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# Peritrophic membrane formation and the midgut barrier to arboviral infection in the mosquito, *Culex tarsalis* Coquillett (Insecta, Diptera)

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## Summary

The formation of the peritrophic membrane in adult female mosquitoes, *Culex tarsalis*, was examined by light and electron microscopy. The initial evidence of secretion of peritrophic membrane precursors occurred at 8–12 h after ingestion of the bloodmeal. Morphogenesis of the peritrophic membrane occurred within 12–16 h after the initial secretion; culminating with a fibrous, multilayered peritrophic membrane 20–24 h after bloodmeal ingestion. A discussion of the potential participation of the peritrophic membrane in a midgut barrier to infection by arboviruses is presented. The participation of the peritrophic membrane in a midgut barrier to infection of *C. tarsalis*, and many other mosquito species, by arboviruses is considered a moot point.

*Key words:* mosquito peritrophic membrane; midgut barrier; microscopy.

## Introduction

The formation of the peritrophic membrane in adult mosquitoes has been explored by light microscopy (Stohler, 1957; Freyvogel and Stäubli, 1965; Gander, 1968; Richardson and Romoser, 1972; Romoser and Cody, 1975) and electron microscopy (ref. cit., Richards and Richards, 1977). Species of mosquitoes that have been studied include *Aedes aegypti* (Stohler, 1975; Freyvogel and Stäubli, 1965; Gander, 1968, Richards and Richards, 1971), *Aedes triseriatus* (Richardson and Romoser, 1972), *Anopheles stephensi, Anopheles gambiae, Anopheles labranchiae atroparvus, Anopheles maculipennis* (Freyvogel and Jaquet,

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1965; Freyvogel and Stäubli, 1965; Gander, 1968) and *Culex nigrapalpus* (Romoser and Cody, 1975).

Chamberlain and Sudia (1961) suggested that some populations of mosquitoes were resistant to infection by viruses because of an ill-defined "gut barrier". These authors further suggested that the peritrophic membrane could potentially be a component of this gut barrier. This concept has been reiterated by Orihel (1975) and McLintock (1978).

The peritrophic membrane is continuously synthesized and is a preformed barrier in larval mosquitoes, as demonstrated by Stoltz and Summers (1971) with mosquito iridescent virus. However, the adult mosquito does not possess a preformed peritrophic membrane (Clements, 1963). Thus, the time of secretion of precursors and the temporal aspects of peritrophic membrane morphogenesis become important (McLintock, 1978). The significance of this time frame is emphasized when one recognizes that the average porosity of dipteran peritrophic membranes is about 20 nm (Richards and Richards, 1977); much too small for most Togaviruses to penetrate (Fenner et al., 1974).

Hardy et al. (1978) reported that resistance to infection of *Culex tarsalis* by western equine encephalomyelitis (WEE) virus was genetically controlled. In addition, the focus of this genetic effect appeared to be the midgut. This study of peritrophic membrane formation in *C. tarsalis* was undertaken to establish the time frame of morphogenesis in order to evaluate the participation of the peritrophic membrane in the gut barrier in the *C. tarsalis*-WEE virus system.

### Materials and methods

The Knights Landing strain of *C. tarsalis* was maintained under the following laboratory conditions: a) 16 h photophase: 8 h scotophase, b) 25–30° C and c) circa 80% relative humidity. At 4 days post-eclosion, females were allowed to feed to repletion on baby chicks (*Gallus domesticus* var. white leghorn). Immediately upon cessation of feeding and at selected intervals thereafter the mosquitoes were anaesthetized with chloroform, decapitated and prepared for either light or electron microscopy.

*Light microscopy.* Mosquitoes were fixed in phosphate buffered formalin (4% w/v; pH 7.2) for 24 h, dehydrated in an ethanol-xylene series and embedded in paraffin (m.p. 55–56° C). Sections (7–10 $\mu$m) were stained with a modified Mallory's triple stain (Conn et al., 1962).

*Electron microscopy.* Mosquitoes were decapitated and the digestive tract dissected in 5% glutaraldehyde (McLean and Houk, 1973). Fixation was allowed to proceed for 4 h, the tissue rinsed in phosphate buffer (0.2 M; pH 7.2) and post-fixed overnight in 1% osmium tetroxide (Palade, 1952). The tissue was rinsed in buffer, dehydrated in an ethanol-propylene oxide series, with overnight staining in 70% ethanol saturated with uranyl acetate (Milne and deZoeten, 1967) and embedded in araldite-epon (Mollenhauer, 1964). Sections were subsequently stained with 1% lead citrate (Reynolds, 1963) for 10–15 min.

