[Jap. J. Parasit., Vol. 31, No. 6, 487-497, Dec., 1982]

An Electron Microscopic Study of Subtegumental Cells and Associated Structures of *Spirometra erinacei*

YOSUKE YAMANE*, AKIO NAKAGAWA*, YUMIKO MAKINO*
AND KAZUMITSU HIRAI†

(Received for publication; August 11, 1982)

Key words: cestode, basal membrane, *Spirometra erinacei*, SEM, subtegumental cell, tegumentary bodies

Introduction

It has already been revealed that the tegument of cestodes is an anucleated cytoplasmic integument which plays a metabolically important role (Lee, 1966, 1972; Smyth, 1969; Lumsden, 1975). The apical surface of the tegument has microtriches which serve the uptake of nutrients and many studies proved the absorptive mechanism of the microtricheal layer (Wright and Lumsden, 1968; Morris and Finnegan, 1969; Reissig, 1970; Threadgold and Read, 1970; Threadgold and Arme, 1974; Conway-Jones and Rothman, 1978; Oaks and Mueller, 1981; Pappas, 1981; Schroeder, Pappas and Means, 1981).

The significance in function of the basal membrane complex and subtegumental cells remains at present to be proved. Many investigators reported on the tubular infoldings formed by basal plasma membrane (Threadgold, 1965; Lumsden, 1966; Beguin, 1966; Bråten, 1968). Jha and Smyth (1969) speculated that active mitochondrial biogenesis took place in the basal membrane.

Meanwhile, ultramicroscopic studies have been carried out on the morphology and function of various types of tegumentary bodies which were produced in subtegumental cells (Oaks and Lumsden, 1971; Lumsden *et al.*, 1974). Threadgold and Hopkins (1981) described that the three types of tegumentary bodies might represent a constant flux outward of membrane constituents such as glycocalyx. Oaks and Mueller (1981) proved the distribution of carbohydrate among these various tegumentary bodies. However, Conway-Jones and Rothman (1978) suggested that the disc-like tegumentary bodies were not secretory bodies but stacks of discs which play a role in the transport of the material absorbed.

* *Department of Environmental Medicine, Shimane Medical University, Izumo 693, Japan*
† *Department of Parasitology, School of Medicine, Ehime University, Ehime 791-02, Japan*

Materials and Methods

The purpose of this study was to discuss the ultrastructure of the basal membrane complex, tegumentary bodies and subtegumental cells observed by transmission and scanning electron microscopy.

Plerocercoids of *Spirometra erinacei* were obtained from subcutaneous tissue of the snake, *Elaphe quadrivirgata*, and were washed carefully in the physiological saline. Five plerocercoids were cut into small pieces before being fixed for 2 hr in chilled 5% glutaraldehyde (0.2 M phosphate buffer pH 7.2). The specimens were then post-osmi-

cated in 1% OsO_4 for 2 hr at 4 C, dehydrated through ascending alcohol series, and embedded in Epon 812. Ultrathin sections of plerocercoids were cut on a LKB ultramicrotome II-B. Thin sections were stained with uranyl acetate and lead citrate, and viewed in a Hitachi HU-2 transmission electron microscope (TEM).

Results

The styren-cracking technique was used to observed cross fractures of the tegument (Tanaka *et al.*, 1974). Some pieces of plerocercoids were embedded in styren, cracked under a stereoscope after the same procedure as mentioned above. After the styren of cracked specimens was dissolved by being soaked in propylene oxide for 4 hr, the specimens were dried according to the critical point dry method, coated with Pt-Pd alloy and observed with the Hitachi MSM-V or S-450 scanning electron microscope (SEM).

Microtriches, 1–2 μm in length, grew densely on the tegumental surface, forming a brush border. Each microthrix was composed of a tubular, proximal part and a spine-like, distal part. The cross fracture of the distal cytoplasmic layer of the tegument exhibited a porous sponge-like structure consisting of homogenous cytoplasmic matrix, numerous vacuoles (pinosomes) and cytoplasmic membranous structures (Fig. 1, arrowhead).

Transmission microscopic observations revealed numerous electron-lucid vesicles (pinosome) and electron-dense disc bodies in the distal cytoplasmic layer of the tegument. The elongated disc bodies which showed fine striated structure were also distributed in the proximal part of microtriches (Fig. 2). The internal plasma membrane of the basal membrane complex arose in places to form a meandering of membranous structures (Fig. 3, small arrowheads). Several phagosome-like vacuoles (Fig. 3, arrowheads) were observed on the surface of the internal plasma membrane of the basal membrane complex. Some of the vacuoles were observed beneath the basal membrane complex. These vacuoles were surrounded by a limiting membrane which included fine granular matrix distributed diffusely in the distal cytoplasmic layer (Fig. 3).

