FOLIA PARASITOLOGICA (PRAHA) 25: 247–255, 1978.

COMPARATIVE STUDY ON THE HISTOCHEMISTRY AND THE FINE STRUCTURE OF THE SUCKERS AND THE ROSTELLUM OF LARVAE OF MULTICEPS MULTICEPS LESKE, 1780 AND MULTICEPS ENDOTHORACICUS KIRSCHENBLATT, 1948

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Abstract. Variation has been shown in the histochemistry and the fine structure of the rostellum and the suckers of mature scoleces of two polycephalic larvae. The microthrix border of the rostellar tegument and the rostellar cells of the larva of *M. endothoracicus* possesses globular structures which contain proteins and mucopolysaccharides. These are absent in the vacuolated rostellar tegument of the larva of *M. multiceps*. The tegument of the larva of *M. endothoracicus* has no vacuoles and contains mainly rod-shaped bodies. The rostellar tegument of both larvae differs histochemically in the contents of phospholipids and polysaccharides. The suckers of the two larval species differ in the density of myofibrils, in glycogen contents and in the size of microtriches. Secretory activities have been demonstrated in both the tegument of the suckers and that of the rostellum.

The present work is a continuation of our study on enzymes in polycephalic cestode larvae (Hulínská et al. 1976). Although cestode larvae with one scolex have been treated into great detail, there is little information available on the hostochemistry and fine structure of polycephalic larvae except for that contained in a study by Race et al. (1965) on the fine structure of larvae of *Multiceps serialis*. We have studied the fine structure of the rostellum and hooks of the larva of *M. endothoracicus* in an earlier paper (Hulínská and Fedoseenko 1977). In the present paper, an attempt has been made to compare several of these findings with those obtained for the larva of *M. multiceps* in order to enable a differentiation of two larval species of the genus *Multiceps* on the basis of their fine structure and histochemistry. An understanding of the fine structure and histochemistry of the rostellar tegument and of that of the suckers is important in an assessment of secretory activities demonstrated by Shield et al. (1973), Šlais (1958, 1961) for cysticercimand adult forms, and by Smyth (1964, 1973) for *Echinococcus granulosus*.

MATERIALS AND METHODS

We selected mature scoleces with a developed rostellum and hooks from a larval material of *M. multiceps* obtained from the brain of sheep, and from *M. endothoracicus* obtained from the thoracic cavity of *Rhombomys opimus*. Part of the material was identical to that used in an earlier paper (Hulínská et al. 1976). Scoleces of larval *M. multiceps* measured 0.86 mm in length at a width of 0.55 mm, those of larval *M. endothoracicus* 1.2—1.3 mm in length, 0.7—0.8 mm in width. The histochemical methods employed were essentially similar to those used in an earlier paper (Hulínská 1977). For studies on the fine structure, the scoleces were fixed either with phosphate-buffered 6 % glutaraldehyde, or with cacodylate-buffered 4 % glutaraldehyde, pH 7.2, and postfixed with 1 % OsO_4 in a buffered solution. The material was embedded in Epon 812 and Westopal. Ultrasections were stained with uranyl acetate and lead citrate, and examined with JEOL 100 B.

RESULTS

A. HISTOLOGY OF THE ROSTELLUM AND SUCKERS

The rostellum of the larva of *M. multiceps* is composed of a small, cone-shape, rostellar prebulb (26×30 μm) and covered with a hypertrophied tegument of a thickness of 12—16 μm. The bulb is made up of muscle fibrils and varies in shape. A rostellar pad of various shape forms a narrow ring around the bulb. The spacious cavity above the rostellum originates in that small lateral extensions from the basal part of the wall of the spiral canal do not come up close to the prebulb. The prominent hypertrophied tegument of the prebulb and of the lateral extensions bulges into the cavity and fills it to some extent only (Plate I, Fig. 1). The rostellum of the larva of *M. endothoracicus* differs from that of the larva of *M. multiceps* in the bigger size of the prebulb (38×48 μm), and in a thinner tegument (6.1—9 μm). Large lateral extensions come close to the prebulb, leaving above the rostellum a small slit-shaped cavity filled with hypertrophied tegument. (Plate I, Fig. 2). Both the rostellar pad and the bulb are very big and almost twice the size of these organs in *M. multiceps*. Differences in the character of the rostellum of the two larval species have been derived from a different number and size of the hooks. The larva of *M. multiceps* possesses less and smaller hooks than the larva of *M. endothoracicus*. The hypertrophied tegument of the larva of *M. multiceps* contains globular structures measuring 1.5 μm in diameter. Although these structures, and also bigger ones (up to 2.6 μm) are present in the tegument and in the cells of the rostellar prebulb of the larva of *M. endothoracicus*, they are absent in the hypertrophied tegument of the rostellum at the time of hook formation.

