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STUDIES ON THE FINE STRUCTURE OF THE ROSTELLAR HOOKS OF MULTICEPS ENDOTHORACICUS DURING THE ULTIMATE PHASE OF THEIR FORMATION

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Abstract. The present study on the fine structure of the rostellum and hooks of *M. endothoracicus* during the ultimate phase of their formation disclosed a unique structure of the tegument and subtegumental layers which has not been found in any other part of the larval body. Evidence has been obtained that the blade of the definitive hook originated from the conical hooklets in the tegument of the rostellar prebulb by a deposition of tegumental protein on their surface. A greatly increased number of pore canals emerging from the basal membrane and occupying two thirds of the distal cytoplasm together with the presence of ribosomes inside and outside the pore canal indicated that the metabolism of the distal cytoplasm was increased during the synthesis of the hook protein. The hook substance originated directly in the pore canals positioned in the direction of the growth of the hooklets. Inside the hypertrophic tegument of the hook organ, we observed the formation of hook protein in the pore canals which communicated with the base of the hook blade for the time of differentiation of the hook handle and blade. After completed differentiation of the hooks, empty pore canals dilated and the tegument became vesicular in appearance.

The fine structure and hook formation have been described by Mount (1970) for larvae of *Taenia crassiceps*, by Blitz and Smyth (1973) for larvae of *Raillietina cesticillus*. The formation of the hook of various cestode species has been studied by a number of authors. Young (1908), Gläser (1909), Crusz (1940) elucidated various phases in the process of hook formation, yet the process itself remained obscure until Mount (1970) observed in his ultrastructural study on the histogenesis of the rostellar hook of *Taenia crassiceps* that it was being formed from the original hooklet. Conversely, Baron (1971) and Bilques and Freeman (1969) suggested that the hooks formed de novo in the hypertrophied tegument. The role played by the tegument and subtegumental cells in the synthesis and transmission of hook protein is still obscure. Lumsden (1966) maintained that hook protein was produced by subtegumental cells and transferred to the tegument. Mount (1970) contradicted this concept in saying that the principal importance in the synthesis of protein should be ascribed to ribosomes in the distal cytoplasm. These disagreeing views instigated our study on the formation of the hook on the rostellum of the polycephalic larva of *M. endothoracicus*.

MATERIALS AND METHODS

Our study was performed on a more advanced larva of *M. endothoracicus* obtained from the thoracic cavity of *Rhombomys opimus* (Lichtenstein, 1823) on day 45 of experimental infection which had been performed at the Zoological Institute, Academy of Sciences, Kazakh SSR. The scoleces of the polycephalic larva measured 1.8 –2 mm in length, at a width of 1—1.2 mm. The material was

fixed with Millonig's method in glutaraldehyde, postfixed in phosphate-buffered 1 % O_sO_4, dehydrated and embedded in Epon and Vestopal. Several scoleces were fixed in 2 % paraformaldehyde in a phosphate buffer and postfixed in 1 % O_sO_4 in phosphate buffer (Karnowsky's method). Sections were cut on a Reichert microtome and stained with uranyl acetate and lead-citrate. A series of semithick sections were stained with 1 % toluidine blue in 1 % borax carmine. We examined a total of 8 scoleces of the polycephalic larva and arranged these to Group III of our classification scheme (Hulínská 1975).

RESULTS

The differentiated rostellum of the larva of *M. endothoracicus* represents an area closely communicating with the cells of the host passing through the spiral canal to the basal part of the rostellum. The conical rostellar prebulb protrudes into this basal part of the canal. The prebulb is overgrown by a bulb forming a flat rostellar pad. The hypertrophic tegument of the lateral extensions of the canal wall encloses the definitive hooks. The folded tegument of the prebulb measures from 8.1—9.8 μm in thickness.

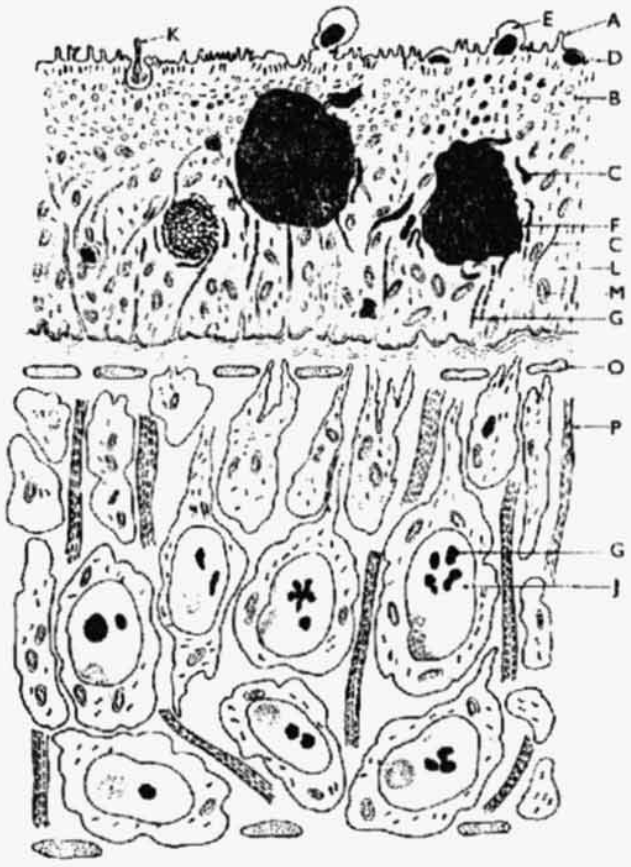

Fig. 1. Rostellar tegument of a larval *M. endothoracicus* reconstructed from a series of ultrasections. The distal cytoplasm contains sections through hooklets. These are surrounded by pore canals, filled with an opaque substance, ribosomal aggregations and numerous mitochondria. Irregularly arranged microtriches and bleb-like extrusions cover the surface of the distal cytoplasm; there is a section through a sensory ending. Rod-shaped bodies and vesicles occupy the anterior third of the cytoplasm. Pore canals emerge from the basal membrane underlaid by a layer of circular muscles. Extensions of subtegumental cells separate this layer from the basal membrane. The nuclei of subtegumental cells contain opaque granules.