The cytoplasmic bridges connected the distal cytoplasmic layer of the tegument and the subtegumental cells which were located beneath the muscle layer, and were recognizable by the presence of various typed bodies. Subtegumental cells also extended prominent cytoplasmic processes (Fig. 4, arrowhead).

The cross fracture of the tegument obtained by the styren-cracking method showed a fibrous complex net work connected with the basal membrane complex (Fig. 5, arrowheads). The basal membrane complex was composed of two structural elements: a thin internal plasma membrane (Fig. 6, arrowheads) and a basal lamina which consisted of finely filamentous fibres (Fig. 6, small arrowheads). Conglomerations of glycogen particles were observed on the surface of the basal membrane complex (Fig. 6). A part of the internal plasma membrane was thrown into the distal cytoplasm (Fig. 6, arrowheads), and also into the underlying basal lamina (Fig. 7, arrow). Protoplasmic extensions (Fig. 7, arrowhead) penetrated the basal membrane complex, extending like canals running between muscle layers into the perinuclear cytoplasm (Fig. 7, arrowhead).

The surface of the basal membrane complex showed small pores, globules and a network of the fibrous basal membrane (Fig. 8). Numerous concave, discoid bodies (Fig. 9, arrowheads) were observed in the pores which were supposed to be the openings of cytoplasmic bridges of subtegumental cells (Fig. 9). Discoid bodies (Fig. 10, arrowheads) conglomerated especially in pores of the basal membrane complex, and showed concave contours similar to the red blood cells of vertebrates.

Subtegumental cells were characterized with large nuclei and developed cytoplasmic processes in which various types of tegumentary bodies were packed. Numerous tegumentary bodies, mitochondria and the Golgi's apparatus were densely distributed in the cytoplasm of subtegumental cells. Various types of tegumentary bodies were classified as follows; "relatively electron-dense, ovoid mottled bodies", "electron-dense, ovoid, not-mottled bodies," "electron-dense, rod- or discoid-shaped bodies", and "electron-lucid, ovoid bodies" which had limiting membranes. Contrary to the dominant distribution of electron-lucid vesicles and electron-dense disc bodies in the distal cytoplasmic layer of the tegument, the electron-dense, ovoid bodies and electron-lucid, ovoid bodies were dominant in the cytoplasm of subtegumental cells. These bodies, especially electron-dense, ovoid mottled bodies and electron-lucid, ovoid bodies were densely observed around the Golgi's apparatus (Fig. 11, arrowheads).

A cracking fracture of subtegumental cells also revealed that various-sized globules were densely packed around a large nucleus in the cytoplasm. Subtegumental cells had cytoplasmic processes which were connected with the tegumental layer (Figs. 12, 13).

Discussion

Different types of bodies in the tegument have been described by many researchers as vacuoles, vesicles, membrane bounded bodies, inclusions, tegumentary bodies, lamellated bodies, rhabdiform organelles, disc-like bodies and rod-shaped bodies. Hayunga and Mackiewicz (1975) classified them into two types of vesicles. One was "rod-shaped bodies." These appeared to be homologous with the "rhabdiform organelles" (Beguin, 1966), "disc-like bodies" (Bråten, 1968), "rods" (Rothman, 1963; Morris and Finnegan, 1969), "type I granules" (Lumsden *et al.* 1974) and with "type I bodies" (Threadgold and Hopkins, 1981). These rod-shaped bodies were suggested as secretory vesicles by Morris and Finnegan (1969). Oaks and Lumsden (1971) described such vesicles might be implicated in the renewal of glycocalyx.

Another group was "lamellated bodies" which were distinctly striated and found in close association with the Golgi's apparatus. This type seems to be correspondent to "type II granules" (Lumsden *et al.*, 1974) and "an unusual laminar body" (Reissig, 1970) and "ovoid granule" (Specian and Lumsden, 1980). Lumsden *et al.* (1974) suggested that the lamellated bodies were also implicated in supplying raw materials for microthrix synthesis. Reissig (1970) supposed that these lamellar bodies played a role in the transport process, and described an interesting resemblance between the structures of these lamellar bodies and the laminar bodies in neurons.

Recently Conway-Jones and Rothman (1978) gave a description of stacks of tegumentary discs. These discs showed a discoid figure with a dense inner core surrounded by lucid boundary, and appeared similar in substance and texture to the distal microtriches. Concave disc bodies were often found as conglomerate discs in pores of the basal membrane complex in the present study. Each disc revealed a concave form which appeared very similar to the red blood cell of vertebrates. The meandering appearance of the stacks of these discs which were described by Conway-Jones and Rothman (1978) could not be defined.

