Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 38 (1981)
Heft: 2

Artikel: Permeability of the midgut basal lamina in the mosquito, "Culex tarsalis" Coquillett (Insecta, Diptera)

Autor: Houk, E.J. / Hardy, J.L. / Chiles, R.E.

DOI: https://doi.org/10.5169/seals-312816

Acta Tropica *38*, 163–171 (1981)

Naval Biosciences Laboratory, School of Public Health, University of California, Oakland, California 94625, USA

Permeability of the midgut basal lamina in the mosquito, *Culex tarsalis* Coquillett (Insecta, Diptera)

E. J. Houk, J. L. Hardy, R. E. Chiles

Summary

The permeability of the basal lamina of the midgut of the mosquito, *Culex tarsalis*, was determined for engorged and unfed mosquito midguts both pre- and postfixed. A range of materials, with diameters from <2nm (lanthanum) through 90 nm (polystyrene spheres), was examined. The permeability of the basal lamina was constant under all experimental conditions; 5–8 nm particles (colloidal thorium) were the largest to consistently permeate. The discussion is centered on the question of how a virus particle, often 10 times the diameter of the established permeability limits of the basal lamina, can traverse this structure. Possible explanations are: 1. The basal lamina is a dynamic, plastic structure that easily distorts under physical and/or biochemical stresses, 2. The virion may possess enzymatic activity that locally alters the structure of the basal lamina, or 3. The extracellular surfaces of the midgut epithelium or the basal lamina itself may possess enzymatic properties that alter the size or possibly the structure of the virion as it passes through.

Key words: mosquito; basal lamina; electron microscopy; tracers; porosity.

Introduction

The basement membrane or basal lamina of insect tissues does not possess fibrous collagen (Ashhurst, 1968; de Biasi and Pilotto, 1976), with the exception of the neural lamella (Treherne and Pichon, 1972) and mesenteric connective tissue (Pipa and Woolever, 1965; Francois, 1978). The insect basement membrane is generally an amorphous association of proteins and glycosaminoglycans (GAGs) functioning as: 1. a barrier to macromolecules and in some instances to cations (Ashhurst, 1968; Treherne and Pichon, 1972) and 2. providing

Correspondence: Dr. E. J. Houk, Naval Biosciences Laboratory, Naval Supply Center, School of Public Health, University of California, Oakland, California 94625, USA

a basis for the structural integrity of epithelia (e.g., digestive tract, Malpighian tubules).

Substantial electron microscopic substructure has been reported for the basal lamina of midguts from several insects (Terzakis, 1967; Holter, 1970; Reinhardt and Hecker, 1973; Houk; Houk et al., 1980). In general, these basal laminae have the appearance of globular structures, perhaps proteoglycans, somewhat rigidly held in place by thin interconnecting fibrils. This multilayered meshwork has been compared to a Millipore filter (Terzakis, 1967). The midgut basal lamina also reveals substantial symmetry when observed in tangential sections; either hexagonal (Terzakis, 1967; Holter, 1970; Richards and Richards, 1968) or cuboidal (Reinhardt and Hecker, 1973; Houk, 1977; Houk et al., 1980).

The mosquito, *Culex tarsalis*, is a vector of western equine encephalomyelitis (WEE) and St. Louis encephalitis (SLE) viruses. This mosquito has been reported to possess a genetically determined 'gut barrier' to WEE viral infection (Hardy et al., 1978). Recently, this barrier to infection has been shown to possess two distinct components: an adsorption component and an inability of the virus to gain access to the hemocoel from infected mosquito midguts (Kramer et al., in press). In view of the obvious porosity of the midgut basal lamina, as outlined above, and the second component of the WEE virus gut barrier, this present study was undertaken. A measure of the occlusion limits of the basal lamina of *Cx. tarsalis* could possibly reveal whether direct interaction of the Millipore filter effect could affect the second component of the WEE virus gut barrier.

