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The Origin and Functions of the Insect Peritrophic Membrane and Peritrophic Gel

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There is a a fluid (peritrophic gel) or membranous (peritrophic membrane, PM) film surrounding the food bolus in most insects. The PM is composed of chitin and proteins, of which peritrophins are the most important. It is proposed here that, during evolution, midgut cells initially synthesized chitin and peritrophins derived from mucins by acquiring chitin-binding domains, thus permitting the formation of PM. Since PM compartmentalizes the midgut, new physiological roles were added to those of the ancestral mucus (protection against abrasion and microorganism invasion). These new roles are reviewed in the light of data on PM permeability and on enzyme compartmentalization, fluid fluxes, and ultrastructure of the midgut. The importance of the new roles in relation to those of protection is evaluated from data obtained with insects having disrupted PM. Finally, there is growing evidence suggesting that a peritrophic gel occurs when a highly permeable peritrophic structure is necessary or when chitin-binding molecules or chitinase are present in food. Arch. Insect Biochem. Physiol. 47:47–61, 2001. **© 2001 Wiley-Liss, Inc.**

Key words: peritrophic membrane; peritrophic gel; PM permeability; perimicrovillar membrane; midgut compartmentalization; digestion

INTRODUCTION AND NOMENCLATURE

In 1762, Lyonet found a membrane surrounding the food bolus in a caterpillar. This was followed by similar findings in different insects and in 1890 Balbiani appropriately named this anatomical structure peritrophic membrane (PM) (Balbiani, 1890). PM is now known to consist of a network of chitin and proteins with which other components associate. The major PM proteins are named peritrophins.

In 1992, Peters proposed to rename peritrophic membrane with peritrophic envelope, taking into account that this structure may be composed of several layers. Nevertheless, in anatomical terminology membrane may be a composite structure, e.g., the tympanic membrane and the nictitating membrane (transparent third eyelid) of birds and reptiles. Thus, the proposed change in name seems unnecessary. A more recent proposal to substitute the term "membrane" with "matrix" (Ramos et al., 1994), on the grounds

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that the PM is not a lipid bi-layer, is not appropriate. The peritrophic membrane is not a cell part, but an anatomical structure, as described above, and hence it is not expected to be a lipid bi-layer. Furthermore, matrix does not convey the idea of a film and suggests it is the fundamental substance of something, usually filling a space (as the mitochondrial matrix). Finally, as extracellular matrix research advances, peritrophic matrix may be mistaken for a structure made up of collagen fibers. I propose to maintain the appropriate and traditional term peritrophic membrane for the film surrounding the food bolus in insects, when it is membranous, and to denote the film peritrophic gel when it is gel-like (Fig. 1).

The recent increase in papers dealing with insect PM is caused mainly by the realization that this structure plays a vital role in gut physiology. Hence, an understanding of the structure, properties, and function of PM is essential when developing methods of control that act via the gut.

The vast literature available on PM is reviewed by Richards and Richards (1977) and more comprehensively by Peters (1992) in his book. More recently 4 other reviews appeared: one dealing with hematophagous Diptera PM (Jacobs-Lorena and Oo, 1996) and 3 others emphasizing PM structural aspects and PM roles in midgut epithelium protection (Tellam, 1996; Lehane, 1997; Tellam et al., 1999).

The present review emphasizes PM and peritrophic gel roles in digestion. Furthermore, based on these putative roles and recent molecular data on PM, the evolution of PM function and structure is discussed.

ORIGIN, TYPES, AND OCCURRENCE OF THE FILMS SURROUNDING THE FOOD BOLUS

The consistency of the fully formed film surrounding the food bolus is variable. In this review, the film is considered to be a peritrophic membrane or peritrophic gel depending on whether or not the film surrounding the food bolus can be picked up with a pair of fine forceps (Fig. 1). Thus, the type of film is identified by dissection. Micrographs usually do not show a peritrophic gel because during fixation it is frequently solubilized (see below). Recently formed PM and even mature PM, at least in Culicidae, may also solubilize during fixation and be easily detected only by dissection of fresh material (Freyvogel and Stäubli, 1965). Nevertheless, in all these cases a texture-less mass is seen over midgut cells in some photographs (Freyvogel and Stäubli, 1965) and may be considered to be the peritrophic gel or a forming PM.

A prerequisite for the study of PM and peritrophic gel function is a detailed understanding of PM types and their occurrence. This understanding developed from a speculative model of the origin and evolution of PM. According to this model, ancestral insects had their midgut cells covered with a mucus similar to that found in most animals. This gastrointestinal mucus, at least in vertebrates, is a gel-like substance composed of mucins (Allen, 1983; Forstner and Forstner, 1986). It is proposed that during evolution PM derived from the gastrointestinal mucus. According to this hypothesis, the peritrophins, the major PM proteins, evolved from mucins by acquiring chitin-binding domains. The concomitant

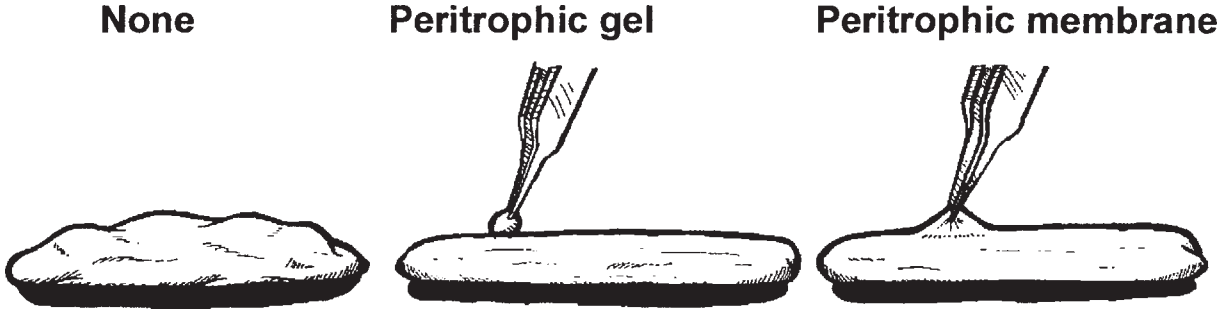

Fig. 1. Identification of film types surrounding the food bolus. After dissection, the midgut contents maintain their form (usually cylindrical) when a surrounding film is present. The film is considered to be a peritrophic membrane or a peritrophic gel depending whether fine forceps are able or not to pick up pieces of the film surrounding the food bolus.

evolution of chitin secretion by midgut cells permitted the formation of the chitin-protein network characteristic of PM structure. In favor of this hypothesis is the fact that peritrophins have, in addition to chitin-binding domains, other domains with overall structure similar to various mucins (Tellam, 1996; Tellam et al., 1999). If the hypothesis that PM is derived from the gastrointestinal mucus is correct, it should have originally been synthesized by all midgut cells and had the properties of the mucus. Later in evolution, insect species would have appeared with a chitin-protein network resulting in PM formation. Therefore, the formation of PM by the whole midgut epithelium is the ancestral condition, whereas the restriction of PM production to midgut sections, or the lack of PM and its replacement by the peritrophic gel, are derived conditions.