In the present study the tegumental bodies showed different five types. Electron-lucid vesicles in the distal cytoplasmic layer of the tegument are supposed to be pinocytotic vesicles or pinosomes. These vesicles were distributed abundantly in the distal cytoplasmic layer of the tegument, but the majority disappeared in the cytoplasm of subtegumental cells. Blitz and Smyth (1973) described that the fluffy vesicles in the tegument could be pinocytotic vesicles, for they were clearly seen fusing with "giant" vesicles in the peri-

nuclear cytoplasm. Other types of bodies are supposed to be secretory bodies, and are probably identical with the concave disc bodies which were confirmed stereoscopically in SEM micrographs (Fig. 10), for the concave disc bodies exhibited round, discoid contours on the whole and rod-shaped contours in a side view in TEM micrographs (Fig. 2).

Functions of these bodies are unknown. Some investigators speculated that these bodies serve the purpose of maintenance of the tegumental surface (Bråten, 1968; Grammeltvedt, 1973; Threadgold and Hopkins, 1981). Trimble III and Lumsden (1975) determined autoradiographically the cytochemical localization of the material containing carbohydrate within tegumental vesicles. Conway-Jones and Rothman (1978) described that the discs appeared similar in substance and texture to the distal microtriches. Oaks and Lumsden (1971) and Lumsden *et al.* (1974) also suggested that tegumentary granules proceeded to become a portion of the distal microtriches.

The distribution and the presence of morphologically different types of the tegumentary bodies in the present study might represent either the discontinuous, sequential synthesis or continued differentiation of various types of bodies. These secretory bodies may be responsible for the maintenance and addition of new, free-surface plasmalemma, or glycocalyx on the superficial cytoplasmic layer of microtriches, or transport of some kinds of enzymes which might serve the purpose of membrane digestion on the superficial cytoplasmic layer or of the protective enzymatic activity to the host-parasite response.

Functions of the subtegumental cells have been discussed in connection with the tegumentary bodies. Morris and Finnegan (1969) described that subtegumental cells were engaged in production of granular inclusions, rod-shaped bodies for subsequent export to the distal cytoplasmic layer of the tegument. Bråten (1968), and Oaks and Lumsden (1971) indicated that the Golgi's apparatus of subtegumental cells was the major source of carbohydrate incorporation into glycoproteins or mucopolysaccharides. A close relationship between the subtegumental cells and the tegumentary bodies was also ascertained in the present study.

The basal membrane complex was composed of an internal plasma membrane and a fibrous basal lamina. The internal plasma membrane covered the fibrous basal lamina, having many projections towards the distal cytoplasmic layer of the tegument (Threadgold, 1965; Reissig, 1970; Yamane, 1968). Morris and Finnegan (1969), and Threadgold and Read (1970) described the multitubular complex which was a unique specialization of the basal plasma membrane. On the basis of its morphological similarity to the invaginations of the basal plasma membrane in kidneys or salt glands of marine birds, they suggested that it might be involved in water or ion transport.

In the present study fibrous infoldings arose showing a meandering of the unit membrane and a close connection with the phagosome-like tegumentary vacuoles. From the observations that the pinosome and phagosome-like bodies were often attached to the tips of the invaginated basal plasma membrane, it can be speculated that the basal membrane complex could serve itself for the active uptake of materials and the transportation from the distal cytoplasmic layer into the perinuclear cytoplasm.

Summary

Using a scanning and a transmission electron microscope, various types of tegumentary bodies, the basal membrane complex and subtegumental cells of the plerocercoid of *Spirometra erinacei* were observed. The tegumentary bodies showed various different types, but were supposed to be pinosomes and secretory vesicles. The differences of electron density, form and distribution of these bodies may be attributable to the degree of maturi-

ty or differentiation of the bodies themselves. The subtegumental cells which produced tegumentary bodies and had the characteristic cytoplasmic processes were stereoscopically observed by the scanning electron microscopy (SEM).