Materials and methods

Mosquitoes, *Cx. tarsalis*, were maintained under developmental conditions described by Houk (1977). Tracer substances were applied to adult female midguts (3–5 days of age) as follows: 1. Engorged and unfed mosquitoes had their digestive tracts dissected directly into tracer materials diluted in Dulbecco's phosphate buffered saline (Na/PBS) and 2. Engorged and unfed mosquitoes were dissected directly into glutaraldehyde (2% or 5% in 0.2 M phosphate buffer; pH 7.2) and prefixed for 30 min, rinsed in Na/PBS and then incubated in the tracer.

Midguts were incubated in the presence of the appropriate tracer for 4–8 h, fixed for 1 h in 2% glutaraldehyde, rinsed thoroughly in Na/PBS and exogenous tracers detected by established methods. Enzyme tracers were detected as described: microperoxidase and cytochrome c (Karnov-

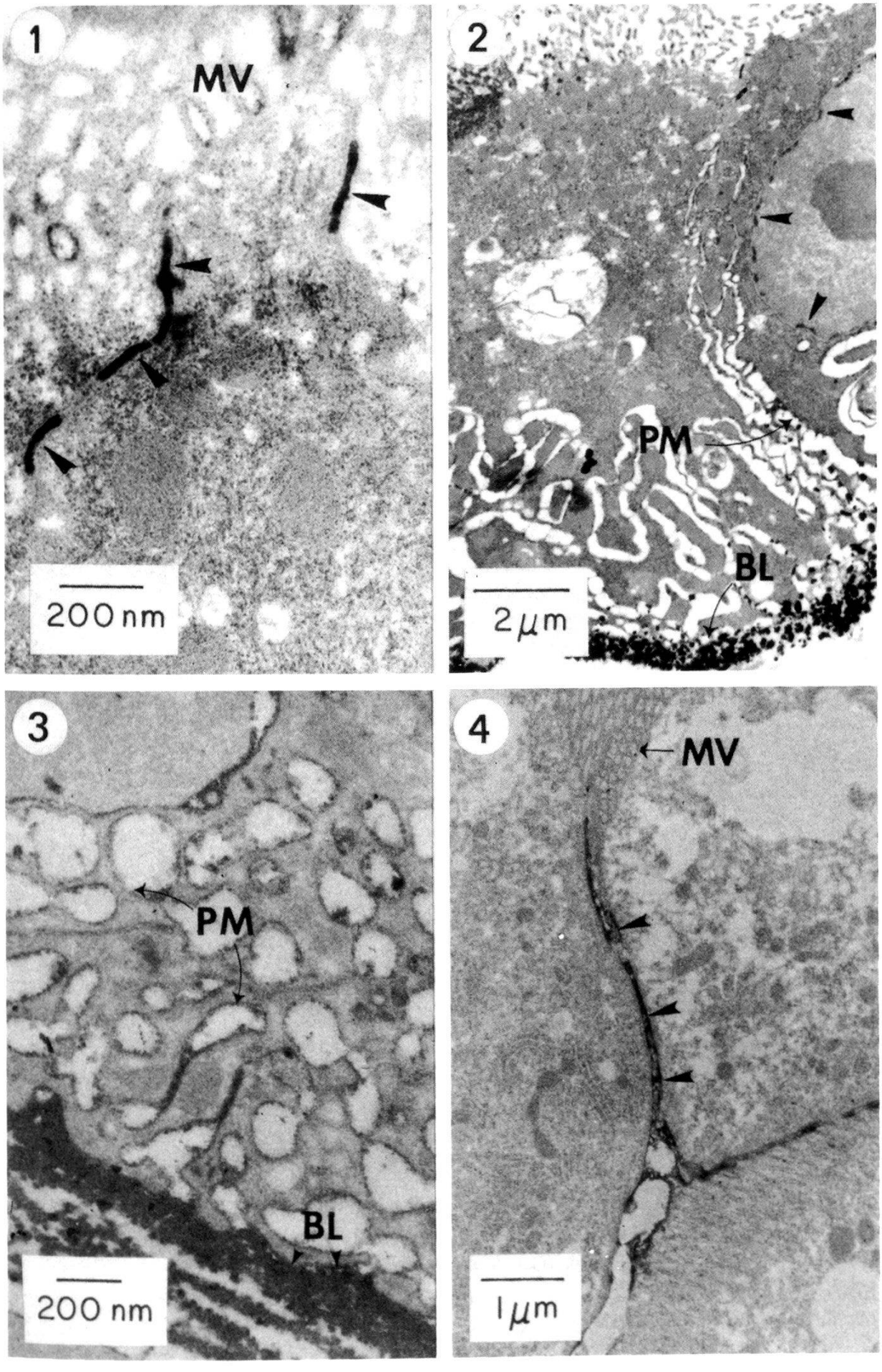
---

Fig. 1. Lanthanum within intercellular spaces (arrows) immediately subjacent to midgut microvilli (MV). ×52,700.

