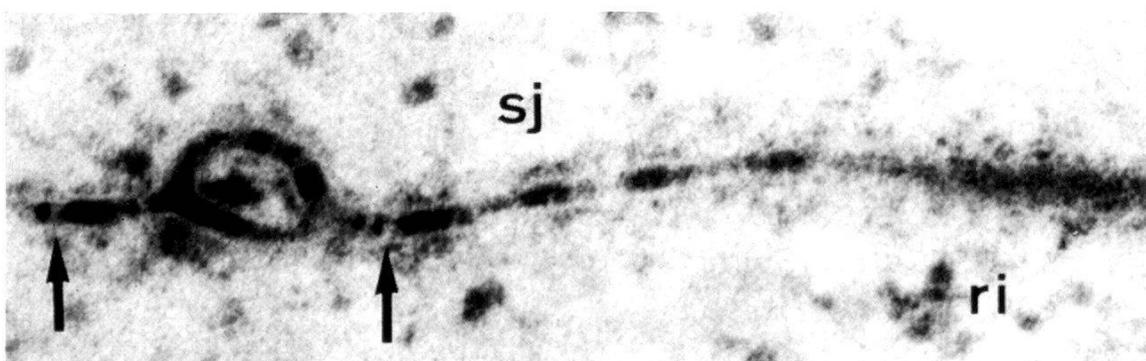
Fig. 14. Stomach, 3 days after emergence. The septa (→) of a septate junction (sj) in the apical cell zone are not stained following incubation procedures with e-PTA solution. Intercellular space electron dense. 5% G/2% O. 130,000 ×.



12



13



14

changes observed during blood digestion make clear that the grids are, at least partially, reversibly elastic.

The electron lucent “holes” enlarge during stomach engorgement; they may become more permeable for ions and macromolecules. Several authors suggest that the BL functions like a millipore filter or as a diffusion barrier for larger macromolecules (TERZAKIS 1967, ASHHURST 1968, BERRIDGE 1970, SPIRO 1970, WOLFF & HÖNIGSMANN 1971, EICHELBERG & WESSING 1971). A more permeable midgut BL could facilitate the transport of products between midgut and haemolymph.

During digestion of the first bloodmeal, new BL material must be synthesized because the BL is markedly thicker after completion of digestion. Hemidesmosomes are thought to take part in this synthesis (TONER & CARR 1968, BRIGGAMAN et al. 1971). Twenty four-day-old female *A. aegypti* show a thick BL with an increased number of structured layers (HECKER et al. 1971). Perhaps this thickening leads to a strengthening of the BL.

1.1.2. Cytochemistry of the BL

Polysaccharides could not be demonstrated within the BL by the TCH-silver method. However, polysaccharides might be combined with proteins (ASHHURST 1968, SPIRO 1970) in such a way that they cannot react with periodic acid to yield aldehyde groups (THIÉRY 1969). The negative e-PTA reaction in the BL points to an absence of alkaline proteins (PFENNINGER 1971). It is possible, however, that cryptic basic proteins are present in the BL but that they are masked by other molecules.

