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Topography of the Tegument and Basement Membrane Complex of *Spirometra erinacei* Plerocercoid by Scanning Electron Microscopy

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Abstract

Three-dimensional architecture of the tegument and basement membrane complex of *Spirometra erinacei* plerocercoid was observed by scanning electron microscopy. Epithelium removal methods developed for the observation of mammalian tissue surface structures were successfully applied to examine the topography of the tegument and basement membrane complex of the cestode. Pinocytotic vesicle-like bodies were seen on the basal face of the external plasma membrane, on which crater-like pores were also observed. As the constituents of the basement membrane complex three layers were defined as follows: 1) the layer formed of conglomerated granules, 2) the thin amorphous layer, 3) the thick fibrous zone. It was suggested that each layer had a peculiar role for metabolic function as the transporting epithelium.

Key words: Basement membrane, scanning electron microscopy *Spirometra erinacei*, tegument, transporting epithelium

Intorduction

The function of the cestode tegument has aroused increasing attentions of parasitologist, because the cestode tegument is analogous to the vertebrate intestinal epithelium. It has been known that cestodes have an anucleated cytoplasmic tegument which play an important role in metabolic function (Rothman, 1963; Lee, 1966; Lumsden, 1966a, 1966b, 1975; Yamane, 1968; Smyth, 1969; Kwa, 1972; Hopkins *et al.*, 1978; Oaks and Muller, 1981; Threadgold and Hopkins, 1981). Previous studies of the cestode tegument through transmission electron microscope (TEM) have not necessarily referred to the tegumental function in conjunction with the basement membrane or the subtegumental layer. So far, Yamane *et al.* (1982) and Lindroos (1984) have attempted to study functions of the tegument systematically.

Recently some new preparation techniques of specimens have been applied effectively to three-dimensional observations of various tissue surfaces by scanning electron microscope (SEM). For example, Naguro *et al.* (1983) showed the basal lamina of intestinal villi of the rat by mechanical removal method after fixation with glutarardehyde-osmic acid. Komuro (1985) also demonstrated the same image by the use of osmic acid maceration, and Kawabe *et al.* (1985) observed the basement membrane topography in human thick skin with PBS-EDTA incubation method.

In the present study, three-dimensional structure of the tegument and basement membrane complex of *Spirometra erinacei* plerocercoids was examined by these methods.

Materials and Methods

Plerocercoids of *Spirometra erinacei* were

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collected from a snake, *Elaphe quadrivirgate,* and were kept living in the mice infected. Plerocercoids removed from the mice subcutaneous tissues were rinsed with 1/15M PBS-buffer, pH7.4 before preparation. Tegument were separated into the components by three following methods; 1) Mechanical removal after osmic acid fixation, 2) PBS-EDTA incubation, 3) Osmic acid maceration.

Mechanical removal method: Plerocercoids were cut into small pieces. A light incision was made with a razer on the surface of each piece, which was then fixed for 2 hrs at 4℃ in 1% OsO_4 in 1/15 M phosphate buffer, pH7.4. After rinse with a buffer solution, the distal cytoplasma of specimens were cautiously separated from the fibrous zone with a fine forceps under the dissecting microscope. These specimens were fixed again for 1 hr at 4℃ in 1% OsO_4 after a brief rinse with the same buffer. The specimens were then treated with 2% tannic acid solution over night. They were immersed again in 1% OsO_4 for 1 hr at 20℃ after rinse with buffer solution (several changes for 15 min each). They were rinsed again, and then dehydrated in a graded series of ethanol, soaked in amylacetate, transfered to critical point drying, coated with gold-palladium alloy, and examined through a Hitachi S-450 scanning electron microscope.