Fig. 2. Sporatic association of lanthanum with the basal plasma membrane (PM) and extensive adsorption to the basal lamina (BL). Chromatin associated precipitate within the nucleus (arrows). ×6200.

Fig. 3. Adsorption of microperoxidase to the basal lamina (BL) and basal plasma membrane (PM). ×37,800.

Fig. 4. Microperoxidase (arrows) within the area of midgut microvilli (MV) in a postfixed preparation. ×10,800.


1
MV
200 nm
2
PM
BL
2 μm
3
PM
BL
200 nm
4
MV
1 μm

sky and Rice, 1969), horseradish peroxidase (Graham and Karnovsky, 1966) and catalase (Venkatachalam and Fahimi, 1969). Polystyrene spheres were diluted in Na/PBS containing 1 mg/ml of bovine serum albumin or microperoxidase. The staining of adsorbed protein by combined en bloc uranyl acetate (Milne and de Zoeten, 1967) and post-staining with lead citrate (Reynolds, 1963), or by enzyme detection methods (Karnovsky and Rice, 1969), served to delineate the spheres.

Lanthanum and thorium are both inherently electron opaque. Colloidal thorium (Thoria-Sol, Polysciences, Inc.) was diluted to 0.01% in Na/PBS. Lanthanum hydroxide (100 mg) was dissolved in a minimum volume of concentrated HCl. This solution was then brought to approximate neutrality (i.e., slight precipitation) with sodium hydroxide. The neutral lanthanum solution was added to Tris (0.2 M; pH 7.2) buffered glutaraldehyde (5%) to yield a final concentration of 0.5% (w/v). Lanthanum was then precipitated in situ after the removal of the Tris-glutaraldehyde by the addition of phosphate buffer (0.2 M; pH 7.2).

Results

A summary of the tracers examined and an indication of their ability to traverse the midgut basal lamina, in both pre- and post-fixed midguts is presented in Table 1.

Lanthanum (<2 nm) passed through the basal lamina and cellular junctions with no apparent restrictions (Figs. 1, 2). When lanthanum was precipitated in situ with phosphate buffer, sporatic association with the basal lamina and

Table 1. Permeability of mosquito midgut basal lamina

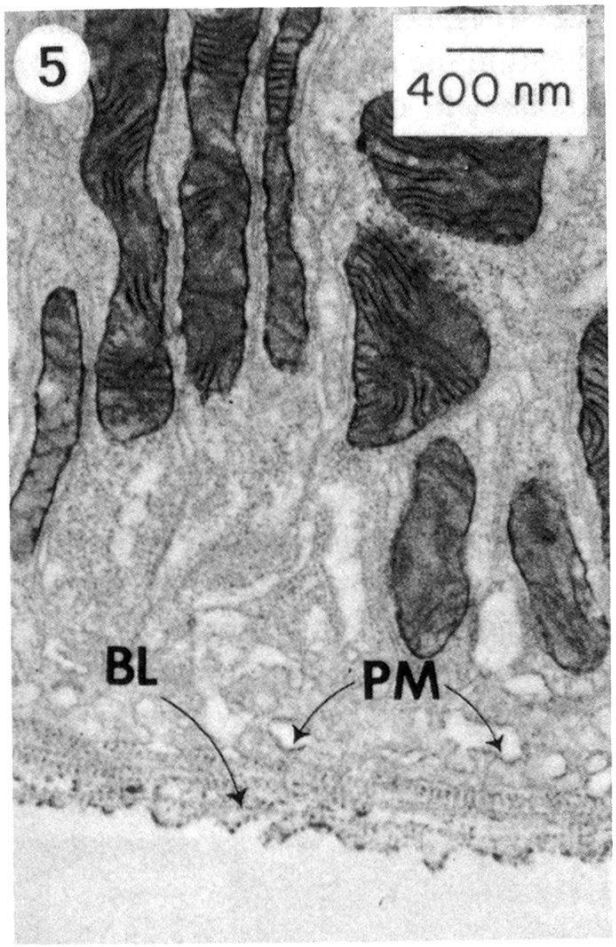
Tracer	Particle size (nm)	Midgut	
		prefixed	postfixed
Lanthanum	<2	+	+
Microperoxidase	2	+	+
Cytochrome c	3	+	+
Horseradish peroxidase	4–6	+	+
Colloidal thorium	5–8	+	+
Catalase	10	–	–
Polystyrene spheres	66	–	–
Polystyrene spheres	90	–	–