## Results

Immediately following a bloodmeal, a finely granular, blue-staining material was observed interspersed with erythrocytes throughout both the an-

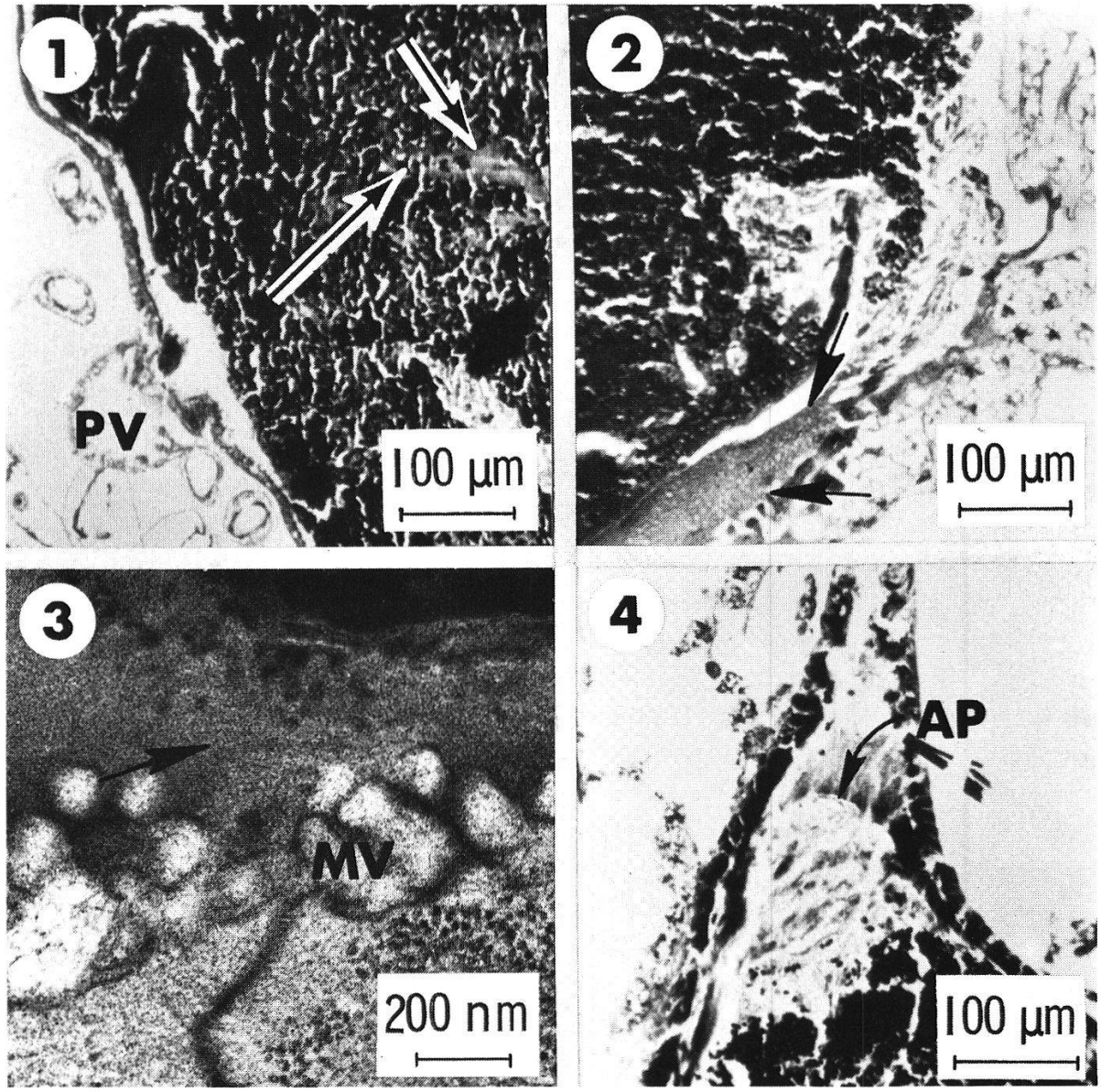


Fig. 1. Posterior midgut in the region of the pyloric valve (PV), immediately following bloodmeal, revealing erythrocytes interspersed within a light (blue-staining) matrix (arrows) thought to represent serum.

Fig. 2. Initial signs of secretion of peritrophic membrane precursors 12 h after bloodmeal ingestion (arrows).

Fig. 3. Electron micrograph of 8 h post-bloodmeal midgut reveals granular secretion adjacent to the microvilli (MV).