Except for Hemiptera and Thysanoptera, which have perimicrovillar membranes in their midgut cells (Silva et al., 1995), PMs are present in most insects. Negative reports are to be viewed with caution as in some insects (bloodsucking mosquitoes) PM is secreted only after distension of the gut (Richards and Richards, 1977) and in others PM is partly solubilized during fixation and can be easily detected only by dissection (see above). PMs are usually classified into two types (Peters, 1992) (Fig. 2). Lists of insects possessing different types of PM may be found in several papers (Waterhouse, 1953a, 1953b; Richards and Richards, 1977; Wigglesworth, 1972; Ferreira and Terra, 1989; Jimenez and Gilliam, 1990; Ferreira et al., 1990; Peters, 1992). PM types vary among the major insect taxa. Type I PM is found in cockroaches (Dictyoptera), grasshoppers (Orthoptera), beetles (Coleoptera), bees, wasps and ants (Hymenoptera), moths and butterflies (Lepidoptera), and in hematophagous adult mosquitoes (Diptera). Type II PM occurs in larval and adult (except hematophagous ones) mosquitoes and flies (Diptera), and in a few adult Lepidoptera. PM production limited to a belt in the middle third of the midgut (the beetle *Ptinus*) or a belt at the posterior end of the midgut (the weevil *Cionus*) (Richards and Richards, 1977) may be considered a particular case of type I PM.

Type I PM is formed either by the whole midgut epithelium, or by only part of it (anterior or posterior regions). PM produced by the whole or anterior midgut epithelium envelops the food along the whole midgut (Fig. 2A, C). When PM is produced only by the posterior part, the anterior midgut epithelium is usually covered with a viscous material, the peritrophic gel (Fig. 2B), as observed in carabid beetles (Ferreira and Terra, 1989) and bees (Jimenez and Gilliam, 1990). This gel is also observed in the anteriorly placed midgut caeca of some insects and along the whole midgut of others (Table 1).

During the formation of type I PM, chitin precursors are thought to be secreted by midgut cells and, after being self-organized into fibers, are interlocked by protein molecules (Reid and Lehane, 1984; Blackburn et al., 1988; Martin and Kirkham, 1989; Weaver and Scott, 1990; Spence and Kawata, 1993; Walters et al., 1993; Ryerse et al., 1994; Tellam et al. 1999). Peritrophins are released by microapocrine secretion (Bolognesi et al., 2001). The formation of these PMs is frequently induced by the distension of the gut caused by food ingestion (Richards and Richards, 1977). Type I PM may be synthesized only after food ingestion, without affecting digestion efficiency, because this PM may be formed quickly due to the cooperation of a large number of cells.

Type II PM is secreted by a few rows of cells at the entrance of the midgut (cardia) and is usually found in insects irrespective of food ingestion (Fig. 2E). Peritrophins are secreted by exocytosis (Eisemann et al., 2001). Type II PM is molded to the diameter of the anterior midgut. *Stomoxys calcitrans* adults, which store ingested blood in the anterior midgut, is one example of a species with type II PM. Hematophagous mosquitoes, which store ingested blood in the dilated posterior midgut, possess a type I PM (Fig. 2G), in spite of their larvae having type II PM. Clearly, PMs molded in the narrower anterior midgut (type II PM) would be unable to expand to accommodate an ingested blood meal such as that found in the posterior midgut of a mosquito (Fig. 2F).

There are a few insects that apparently do not have a PM, such as some adult ants (Hymenoptera), most adult moths and butterflies (Lepidoptera), lice (Phthiraptera), book lice (Psocoptera), Zoraptera, Strepsiptera, Raphidioptera, Megaloptera, adult fleas (Siphonaptera), and Bruchid beetles (Peters, 1992). The Hymenopteran and Lepidopteran insects that lack a PM seem to only feed on low molecular