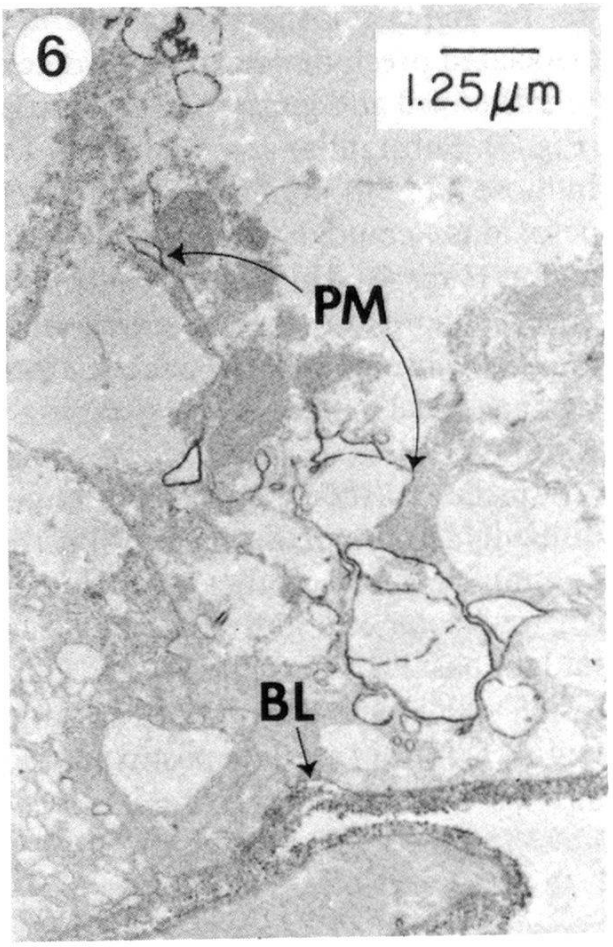
Fig. 5. Cytochrome c apparent along the basal plasma membrane (PM) and adsorbed to the basal lamina (BL). ×23,000.

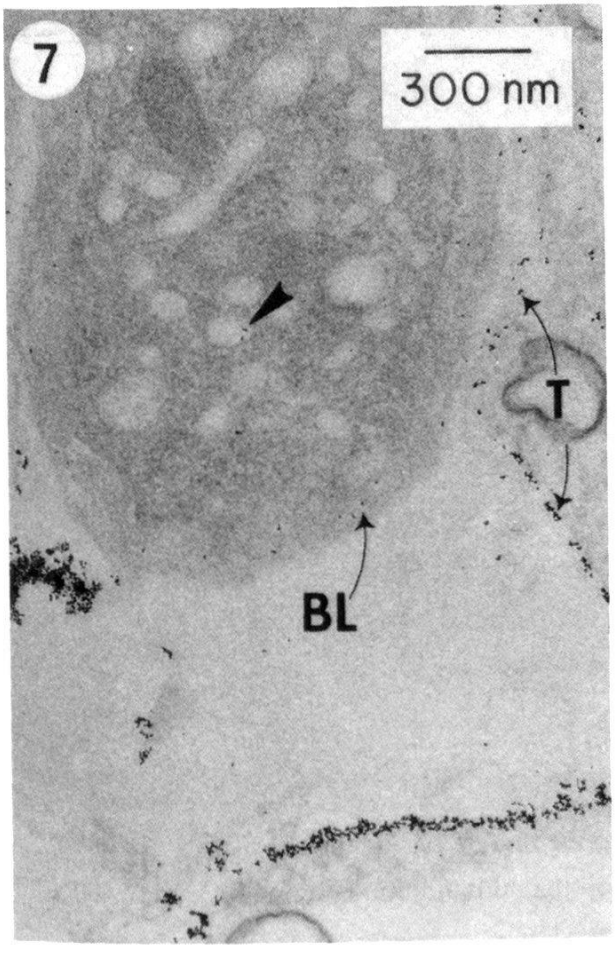
Fig. 6. Horseradish peroxidase along the basal plasma membrane (PM) and the basal lamina (BL). ×7200.

