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The peritrophic membrane of *Ixodes ricinus**

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Abstract. A peritrophic membrane was found in all three stages of *Ixodes ricinus* at no later than 18 h after their placement on rabbits. It was found to remain intact until at least 11, 30 and 10 days after repletion in larvae, nymphs and females, respectively. In blood-feeding *I. ricinus*, the peritrophic membrane is an uneven single layer with a thickness of about 0.03–0.48 μm in larvae, 0.03–0.79 μm in nymphs and 0.04–0.93 μm in females. It covers the whole surface of the midgut epithelium at a distance of about 0.2–0.8 μm. After repletion, the peritrophic membrane becomes thicker and thicker and more and more winding and simultaneously becomes multi-layered mainly in its arched parts. The distance between the peritrophic membrane and the midgut epithelium increases considerably and in the arched parts can reach as much as about 13 and 16 μm in metamorphosing larvae and nymphs, respectively and 25 μm in ovipositing females.

Peritrophic membranes (PMs) are found in members of almost all animal phyla (Peters 1968) and have been most extensively investigated in arthropods. They can function as a physiological barrier possessing a selective permeability (Zhuzhikov 1970; Rudzinska et al. 1982; Peters and Wiese 1986) or as a mechanical barrier protecting the midgut epithelium from damage afflicted by hard food particles. The latter function of the PM is most obvious in arthropods feeding on solid foods and is generally accepted. In blood-sucking vector arthropods that exhibit a PM, some correlations have been found between the PM and the infection process of a pathogenic agent (Stohler 1957, 1961; Esslinger 1962; Laurence 1966; Harmsen 1973; Steiger 1973; Ellis and Evans 1977; Rudzinska et al. 1982; Ponnudurai et al. 1988). Thus, the findings involving the PM in a blood-sucking vector are of particular significance for an understanding of the behavior of an etiologic agent within its vector.

Amongst the blood-sucking arthropods, most form a PM (Ito et al. 1975). Members of the suborder Ixodida (Krantz 1978) are hematophagous ectoparasites of terrestrial vertebrates and transmit numerous pathogenic agents to man and terrestrial vertebrates, including spirochetes, bacilli, viruses, and rickettsiae, as well as protozoa. To date, of some 800 species (Krantz 1978; Aeschlimann and Gern 1990) of the suborder Ixodida (Acari), a PM has been reported only in *Ixodes dammini* (Ixodidae; Rudzinska et al. 1982) and in *Ornithodorus moubata* (Argasidae; Grandjean 1984). Although a membrane structure similar to the PM of insects has been mentioned by Samson (1909) and Girardin (1986, unpublished data), the existence of a PM in *I. ricinus* has not yet been confirmed. In the present study the PM of *I. ricinus* is described. Part of the results were presented at the 8th International Congress of Acarology in Ceské Budéjovice, Czechoslovakia, on August 6–11, 1990 (Zhu et al. 1990).

Materials and methods

Larvae, nymphs and adults (female/male ratio, 1:1) of *Ixodes ricinus* from a laboratory colony were fed on New Zealand white rabbits (Graf 1978). Ticks were sampled at different intervals after their placement on rabbits and after repletion. The repleted ticks were kept at 18°–20° C and saturated humidity. To avoid confusing the PM material secreted during the blood meal with that secreted soon after repletion (Rudzinska et al. 1982) and to determine the thickness of the PM at the end of the rapid feeding phase (Lees 1952; Kheisin and Lavrenko 1956; Balashov 1968; Aeschlimann and Grandjean 1973), ticks that appeared to be fully engorged but remained attached to the rabbits were also sampled. In addition, unfed larvae, nymphs and adults were used as controls. The sampled ticks were halved longitudinally in cold Karnovsky' fixative (Karnovsky 1965) and then kept in fresh cold fixative at 4° C for 1–4 h. After the tick viscera had hardened, pieces of the midgut were isolated and processed for optical and transmission electron microscopy (Agbede et al. 1986). Semi-thin sections were stained

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with toluidine blue and observed with an Olympus Vanox-S (Olympus Optical Co., Ltd) optical microscope. Ultra-thin sections were stained with uranyl acetate and with lead citrate and then examined with a Philips EM 201 electron microscope. PMs of different parts of the midgut lumen of ticks were photographed and measured.