EXPLANATIONS FOR BOTH FIGURES AND PLATES:

A — microtriches, B — rod-shaped bodies, C — pore canals, D — limiting plasma membrane, E — bleb-like extrusions, F — hooklets, G — condensed, electron-opaque substance, H — hooks, I — vesicles, J — nucleus, K — sensory endings, L — ribosomes, M — mitochondria, N — membranous formations, O — circular muscles, P — longitudinal muscles, R — rostellar cone, T — hypertrophied tegument.

With toluidine blue we disclosed in the tegument yellow coloured sections through conical hooklets measuring 5.2—6.2 μm in diameter. Elongate cells underlying the tegument contained spherical nuclei (2.4×2.5 μm). Granules (0.2—0.8 μm) of a similar colour to that of the hooklet sections were observed in the tegument (Plate I, Fig. 1). A number of these granules were found also in some nuclei of tegumental cells.

Our electron-microscopic examination disclosed organelles in the rostellar tegument which could not be found anywhere else in the larval body (Fig. 1). There was also a difference in the make-up of microtriches of the loose microthrix border lining the tegument of the prebulb in that some had a thin base and a fibrous point, other had no point. The confining, superficial, electron-dense plasma membrane attained a thickness of up to 200 Å by contrast to a standard thickness of 100—110 Å of this membrane in the spiral

canal. The superficial membrane was underlaid densely by rod-shaped bodies (Plate I, Fig. 2). Mitochondria, rod-shaped bodies and vesicles confined by a membrane were present in the proximal 1/3rd of the cytoplasm of the prebulbar tegument. Long mitochodria with longitudinal cristae and numerous pore canals (Plate II, Fig. 2), the latter being differently long and differently arranged evaginations of the basal membrane, were observed in the central and basal part of the distal cytoplasm. The diameter of the pore canals was 380 Å; sometimes, the diameter of their dilated ends was 460 Å (measured in cross-section). Ribosomes were distributed regularly on the inner side of the wall of the pore canal. A communication was established between pore canals and mitochondria (Plate III, Fig. 1). The heavy multiplication of evaginated pore canals emerging from the basal membrane, and the presence of ribosomes resembling an RES of nucleic cells indicated an increase in the metabolism of the distal cytoplasm. The pore canals joined into thick tubular formations mainly in the vicinity of the hooklets. Spiral formations in the distal cytoplasm (Plate III, Fig. 2) had apparently been formed from dystrophic changes in the tissue. The distal cytoplasm enclosed primary hooklets (Plate IV, Fig. 1). The highly electron-opaque part of the hooklet measured 6.2 μm and more in diameter. A cross-section through the basal part of the hooklet disclosed its irregular ovoid shape and a membrane limiting it from the distal cytoplasm. Pore canals of the distal cytoplasm were lying in concentric lines around the hooklet. Larger pore canals were filled with an electron-opaque substance. Both hooklet and pore canals were surrounded by numerous ribosomes. Smaller formations representing sections through the blade of the hooklet were vacuolized (Plate IV, Fig. 2). The central vacuoles were empty, those closer to the surface contained an electron-opaque substance. An undulate basal membrane lined the distal cytoplasm which was traversed by pore canals. The fibrillar layer was underlaid by circular muscles separated by extensions of subtegumental cells. A concentration of ribosomes, an endoplasmic reticulum and mitochondria were present in the cells of the cytoplasm. The shape of the nuclei was ovoid. The number of pores in the nuclear membrane of these cells was higher than that observed in cells of other parts of the larval body. There was a remarkable concentration of chromatin near the nuclear membrane (Plate V, Fig. 1). The electron-opaque substance inside the nuclei was condensed in spherical formations of different size (Plate V, Fig. 2). Cells with this substance in their nuclei occurred mainly in the central part of the rostellar prebulb. The rostellar tegument was the site of a frequent incidence of sensory endings. In sections, the sensory organelle was shaped as a sensory sac attached to the superficial plasma membrane. It contained microvesicles and long mitochondria. The ciliary extension, of similar length to that of the microthrix but thicker (0.35 μm), consisted of microtubules (Plate V, Fig. 3). The hypertrophied tegument on the lateral extensions of the rostellum in the basal part of the canal enclosed the definitive hooks. An electron-opaque substance (hook protein) produced in the pore canals, could be seen to be deposited on the lateral margin of the hooks (Plate VI, Fig. 1). Typical of the remaining part of the hypertrophied tegument was the presence of ribosomal aggregations, mitochondria and thickened pore canals positioned in the direction of growth of the hook base. The base of the hook was thickened by the deposition of hook protein along the basal plate, then followed the development of the guard and handle of the hook. It appeared that at least a portion of the protein utilized in handle and guard formation have been synthesized in pore canals joined to the basal plate. Rostellar cells and ribosomal aggregations were active in the synthesis of the remaining portion of protein (Plate II, Fig. 2). The pore canals dilated after the completion of hook differentiation. While the distal cytoplasm contained dilated and mostly empty vesicles, the narrow strip of cytoplasm surrounding the hook still contained rod-shaped bodies, and the remaining pore canals were filled with an electron opaque substance.