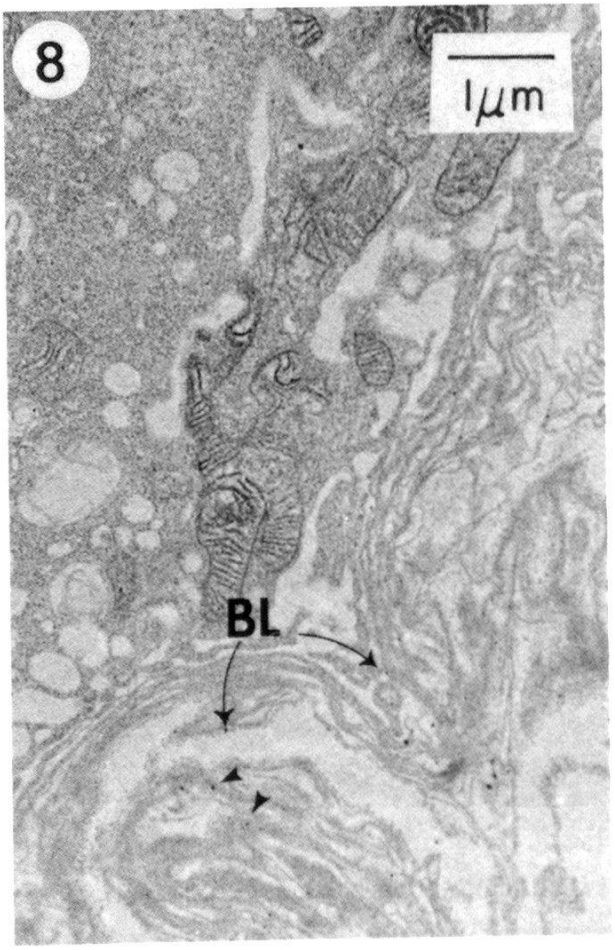
Fig. 7. Colloidal thorium reveals substantial adsorption to the basement membranes of tracheoles (T), limited association with the midgut basal lamina (BL) and limited access to midgut extracellular spaces proximal to the basal lamina (arrows). ×32,400.

Fig. 8. Catalase reveals sporatic association with midgut basal lamina (BL) and adjacent basement membranes (arrows). ×10,200.


5
400 nm
BL
PM


6
1.25 μm
PM
BL


7
300 nm
T
BL


8
1 μm
BL

the basal plasma membrane was observed (Fig. 2). In addition, chromatin associated precipitation could be detected within the nucleus (Fig. 2).

Prefixed midguts allowed microperoxidase to traverse the basal lamina (Fig. 3). Substantial adsorption was found along the basal plasma membrane. In those midguts preincubated in Na/PBS-tracer without prior fixation, microperoxidase could be detected in occasional sections within the microvillar region (Fig. 4). This may be attributed to an unbalanced osmolarity of the incubation medium with respect to the mosquito midgut epithelial cell.

Cytochrome c and horseradish peroxidase were both adsorbed to the basal lamina (Figs. 5, 6). Both molecules were able to cross the basal lamina with and without prior fixation. There was no indication of junctional permeability.

Colloidal thorium was able to penetrate the basal lamina with apparent difficulty in both prefixed (Fig. 7) and postfixed midguts. There was substantial accumulation/adsorption of thorium within the basement membranes of muscle, tracheolar cells and axons adjacent to the midgut but little found within the midgut basal lamina (Fig. 7).