Fig. 4. Formation of anterior plug (AP) of peritrophic membrane at the juncture of the nondistensible anterior midgut and the posterior midgut.

terior and posterior regions of the midgut (Fig. 1). By 1 h post-bloodmeal, the granular, blue-stained material (i.e., serum) was absent presumably because of the mosquito's diuretic activity (Boorman, 1960; Stobbart, 1977).

Significant peripheral loss of erythrocytic integrity in the bloodmeal was apparent by 4 h post-bloodmeal. However, there was no evidence of peritrophic

membrane precursor secretion until about 8 h after the bloodmeal was ingested. At this time, a fine blue-staining line was apparent, completely encompassing the blood-clot. By 12 h, there was a large amount of this material, perhaps more in the regions of the pyloric valve and the anterior-posterior midgut juncture (Fig. 2). However, secretion appeared to be uniformly distributed throughout the cells of the posterior midgut.

The first evidence of peritrophic membrane precursor secretion as observed by electron microscopy occurred at 8 h post-bloodmeal also. A finely granular material was observed at the periphery of the blood-clot adjacent to the microvilli (Fig. 3).

The secretory activity by 16 h was quite significant and the anterior portion of the blood-clot had begun to reveal an anterior plug, as described previously (Richardson and Romoser, 1972; Romoser and Cody, 1975) (Fig. 4). The thickness of the secretion was up to 100 $\mu$m as measured from light micrographs. Correlative electron microscopy did not reveal a membranous structure but rather two amorphous layers (Fig. 5). The material adjacent to the blood-clot was composed of polymorphic vesicles, some containing electron opaque material. Immediately adjacent to the midgut epithelial cells a disoriented fibrous layer was observed. It was thus presumed that most or all requisite materials for the formation of a peritrophic membrane were present within the midgut lumen at 16 h.

The peritrophic membrane was indistinguishable around the brown-stained blood residue 24 h after a bloodmeal by light microscopy (Fig. 6). However, the electron microscope revealed a distinct multilayered peritrophic membrane (Fig. 7).

The midgut was devoid of bloodmeal residues and all remnants of the peritrophic membrane between 48 and 72 h post-bloodmeal.

## Discussion

Peritrophic membranes in mosquitoes are formed by merocrine secretion of precursor materials (Gander, 1968) with subsequent macromolecular synthesis in the midgut lumen. The time frame through which this secretion occurs varies with the species of mosquito studied. In the *Aedes* sp. (Freyvogel and Jaquet, 1965; Freyvogel and Stäubli, 1965; Richardson and Romoser, 1972), peritrophic membrane formation was reported to be complete within 4 h for *A. triseriatus* and within 4–6 h in *A. aegypti. A. stephensi* did not form a peritrophic membrane until about 15–20 h after a bloodmeal (Freyvogel and Jaquet, 1965). Gander (1968) reported that secretion of peritrophic membrane precursors took several hours in *A. stephensi;* thus, the delay in peritrophic membrane formation. As a comparison, the precursors of the peritrophic membrane in *A. triseriatus* have been reported to be secreted within 50 min of bloodmeal ingestion (Richardson and Romoser, 1972). *C. nigrapalpus* demonstrated