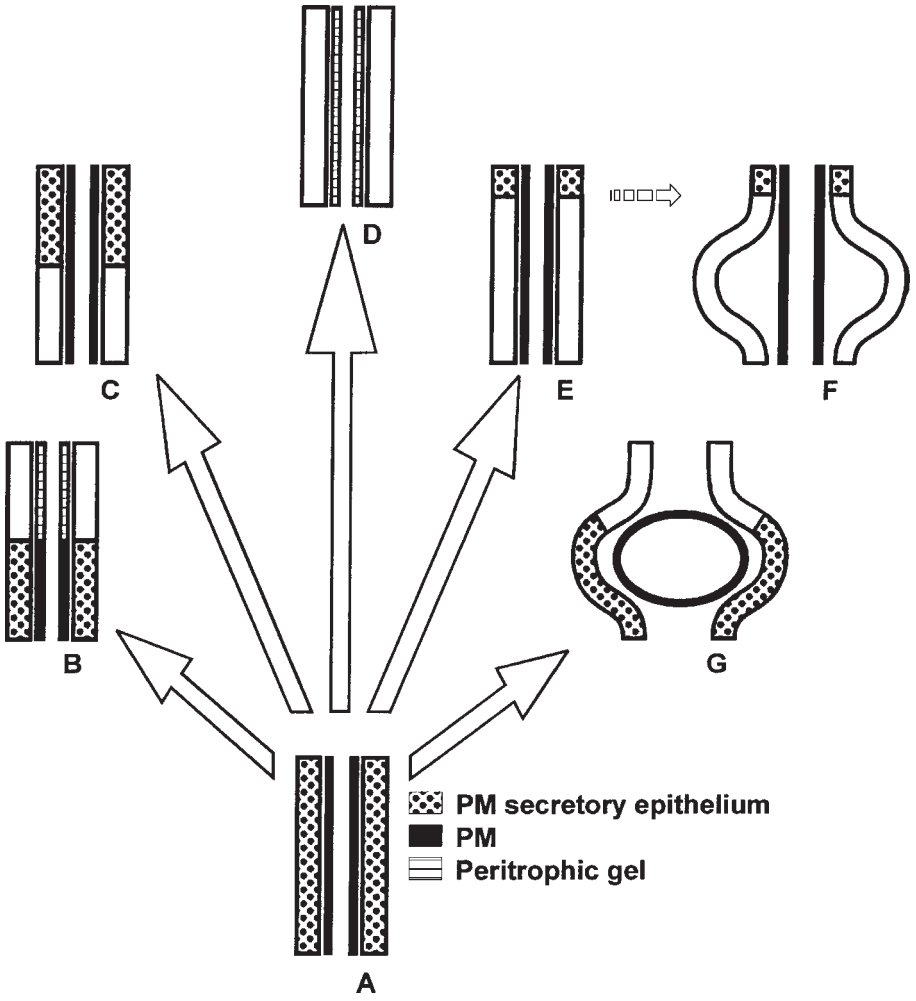

Fig. 2. Occurrence of peritrophic gel and peritrophic membrane (PM) in insects. Type I PM (A–C, D, G) is formed by most midgut epithelium, whereas type II PM (E,F) is secreted by a few row of cells at the entrance of the midgut (cardia). F is a hypothetical condition where a type II PM is formed in an insect with a dilated posterior midgut (like an hematophagous mosquito). The distinction between the two types of PM at the molecular level is less clear. Larvae of *Lucilia cuprina*, which produces type II PM, synthesize two peritrophins in the cardia and also, to a lesser extent, in the midgut epithelium (Tellam et al., 1999).

TABLE 1. Insects With Peritrophic Gel in Different Midgut Regions*

Anterior midgut	**Midgut caeca**
Apis mellifera[a]	*Locusta migratoria*[d]
Pheropsophus aequinoctalis[b]	*Abracis flavolineata*[e]
Tenebrio molitor[c]	
Migdolus fryanus[c]	**Whole midgut**
Sphenophorus levis[c]	*Zabrotes subfrasciatus*[c]
Dermestes maculatus[c]	*Anopheles maculipennis*[f]

*Although Ferreira et al. (1990) described the caeca PM of *Abracris flavolineata* as "solid", that PM is actually a gel if the operational definition given in Figure 1 is taken into account.
[a]Jimenez and Gilliam (1990).
[b]Ferreira and Terra (1989).
[c]Terra et al. (unpublished results).
[d]Baines (1978).
[e]Ferreira et al. (1990).
[f]Freyvogel and Stäubli (1965).

weight substances such as sugars, which render luminal digestion unnecessary (see Peritrophic Membrane Functions). Perhaps the other insects, such as *Zabrotes subfasciatus* (Bruchidae), lack a PM because they have a peritrophic gel along the whole midgut (Table 1).

A peritrophic gel occurs along the whole midgut in relatively highly evolved insects such as Bruchidae, suggesting that it corresponds to a derived component and not to the primitive mucus thought to have occurred in the putative insect ancestors. If this is true, the peritrophic gel should be composed of peritrophins different from those of PMs, as they would be able to form gel-like films in the absence of chitin. The absence of

chitin in the gel film surrounding the food bolus in the anterior midgut of *Dermestes maculatus* is indicated by the lack of binding of wheat-germ aglutinin, a chitin-binding lectin, in the presence of excess N-acetylglucosamine (Caldeira et al., unpublished results). Excess N-acetylglucosamine hinders wheat-germ agglutinin binding with glycoproteins, but not with chitin. In contrast to the gel, in the absence of chitin microfibrils, a PM is not formed from PM peritrophins, as judged by the fact that food is not enveloped by a PM in the midgut lumen of calcofluor-treated Lepidoptera larvae (Wang and Granados, 2000; Bolognesi et al., 2001) (see also Functional Effects of PM Absence or Modification).

PERITROPHIC MEMBRANE FUNCTIONS

Overview

The study of PM function is frequently approached by the study of its chemical structure. This approach is logically inconsistent, as summarized below and discussed in detail by Nagel (1961) in his book on the logic of scientific explanation. Structure refers to the spatial organization of physical systems, whereas function refers to the temporal patterns of activities of these constituents. Therefore, structure does not logically determine function, though the specific structure possessed by a system does set bounds to the kinds of activities the system can engage. Thus, PM functions are elucidated by studying how PM affects midgut events (digestion and absorption). The chemical structure of PM define its properties (such as strength, elasticity, and porosity), which set limits to the kinds of functions PM can play.

As mentioned before, gut cells in most animals are covered with a gel-like coating of mucus, which was most thoroughly studied in mammals (Forstner and Forstner, 1986). In these animals, the mucus is supposed to lubricate the mucosa, protecting it from mechanical damage, and to trap bacteria and parasites. Since the insect midgut epithelium lacks a mucus coating, PM functions were supposed to be analogous to that of mucus, but until recently, only a role in protection against mechanical damage of the midgut was emphasized (Peters, 1992). This may be a significant function of PM in some insects, but is unlikely to be its sole function, as PMs are found in many fluid-feeding insects. More recently, a PM function as a barrier to microorganisms was fully recognized (Peters, 1992; Jacobs-Lorena and Oo, 1996; Tellam, 1996; Lehane, 1997). Since then, the function of PM as a microorganism barrier has been considered so important that some authors (Lehane, 1997) suggested it as the main function of PM. There is, however, evidence based on calcofluor data suggesting that PM compartmentalization of digestive events is even more important (see Functional Effects of PM Absence or Modification).

A broadly protective role towards the epithelia is played both by gastrointestinal mucus and PM. This is to be expected as PM probably evolved from a mucus-like substance (see Origins, Types, and Occurrence of the Films Surrounding the Food Bolus). Thus, the specific functions of PM (those not played also by mucus) must depend on the fact that PM compartmentalizes the midgut lumen into an endoperitrophic space (inside PM) and an ectoperitrophic space (space between PM and midgut epithelium). The specific functions of compartmentalization and selective permeability are reviewed below.