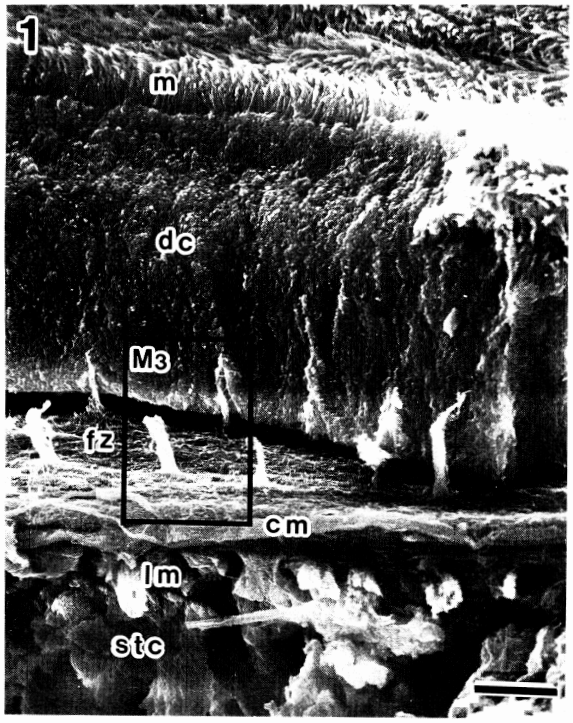
PBS-EDTA incubation method: Incubating the specimens in PBS-EDTA for 50 min at 37℃ (Scaletta and MacCallum, 1972), the macerated epithelium were gently separated from the underlying basal lamina with a fine forceps under the dissecting microscope. Samples for SEM observation were prepared through the same process as mentioned above.

Osmic acid maceration method: After fixation for 2 hrs in 3% glutarardehyde with cacodylate buffer, pH7.2, specimens were immersed for 3 days in 2% OsO_4. Thereafter, they were rinsed briefly with the same buffer. Before processing further, the specimens were agitated vigorously to facilitate removal of the external plasma membrane. The specimens were then rinsed with distilled water, and were prepared for SEM through the prescribed process.

Result

Through the removal of the distal cytoplasma by the mechanical removal method, the surface of fibrous zone was exposed, and three-dimensional architecture of the tegument and subtegumental cell layer was revealed simultaneously (Fig. 1). The fibrous zone showed a reticular structure composed of the fibers. Each fiber was about 30nm in diameter. The surface of the zone exhibited porous, sieve-like structure (Fig. 2). Many fibrous protrusions were observed on the surface of the zone and they contained granules in their canals (Fig. 3).