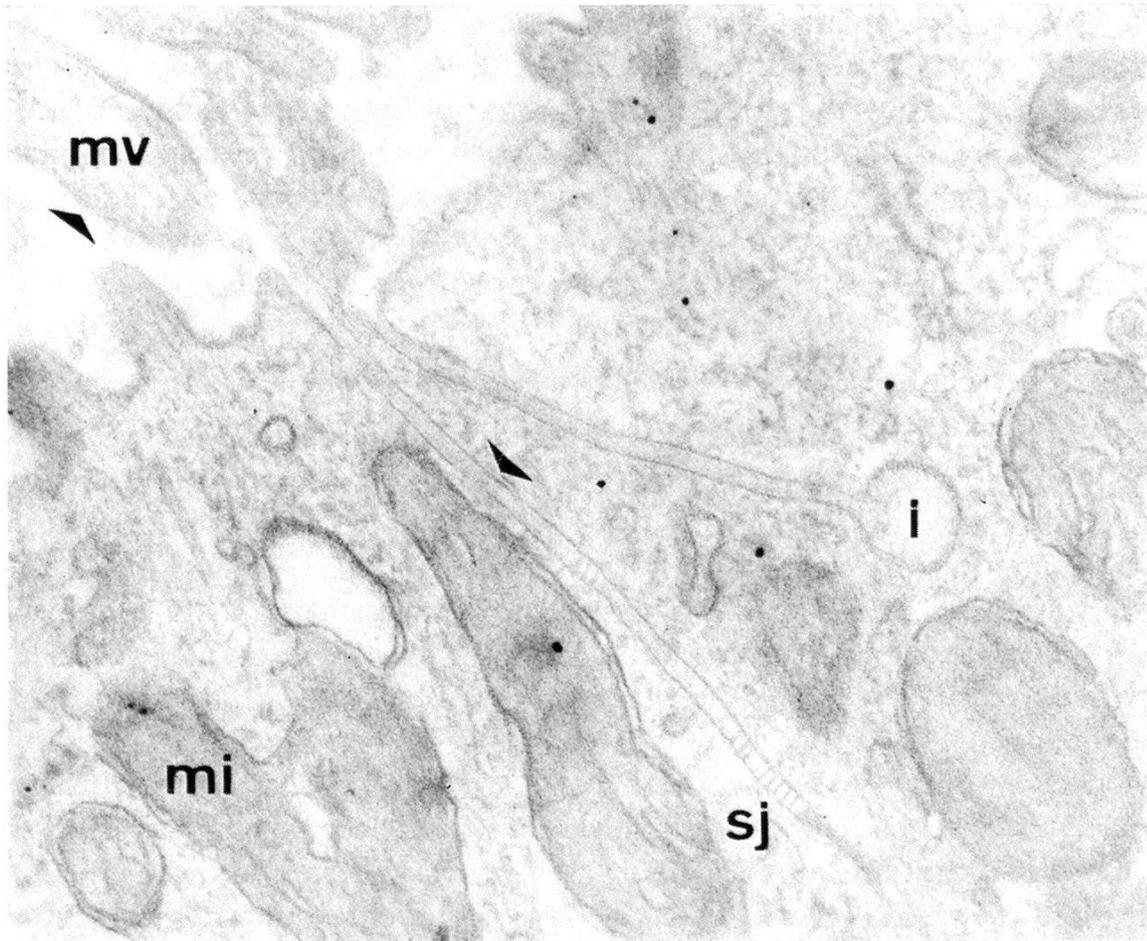
1.2. Junctions

1.2.1. Function of the junctions during bloodmeal and digestion

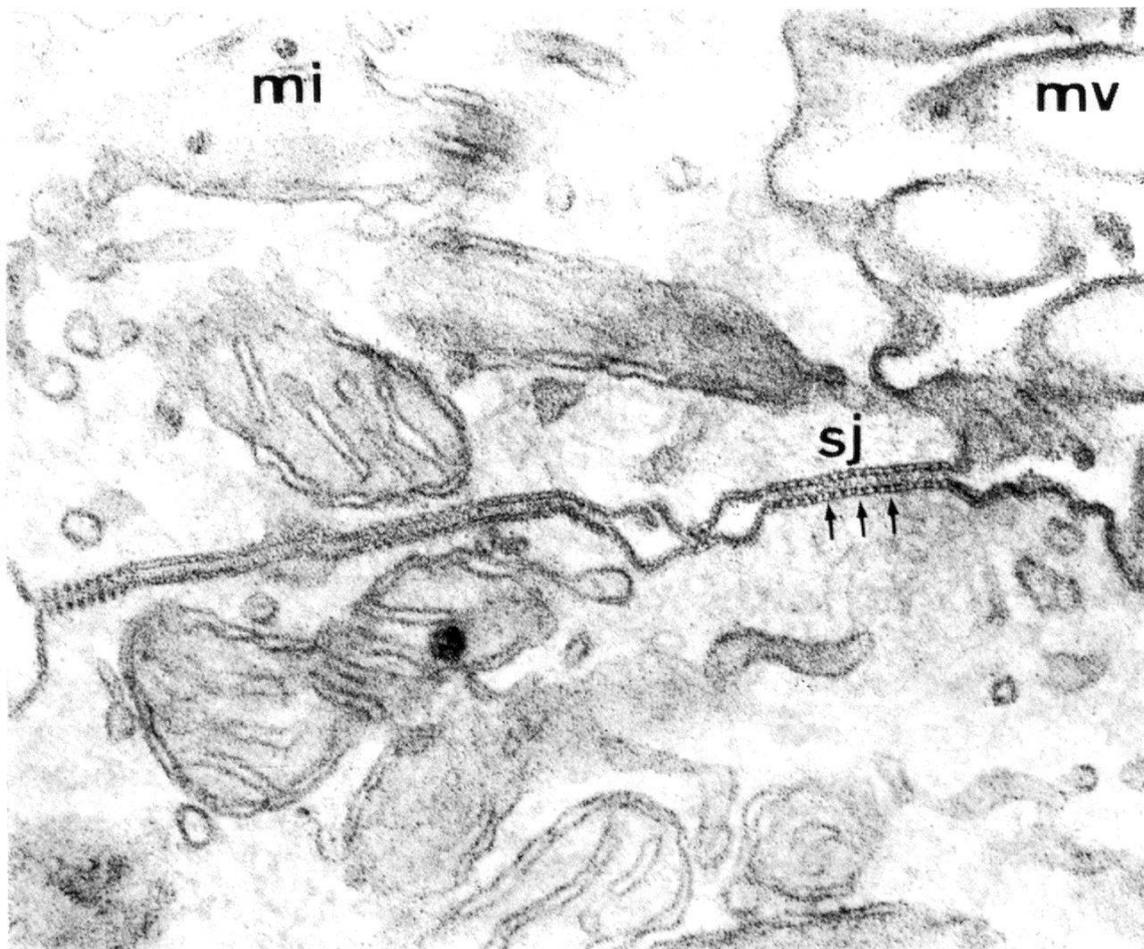
Gj and sj intercellular cleft widths are not changed as a result of engorgement nor do they change during the course of digestion. There are no visible structural modifications which point to altered functions connected with blood digestion.