DISCUSSION

The rostellum of a larval *M. endothoracicus* is the site of contact with the tissue of the host, and there is evidence of a host-parasite relationship on its surface. The contractile surface of the rostellum has most unique properties not to be found in any other part of the larval body. Blitz and Smyth (1973) observed that the tegument of the rostellum was much thinner (around 1 μm) than that of the strobila and therefore capable of contraction. Similar differences have been found by Sakamoto and Sugimura (1969) in *Echinococcus multilocularis*. Featherstone (1972) reported for the rostellar tegument of *T. hydatigena* a thickness of 6—8 μm which is similar to that found in the larva of *M. endothoracicus*. Our evidence of a sensory function of the rostellum due to the presence of sensory endings in its tegument has been supported by Jha and Smyth (1971) and Sakamoto and Sugimura (1969). Blitz and Smyth (1973) ascribed a sensory function even to pore canals described by Threadgold (1962) for *Dipylidium caninum* and by Morseth (1966) for *T. pisiformis*. We found in the rostellar tegument of a larval *M. endothoracicus* both sensory endings of a standard type with a distal ciliary extension and an increased number of tubules known as "pore canals" in the literature which emerged from the base of the distal cytoplasm.

Pore canals originate from an evagination of the basal membrane and, hence, are not sensory endings as suggested by Threadgold (1962) and other authors. The vehement development of these canals which are filled with an electron-opaque substance and located in the dista leytoplasm of the rostellum along the hooklets, and in the hypertrophied tegument covering the base of the hooks indicates that they participate in the differentiation of the hooks. Hook protein synthetized by the nuclei of subtegumental cells and ribosomes is transported through the pore canals to the surface of the growing hook. Tegumental protein recorded by Lumsden (1966) for *Hymenolepis diminuta* is synthetized in subtegumental cells and transferred to the tegument. Mount (1970) reported that the hypertrophied tegument of the hook organ contained an electron-opaque substance (protein) in vesicles, and ribosomal aggregations. He suggested that ribosomal aggregations in the tegument may participate, to a certain extent, in the synthesis of hook protein. He maintained that the secondary thickening of the hook base was caused by the electron-opaque substance inside the vesicles. In our material, the electron-opaque substance was concentrated in pore canals of which several communicated with the hook surface. In the vicinity of the definitive hook complete with a handle and a guard, the pore canals were dilated into empty vesicles.

ИЗУЧЕНИЕ УЛЬТРАСТРУКТУРЫ ХОБОТКОВЫХ КРЮЧЬЕВ *MULTICEPS ENDOTHORACICUS* В ПОЗДНЕЙ ФАЗЕ ИХ ОБРАЗОВАНИЯ

Д. Гулинска и В. М. Федосеенко

Резюме. Исследована ультраструктура хоботка и хоботковых крючьев *M. endothoracicus* в поздней фазе их развития. Обнаружено необыкновенное строение тегумента и субтегументальных слоев, до сих пор не обнаруженное в других частях тела личинки. Доказано, что лезвия дефинитивных крючьев образованы из конических крючочков в тегументе хоботкового пребульбуса путем депонирования протеина тегумента на их поверхности. Увеличение числа каналов выходящих из базальной мембраны и выполняющих 2/3 дистальной цитоплазмы, также как и присутствие рибосом внутри и снаружи каналов,

показывают, что метаболизм дистальной цитоплазмы увеличился во время синтеза протеина крючьев. Крючьевое вещество образуется прямо в каналах, которые ориентированы в направлении роста крючочков. Внутри гипертрофического тегумента органа крючьев образуется протеин крючьев в каналах соединенных с основой лезвия только в течение дифференцировки их рукоятки и лезвия. После окончания дифференцировки крючьев пустые каналы расширяются и тегумент принимает везикулярный вид.

REFERENCES

BARON P. J., On the histology, histochemistry and ultrastructure of the cysticercoid of *Raillietina cesticillus* (Molin, 1858) Fuhrmann 1920 (Cestoda, Cyclophyllidea). Parasitology 62: 233–245, 1971.

BLITZ N. M., SMYTH J. D., Tegumental ultrastructure of *Raillietina cesticillus* during the larval–adult transformation, with emphasis on the rostellum. Int. J. Parasitol. 3: 561–570, 1973.

BILQEES F. M., FREEMAN R. S., Histogenesis of the rostellum of *Taenia crassiceps* (Zeder, 1800) (Cestoda), with special reference to hook development. Can. J. Zool. 47: 215–261, 1969.

CRUSZ H., Further studies on the development of *Cysticercus fasciolaris* and *Cysticercus pisiformis*, with special reference to the growth and sclerotization of the rostellar hooks. J. Helminthol. 22: 179–198, 1948.

FEATHERSTONE D. W., *Taenia hydatigena* IV. Ultrastructure study of the tegument. Z. Parasitenk. 38: 214–232, 1972.

GLÄSER H., Zur Entwicklungsgeschichte des *Cysticercus longicollis* Rud. Z. Wiss. Zool. 92: 540–561, 1909.

HULÍNSKÁ D., Morphogenesis of the larval stages of *Multiceps endothoracicus* Part I. Zool. Anz., Jena 195: 201–209, 1975.

JHA R. K., SMYTH J. D., Ultrastructure of the rostellar tegument of *Echinococcus granulosus* with special reference to biogenesis of mitochondria. Int. J. Parasitol. 1: 169–177, 1971.

LUMSDEN R. D., Cytological studies on the absorptive surfaces of cestodes. II. The synthesis and intracellular transport of protein in the strobilar integument of *Hymenolepis diminuta*. Z. Parasitenk. 28: 1–13, 1966.

MORSETH D. J., The fine structure of the tegument of adult *Echinococcus granulosus*, *Taenia hydatigena*, and *Taenia pisiformis*. J. Parasitol. 52: 1074–1085, 1966.

MOUNT P. M., Histogenesis of the rostellar hooks of *Taenia crassiceps* (Zeder, 1800) (Cestoda). J. Parasitol. 56: 947–961, 1970.

SAKAMOTO T., SUGIMURA M., Studies on echinococcosis XXI. Electron microscopical observations on general structure of larval tissue of multilocular *Echinococcus*. Jap. J. Vet. Res. 17: 67–80, 1969.