The basal face of the external plasma membrane turned over through osmic acid maceration method was shown in Fig. 4. Several pinocytotic vesicle-like bodies and crater-like pores, through which secretion granules might be discharged, were seen on the basal face. On removal of the external plasma membrane through osmic acid maceration, microtriches connecting with the external plasma membrane were clearly shown (Fig. 4). Conglomerate granules were in layers on the apical surface of the syncytium right under the external plasma membrane. Sink holes arranging with regular intervals were also observed on the apical surface of the syncytium, and the capsules of secretion granules covered with a membrane peeped in the center of the sink holes (Figs. 5, 6). The granular layer was mostly composed of discoid bodies holding their major axes in the vertical position to the apical surface (Fig. 7). An aggregation of vesicles, secretion granules, and discoid bodies were shown in the lower syncytium layer (Fig. 8). When the distal cytoplasma was separated from the underlying basal membrane by EDTA-incubation method, the same surface of the fibrous zone as observed by the mechanical removal method were exposed. The basal lamina, probably lamina densa, was also revealed by the method (Fig. 9). Some holes were observed on the basal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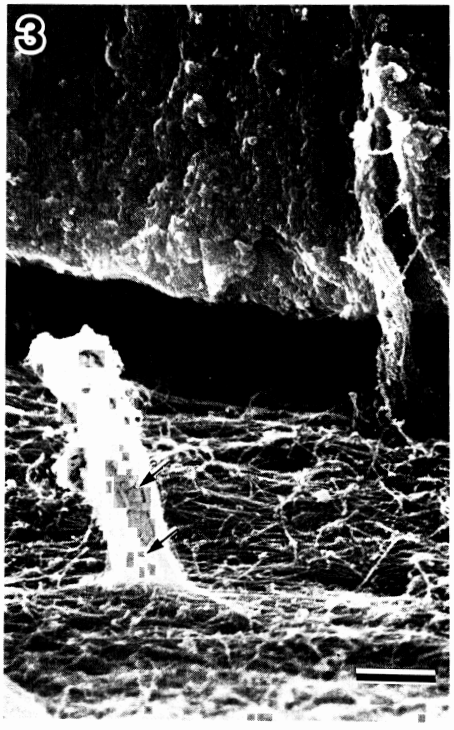
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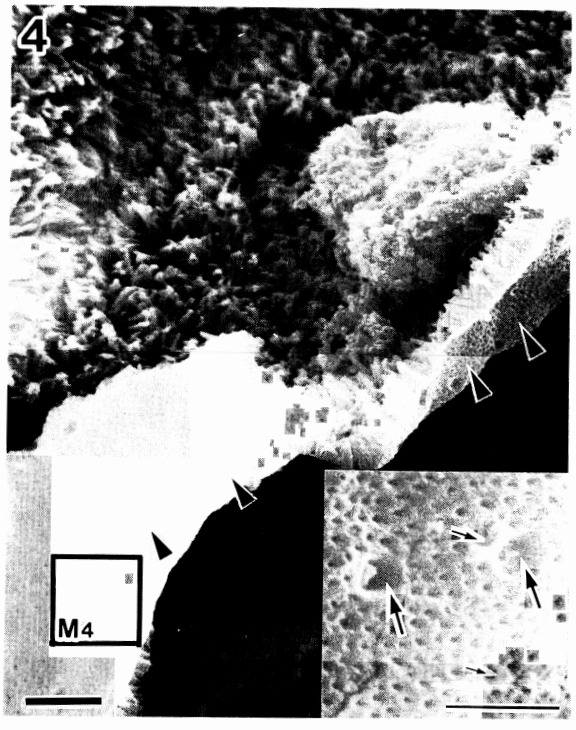
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M4

face of the internal plasma membrane (Fig. 10). It is suggested that they should be pores communicating with basal infold or with the cytoplasmic bridges. Many fenestrations of various sizes were also found on the surface of the basal lamina (Fig. 11).