Catalase and both sizes of polystyrene spheres could not traverse the basal lamina in either pre- or postfixed midguts (Figs. 8, 9, 10). Catalase could be

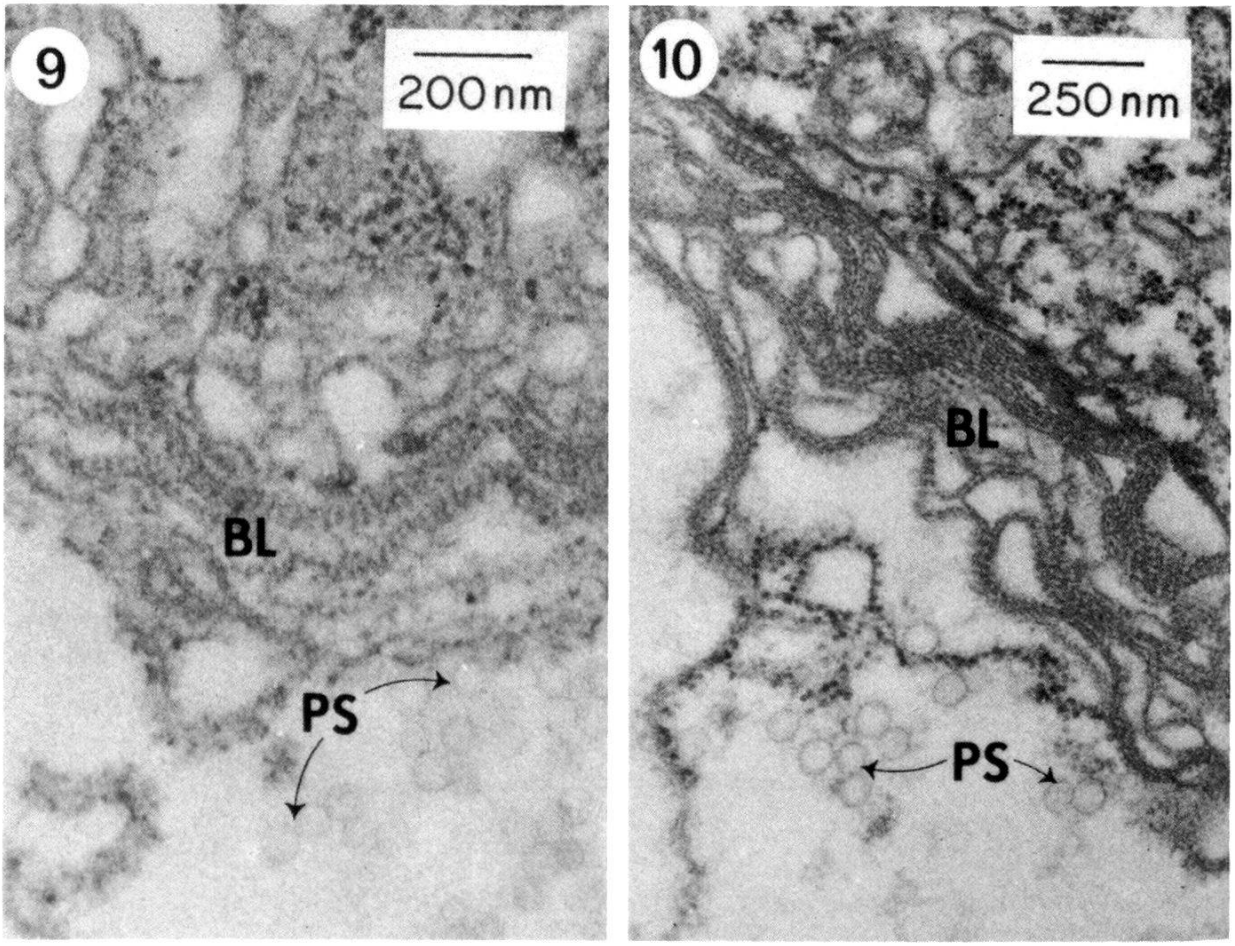


Fig. 9. Polystyrene spheres (PA; 66 nm) adjacent to the midgut basal lamina (BL). ×51,800.