Compartmentalization of Digestive Events

Any description of the spatial organization of digestion in an insect must relate midgut compartments (cell, ecto-, and endoperitrophic spaces) to each phase of digestion and, hence, to the corresponding enzymes. To accomplish this, enzyme determinations must be performed in each midgut luminal compartment and in the corresponding tissue.

The first attempt to relate midgut compartments with each phase of digestion was accomplished with larvae of *Rhynchosciara americana* (Diptera), which possess type II PM (Terra et al. 1979) (Fig. 3). Trypsin is found in luminal contents inside and outside PM, with small amounts being recovered in cells. Hence, trypsin is secreted by cells and is able to cross the PM. Aminopeptidase is not found inside PM, but is found outside PM. Thus, aminopeptidase is secreted into luminal contents but it does not pass through the PM. Large amounts of aminopeptidase are also found in caeca cells. Amylase has a midgut distribution similar to trypsin, whereas maltase is restricted to midgut cells, mainly those of caeca.

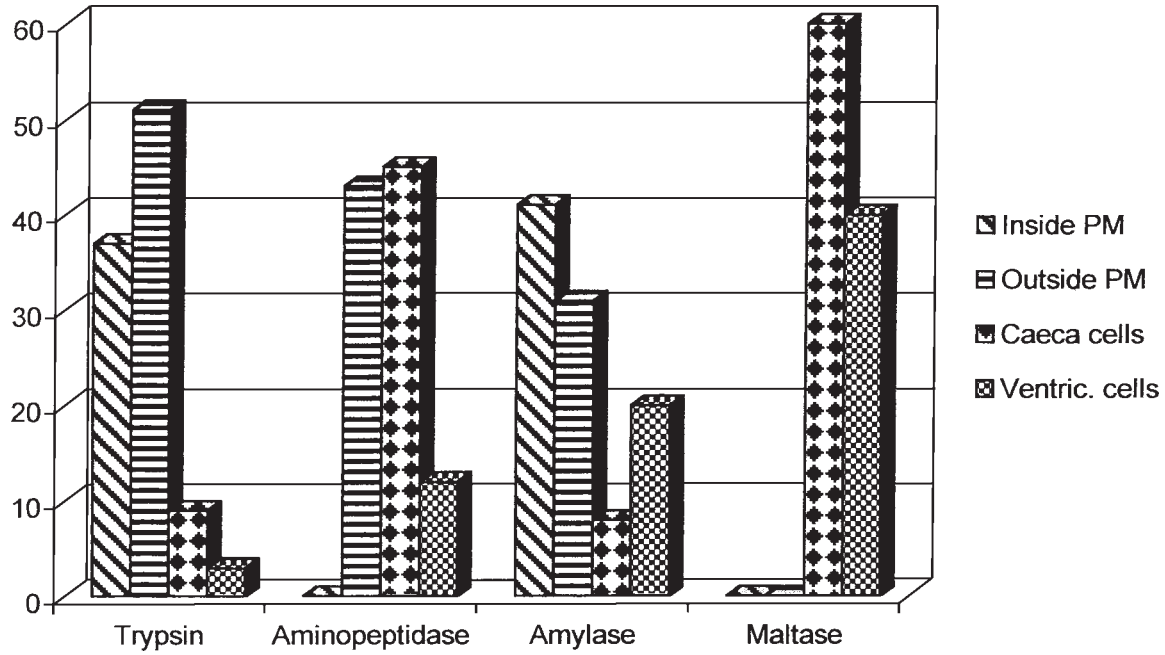

Fig. 3. Enzyme activities (%) in midgut compartments of *Rhynchosciara americana*. Enzymes were sampled from these compartments by aspiring the ectoperitrophic fluid from caeca with the aid of a capillary, then by dissection the contents of the peritrophic membrane and caeca and ventriculus tissue were collected. Ectoperitrophic fluid is near-quantitatively recovered because this insect has large caeca freely opened to the ventriculus. According to Terra et al. (1979).

These results, together with subcellular fractionation data (Ferreira and Terra, 1980; Klinkowstrom et al., 1994), led to the proposal that initial digestion occurs inside the PM by the action of enzymes such as amylase and trypsin. Intermediate digestion takes place in the luminal contents outside the PM, involving enzymes such as amylase and luminal aminopeptidase, and final digestion occurs in the surface of midgut cells, mainly of caeca cells, depending on enzymes exemplified by microvillar aminopeptidase and maltase.

An understading of the spatial organization of digestion requires knowledge of midgut fluid fluxes, in addition to the distribution of digestive enzymes. The determination of midgut fluid fluxes is usually performed with the aid of dyes. Secretory regions transport injected dye into the gut lumen, whereas absorbing regions accumulate orally fed dyes.

After studying the compartmentalization of digestive enzymes and midgut fluxes in the larva of *Rhynchosciara americana*, the following model was proposed (Ferreira et al., 1981; Terra and Ferreira, 1981) (Fig. 4C). According to the model, food flows inside the PM from the anterior midgut to the posterior midgut, whereas outside the PM water flows from the posterior midgut to the caeca. The enzymes involved in initial digestion should penetrate and be retained within the anterior endoperitrophic space, since the enzyme-substrate complexes may be too large to diffuse back across the PM. Nevertheless, as the food progresses along the endoperitrophic space, the molecular size of the food particles decrease until they are able to pass through the PM together with the enzymes to which they are bound. The enzymes and oligomeric molecules are then displaced towards the caeca, where terminal digestion and absorption occur. The enzymes may diffuse back to the anterior ventriculus and enter the endoperitrophic space again and a new cycle starts.