Discussion

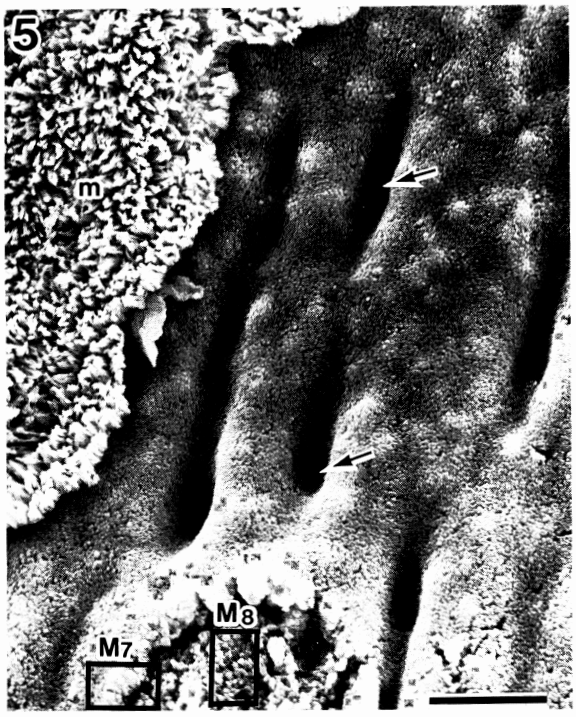
Basement membranes are found in all organs at the interface between highly specialized cell (epithelial, endothelial and certain mesenchymal cells, such as smooth muscle cells) and connective tissue stroma (Furthmayr, 1986). The so-called basement membrane is also found at the interface between the tegument and subtegumental cell layer in cestodes. Yamane *et al.* (1982) reported that the basement membrane complex was composed of two structural elements: a thin internal plasma membrane and a basal lamina consisting of finely-filamentous fibers. The basement membrane is defined, on the basis of electron microscopy, as thin, amorphous, sheet-like structure separated into several distinct layers, including the lamina lucida (close to the cell), lamina densa, and a reticular layer (close to the connective tissue stroma) (Furthmayr, 1986). The basal lamina (basement membrane) was also observed by SEM (Naguro, *et al.*, 1983; Komuro, 1985) as the thin amorphous sheet-like structure under the epithelial cells of the rat intestinal villi. As the result of the present study, three layers were clearly defined between the internal plasma membrane and the muscle layer as follows: a layer of conglomerated granules, which may be consistent with electron lucid layer (lamina lucida), a layer of thin amorphous sheet like-structure, which may be consistent with electron dense layer (lamina densa), and a fibrous zone which was considered as a connective tissue stroma. The so-called reticular layer could not be recognized in our observation. Yamane *et al.* (1982) reported that the basal lamina was a layer consisting of finely-filamentous fibers, whereas the layer was observed as the fibrous zone in our study. TEM and SEM observations have recently come to be used effectively for studying on functional morphology of the tegument in cestodes (Arme and Threadgold, 1976; Belton, 1977; Yamane *et al.*, 1978, 1982; Conder *et al.*, 1983). These studies were generally concerned with ultrastructure in regard to metabolic function of the cestode tegument. Threadgold and Brennen (1978) gave an attention to the mode of operation of the tegument as a transporting epithelium in the trematode, *Fasciola hepatica*. They ascertained that Na^+K^+-ATPase was associated with the membrane of the basal infolds and the apical and basal plasma membranes. Basal infolds were also found in diphyllobothriid cestodes (Bråten, 1968). This function associated with the activity of Na^+K^+-ATPase in platyhelminthes is one of the mechanism of absorbtion through the transporting epithelium, which has been known already in mamalian intestinal epithelium (Sculz and Zalusky, 1964). Lindroos (1984) demonstrated that the subtegument of the tape worm play a role to control an efficient distribution of nutrients, using the method of lanthanum nitrate infiltration. In the present

Fig. 1. Three-dimensional architecture of the tegument.
Cylindric protrusions are seen on the surface of the fibrous zone. Bar: 0.5 um, cm: circular muscle, dc: distal cytoplasma, fz: fibrous zone, lm: longitudinal muscle, m: microtriches, stc: subtegumental cell layer

Fig. 2. Surface of the fibrous zone.
Pores arranging with regular intervals are seen on the surface (arrows). Bar: 1 um

Fig. 3. Higher magnification of the part M3 in Fig. 1.
Fibrous protrusions contain granules (arrow). Bar: 1 um