Fig. 15. Apical part of a stomach cell, 7 days after BM. Control to the polysaccharide-silver method (H₂O₂-oxidation instead of PA, 67 hours TCH). Glycocalyx and intercellular membrane “coats” are not contrasted (►). Vesicular region of loose contact (i), septate junction (sj), mitochondria (mi), microvilli (mv). 5% G/2% O. 69,000 ×.

Fig. 16. Apical part of a stomach cell, 7 days after BM. Demonstration of polysaccharides (PA, 67 hours TCH). Cross sectioned septate junctions (sj) exhibit dotted stained cell membranes, presumably representing attachment contacts between septa and cell membrane (⇔). The septa are not stained. Mitochondria (mi) microvilli (mv). 5% G/2% O. 69,000 ×.



15



16

The *ma*, postulated to be cell attachment zones (FAWCETT 1966, ARMSTRONG 1970, HAGOPIAN 1970, SHEFFIELD 1970), show a more narrow intercellular cleft during the period of highest mechanical stress. This narrowing could be interpreted as a sign of firmer mechanical adherence in conjunction with a changed molecular array in the intercellular cleft and/or as an altered state of ion permeability leading perhaps to a more intense cell communication.

1.2.2. Cytochemistry of the junctions

Polysaccharides are clearly visible in gj, where they fill up the intercellular gap (fig. 10). This points to a polysaccharide nature for the hexagonal arrayed particles of the gj. These polysaccharides could function as “cement” substance for cellular adhesion. In the sj regions the polysaccharide positive “coats” of adjoining cell membranes do not fuse; the septa themselves appear to lack polysaccharides (fig. 10, 16). The pattern of alternate positive and negative staining within membrane leaflets could represent polysaccharide-rich “cement” particles or adhesion points for the septa. These particles may be correlated with the particles arranged in regular rows that have been found in freeze-etched preparations of sj (FLOWER 1971). This interpretation could also explain why TCH-silver treated sj were positively marked in tangential sections (fig. 10). The dense contrast of the interseptal spaces after e-PTA incubation (fig. 14) indicates the presence of alkaline proteins. *Ma* and *hemidesmosomes* are not contrasted by the TCH-silver method. The intercellular cleft of *ma* seems not to contain the same polysaccharide rich “cement” substances found in gj and sj. “Maculae” of *ma* as well as hemidesmosomal patches exhibit e-PTA positive material (fig. 8, 9). This material is presumably basic amino acid rich protein as it occurs in synaptic densities (BLOOM & AGHAJANIAN 1968, MEYER 1969, PFENNINGER 1971).

2. Mechanical function of the BL and of the junctions in the stomach of female *A. aegypti* as compared with other midgut epithelia

2.1. BL

In the forepart of the female *A. aegypti* midgut, the BL layers are similar to those seen in the stomach but are rarely as distinctly outlined. Tangential sections of the fore part also show a grid structure (mesh width $305 \pm 5 \text{ \AA}$). It is of interest that this part of the midgut is barely distended during a bloodmeal (CHRISTOPHERS 1960, GOODING 1972). Ultrastructural differences between the fore part of the midgut and of the stomach are presently undergoing morphometric analysis

(HECKER in prep.).

Although grids are not seen in male mosquito midgut BL, the presence of a scattered few dotted structures in this region (HECKER et al. 1971a) suggests a potential for grid formation. It is possible that grids are not necessary for non-blood sucking males since there are no mechanical pressures resulting from blood engorgement.

Studies on the ultrastructure of the midgut BL in other blood sucking insects, such as female *Anopheles* and male and female fleas (Lit. cit.) have revealed grid-like substructures. PACHECO & OGURA (1966) and PACHECO (1970), however, did not find grids in the midgut BL of *Rhodnius*.

There are non-blood sucking insects that possess regularly structured midgut BL (e.g. cockroaches, coleopterans; lit. cit.) thereby demonstrating that grid-like structures are not restricted to hematophagous forms.