THREADGOLD L. T., An electron microscope study of the tegument and associated structures of *Dipylidium caninum*. Quart. J. micr. Sci. 103: 135–140, 1962.

YOUNG R. T., The histogenesis of *Cysticercus pisiformis*. Zool. Jahrb. Anat. 26: 183–254, 1908.

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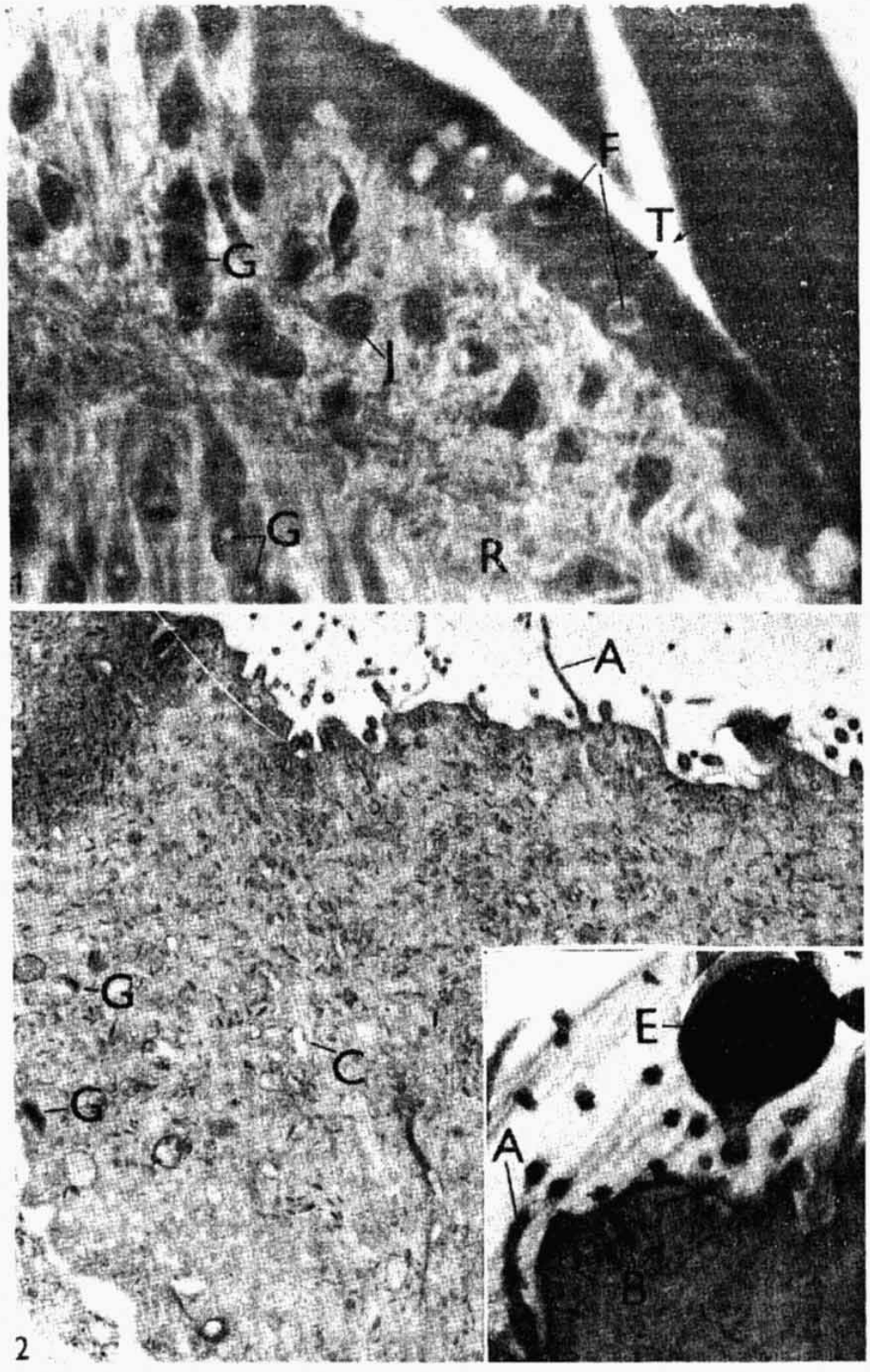


Fig. 1. Semithick section through part of the rostellum of a larval *M. endothoracicus*. Sections through hooklets in the tegument of the conical prebulb. Granules of different sizes present in subtegumental cells (×650, toluidine blue). **Fig. 2.** Tangential section through the tegument of the apical prebulbar portion. An occasional fibrous microthrix can be seen in the folded surface. The distal cytoplasm contains rod shaped bodies, vesicles, pore canals and mitochondria (×12,000). Insert: The surface of the distal cytoplasm with an opaque thickening of the plasma membrane and an electron-opaque formation. Rod-shaped bodies lying perpendicular to the surface (×25,000).