References

1) Beguin, F. (1966): Étude an microscope électronique de la cuticle et des ses structures associées chez quelques cestodes. Essai d'histologie comparée. Z. Zellforsch., 72, 30–46.
2) Blitz, N. M. and Smyth, J. D. (1973): Tegumental ultrastructure of *Raillietina cesticilus* during the larval-adult transformation, with emphasis on the rostellum. Internat. J. Parasitol., 3, 561–570.
3) Bråten, G. (1968): The fine structure of the tegument of *Diphyllobothrium latum* (L.): A comparison of the plerocercoid and adult stages. Z. Parasitenkd., 30, 104–112.
4) Conway-Jones, P. B. and Rothman, A. H. (1978): *Hymenolepis macrostoma*: Tegumentary discs. Exp. Parasit., 44, 108–115.
5) Grammeltvedt, A. F. (1973): Differentiation of the tegument and associated structures in *Diphyllobothrium dendriticum* Nitsch (1824) (Cestoda: Pseudophyllidea). An electron microscopical study. Internat. J. Parasitol., 3, 321–327.
6) Hayunga, E. G. and Mackiewicz, J. S. (1975): An electron microscope study of the tegument of *Hunterella nodulosa* Mackiewicz and McCrae, 1962 (Cestoidea: Caryophyllidea). Internat. J. Parasitol., 5, 309–319.
7) Jha, R. K. and Smyth, J. D. (1969): *Echinococcus granulosus*: Ultrastructure of microtriches. Exp. Parasit., 25, 232–244.
8) Lee, D. L. (1966): The structure and composition of the helminth cuticle. In Advances in Parasitology, Vol. 4, ed. by Ben Dawes, Academic Press, London and New York, 187–254.
9) Lee, D. L. (1972): The structure of the helminth cuticle. In advances in Parasitology, Vol. 10, ed. by Ben Dawes, Academic Press, London and New York, 347–379.
10) Lumsden, R. D. (1966): Cytological studies on the absorptive surfaces of cestodes. II. The synthesis and intracellular transport of proteins in the strobilar integument of *Hymenolepis diminuta*. Z. Parasitenkd., 28, 1–13.
11) Lumsden, R. D. (1975): Surface Ultrastructure and cytochemistry of parasitic helminths. Exp. Parasit., 37, 267–339.
12) Lumsden, R. D., Oaks, J. A. and Mueller, J. F. (1974): Brush border development in the tegument of the tapeworm, *Spirometra mansonoides*. J. Parasitol., 60, 209–226.
13) Morris, G. P. and Finnegan, C. V. (1969): Studies on the differentiating plerocercoid cuticle of *Schistocephalus solidus*. II. The ultrastructural examination of cuticle development. Can. J. Zool., 47, 957–964.
14) Oaks, J. A. and Lumsden, R. D. (1971): Cytological studies on the absorptive surfaces of cestodes. V. Incorporation of carbohydrate containing macromolecules into tegument membranes. J. Parasitol., 57, 1256–1268.
15) Oaks, J. A. and Mueller, J. R. (1981): Location of carbohydrate in the tegument of the procercoid of *Spirometra mansonoides*. J. Parasitol., 67, 325–331.
16) Pappas, P. W. (1981): *Hymenolepis diminuta*: Partial characterization of membrane-bound nucleotidase activities (ATPase and 5-Nucleotidase) in the isolated brush border membrane. Exp. Parasit., 51, 209–219.
17) Reissig, M. (1970): An unusual laminar structure in the integument of *Hymenolepis diminuta*. J. Ultrast. Res., 31, 109–115.
18) Rothman, A. H. (1963): Electron microscope studies of tapeworms: The surface structures of *Hymenolepis diminuta* (Rudolphi, 1819), Blanchard, 1891. Trans. Amer. Micros. Soc., 82, 22–30.
19) Schroeder, L. L., Pappas, P. W. and Means, G. E. (1981): Trypsin inactivation by intact *Hymenolepis diminuta* (Cestoda): Some characteristics of the inactivated enzyme. J. Parasitol., 67, 378–385.
20) Smyth, J. D. (1969): The physiology of cestodes. Oliver & Boyd, London, 6–23.
21) Specian, R. D. and Lumsden, R. D. (1980): The microanatomy and fine structure of the rostellum of *Hymenolepis diminuta*. Z. Parasitenkd., 63, 71–88.
22) Tanaka, K., Iino, A. and Naguro, T. (1974): Styren resin cracking method for observing biological materials by scanning electron microscopy. J. Elect. Microsc., 23, 313–315.
23) Threadgold, L. T. (1965): An electron microscope study of the tegument and associated structures of *Proteocephalus pollanicoli*. Parasitol., 55, 457–472.
24) Threadgold, L. T. and Arme, C. (1974):

Hymenolepis diminuta: An electron microscope study of ion absorption. Exp. Parasit., 35, 475–491.
25) Threadgold, L. T. and Hopkins, C. A. (1981): *Schistocephalus solidus* and *Ligula intestinalis*: Pinocytosis by the tegument. Exp. Parasit., 51, 444–456.
26) Threadgold, L. T. and Read, C. P. (1970): Ultrastructure of a unique membrane specialization in tegument. Exp. Parasit., 28, 246–252.
27) Trimble III, J. J. and Lumsden, R. . (1975): Cytochemical characterization of tegument membrane-associated carbohydrates in *Taenia crassiceps larva*. J. Parasitol., 61, 665–676.
28) Yamane, Y. (1968): On the fine structure of *Diphyllobothrium erinacei* with special reference to the tegument. Yonago Acta Med., 12, 169–181.
29) Wright, R. D. and Lumsden, R. D. (1968): Ultrastructural and histochemical properties of the acanthocephalan epicuticle. J. Parasitol., 54, 1111–1123.