We suggest that a grid-like substructure may reinforce the BL. Such a BL, together with the gut epithelium, could act as an elastic tube reacting to internal pressure forces. Furthermore, the BL could be responsible for evenly distributing these mechanical forces to the underlying muscle net. Under the mechanical stress of a bloodmeal all the structural elements mentioned are likely to function as a unit.

2.2. Junctions

As is true for the stomach, the *fore part* of the midgut of *A. aegypti* females contains gj, sj and hemidesmosomes, *ma* however, were not found in the fore part. A similar situation exists for the whole midgut epithelium of male mosquitoes (HECKER et al. 1971a).

In a preliminary study *ma* were demonstrated in the flea midgut (REINHARDT et al. 1972). *Ma* have been found in several non-blood sucking insects (lit. cit.), and are therefore not restricted to hematophagous forms.

It was suggested by HECKER et al. (1971a) that *ma* may act as additional junctions for the stronger mechanical attachment which would be necessary for epithelium consolidation (lit. cit.). The fact that their intercellular gap in the stomach of *A. aegypti* females is significantly narrowed upon blood uptake, may support this theory.

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Zusammenfassung

Im hinteren Mitteldarmabschnitt (Magen) von Stechmückenweibchen, *Aedes aegypti*, wurden die Basallamina des Epithels und dessen spezialisierte Zellverbindungen («gap junctions», «septate junctions», Maculae adhaerentes, Hemidesmosomen) auf ihre Struktur und Funktion hin untersucht.

Die Basallamina enthält in einer amorphen Matrix rechtwinklig angeordnete, netzförmige Strukturen in mehreren Schichten. Während einer Blutmahlzeit dehnt sich mit dem Epithel auch die Basallamina. Dabei werden die Netz-Maschenweiten größer, und das ursprünglich quadratische Netz wird rhombisch. Mit dem Nachlassen der Dehnung im Laufe der Blutverdauung erlangt die Basallamina annähernd wieder ihre Ausgangsstruktur. Die Interzellärspalt-Weiten der gap- und septate junctions werden durch die verschiedenen Dehnungszustände des Mitteldarmes nicht beeinflusst. Hingegen wird die Spaltweite der Maculae adhaerentes mit der Blutmahlzeit vorübergehend enger.

Mit Hilfe elektronenmikroskopisch-histochemischer Methoden wurden die untersuchten Strukturen auf Kohlenhydrate und basische Proteine geprüft. Bei gap- und septate junctions sind auf der Außenseite der Zellmembranen Kohlenhydrate vorhanden. Der apicale Interzellulärspalt, die «Maculae» der Maculae adhaerentes und der Hemidesmosomen reagieren positiv auf eine Proteinprüfung. Das sowohl für Kohlenhydrate als auch für Proteine negative Resultat der Basallamina kann jedoch ein maskiertes Vorkommen dieser beiden Grundsubstanzen nicht ausschließen.

Es wird diskutiert, ob – zusammen mit dem Mitteldarm-Epithel und dem Muskelnetz – der besonders strukturierten Basallamina und der spezialisierten Zellverbindungen (speziell den Maculae adhaerentes) Verfestigungsfunktionen bei der Blutmahlzeit zukommen.

Résumé

Les auteurs ont étudié, dans la partie postérieure de l'intestin moyen de femelles d'*Aedes aegypti*, la structure et la fonction de la membrane basale de l'épithélium et de ses connexions intercellulaires («gap junctions», «septate junctions», maculae adhaerentes, hémidesmosomes).

La membrane basale est constituée de plusieurs couches de structures en réseau à mailles rectangulaires, incluses dans une matrice amorphe. Au cours du repas

sanguin la membrane basale suit la dilatation de l'épithélium; les mailles deviennent plus larges et le réseau devient rhombique. Après la digestion du sang la membrane basale reprend presque sa structure initiale. Au cours de ce processus les espaces intercellulaires au niveau des «gap-» et des «septate junctions» ne subissent pas de modifications, tandis que ceux des maculae adhaerentes sont temporairement réduits après l'ingestion du sang.

Les structures décrites ont été testées par des méthodes histochimiques en microscope électronique pour rechercher la présence des carbohydrates et de protéines basiques. Des carbohydrates ont été identifiés sur la surface externe de la membrane, au niveau des «gap-» et des «septate junctions»; et des protéines basiques dans l'espace intercellulaire et dans les «maculae» des maculae adhaerentes et des hémidesmosomes. La constatation d'une réaction négative vis-à-vis des carbohydrates et des protéines basiques dans la membrane basale ne peut pas exclure une présence masquée de ces deux groupes de substances.

Le rôle mécanique de la membrane basale et des connexions intercellulaires (surtout des maculae adhaerentes), en relation avec l'épithélium et la musculature intestinale, est discuté.