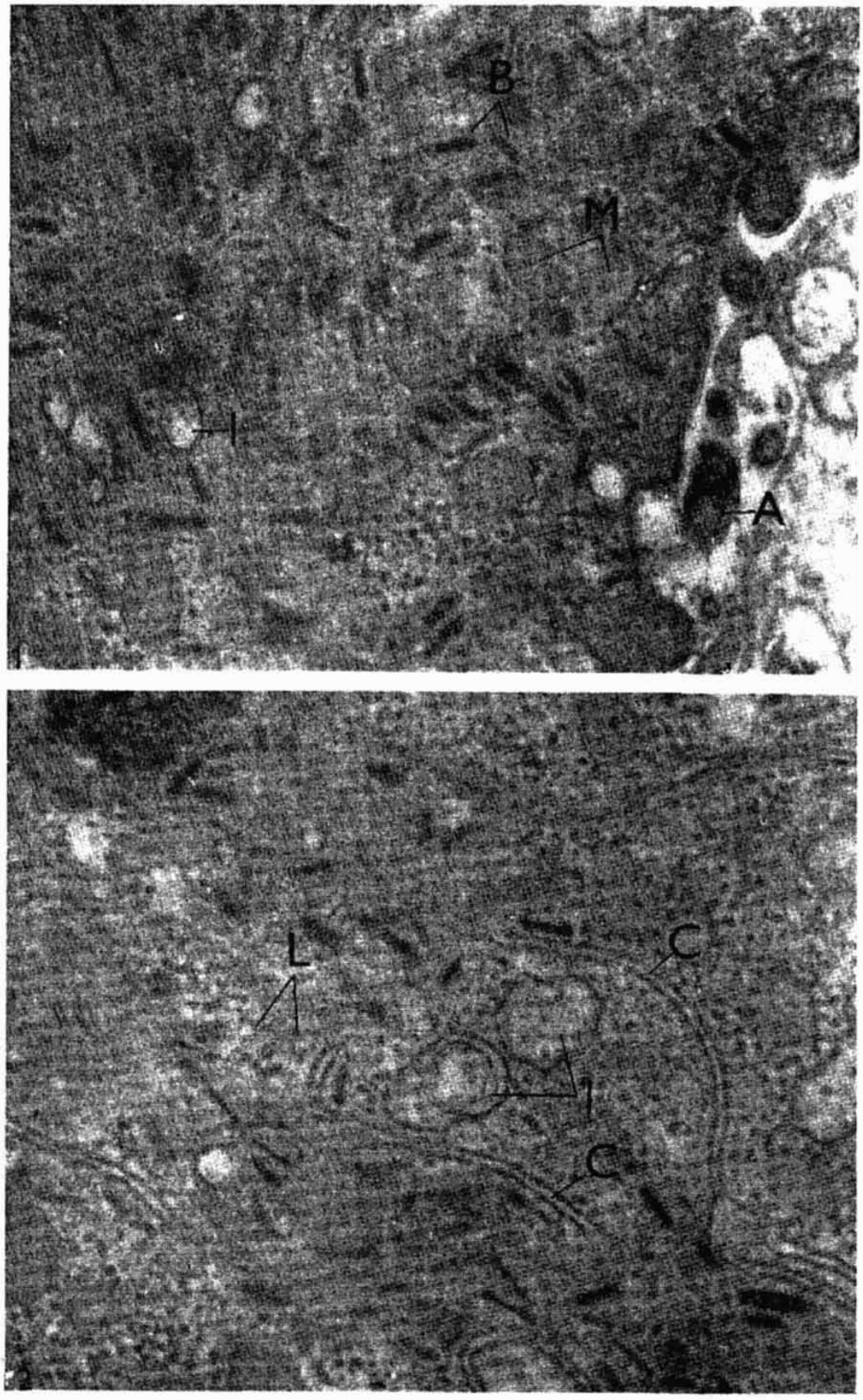
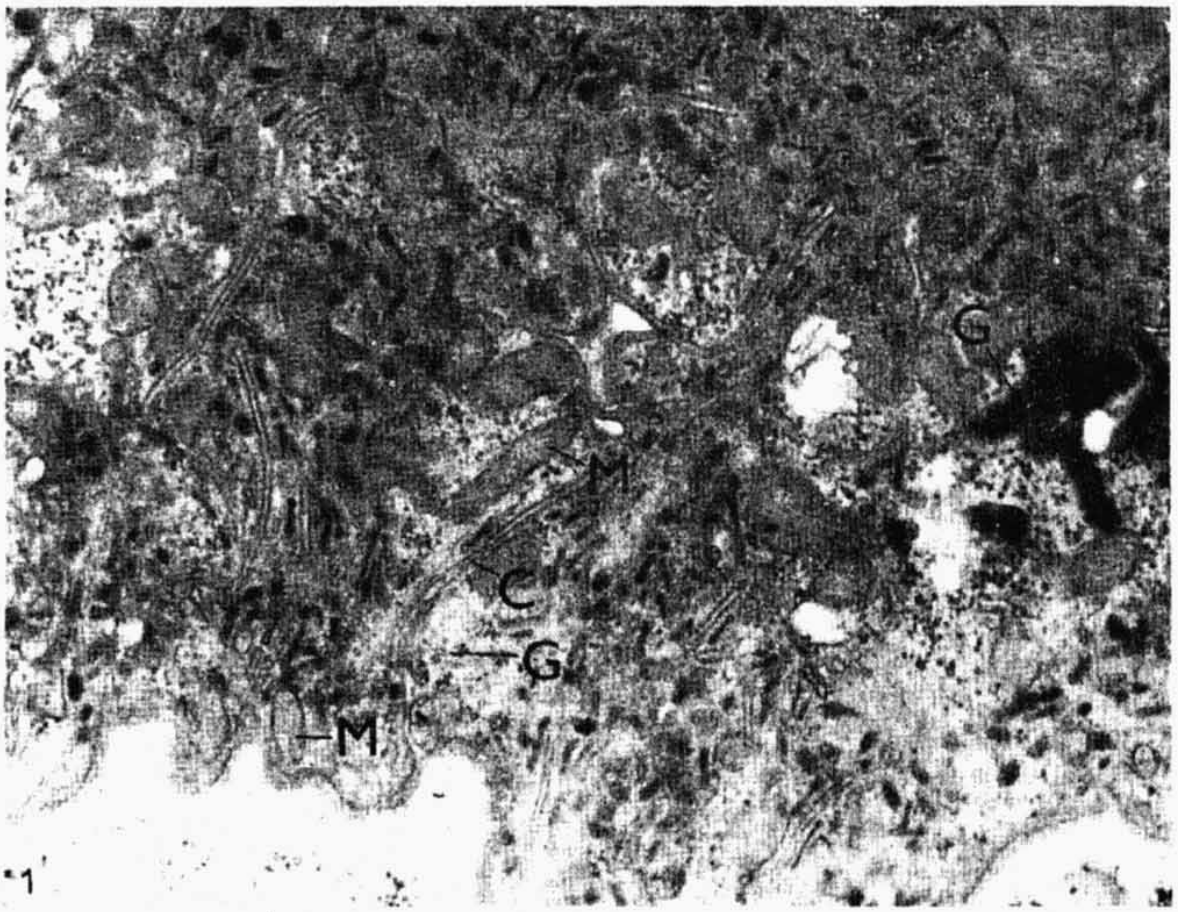