Suckers of the larva of *M. multiceps* measure 0.46 mm in diameter. They contain longer and more densely organized myofibrils than the suckers of the larva of *M. endothoracicus* which measure 0.40 mm in diameter. Myofibrils of the suckers are transverse, circular and oblique. The oval nuclei are positioned in the direction of the course of the myofibrils. The surface of the suckers is covered with a sheath made up of connective tissue which is thicker in larvae of *M. multiceps* than in those of *M. endothoracicus*. The spiral canal opens into the suckers. Its tegument is thickened at the site of entrance into the suckers and this part stains contrastly to the tegument of the remaining part of the spiral canal. At the site of its entrance into the suckers, the wall of the spiral canal has a denser parenchyma with less calcareous corpuscles and more ecxretory canals which fuse into the lateral excretory canal of the scolex below each sucker. The parenchyma situated in the vicinity of the suckers contains fibrils made up of connective tissue and of muscles of the receptaculum. Excretory vesicles and host cells entered by the parasite are visible in the lumen of the suckers.

B. HISTOCHEMISTRY OF THE SUCKERS AND THE ROSTELLUM

As disclosed in both larval species by histochemical tests, the tegument of the spiral canal in the area of the rostellum and the suckers stains contrastly to that covering the remaining part of the canal. It stains dark blue with Giemsa and gives a positive reaction with acid haematein, Luxol's blue and Sudan IIIB (Plate I, Fig. 3). The blue colour of these reactions indicates the presence of phospholipids. In reactions for SS- and SH groups of proteins, the tegument of this part stains similar to that of the remaining parts of the spiral canal. Its surface contains acid mucosubstances with HSO_3 groups. The thick tegument of the prebulb of the larval *M. multiceps* contains tyrosine, less tryptophane, histidine, phospholipids and less neutral mucopolysaccharides (Table 1).

After the differentiation of the hooks, the tegument contains, in addition to phospholipids and proteins with SS groups, PAS-positive globules which display a feeble meta-

chromasia with toluidine blue. With peracetic acid-aldehyde fuchsine, the globules give a weak positive reaction for tyrosine and SS groups of proteins. The thin tegument of the prebulb of the larva of *M. endothoracicus* contains tyrosine, tryptophane, histidine, proteins with SS groups and neutral mucopolysaccharides. After the differentiation

Table 1. Results of histochemical reactions in the rostellum of a larval *M. multiceps*

Reactions	Prebulb				Hypertrophied tegument after hook differentiation	Globules in the hypertrophical tegument
	microtriches	tegument	subteg. cells			
			plasma	nuclei		
PAS	–	+	+	–	–	+++
Acetylation 58 °C + PAS	–	–	–	–	–	–
Desacetylation + PAS	–	+	–	–	–	++
Best carmine	–	–	+	–	–	–
AB PAS	blue	pink	–	–	–	pink
AB pH 2.6	+++	–	–	–	–	+
Sakaguchi	–	+	–	–	–	–
Morel Sisley	++	+++	+	–	++	++
TK (Coupled tetrazonium test)	++	+++	+	–	+++	++
DMAB	+	+	–	–	–	–
DDD	–	–	–	–	–	–
DDD + thioglycollic acid	+	+	–	–	+++	+
Peracetic acid + aldehyde fuchsine	+	+	–	–	++	++
Luxol blue	–	++	–	–	+++	–
Chromatein + acid haematein	+	++	–	–	+++	–
Fettrot 7 B	–	–	–	–	–	–
Sudan black B	+	++	–	–	++	–

of the hooks, the hypertrophied tegument of the rostellum differs from that of the prebulb in the presence of phospholipids and in its more positive reaction for SS groups of proteins (Table 2). Globules are seen on the surface of the tegument of the prebulb and in subtegumental cells; they are PAS-positive, stain with the coupled tetrazonium reaction and can be demonstrated with Masson's trichrome and Mallory's PTH reaction. The tegument of the suckers of both larval species contains phospholipids, less neutral lipids, it is PAS-positive even after desacetylation, positive for tyrosine, tryptophane, histidine and arginine. It stains feebly in a reaction for SH groups of proteins, heavily in a reaction for SS groups of proteins. The muscle myofibrils of the suckers are positive for tyrosine, tryptophane, histidine and phospholipids. The suckers are PAS-positive; Best's reaction discloses a large quantity of glycogen. They give a feeble reaction for proteins with SH groups and a negative reaction for proteins with SS groups. The nuclei of the suckers are positive for phospholipids and tyrosine. The connective tissue sheath of the suckers is highly argyrophile; it stains faintly in a reaction for neutral lipids, strongly for phospholipids, tyrosine, tryptophane, histidine, and is PAS-positive (Table 3).