In *Musca domestica* (Diptera) (with type II PM), the compartmentalization of digestive enzymes is similar to *R. americana*, and an endo-ectoperitrophic circulation of digestive enzymes apparently occurs in the posterior larval midgut. This was confirmed by the finding that an increase in dietary protein fed to larvae led to both a decrease in the

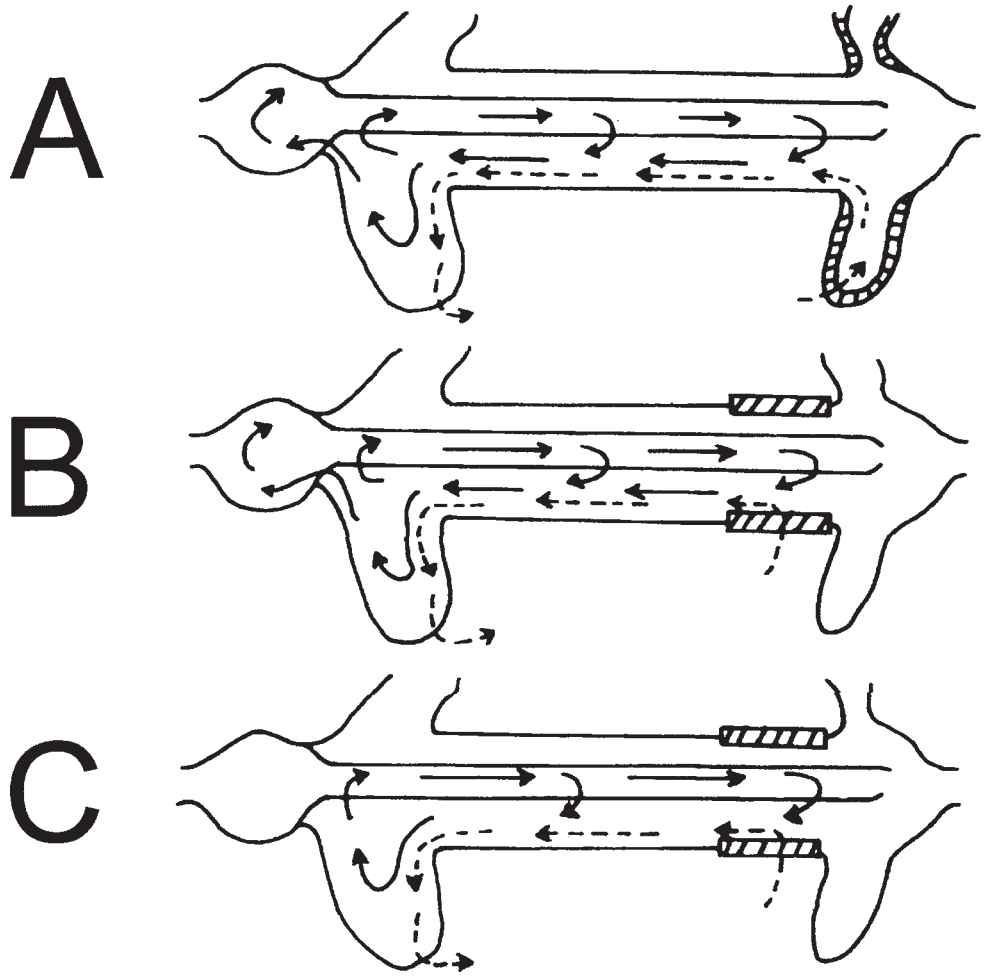

Fig. 4. Diagrammatic representation of water fluxes (dotted arrows) and the circulation of digestive enzymes (solid arrows) in putative insect ancestors (A–C). In Neoptera ancestors (**A**), midgut digestive enzymes pass into the crop. Countercurrent fluxes depend on the secretion of fluid by the Malpighian tubules and its absorption by the caeca. Holometabola ancestors (**B**) are similar, except that secretion of fluid occurs in posterior ventriculus. Panorpoid ancestors (**C**) display countercurrent fluxes like Holometabola ancestors; midgut enzymes are not found in the crop, and only their polymer hydrolases pass through the peritrophic membrane. In evolved insects, the anterior midgut may replace the absent midgut caeca in absorption. Based on Terra (1990).

trypsin gradient along their posterior midgut and to an increase in the trypsin excretory rate. Moreover, dye experiments showed the existence of the appropriate fluid fluxes (Espinoza-Fuentes and Terra, 1987; Terra et al., 1988).

Lepidoptera larval midgut (with type I PM) do not possess caeca; therefore, it is not possible to collect uncontaminated ectoperitrophic fluid from them. Nevertheless, a fluid enriched with the contents of the ectoperitrophic space can be collected by rinsing the luminal surface of the midgut epithelium (Santos et al., 1983, 1984). The use of techniques similar to those described above were used to show that the compartmentalization of digestive enzymes and their endo-ectoperitrophic circulation in Lepidoptera were similar to Diptera (Santos et al., 1983, 1984, 1986; Ferreira et al. 1994a,b).

Studies similar to those described for Diptera and Lepidoptera were accomplished with several species pertaining to different orders and used to support a model on the evolution of insect digestive systems (Terra, 1990).

The spatial organization of digestion in the putative ancestors described in Figure 4 were based on data obtained from the following insects (review: Terra, 1990; Terra and Ferreira, 1994): (1) Orthoptera, *Schistocerca gregaria*, *Abracris*

flavolineata; (2) Coleoptera, *Pheropsophus aequinoctialis, Tenebrio molitor, Pyrearinus termitilluminans, Attagenus megatoma, Cylas formicarius;* (3) Diptera, *Rhynchosciara americana, Trichosia pubescens, Musca domestica*; (d) Lepidoptera, *Erinnyis ello* and *Spodoptera frugiperda.*

Peritrophic Membrane Permeability

The capacity of the PM to compartmentalize the luminal digestive enzymes depends on its permeability. Pore sizes of the PM may be determined by comparing molecular weights of enzymes restricted to the ectoperitrophic fluid (e.g., luminal aminopeptidase, Fig. 3) with those of enzymes present in the endoperitrophic space (e.g., trypsin and amylase, Fig. 3). Such studies led to the conclusion that PM of larvae of the sciarid flies *Rhynchosciara americana* (Terra and Ferreira, 1983) and *Trichosia pubescens* (Espinoza-Fuentes et al., 1984) have pore diameters of 7.5–8 nm.

When ectoperitrophic fluid is not quantitatively recovered (e.g., in Lepidoptera larvae), it is not possible to use the amounts of luminal enzymes inside and outside PM to identify which enzymes are restricted to the ectoperitrophic space. In these circumstances, it is necessary to use another procedure. Table 2 shows that acetylglucosaminidase, carboxypeptidase A, and trypsin are necessarily secreted into the ectoperitrophic fluid, as their specific activities in fluid are higher than in midgut tissue. Amylase and trypsin, known to occur in major amounts inside PM (activities in PM contents are about 70% of activities in midgut homogenates, Santos et al., 1983), have specific activities in PM contents higher or equal to specific activities in ectoperitrophic fluid. Since acetylglucosaminidase and carboxypeptidase are stable if mixed with midgut contents, the fact that their specific activities in PM contents are lower than in ectoperitrophic fluid indicate that they are restricted to the last mentioned compartment. A comparison of molecular weights of the enzymes (Table 2) led to the conclusion that PM pores in *E. ello* larvae have diameters of 7.0–7.5 nm. The same procedure was used for another Lepidoptera larvae (*Spodoptera frugiperda*) and the diameters found for PM pores were 7.5–8 nm (Ferreira et al., 1994a).