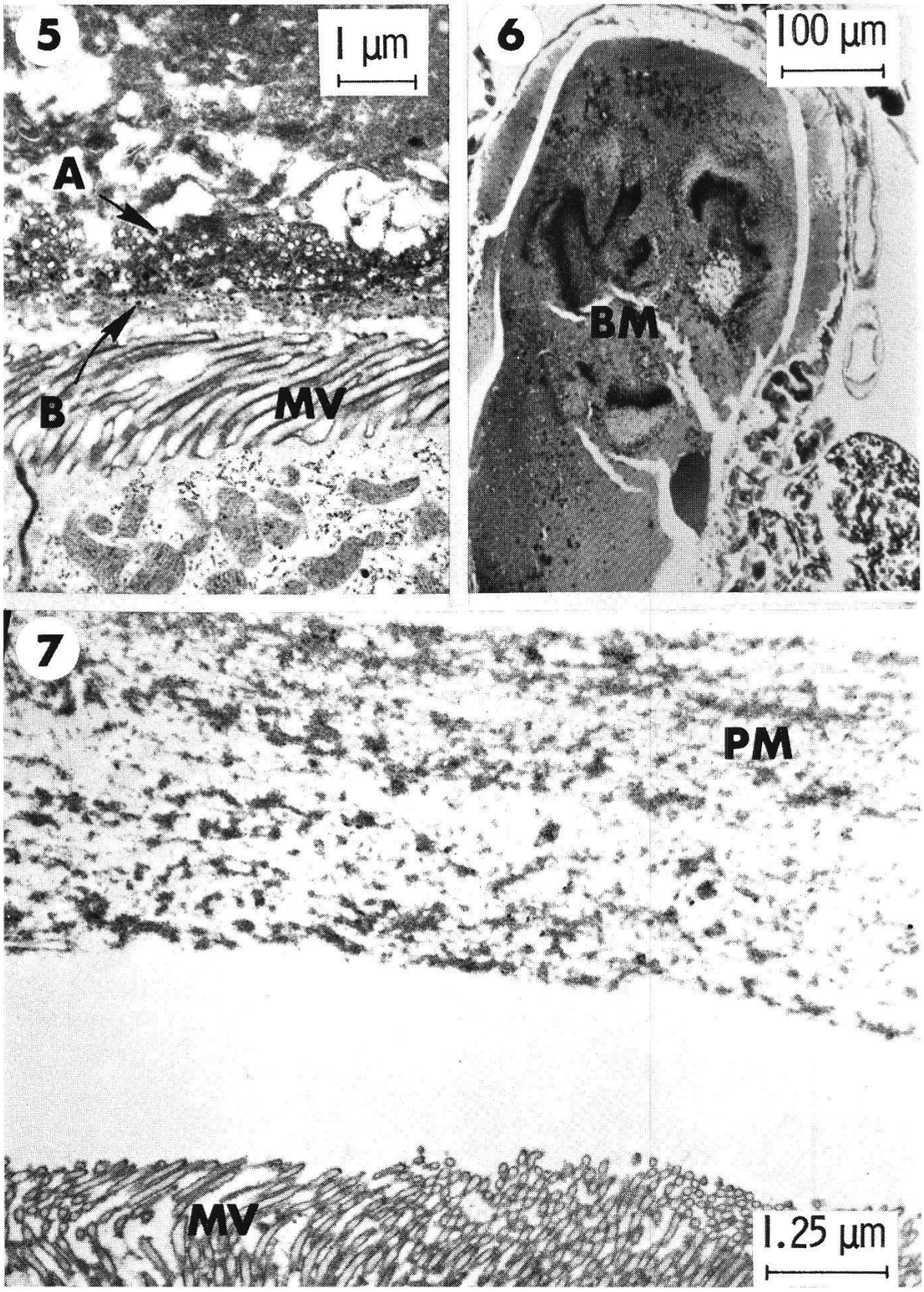


Fig. 5. 16 h post-bloodmeal; two distinct layers of presumed peritrophic membrane precursors are seen: (A) polymorphic vesicles and (B) fibrous material.

Fig. 6. 24 h post-bloodmeal light micrograph fails to resolve the peritrophic membrane around the bloodmeal residue (BM).

Fig. 7. 24 h post-bloodmeal multilayered fibrous peritrophic membrane (PM) adjacent to microvilli (MV).

secretory activity within 6 h of a bloodmeal and complete formation of the peritrophic membrane by 24 h (Romoser and Cody, 1975). Similarly, Whitfield et al. (1973) observed no evidence of peritrophic membrane formation until at least 12 h after a bloodmeal in *C. pipiens pipiens*. The *Culex* sp. all seem to reveal the same time frame of peritrophic membrane morphogenesis (Romoser and Cody, 1975; Whitfield et al., 1973; data herein).

Virus adsorption to and/or infection of midgut epithelial cells of mosquitoes, if it is to occur, probably occurs within 4 h of the ingestion of a bloodmeal (Whitfield et al., 1973). At approximately 4 h post-bloodmeal, peripheral hemolysis and the initial stages of blood-clot formation are observed (ref. cit., Gooding, 1972). Virus "trapped" within a blood-clot can, in all probability, be considered non-infectious for two obvious reasons. First, virus particles initially immobilized by the blood-clot are subsequently surrounded by a peritrophic membrane; a 20 nm meshwork (Richards and Richards, 1977) that would preclude access to epithelial membranes to all but the smallest Togaviruses (Fenner et al., 1974). Second, following peritrophic membrane formation, proteolytic enzymes are secreted to digest the bloodmeal (ref. cit., Gooding, 1972). Arboviruses, including WEE, have been shown to be sensitive to vertebrate proteases, in vitro (Takehara and Hotta, 1961) but have not been tested against invertebrate enzymes. The point is however that secretion of proteases occurs so late in the viral infection process as to preclude their influence in either a positive or negative manner (ref. cit., Gooding, 1972; Houk, unpubl. obs.). Thus, the peritrophic membrane, and most probably mosquito proteases, as barriers to infection of midgut epithelial cells in *C. tarsalis* and several other species of mosquitoes is a moot point.

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