Results

A PM was detected in all three stages of *Ixodes ricinus* at no later than 18 h (Fig. 1) after their placement on rabbits for blood feeding. It was found to remain intact until at least days 11 (264 h; Figs. 2, 3), 30 (720 h) and 10 (240 h) after repletion in larval, nymphal and female *I. ricinus*, respectively. The PM in an ovipositing female examined at 14 days (336 h) after repletion was found to have been partially displaced and broken (Fig. 9). No PM was found in unfed larvae, nymphs or adults or in fed males.

In blood-feeting *I. ricinus*, the PM is an uneven single layer with a thickness of about 0.03–0.48 μm in larvae, 0.03–0.79 μm in nymphs and 0.04–0.93 μm in females (Table 1). It covers the whole surface of the midgut epithelium at a distance of about 0.2–0.8 μm. At 18 h after placement, the PM was clearly visible under the electron microscope and seemed to be a relatively broad zone without well-defined boundaries (Fig. 1). At about 2–6 h after its appearance, this broad zone became a definite thin layer (Table 1). During the remaining part of the slow feeding phase, the thickness of the PM appeared to increase (Table 1), whereas during the rapid engorging phase, it decreased considerably (Table 1, Fig. 4).

After repletion, the PM became thicker and thicker and more and more winding and simultaneously became multi-layered mainly in its arched parts (Figs. 2, 3, 5–9). There was a large increase in the thickness of the PM during the first 12 h after detachment of ticks (Table 1, Fig. 5). The PM in repleted females seemed to be less winding and the multi-layered phenomenon, less obvious than in larvae and nymphs (Figs. 2, 3, 5–9). The distance between the PM and the epithelium increased greatly and in the arched parts of the PM it reached as much as about 13 and 16 μm in metamorphosing larvae (Fig. 3) and nymphs (Fig. 6), respectively, and 25 μm in ovipositing females (Fig. 9). The PM appeared spongy and its electron density was generally lower than that of the gut lumen contents. However, a PM whose electron density was higher than that of the lumen contents was found in an ovipositing female fixed at day 14 (336 h) after repletion (Fig. 9).

Table 1. Thickness of the PM in *Ixodes ricinus*

Sampling Time (h after placement/after repletion)	Thickness of the PM (minimum-maximum, μm)		
	Larvae (numbers of examined ticks)	Nymphs (numbers of examined ticks)	Females (numbers of examined ticks)
18 h	0.117–0.477 (1)	0.078–0.708 (1)	0.078–0.247 (1)
20 h	0.04 –0.141 (1)	0.105–0.526 (2)	
22 h	0.051–0.135 (1)	0.116–0.287 (1)	0.071–0.116 (2)
24 h		0.272–0.39 (1)	
26 h	0.136–0.194 (1)	0.233–0.486 (1)	
28 h		0.261–0.351 (1)	
30 h	0.116–0.214 (1)	0.246–0.351 (1)	
48 h	0.038–0.092 (1)[a]	0.292–0.789 (2)	
72 h	0.027–0.076 (2)[a]	0.039–0.105 (1)[a]	0.067–0.298 (3)
72 h/12 h	0.082–0.682 (2)	0.067–0.356 (1)	
72 h/24 h	0.062–1.05 (1)	0.146–0.898 (1)	
72 h/48 h	0.146–1.169 (1)	0.195–1.679 (1)	
72 h/72 h	0.195–0.937 (1)		
72 h/264 h	0.265–6.937 (1)		
96 h		0.026–0.117 (1)[a]	
96 h/12 h		0.165–1.462 (1)	
96 h/24 h		0.173–1.403 (1)	
96 h/48 h		0.506–1.374 (1)	
96 h/96 h		0.273–2.02 (1)	
96 h/168 h		0.308–4 (1)	
96 h/336 h		0.313–9.333 (2)	
96 h/720 h		0.312–9.091 (1)	
120 h			0.04 –0.93 (2)
168 h			0.056–0.251 (2)[a]
168 h/12 h			0.085–1.563 (1)
168 h/54 h			0.117–1.162 (1)
168 h/90 h			0.307–3.203 (1)
168 h/240 h			0.208–1.015 (1)
168 h/336 h			0.349–4.437 (1)
Total tick numbers	(14)	(23)	(15)