マンソン裂頭条虫（*Spirometra erinacei*）の表皮下細胞及び関連組織構造の電子顕微鏡的研究

山根洋右　中川昭生　牧野由美子
（島根医科大学環境保健医学教室）
平井和光
（愛媛大学医学部寄生虫学教室）

走査型電子顕微鏡及び透過型電子顕微鏡を用い，マンソン裂頭条虫（*Spirometra erinacei*）のプレロセルコイドについて，いろいろな形態を示す表皮層内の小体，基底膜構造，表皮下細胞を観察した．表皮層内の小体は形，電子密度，分布領域などいろいろ異なるが，ひとつは表皮最表面膜のとりこみによる pinosome と考えられ，他はいずれも同一物で表皮下細胞で産生される分泌顆粒，いわゆる表皮層内小体 tegumentary bodies と思われる．この分泌顆粒は表皮層内では脊椎動物の血球に似た中央部が陥凹した円板状の形態を呈し，成熟度や分化などに応じて異なる電子密度や形態をとりうるものと推察される．

基底膜層の形質膜の一部は，ところどころ内方にむけて折れ込み，pinosome や phagosome 様顆粒のとりこみへの関与を示唆する所見を観察した．また複雑な樹枝状突起を有し表皮層内小体を分泌する表皮下細胞の立体像を明らかにした．

Explanation of Figures

Fig. 1 A cracking fracture of the tegument, showing dense growth of microtriches (M), vacuoles (V) and the membranous structure (arrowhead) in the distal cytoplasmic layer. (×9,500).

Fig. 2 A TEM micrograph of the tegumentary bodies in the distal cytoplasmic layer. Some elongated discoid bodies (arrow) and pinosomes (arrowhead) distributed in the proximal part of microtriches (M). (×32,500).

Fig. 3 A TEM micrograph of the tegument, showing the distal cytoplasmic layer (DC), and the basal membrane complex (BM). Note the phagosome-like vacuoles (arrowheads) and meandering of the internal plasma membrane complex. (×8,500).

Fig. 4 A TEM micrograph of the perinuclear, cytoplasm immediately beneath the basal membrane complex, showing the cytoplasmic processes (arrowhead), cytoplasmic bridges (CB), muscle layers (ML) and glycogen particles (G). (×9,100).

Fig. 5 A cracking fracture of the basal membrane complex (BM) and the muscle layers (ML). Note the fibrous network of the internal plasma membrane (arrowheads) in connection with the distal cytoplasmic layer (DL). (×12,500).

Fig. 6 The basal membrane complex (BM), showing an internal plasma membrane (arrowheads), glycogen particles (G) and fibrous basal lamina (small arrowheads). (×18,000).

Fig. 7 The basal membrane complex (BM), showing the invagination of the internal plasma membrane (arrow) and the canalicular cytoplasmic bridge (arrowhead) of the subtegumental cell. (×21,000).

Fig. 8 A SEM micrograph of the basal membrane complex (BM), showing the fine network and globules (G). (×4,500).

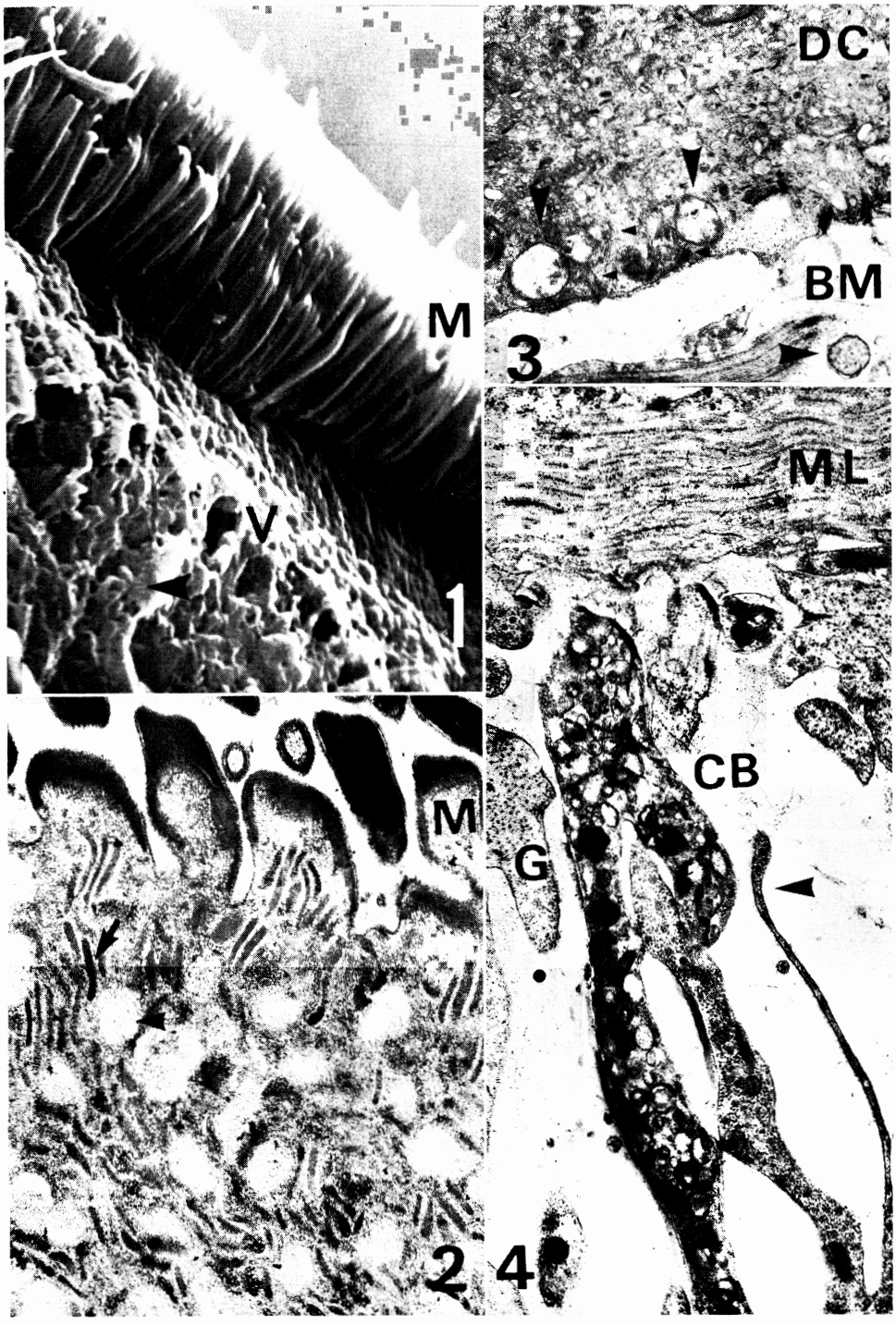
Fig. 9 A SEM micrograph of the pores of the basal membrane complex. Note the concave discoid bodies (small arrowheads) and round globules (G). (×12,000).