Fig. 1. Proximal portion of the distal cytoplasm of the prebulb. The undulate surface is limited by a thick membrane. This portion of the cytoplasm contains rod-shaped bodies, membranebound vesicles and mitochondria (×26,500). **Fig. 2.** In the central part of the distal cytoplasm there are pore canals, ribosomes and mitochon dria in the finely granular cytoplasm with rodshaped bodies (×26,500).



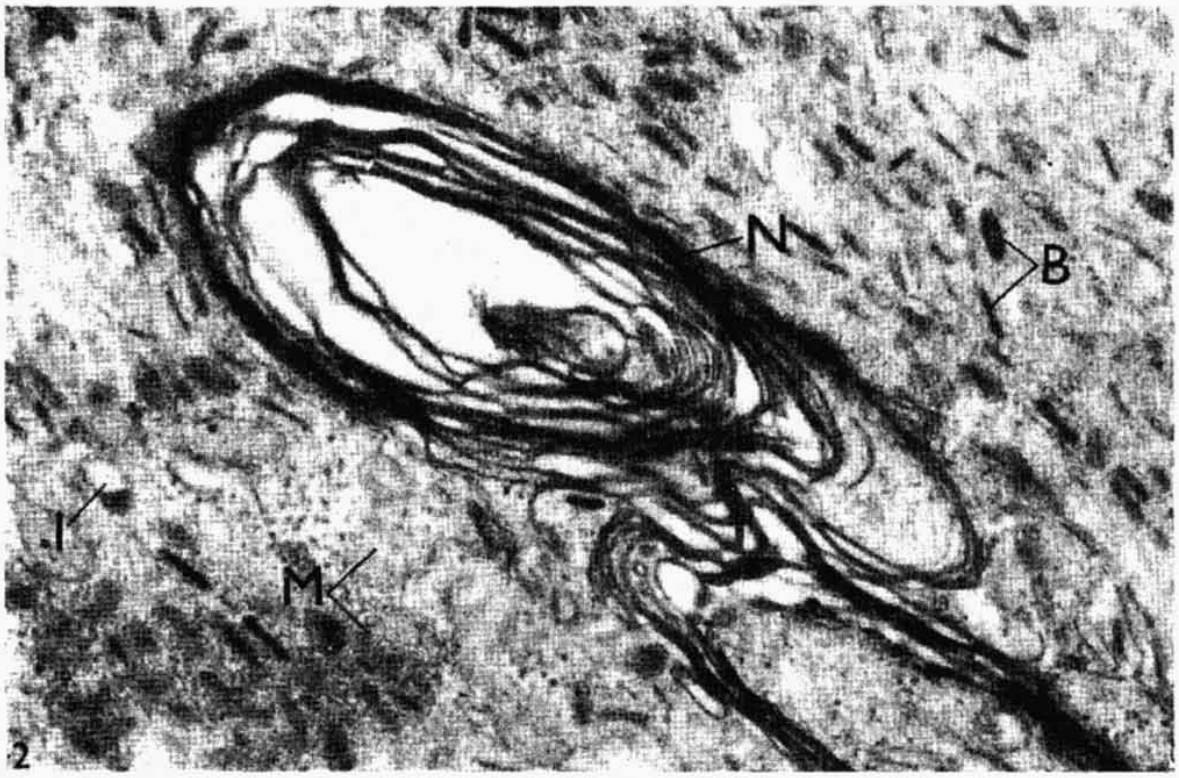


Fig. 1. The central and basal parts of the distal cytoplasm are traversed by numerous pore canals, vesicles filled with an opaque substance and large mitochondria. Ribosomal aggregations are present around the pore canals (× 18,500). **Fig. 2.** Membranous, spiral formations in the distal cytoplasm of the prebulb are surrounded by ribosomes, mitochondria and rod-shaped bodies (× 23,600).