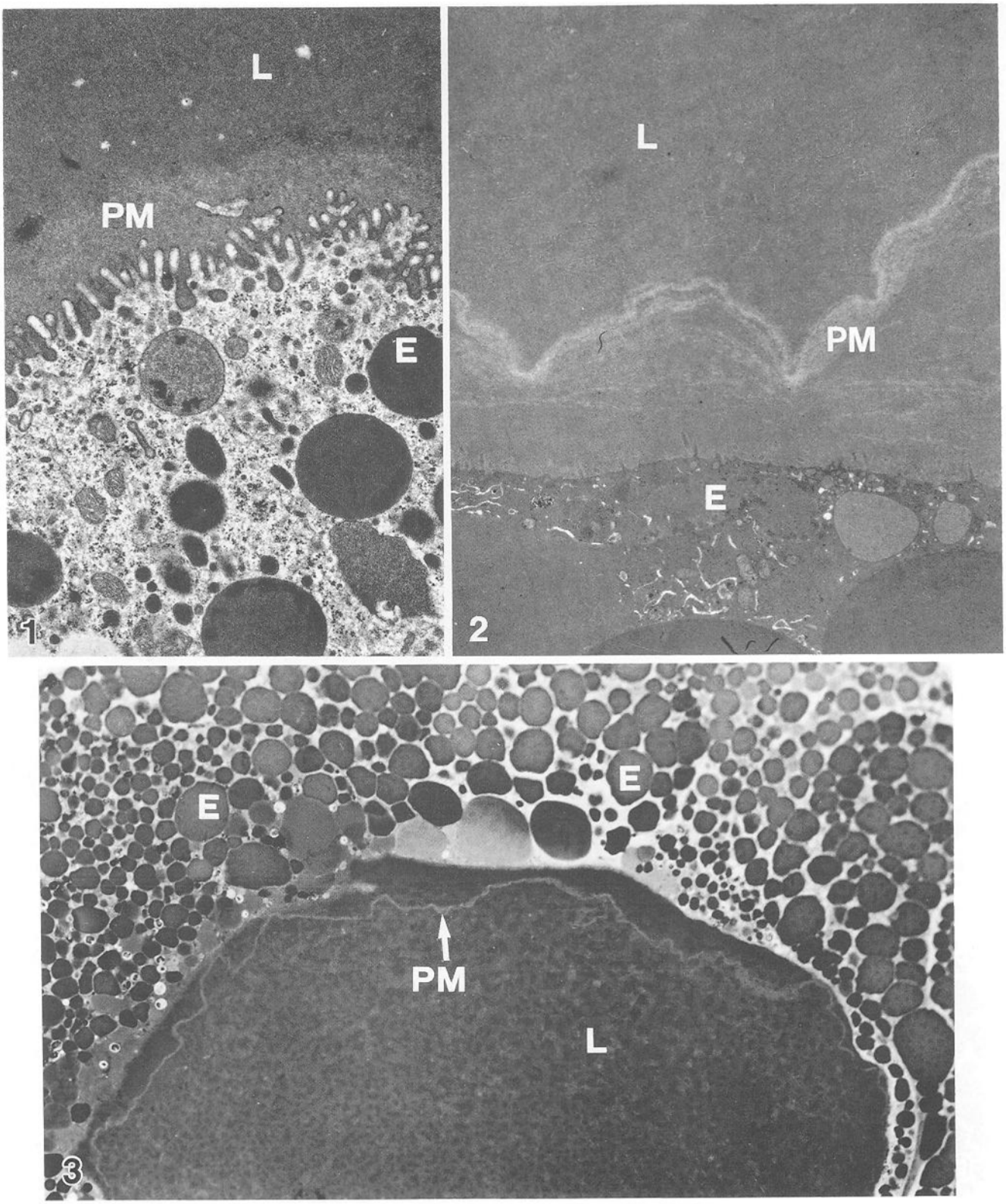
[a] Ticks appearing to be engorged but remaining attached when sampled