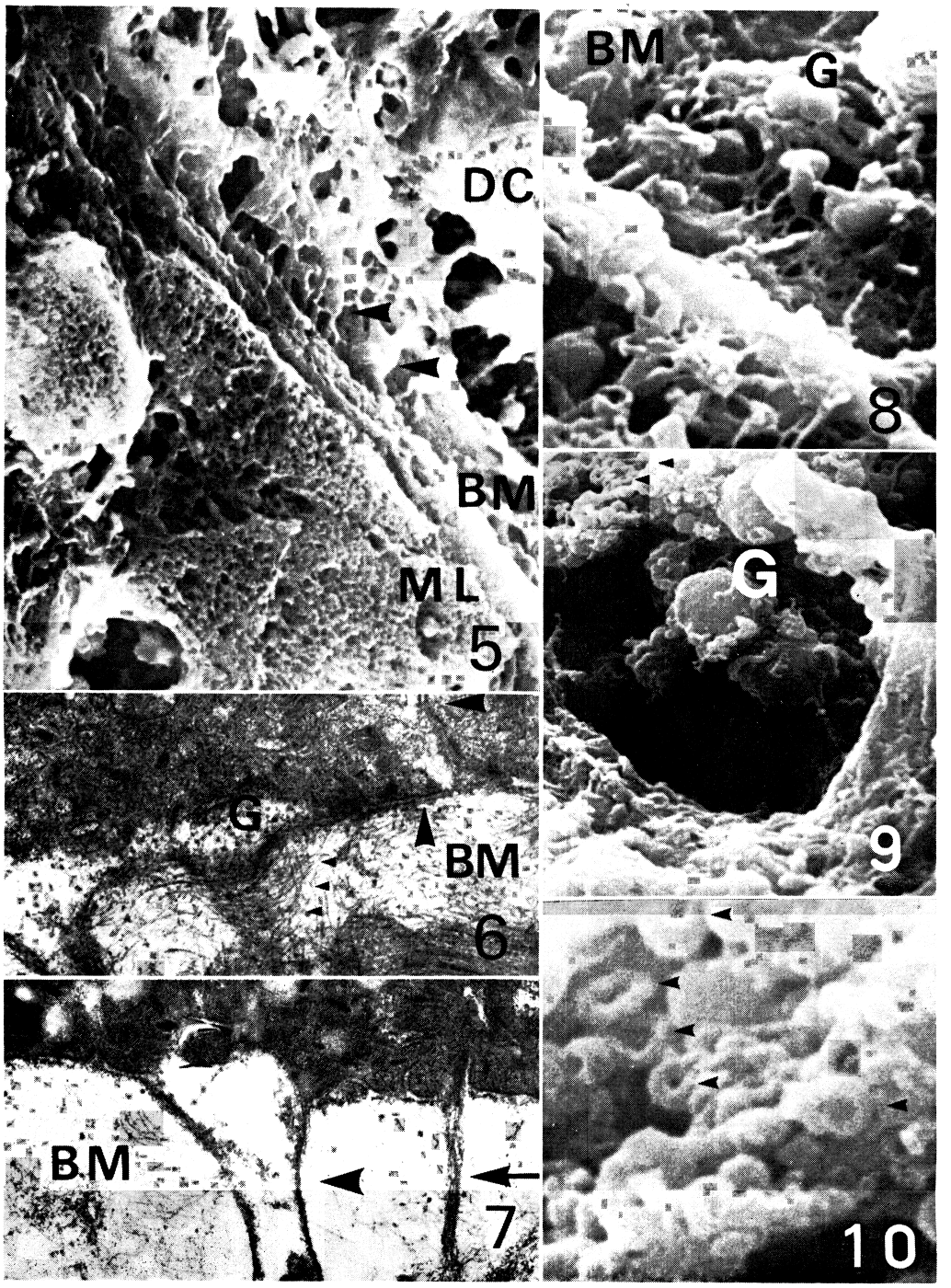
Fig. 10 A higher magnificaiton of the concave discoid bodies (arrowheads), showing similar contours to the red blood cells. (×24,000).