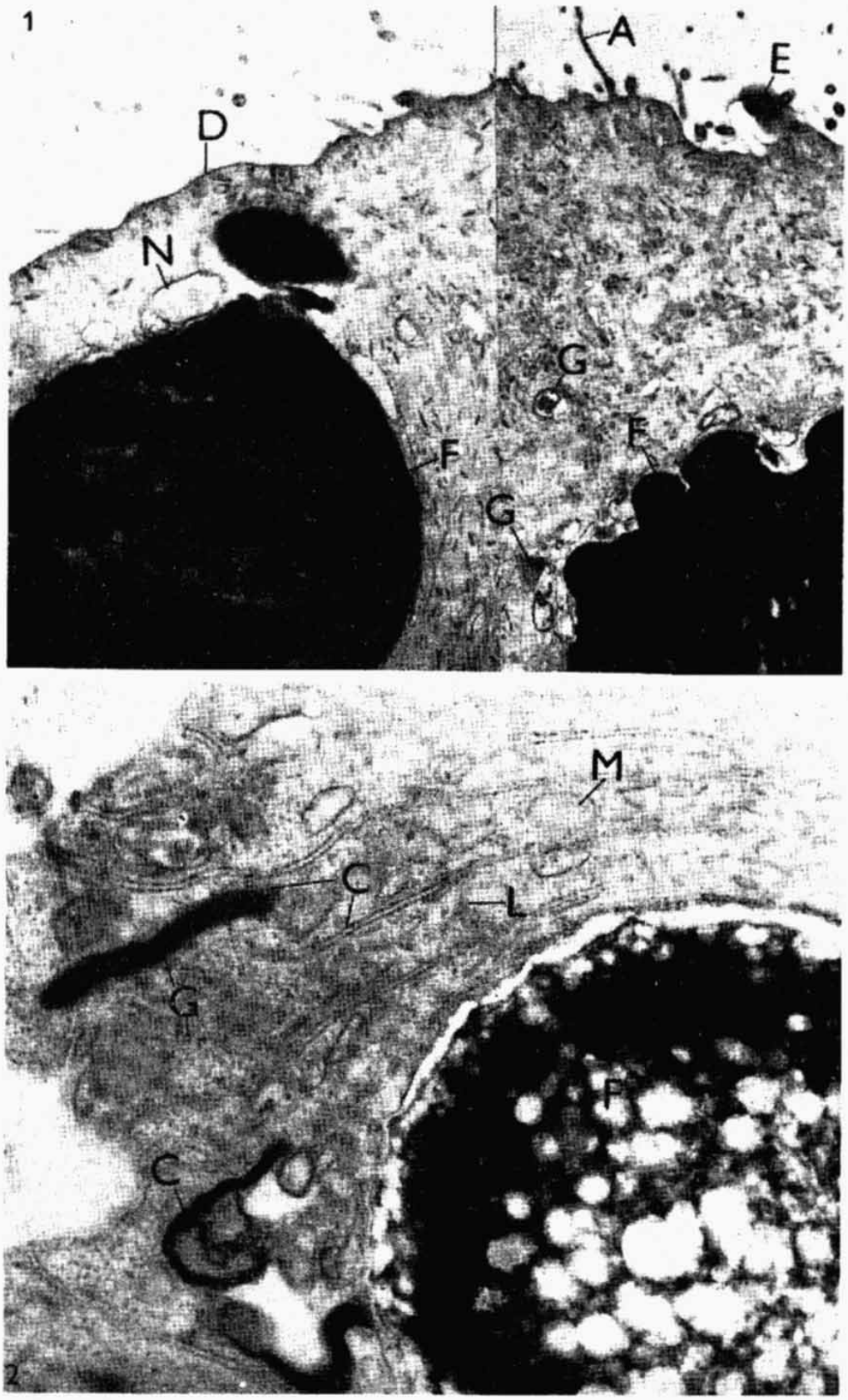


Fig. 1. Tangential section through the tegument of the lateral part of the prebulb. Sections through hooklets are visible in the distal cytoplasm. An electron-opaque substance inside vesicles and pore canals causes a secondary thickening of the hooklet (×12,100). **Fig. 2.** Transverse section through a vacuolized point of a hooklet. An electron-opaque substance is present in the pore canals and on the surface of the hooklet. Ribosomal aggregations occur around the pore canals (×22,600).