Discussion

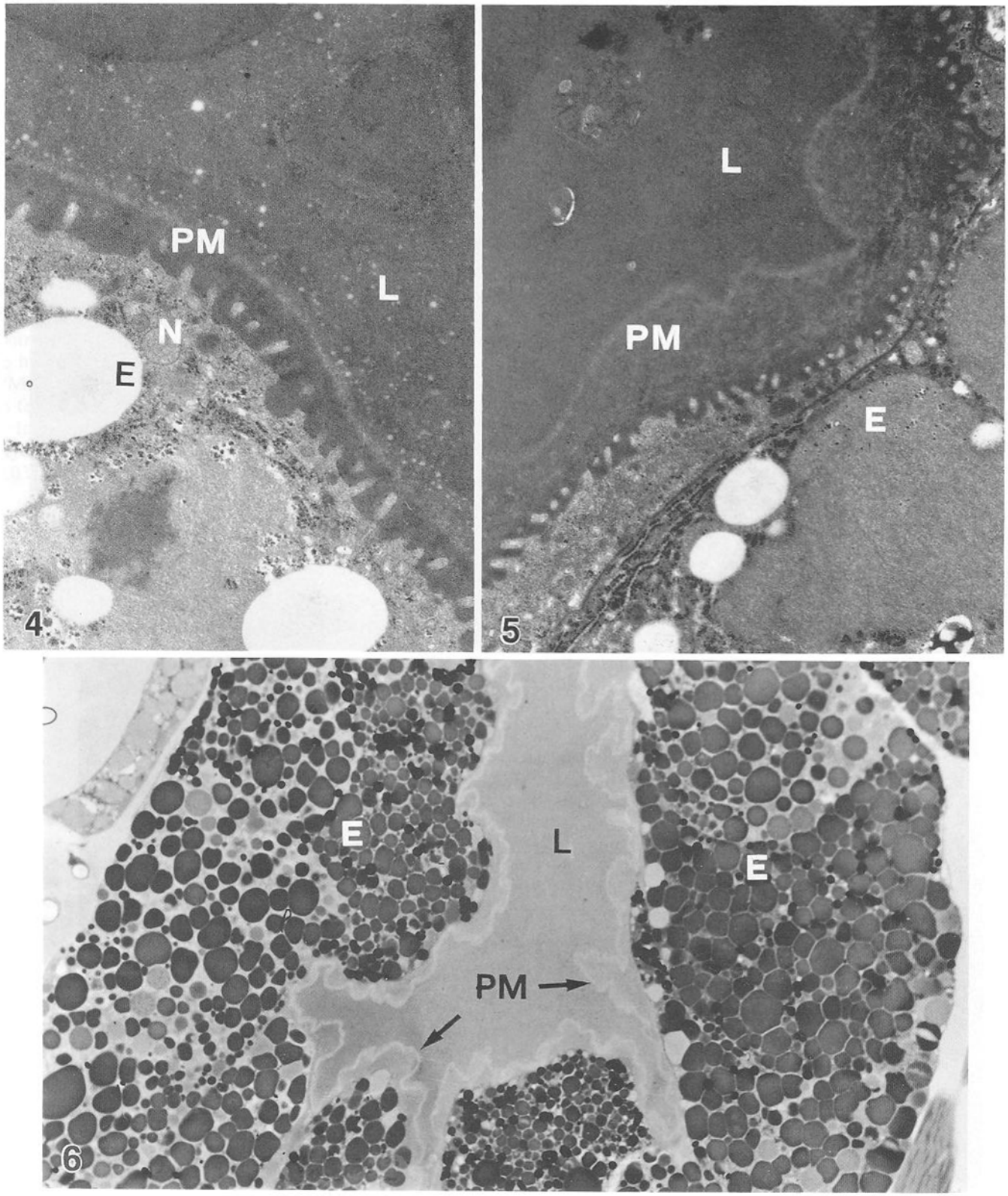
The PM of *Ixodes ricinus* feeding on rabbits resembles that of *I. dammini* feeding on hamsters (Rudzinska et al. 1982). However, the PM of all three stages of *Ixodes ricinus* appears at the same time (18 h after placement of the ticks on rabbits) and about 9 h earlier than that of *I. dammini* (Rudzinska et al. 1982). Whether these discrepancies result from the different tick species used and/or from the different experimental conditions applied remains to be determined.