Fig. 4. Basal face of the external plasma membrane (arrowheads).
(Inset) Higher magnification of the part M4.
Pinocytotic vesicle-like bodies (small arrows) and crater-like pores (large arrows) are seen. Bar: 1 um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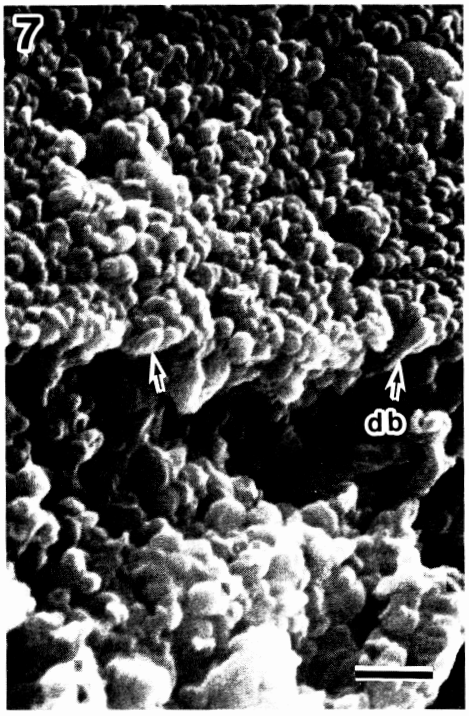
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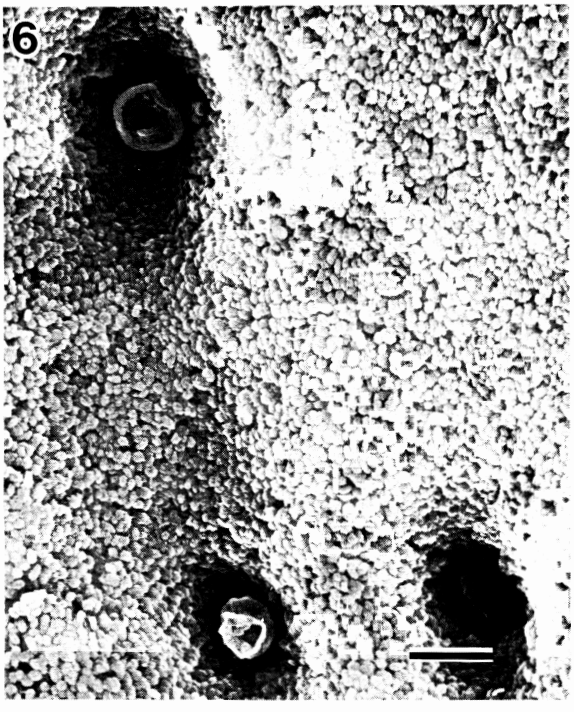
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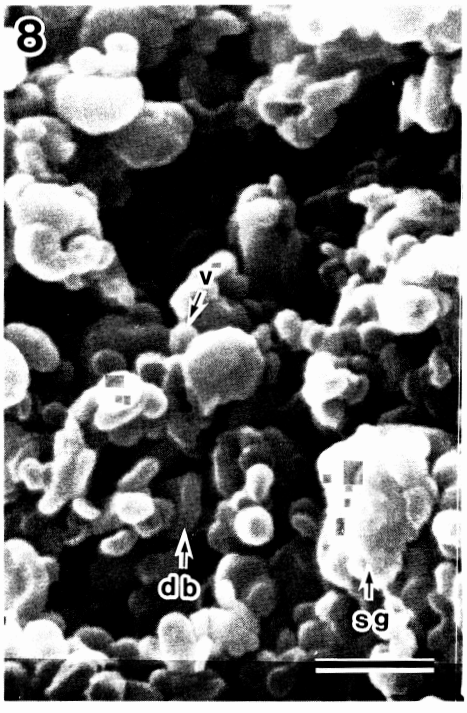
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study three-dimensional architecture of the tegument and basement membrane complex was observed by the method of removing epithelium. The basal face of the external plasma membrane with hollow microtriches was clearly shown. The image is similar to the basal face of the cytoplasmic membrane with microvilli in the rat intestine (Inoue *et al.*, 1984; Osatake *et al.*, 1985). Holy and Oaks (1986) observed the filamental structures in a microthrix core by TEM. Such structures were not observed in our samples by the process of osmic acid maceration. Conder *et al.* (1983) studied the P face of the proximal tegument membrane of *Taenia taeniaeformis* by the freeze-etching technique. The structure described as "pore like holes" by Conder (1983) seems to be a cut face of the filaments in the microthrix core. A more improved device should be required to observe the microthrix filament by SEM. Conglomeration of discoid bodies on the apical surface right under the external plasma membrane may be an artifact due to the long process of osmic acid maceration. Numerous projections of secretion mass in the center of the sink holes under the external plasma membrane may be formed by invaginations of the basal plasma membrane when the secretion was discharged into the tegument. A shematic diagrame of tegumental architecture was proposed as Fig. 12. It is presumed that each layer plays a peculiar role in metabolic function as the transporting epithelium.

Newly developed techniques in SEM will contribute effectively towards the elucidation of the mechanism of the metabolic and protective function in cestodes.