PM may have a large range of pore sizes: some small or very large and most of them in the middle range. The pore sizes determining enzyme distribution, as detailed above, necessarily correspond to the most frequent pore sizes, putatively those of intermediate size. Enzymes larger than the calculated pore sizes may occasionally be found inside PM, but in non-significant amounts. Thus, the mentioned pores are those responsible for maintaining different types of digestive enzymes inside and outside PM.

Pore sizes were also determined using varied molecules of known molecular weights with different techniques (Table 3). Most of the data agree with those obtained from enzyme distribution studies. Pore sizes of 300–800 nm are surprisingly large and may be due do damage of PM during its manipulation. Pore sizes (Table 3) in the range of 17–36 nm were obtained with methods able to detect very small amounts of substances traversing PM and, for this, they probably correspond to the large pores putatively occurring in low frequency in PMs. Although these large pores are supposed to be of no importance regarding digestive events, they set the size limits that

TABLE 2. Diameters and Specific Activities (mU/mg protein) of Digestive Enzymes in Midgut Cells and in the Endo- and Ectoperitrophic Spaces from *Erinnysis ello* larvae*

Enzyme	Midgut tissue	Ectoperitropic fluid	PM contents	Main site	Diameter (nm)
N-acetylglucosaminidase	1.8	2.8	0.41	Out PM	8.6
Amylase	150	970	1,400	In PM	6.2
Carboxypeptidase A	23	50	17	Out PM	7.6
Trypsin	1.9	10	10	In PM	6.5
α-Galactosidade	1.02	0.45	0.129	Tissue	7.3
Trehalase	32	16.1	7.1	Tissue	7.9

*Ectoperitrophic fluid was collected by rinsing with saline the luminal surface of the midgut tissue. Diameters of the hydrated enzymes were interpolated in a plot of log (M_r) against Stoke's radius for 11 proteins. M_r values were determined by centrifugation and electrophoresis. According to Santos and Terra (1986).

TABLE 3. PM Pore Sizes*

Order	Number of species	Diameter (nm)	Technique
Diptera	1	8–9	Colloidal gold[a]
Diptera	2	7.5–8	Enzyme distribution[b]
Diptera	6	8–9	Dextran distribution[c]
Diptera	1	8–9	Diffusion rate (PM mounted as a sac)[d]
Diptera	2	>17	Dextran distribution[e]
Lepidoptera	2	7–8	Enzyme distribution[f]
Lepidoptera	1	7–8	Diffusion rate (PM mounted as a sac)[g]
Lepidoptera	1	300–800	Diffusion rate (PM fragments between chambers)[h]
Lepidoptera	4	21–29	Dextran distribution[i]
Orthoptera	3	24–36	Dextran distribution[i]

*Pore sizes of 300–800 nm in diameter may result from damages during PM manipulation, those in the range 17–36 nm probably correspond to rarely-occurring pores, whereas the 7–9 nm pores are the most frequent ones. See text for details.
[a]Zhuzhikov (1964).
[b]Terra and Ferreira (1983), Espinoza-Fuentes et al. (1984).
[c]Peters and Wiese (1986).
[d]Miller and Lehane (1990).
[e]Edwards and Jacobs-Lorena (2000).
[f]Santos and Terra (1986), Ferreira et al. (1994a).
[g]Wolfersberger et al. (1986).
[h]Adang and Spence (1983).
[i]Barbehenn and Martin (1995).

an infecting particle must have to successfully pass through PM.

It is interesting to note that *Glossina morsitans, R. americana, T. pubescens*, and the other Diptera larvae studied by Peters and Wiese (1986) possess type II PM (Wigglesworth, 1972), whereas *Manduca sexta*, *E. ello*, and *S. frugiperda* larvae display type I PM (Wigglesworth, 1972). Hence, the mechanism of secretion of PM seems to be of no major importance in determining pore size.

The chitin-peritrophin matrix of PM may be responsible for the strength and elasticity of this membrane, in addition to the role in defining its permeability. In accordance with this hypothesis, inhibition of protein synthesis resulted in disturbed formation of PM, whereas inhibition of chitin synthesis resulted in a pronounced enhancement of permeability (Zimmermann and Peters, 1987). Moreover, peritrophin antiserum (Willadsen et al., 1993) and peritrophin-binding lectins (Eisemann et al.,1994) ingested by *Lucilia cuprina* decrease the permeability of PM.

Although the peritrophin-mediated chitin cross-linking process seems to regulate the highly defined porosity present in most PMs, the detailed molecular mechanisms determining this porosity are not clear. The permeability of PM may change in some cases depending on pH and ionic concentration (Miller and Lehane, 1993). This and the fact that anionic charged proteoglycans are common in the PM led Lehane (1997) to propose a proteoglycan role in PM permeability. Nevertheless, since most PM permeability determinations (see above) showed a dependence on molecular size, not on molecular charge, a proteoglycan role in PM permeability, at least in relation to proteins, may not be a major one. Furthermore, by determining the rate of mono- and polyanionic dextrans passing through PMs of Lepidoptera and Orthoptera species, Barbehenn and Martin (1997) showed that polyanion exclusion does not contribute significantly to PM permeability.

Peritrophic Membrane Functions Distinct From Those of Gastrointestinal Mucus

Protection against food abrasion and microorganism invasion are functions shared by PM and gastroingestinal mucus (see Overview). The studies on the spatial organization of digestion (Compartmentalization of Digestive Events) and PM permeability (Peritrophic Membrane Permeability) have led to the proposal that PM plays several roles in digestion (Terra, 1990; Terra et al., 1994) that are summarized in Table 4 and are detailed below.