Our observations show that at the onset of its occurrence, the PM seems to be a relatively broad zone without clear boundaries that becomes definite only at about 2–6 h after its appearance. We suggest that it is this broad zone that condenses into a real PM at 2–6 h after its occurrence, since it displays the same spongy appearance and the same electron density as the PM that occurs later.

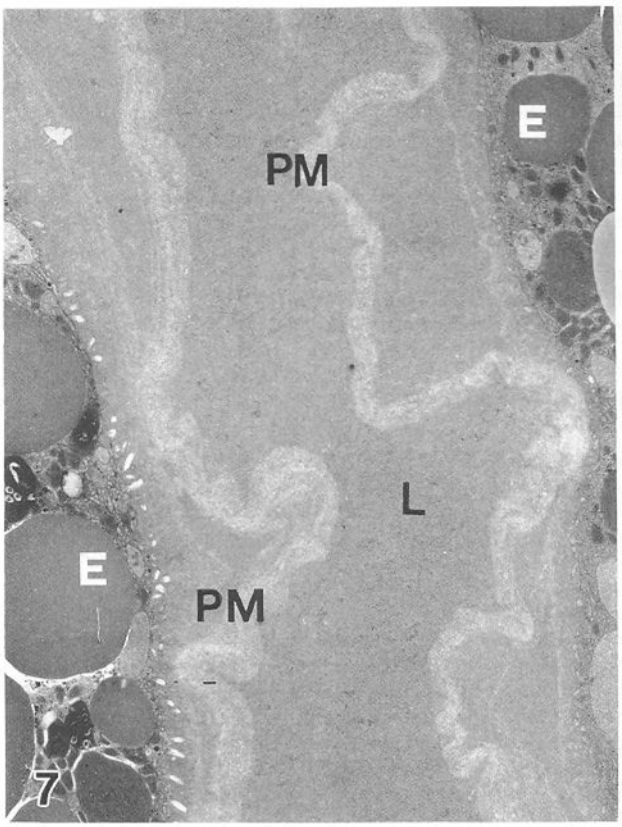
It has generally been considered that the PM is derived from the secretion of the midgut epithelial cells (Richards and Richards 1977). No PM was found at 16 h after tick placement, but it was evident and relatively broad at 18 h after placement. Therefore, the secretion of PM material must be very rapid beginning at the moment in which the midgut begins to form a PM. On the other hand, unlike that of most other blood-sucking arthropods, the midgut of feeding *Ixodes ricinus* females usually contains no erythrocytes. In the midgut of *Ixodes ricinus* larvae and, occasionally, of *Ixodes ricinus* nymphs, there are many erythrocytes and erythrocyte