Acknowledgements

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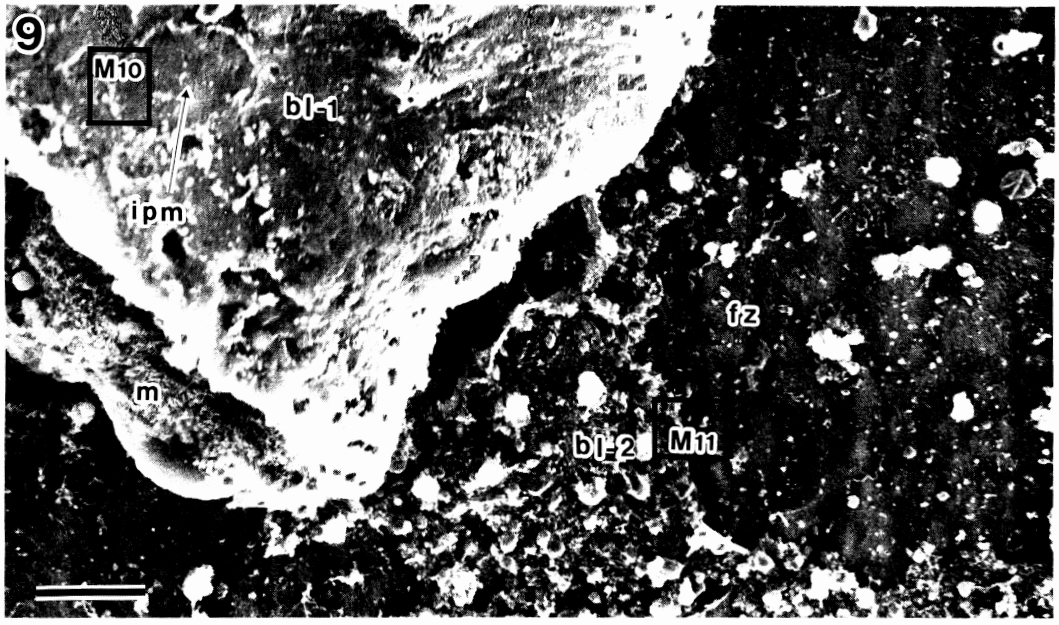
Fig. 5. Surface of the tegument right under the external plasma membrane.
Sink holes (arrows) are seen on the surface. Bar: 0.5 um

Fig. 6. Higher magnification of the surface of the tegument stript off the external plasma membrane.
Secretion granules covered with membrane peep in the center of the sink holes. Bar: 1 um

Fig. 7. Higher magnification of the part M7 in Fig. 5.
Distal part of the tegument right under the external plasma membrane is almost occupied with discoid bodies. Bar: 0.5 um