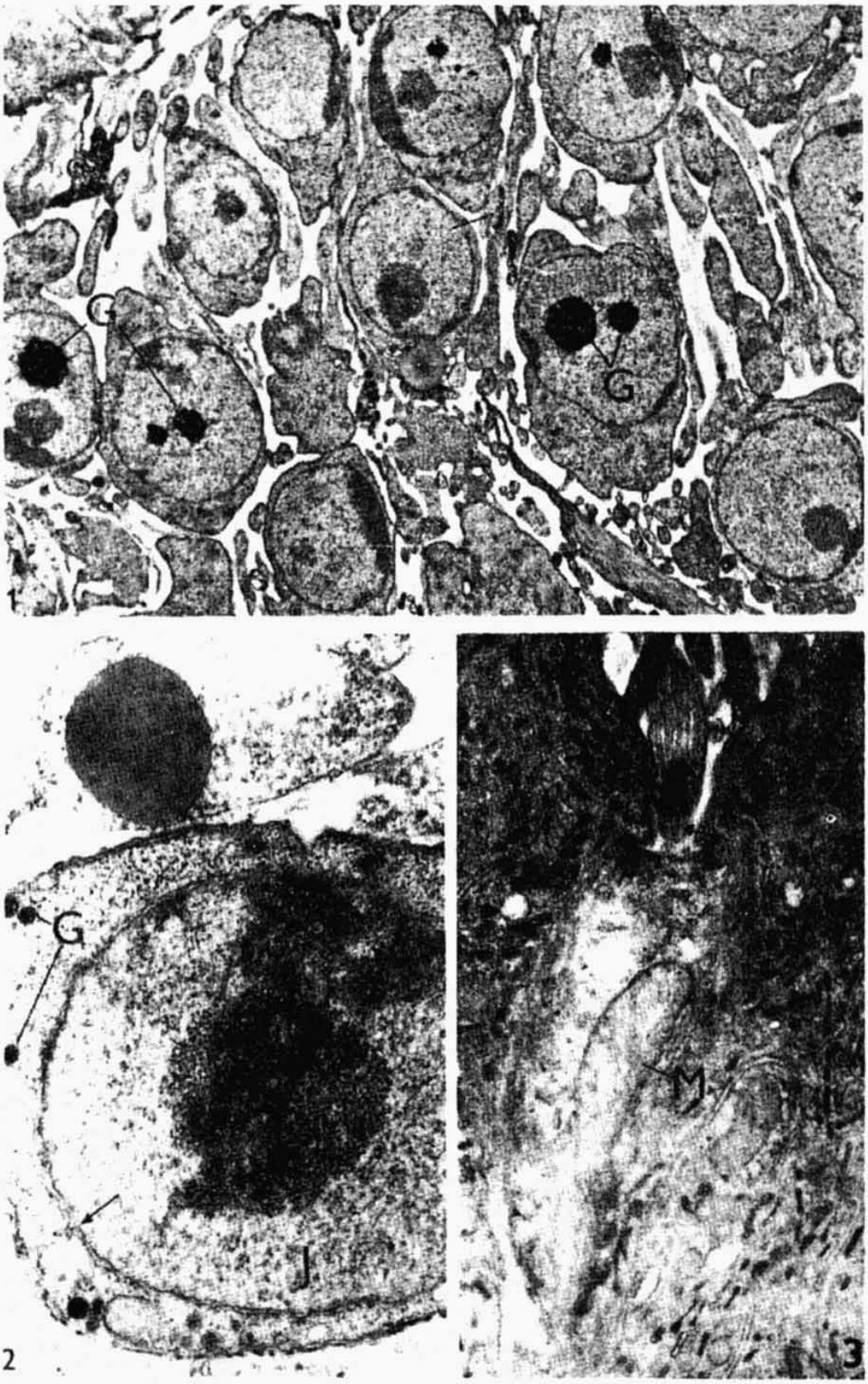


Fig. 1. The spherical nucleus of subtegumental cells of the prebulb contains an opaque substance. Mitochondria, ribosomes and rod-shaped bodies are present in the cytoplasm of the cells (× 13,000). **Fig. 2.** Part of a subtegumental cell with an electron-opaque substance in the plasma. Pores are seen in the nuclear membrane (× 18,300). **Fig. 3.** Sensory endings in the rostellar tegument with a distal ciliary extension. The sensory sac contains a large mitochondrion. The sac communicates with the plasma membrane (× 18,300).

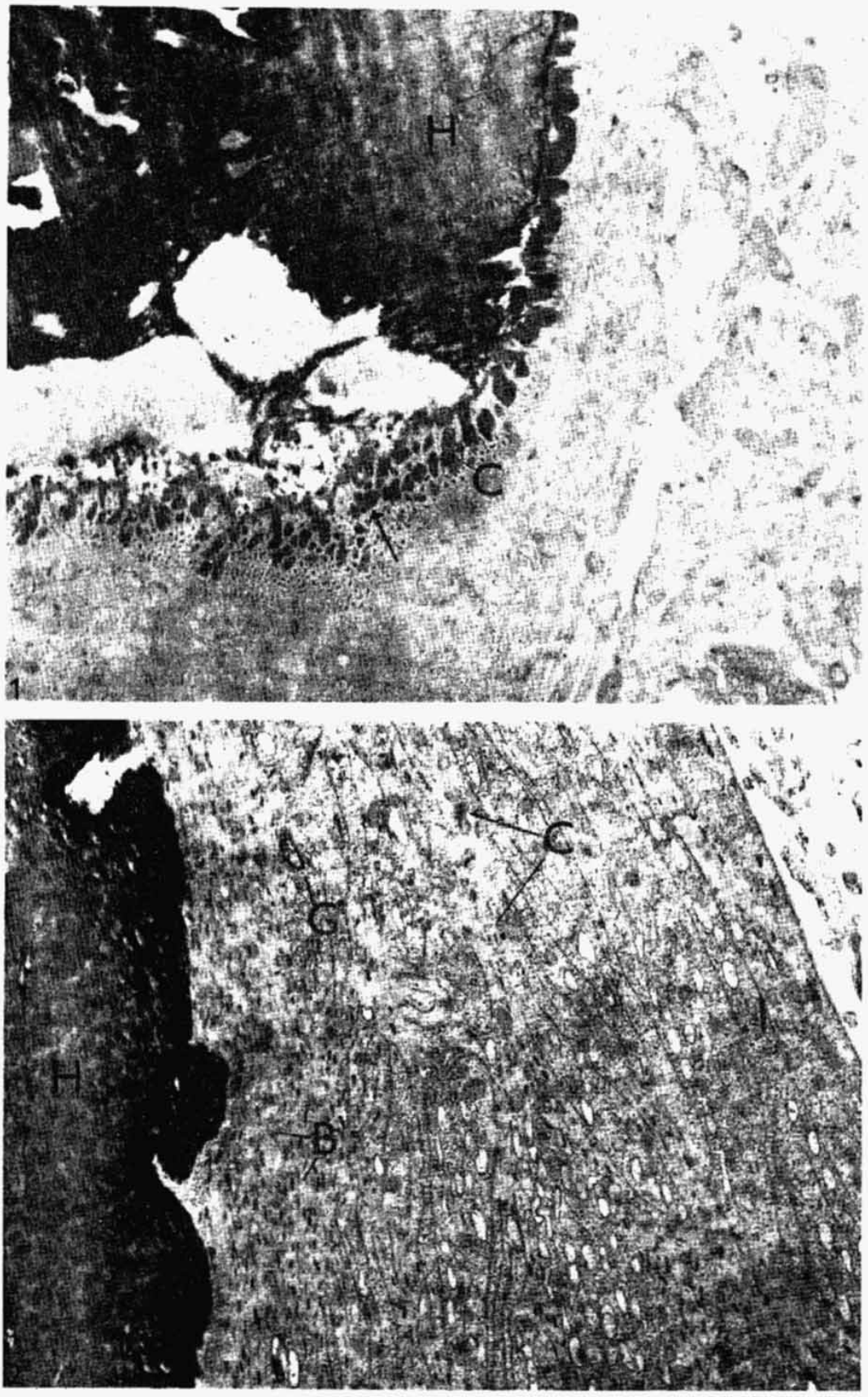


Fig. 1. The micrograph shows an accumulation of an opaque substance in the hypertrophied tegument, and ribosomes in the vicinity of the basal plate of the hook. The guard of the hook is being formed by a deposition of the opaque substance (×12,800). **Fig. 2.** The hypertrophied tegument contains vesicles and pore canals filled with an opaque substance, and aggregations of ribosomes. On the left, part of the opaque guard of the hook (×13,500).