Fig. 10. Polystyrene spheres (PA; 90 nm) adjacent to the midgut basal lamina (BL). ×37,400.

observed in sporatic association with the extreme hemocoelar margin of the basal lamina.

There was no apparent alteration in basal lamina permeability in response to bloodfeeding.

Discussion

The results for the permeability of the midgut basal lamina of the mosquito, *Cx. tarsalis* (Table 1), were a reflection of the data for basement membranes from other insect tissues (Table 2). Horseradish peroxidase has been demonstrated to penetrate the basement membrane in all tissues examined to date, including the mosquito midgut basal lamina (Fig. 7). Colloidal thorium was able to traverse the midgut basal lamina with difficulty (Fig. 8). This would indicate that the limits of occlusion for the midgut basal lamina are in the range of colloidal thorium (5–8 nm), slightly above the average diameter for horseradish peroxidase (4–6 nm; Table 1).

How then does something as substantial as WEE and SLE virions, about 5 times the apparent midgut occlusion limit, traverse this seemingly insurmountable basal lamina barrier? Three explanations seem plausible. First, the basal lamina is probably not a static structure in the sense of a Millipore filter, but rather a dynamic structure. This dynamic status may allow for substantial distortion of the basal lamina in response to physical and/or biochemical stress. In fact, the porosity observed in the electron microscope might conceivably be a minimum porosity induced by glutaraldehyde fixation, but our studies with pre- and postfixed midguts do not support this concept (Table 1). However, in a

Table 2. Permeability of the basement membrane of several insect systems

System	Particle size (nm)	References
Reproductive		
Ovaries – *Aedes* (Mosquito)	11	Anderson and Spielman, 1971
Ovaries – *Galleria* (Moth)	4–6	Przelecka and Dutkowski, 1975
Ovaries – *Libellula* (Dragonfly)	4–6	Kessel and Ganion, 1979
Testes – *Locusta* (Locust)	4–6	Szollosi and Marcaillou, 1977
Excretory – Malpighian tubules		
Calliphora (Blowfly)	4–6	Berridge and Oschman, 1969
Calpodes (Skipper)	4–6	Locke and Collins, 1968
Libellula (Dragonfly)	4–6	Kessel, 1970
Nervous – Brain		
Manduca (Moth)	4–6	Lane, 1972
Ostrinia (Moth)	4–6	Houk and Beck, 1975
Periplaneta (Cockroach)	4–6	Lane and Treherne, 1972

qualitative sense one could discern changes in the amount of horseradish peroxidase and cytochrome c that crossed the basal lamina in prefixed midguts as compared to postfixed. Second, perhaps the virion itself is altered before or during the process of penetrating the basal lamina. It is conceivable that a nonenveloped particle, whose diameter is reduced by about 30–40%, would be more easily transported through this maze. In fact, we have observed nonenveloped particles that appeared to be in the process of traversing the basal lamina in several strains of *Cx. tarsalis*. Third, the virion could possess enzymatic properties that assist in its penetration of the basal lamina.

We are currently attempting to identify the nature of the virion that crosses the basal lamina. In addition, we are testing the hypothesis that nonenveloped particles might be infectious to the mosquito when inoculated directly into the hemocoel.

Acknowledgments. This research was supported by a U.S. Army Contract/Grant, DAMD 17-77-C-7018, U.S. Army Medical Research and Development Command, Washington, D.C., and by the Office of Naval Research.

Anderson W. A., Spielman A.: Permeability of the ovarian follicle of *Aedes aegypti* mosquitoes. J. Cell Biol. *50*, 201–221 (1971).

Ashhurst D. E.: The connective tissue of insects. Ann. Rev. Ent. *13*, 45–74 (1968).

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

de Biasi S., Pilotto F.: Ultrastructural study of collagenous structures in some Diptera. J. submicrosc. Cytol. *8*, 337–345 (1976).

Francois J.: The ultrastructure and histochemistry of the mesenteric connective tissue of the cockroach *Periplaneta americana* L. (Insecta, Dictyoptera). Cell Tissue Res. *189*, 91–107 (1978).