TABLE 4. PM Functions Distinct From Those of Gastrointestinal Mucus*

Primary Functions
All orders
(a) Prevention of non-specific binding onto cell surface
(b) Prevention of excretion by allowing enzyme recycling
(c) Removal of oligomeric molecules from inside PM
Panorpoidea
(d) Restriction of oligomer hydrolases to ectoperitrophic space
(e) Restriction of monomer production to cell surface
Secondary functions
(f) Enzyme immobilization
(g) Toxin binding

*Primary functions are those probably evolved under selective pressures, whereas secondary ones are consequences of the chemical properties of PM components. Functions a–c are more basic and found in all insect orders, whereas functions d and e are more sophisticated and occur only among the Panorpoidea, which includes Diptera and Lepidoptera. See text for details.

1. Prevention of non-specific binding of undigested material onto midgut cell surface:

   Any chromatographic separation of proteins from tissue homogenates often results in the problem of non-specific binding of polar material onto the column resin. Similarly, unless isolated from undigested midgut contents by PM, the pollar luminal surface of midgut cells is subjected to such non-specific binding. A consequence of this may be the impairment of membrane-bound hydrolases (as aminopeptidase) and amino acid, glucose, and other carriers, leading to a decrease in the efficiency of terminal digestion and absorption. The gastrointestinal mucus of vertebrates provide some protection against non-specific binding by acting as a diffusion barrier for large molecules. Nevertheless, PM is expected to be much more efficient than mucus because only molecules with diameters of less than 8–9 nm are able to move across it.
2. Prevention of enzyme excretion by allowing the endo-ectoperitrophic circulation of digestive enzymes: Although the enzyme recycling model (see Compartmentalization of Digestive Events) has been widely accepted, since 1986 (e.g., Dow, 1986) some authors questioned its validity in insects having type I PM, particularly the Lepidoptera. They criticize the model on the grounds that the ectoperitrophic space in these insects is virtually absent, and, therefore, unable to contain a countercurrent flux of fluid. In spite of this, there is growing evidence that insects with type I PM, including Lepidoptera, possess enzyme recycling mechanisms. Thus, as expected by the model, most trypsin activity is found in the lumen of *Manduca sexta* (Lepidoptera, type I PM) anterior midgut, whereas trypsin m-RNA predominates in middle midgut (Peterson et al., 1994). Furthermore, immunocytochemical data showed the occurrence in the anterior midgut of significant amounts of a 41-kDa protein and a trypsin secreted by the posterior midgut of *Manduca sexta* (Borhegyi et al., 1999) and *Tenebrio molitor* (Coleoptera, Type I PM) (Cristofoletti et al., 2001), respectively. Finally, the decreasing trypsin and chymotrysin gradient along *S. frugiperda* midgut contents (putatively generated by the recycling mechanism, see Compartmentalization of Digestive Events) was shown to disappear in calcofluor-treated larvae lacking a PM (Bolognesi et al. 2001) (see also Functional Effects of PM Absence or Modification).
3. Increase in the efficiency of digestion of polymeric food by allowing the removal of the resulting oligomeric molecules from the endoperitrophic space, powered by the recycling mechanism: Since oligomers may be substrates or inhibitors for some polymer hydrolases, their presence should decrease the rate of polymer degradation. A fast polymer degration assures that polymers are not excreted and hence increases their digestibility.
4. Increase in the efficiency of oligomeric food hydrolysis by allowing the transference of oligomeric molecules to the ectoperitrophic space (see 3) and by restricting oligomer hydrolases to this compartment: In these conditions, oligomer hydrolysis occur in the absence of probable partial inhibition (because of non-productive binding) by polymeric food and putative non-specific binding by non-dispersed undigested food.
5. Restriction of food monomer production to the neighborhood of the midgut cell surface: This is a consequence of (4) and causes an increase in the concentration of the final products of digestion close to the carriers responsible for their absorption.
6. Enzyme immobilization: Midgut luminal enzymes, in addition to occurring in the endoperitrophic and ectoperitrophic spaces, may be associated with PM (Terra et al., 1979; Walker et al., 1980; Peters and Kalnins, 1985; Ramos et al., 1993; Jordão et al., 1996a,b). Nevertheless, the only quantitative determination of digestive enzymes associated with washed PMs was performed in *Spodoptera frugiperda* (Lepidoptera) larvae. Results showed that the PMs may contain up to 13 and 18% of the midgut luminal activity of amylase and trypsin, respectively (Ferreira et al., 1994b). Luminal enzymes, such as trypsin, may be found by immunocytochemical techniques predominating in the ectoperitrophic space and at the tissue face of the PM. This result is an artefact caused by the preferential removal of endoperitrophic proteins during fixation (Jordão et al.,

1996a,b). But, although the alleged importance of enzyme immobilization in PM may be partly due to artefacts, microscopic and biochemical data suggest that the phenomenon exists. Hence, enzyme immobilization may play a role in digestion, although a minor one. The attachment mechanism of enzymes in PM is not well known. Nevertheless, there is evidence, at least in *S. frugiperda*, that trypsin, amylase, and microvillar enzymes are incorporated into the jelly-like substance associated with PM when the enzymes, still bound to membranes, are released from midgut cells by a microaprocrine process (Jordão et al., 1999; Bolognesi et al., 2001). The lack of domains in insect trypsins (and other digestive enzymes) able to bind with PM molecules (Tellam et al., 1999) also favors this hypothesis.

7. Toxin binding: Since the finding of Bernays and Chamberlain (1980) that up to 30% of the potentially toxic dietary tannins are attached to and excreted with *Schistocerca gregaria* PM, a toxin binding role has been proposed for PM. Nevertheless, detailed studies with *Manduca sexta* showed that tannins are maintained in the endoperitrophic space because they form high molecular weight complexes, in the presence of calcium and magnesium ion concentrations similar to those found in midgut contents (Barbehenn and Martin, 1998). In a similar way, some lipophilic and amphiphilic noxious substances are also contained by *Melanoplus sanguinipes* PM, as they associate with large lipid aggregates formed during digestion (Barbehenn, 1999). Thus, toxin binding by PM seems to be a less widespread phenomenon than once expected. In spite of this, recently, hemin was shown to bind to the PM of *Aedes aegypti*, suggesting a role in heme detoxification in this hematophagous insect (Lemos et al., unpublished data).