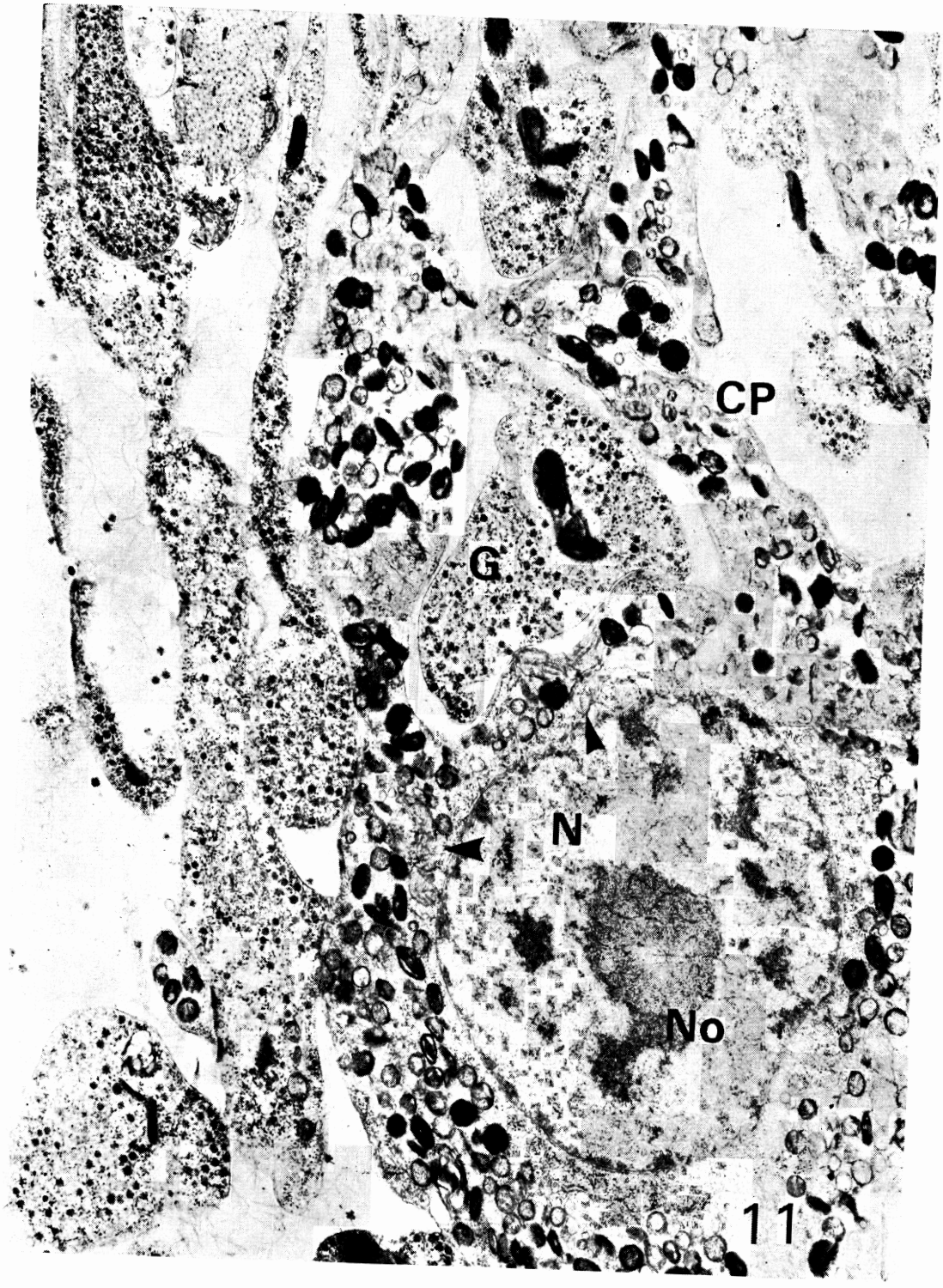
Fig. 11 The subtegumental cells with complicated cytoplasmic processes (CP), showing a large nucleus (N) with a nucleolus (No). Note conglomerations of glycogen particles (G) and the various types of bodies around the Golgi's apparatus (arrowheads) in the cytoplasm. (×13,500).