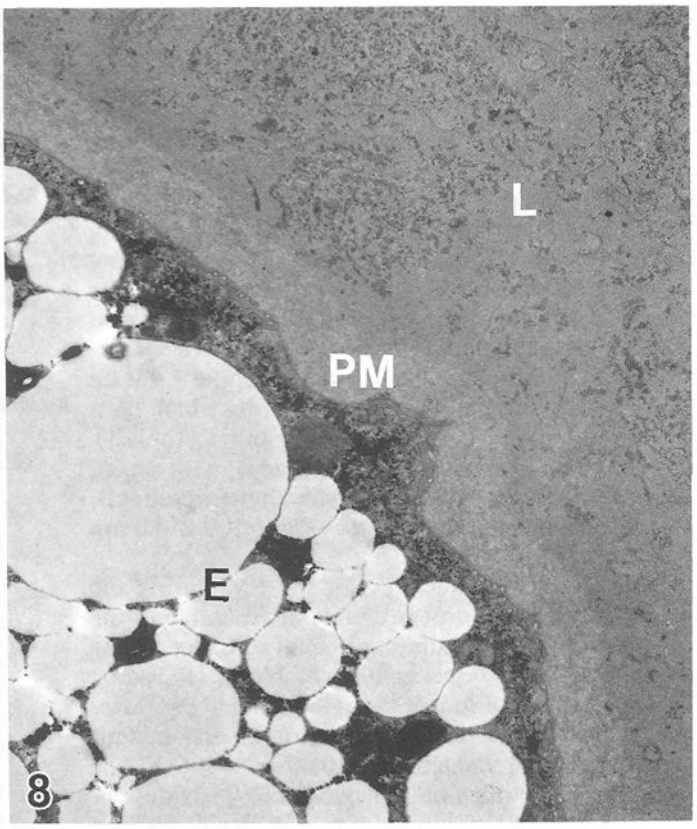


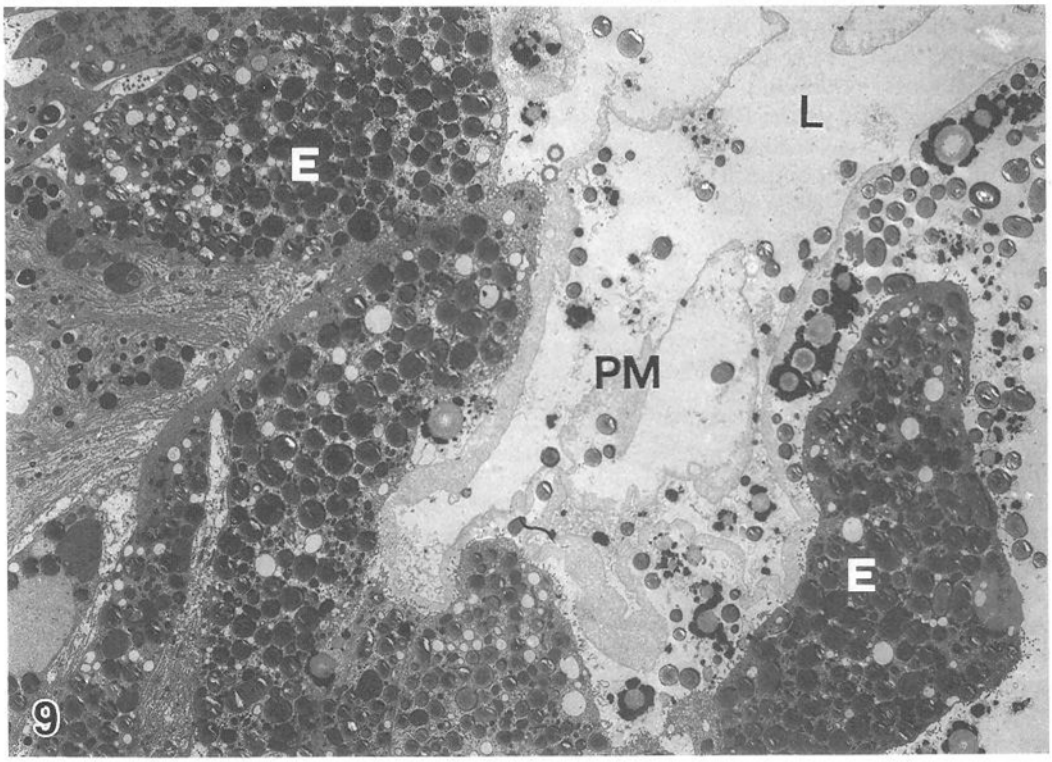
Figs. 1–3. Peritrophic membrane (*PM*) in the midgut lumen (*L*) of a larva and a nymph. *E*, Midgut epithelial cells. **Fig. 1.** Ultra-thin section of the midgut from a nymph at 18 h after placement on the rabbit. Note the thick PM without clear boundaries. Transmission electron micrograph, ×17100. **Figs. 2, 3.** Larva at 11 days (264 h) after repletion. **Fig. 2.** Ultra-thin section. Note the thick, winding and multilayered PM. Transmission electron micrograph, ×8,650. **Fig. 3.** Semi-thin section. Note the winding PM and the large space between the PM and the midgut epithelium. Optical micrograph, toluidine blue, ×750



Figs. 4–6. Peritrophic membrane (*PM*) in the midgut lumen (*L*) of nymphs. *E*, Midgut epithelial cells. **Fig. 4.** Midgut from a nymph at 4 days (96 h) after placement on the rabbit. When sampled, this nymph appeared to be fully engorged but remained attached. Transmission electron micrograph. ×25650. **Fig. 5.** Midgut from a nymph at 12 h after repletion. Note the PM with a winding inner margin and an indefinite outer margin. Transmission electron micrograph, ×17100. **Fig. 6.** Midgut of a nymph at 14 days (336 h) after repletion. Note the thick and winding PM and the large space between the PM and the midgut epithelium. Optical micrograph, toluidine blue, ×750