Graham R. C. jr., Karnovsky M. J.: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney; ultrastructural cytochemistry by a new technique. J. Histochem. Cytochem. *14*, 292–302 (1966).

Hardy J. L., Apperson G., Asman S. M., Reeves W. C.: Selection of a strain of *Culex tarsalis* highly resistant to infection following ingestion of western equine encephalomyelitis virus. Amer. J. trop. Med. Hyg. *27*, 313–321 (1978).

Holter P.: Regular grid-like substructures in the midgut epithelial basement membrane in some Coleoptera. Z. Zellforsch. *110*, 373–385 (1970).

Houk E. J.: Midgut ultrastructure of *Culex tarsalis* (Diptera: Culicidae) before and after a bloodmeal. Tissue Cell *9*, 103–181 (1977).

Houk E. J., Beck S. D.: Comparative ultrastructure and blood-brain barrier in diapause and nondiapause larvae of the European corn borer *Ostrinia nubilalis* (Hübner). Cell Tissue Res. *162*, 499–510 (1975).

Houk E. J., Chiles R. E., Hardy J. L.: Unique midgut basal lamina in the mosquito, *Aedes dorsalis* (Meigen) (Insecta: Diptera). Int. J. Insect Morphol. Embryol. *9*, 161–164 (1980).

Karnovsky M. J., Rice D. F.: Exogenous cytochrome c as an ultrastructural tracer. J. Histochem. Cytochem. *17*, 751–753 (1969).

Kessel R. G.: The permeability of dragonfly Malpighian tubules to protein using horseradish peroxidase as a tracer. J. Cell Biol. *47*, 299–303 (1970).

Kessel R. G., Ganion L. R.: Localization of horseradish peroxidase in the panoistic dragonfly ovary. J. submicrosc. Cytol. *11*, 313–324 (1979).

Kramer L. D., Hardy J. L., Presser S. B., Houk E. J.: Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. Amer. J. trop. Med. Hyg. (in press).

Lane N. J.: Fine structure of a lepidopteran nervous system and its accessibility to peroxidase and lanthanum. Z. Zellforsch. *131*, 205–222 (1972).

Lane N. J., Treherne J. E.: Studies on perineurial junctional complexes and the sites of uptake of microperoxidase and lanthanum in the cockroach central nervous system. Tissue Cell *4*, 427–436 (1972).

Locke M., Collins J. V.: Protein uptake into multivesicular bodies and storage granules in the fat body of an insect. J. Cell Biol. *36*, 453–483 (1968).

Milne R. G., de Zoeten G. A.: A comparison of some methods of preparation of thin sections of virus infected leaves for electron microscopy. J. Ultrastruct. Res. *19*, 398–407 (1967).

Pipa R. L., Woolever P. S.: Insect neurometamorphosis. II. The fine structure of perineurial connective tissue, adipohemocytes, and the shortening ventral nerve cord of a moth, *Galleria mellonella* (L.). Z. Zellforsch. *68*, 80–101 (1965).

Przelecka A., Dutkowski A. B.: The affinity of the basement membrane in the ovarial sheath of *Galleria mellonella* (Lepidoptera) for concanavalin A under normal and hormonally changed conditions. J. Microsc. Biol. cellul. *23*, 229–236 (1975).

Reinhardt C., Hecker H.: 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. (Basel) *30*, 213–236 (1973).

Reynolds E. S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. *17*, 208–212 (1963).

Richards A. G., Richards P. A.: Flea *Ctenophthalmus:* heterogeneous hexagonally arranged layer in the midgut. Science *160*, 423–425 (1968).

Szollosi A., Marcaillou C.: Electron microscope study of the blood-testis barrier in an insect: *Locusta migratoria.* J. Ultrastruct. Res. *59*, 158–172 (1977).

Terzakis J. A.: Substructure in an epithelial basal lamina (basement membrane). J. Cell Biol. *35*, 273–278 (1967).

Treherne J. E., Pichon Y.: The insect blood-brain barrier. Advanc. Insect Physiol. *9*, 257–313 (1972).

Venkatachalam M. A., Fahimi H. D.: The use of beef liver catalase as a protein tracer for electron microscopy. J. Cell Biol. *42*, 480–489 (1969).