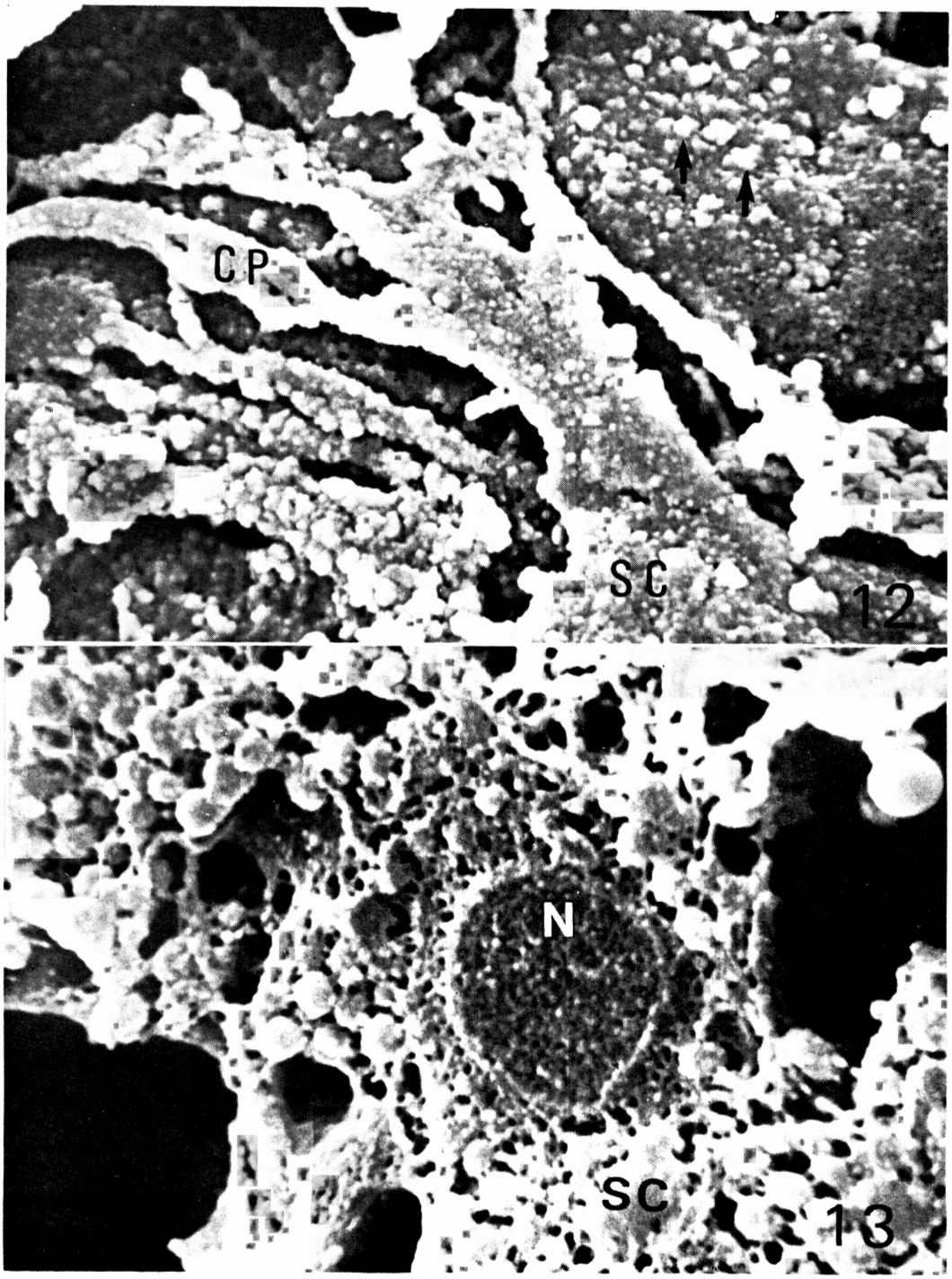
Fig. 12 A cracking fracture of the subtegumental cells (SC), showing complicated cytoplasmic processes (CP) and many globules (arrows) on the cell surface. (×16,900).

Fig. 13 A cracking fracture of the subtegumental cell (SC), showing numerous globules around large nucleus (N) in the cytoplasm. (×17,400).


M
V
1
DC
BM
3
ML
CB
G
4
M
2


DC
BM
ML
5
BM
G
8
G
9
G
BM
6
BM
7
10


CP
G
N
No
11


CP
SC
12
N
SC
13