Figs. 7–9. Peritrophic membrane (*PM*) in the midgut lumen (*L*) of a nymph and females. *E*, Midgut epithelial cells. **Fig. 7.** Ultra-thin section of the midgut from a nymph at 14 days (336 h) after repletion. Note the thick, winding and multi-layered PM. Transmission electron micrograph, ×4300. **Fig. 8.** Ultra-thin section of an extremely enlarged digestive cell of the midgut epithelium from a female at 54 h after repletion. Transmission electron micrograph, ×8650. **Fig. 9.** Ultra-thin section of the midgut from an ovipositing female at 14 days (336 h) after repletion. Note that the PM has obviously been displaced and its electron density appears to be higher than that of the lumen contents. Also note the disintegrating digestive midgut epithelial cells and the hematin granules in the ectoperitrophic spaces and in the midgut lumen proper. Transmission electron micrograph, ×1900

"ghosts", but these are seen only during the last 24 h of gorging and for 1–2 days after repletion (Balashov 1968). The above phenomena result from the "non-blood" components of ingested food at the early stage of feeding activity as well as from the immediate hemolysis characterizing ixodids (Balashov 1968; Stevens 1968). Consequently, no erythrocytes are available to form a template for the developing PM (Perrone and Spielman 1988) in blood-feeding *Ixodes ricinus*. This factor and the rapid secretion of PM material might result in the formation of a relatively broad PM without well-defined boundaries at the onset of its appearance. The occurrence of a PM without a clear outer boundary during the first 12 h after repletion (Figs. 5), during which period the secretion of PM material is very rapid (Table 1), supports the above suggestion. Afterwards, with an increase in the pressure exerted by the lumen contents, the PM is condensed at a distance of about 0.2–0.8 μm from the epithelium.

The decrease in the thickness of the PM during the rapid feeding phase is most probably attributable to the rapid extension of the midgut epithelial cells due to the rapid ingestion of a large volume of blood (Balashov 1968), and the formation of a highly folded PM after repletion may be due to the reduction in the midgut lumen volume after detachment (Balashov 1968). The above phenomena may be considered to provide evidence for the elastic nature of the PM (Richards and Richards 1977). The occurrence of the multi-layered PM after detachment might implicate the intermittent secretion of PM material in *Ixodes ricinus* after repletion.

Our results reveal that the electron densiy of the PM is generally lower than that of the gut lumen contents. However, the electron density of the PM in an ovipositing female fixed at day 14 (336 h) after repletion was found to be higher than that of the gut lumen contents. This phenomenon may be related to the low concentration of hemoglobin in the gut lumen (Roesler 1934; Balashov 1968) of the ovipositing female.

Although we could not determine the time at which the PM disintegrates and disappears in *Ixodes ricinus*, we found that the PM in an ovipositing female examined at 14 days after repletion was partially displaced and broken. This phenomenon may result from the disintegration of digestive cells and the shrinkage of the gut lumen (Balashov 1968). The presence of hematin granules in the ectoperitrophic spaces and in the midgut lumen proper (Fig. 9) supports this hypothesis.

To date, our knowledge of the way in which the PM is secreted and of exactly what is secreted remains limited, despite several studies previously conducted on this subject (Moloo et al. 1970; Richards 1975; Becker et al. 1976; Perrone and Spielman 1988). As to the PM of ticks, the process of its secretion and its chemical composition remain to be determined.

As a vector of diverse diseases, *Ixodes ricinus* transmits numerous pathogenic agents to man and animals, including *Borrelia burgdorferi*, the spirochetal agent of Lyme borreliosis in Europe (Barbour et al. 1983; Burgdorfer et al. 1983; Gern et al. 1990; Aeschlimann and Gern 1990). The present findings on the PM of *Ixodes ricinus* may provide some clues to enhance our understanding of the behaviour of these pathogenic agents in their vector. We have recently investigated the interaction between *B. burgdorferi* and the PM of *Ixodes ricinus*. The results will be reported in another paper.

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