Current data suggest that PMs of all insects have functions (1–3), whereas functions 4 and 5 are demonstrable only in PMs of Panorpodea (taxon that includes Diptera and Lepidoptera). PM function (6) may occur in all insects but this needs further confirmation. Function 7, although it may be important for some insects, should be viewed as opportunistic. In other words, PM probably evolved from a protective role to more sophisticated functions (1–6) under selective pressures and, due to the chemical properties of their constituents, PM also developed the ability to bind different compounds including toxins.

Functional Effects of PM Absence or Modification

Calcofluor inhibits the formation of chitin-containing microfibrils by binding the polysaccharide (Maeda and Ishida, 1967). When Calcofluor is injected into a Dipteran species (*Calliphora erythrocephala)*, PM permeability is enhanced (Zimmermann and Peters, 1987), and when fed to a Lepidoptera, an increase in baculovirus-induced infection is observed (Shapiro and Vaughn, 1995). However, Wang and Granados (2000), after feeding larvae of the moth *Trichoplusia ni* with calcofluor, observed an inhibition of PM formation and noted high larval mortality. Examination of dead larvae showed no signs of microbial infection. As previous work suggested that calcofluor does not affect midgut cells (Adams et al., 1994), the results with *T. ni* probably mean that in normal conditions PM is less important to prevent microorganism infections than to compartmentalize the digestive events (see Compartmentalization of Digestive Events). As mentioned before (see Peritrophic Membrane Functions Distinct From Those of Gastrointestinal Mucus), PM lack caused by calcofluor ingestion abolishes midgut enzyme recycling in the larvae of *S. frugiperda* (Bolognesi et al., 2001).

Taking the physiological roles proposed for the PM into account, this membrane should only be absent in insects that do not display luminal digestion. One such example are the Hemiptera, which feed on phloem sap (composed mainly of sugars, amino acids, and amines) and do not have a PM (Terra and Ferreira, 1994; Silva et al. 1995). Nevertheless, several Lepidoptera adults have a PM (Waterhouse, 1953a, 1953b), although many of them apparently feed only on nectar (sugars) that do not need luminal digestion. It should be noted, however, that some Lepidoptera adults are known to visit carrion, dung, and rotten fruit and a few need a protein diet to complete vitellogenesis in the form of blood or pollen (Terra and Ferreira, 1994). Moreover, even nectar-feeding Lepidoptera adults such as *Erinnyis ello* have the

capacity to digest macromolecules such as starch and protein (Terra and Ferreira, 1994). Thus, the maintenance of the ability to digest food, which is seldom ingested by some adult Lepidoptera, may be related to the improvement of egg production. It will be interesting to see if there is a correlation between the presence of PM in Lepidoptera adults and true luminal digestion. It was suggested that hematophagous Hemiptera do not have a PM because they are derived from sap-sucking Hemiptera (Terra and Ferreira, 1994), whereas insects displaying pre-oral digestion possesss a PM, probably related to the fact that pre-oral digestion consists only of initial digestion, with the subsequent phases of digestion taking place in the midgut (Colepicolo Neto et al., 1986).

Mosquitoes lacking PM are able to complete bloodmeal digestion (Billingsley and Rudin, 1992). Nevertheless, unless the lack of PM is proved to have no effect on quantitative nutritional parameters (digestibility, efficiency of conversion of food into body mass, and growth rate), there is no reason to suppose that functions of the PM in mosquitoes are different from those in other insects, as suggested by Billingsley and Rudin (1992).

PERITROPHIC GEL FUNCTIONS

Peritrophic gel differs from PM in two important aspects: lack of mechanical resistance, which needs no further comment, and permeability properties. Based on the size of the enzyme molecules passing through the peritrophic gel from the midgut caeca into the crop of *Abracris flavolineata* (Ferreira et al., 1999) and from anterior midgut cells into the crop of *Pheropsophus aequinoctialis* (Ferreira and Terra, 1989), the gel pores were estimated to be larger than 8.6 nm. As discussed in Peritrophic Membrane Permeability, PM pore sizes are less than 8 nm, if measured with similar techniques. Thus, it seems that a peritrophic gel separates the midgut cells from the food when a forward movement of enzymes and fluid (associated with crop digestion) is necessary. It is interesting to note that in both insects, a PM is found in posterior midgut, probably a necessary condition to improve the compartmentalization of digestive events and, hence, the efficiency of digestion.

Another explanation, at least for beetles, takes into account their presumed ancestors (Terra, 1990) that had an acidic anterior midgut (with a peritrophic gel and high carbohydrase activity) and an alkaline posterior midgut (with PM and a high proteinase activity). This characteristic is thought to be an adaptation to a diet consisting of plant material with associated fungi and bacteria, in which part of a beetle's enzymes (putatively including chitinase) are unstable in the presence of their own proteinases. In these conditions, a peritrophic gel in the anterior midgut is not affected by the hypothetical chitinase that is inactivated by the proteinases in the posterior midgut.

The occurrence of a peritrophic gel in the anterior midgut of the beetles Curculionidae, Cerambycidae, and Dermestidae and in bees (Hymenoptera) probably corresponds to an unchanged ancestral condition. None of these insects have a crop digestion.

The lack of PM and its replacement by a peritrophic gel in Bruchidae larvae deserve attention. Bruchid beetles exploit legume seeds that are rich in the storage protein vicilin. This protein is detrimental to the bruchid *Callosobruchus maculatus* (Macedo et al., 1993), probably because vicilins are able to bind with chitinous structures in the insect midgut (Sales et al., 1996). The presence of a peritrophic gel lacking chitin putatively compartmentalizes digestive events in bruchid larvae and decreases the detrimental effect of vicilin.

CONCLUDING REMARKS

During the last 10 years, research has progressed far enough to permit a fairly complete description of the main functions of PM. There is a large body of data showing that PM combines a protective function, similar to a gastrointestined mucus, with those related to the compartmentalization of digestion. Nevertheless, in spite of existing hypotheses, it is not perfectly clear what physiologically determines if the peritrophic film will be a PM or a peritrophic gel. In the light of current research, the distinction between PM and peritrophic gel seems more important than the traditional distinction between type 1 and type 2 PMs, which is based only on their site of synthesis.

Other major points needing clarification are how the chemical nature of peritrophic gel and PM define their strength, elasticity, and porosity

and how these structures are self-assembled in the midgut lumen. These points are now being addressed with the powerful methods of modern protein chemistry and molecular biology and should soon lead to a comprehensive description of peritrophic gel and PM structure and formation.

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