Fig. 8. Higher magnification of the part M8 in Fig. 5.
Granules of various size and shape are seen under the conglomeratic granular layer. Bar: 0.5 um, db: discoid body, sg: secretion granule, v: vesicle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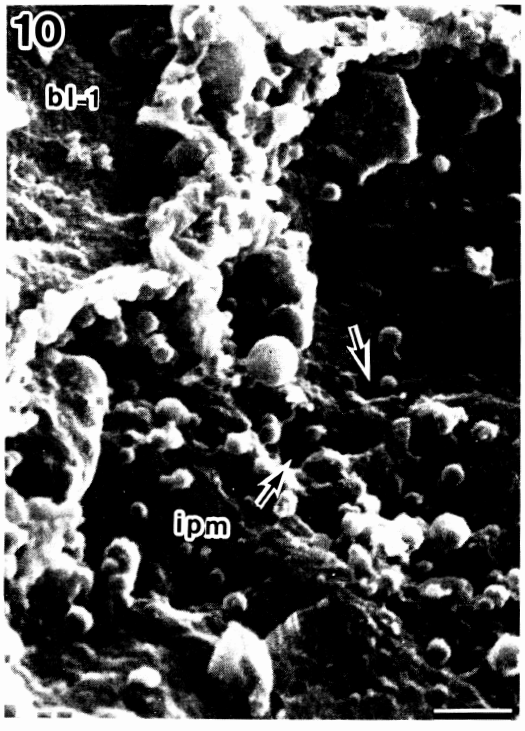
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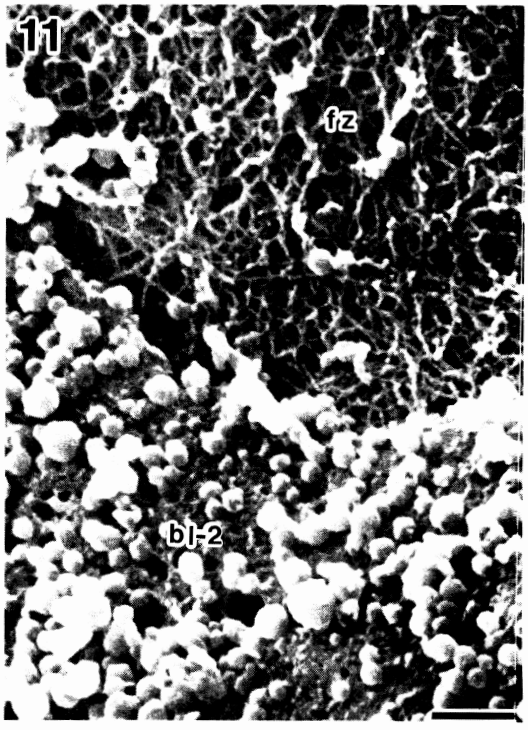
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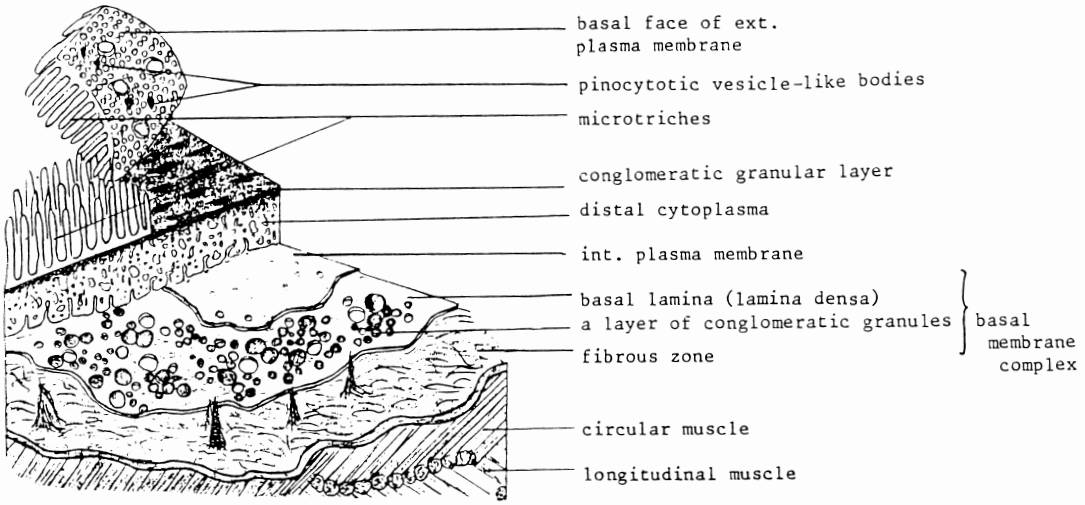

Fig. 12. Schematic diagram of tegumental architecture.

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Fig. 9. Basal face of the tegument with a part of the basal lamina.
Fibrous zone and outside of the basal lamina are seen. Bar: 10 um, b1-1: inside of the basal lamina, ipm: internal plasma membrane, b1-2: outside of the basal lamina.

Fig. 10. Higher magnification of the part M10 in Fig. 9.
Inside of the basal lamina and of the internal plasma membrane are seen. Some holes are seen on the basal face of the internal plasma membrane (arrows). Bar: 0.5 um

Fig. 11. Higher magnification of the M11 in Fig. 9.
Outside of the basal lamina and the surface of the fibrous zone are seen. Granules of various size are conglomerated on the outside of the basal lamina. Bar: 0.5 um.

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