C. THE FINE STRUCTURE OF THE ROSTELLUM AND THE SUCKERS

The tegument of the rostellar prebulb of both larval species is covered with long, fine, microtriches which have a partly reduced thin point and a thin base. The length of the microtriches varies; sometimes, it is more than 4 μm, sometimes it ranges from 1—2 μm. Microtriches of the larva of *M. multiceps* have a very thin (0.2 μm), spine-like, elongate point which is feebly electron-dense at the site of its entrance into the base. The microthrix border of the larva of *M. endothoracicus* contains secretory, highly electron-dense, globules situated in the evaginations from the distal cytoplasm. The evaginations are covered with a plasmic membrane. Globules are located in the basal part of the evaginations which are electron-lucid and do not contain bodies of the distal cytoplasm (Plate II, Fig. 1). The globules disappear after the differentiation of the hooks

Table 2. Results of histochemical reactions in the rostellum of a larval *M. endothoracicus*

Reactions	Prebulb					Hypertrophied tegument after hook differentiation	Globules on the tegument
	microtriches	tegument	subteg. cells				
			plasma	nucleus	globules		
PAS	−	+++	+	−	+++	+	+++
Acetylation 58 °C + PAS	−	−	−	−	−	−	−
Desacetylation + PAS	−	++	−	−	++	−	++
Best carmine	−	−	++	−	−	−	−
AB + PAS	blue	pink	pink	−	pink	blue	pink
AB ph 2.6	++++	+	−	−	+	++	+
AB + methylation	−	−	−	−	−	−	−
AB + demethylation	++	−	−	−	−	+	−
Sakaguchi	−	++	−	−	−	−	−
Morel-Sisley	+++	++++	+	−	++	++	++
Tk (Coupled tetrazonium test)	+++	++++	+	+	++	+++	++
DMAB	+	++	−	−	−	+	−
DDD	−	++	+	−	−	+	−
DDD + thioglycollic acid	++	++	+	−	+	+	+
Peracetic acid + aldehyde fuchsine	++	++	+	−	++	+	++
Aldehyde fuchsine	+	+	−	−	++	+	++
Luxol blue	−	−	−	−	−	++++	−
Chloroform extraction + Luxol blue	−	−	−	−	−	+	−
Chromation + acid haematein	+	+	−	−	−	+++	−
Fettrot 7 B	−	−	−	−	−	+	−
Sudan black B	+	−	−	−	−	+++ (blue)	−

from the tegument of the rostellum of *M. endothoracicus*. The slit representing the remnant of the cavity of the spiral canal, which is filled with a hypertrophied tegument, contains in its lumen globules inside blebs separated from the tegument by cutting sections (Plate II, Fig. 2). The tegument of the rostellum contains electron-dense rod-shaped bodies, larger oval bodies and occasional electron-lucid vesicles. In the larva of *M. multiceps*, the distal cytoplasm of the tegument of the prebulb is heavily vacuolated and contains mainly larger oval bodies. The microtriches are slender with a long point

measuring 2—3 μm in length. Among the microtriches there are irregularly shaped, feebly electron-dense structures with numerous vesicles, which are not bound by a plasmic membrane (Plate II, Fig. 3). After the differentiation of the hooks, the tegument of the rostellum contains vacuoles, vesicles, electron-dense globules and layered, membranous structures (Plate II, Fig. 4). In the vicinity of the hooks of both larval species, the hypertrophied tegument contains tubular structures filled with an electron-dense substance (Plate III, Fig. 1). In the larva of *M. multiceps*, these tubes are thickened and very big; after their contents have been used up, they change into membranous structu-

Table 3. Results of histochemical reactions in the suckers of larval *M. multiceps* and *M. endothoracicus*

Reactions	Suckers				
	myofibrils	nuclei of myofibrils	tegument outer layer	tegument inner layer	Connective tissue sheath
PAS	+++	–	++	+	+++
Schiff	–	–	–	–	–
Acetylation 58 °C + PAS	–	–	–	–	–
Desacetylation + PAS	++	–	++	+	+++
Best carmine	++++	–	–	–	–
Saliva test + Best carmine	–	–	–	–	–
AB PAS	pink	–	pink	blue	pink
AB pH 2.6	–	–	–	+++	–
AB + methylation	–	–	–	–	–
AB + demethylation	–	–	–	+	–
Sakaguchi	+	–	–	+++	+
Morel Sisley	++	+	+++	+++	+++
TK (Coupled tetrazonium test)	+++	++	+	+++	+++
DMAB	+	–	+	+++	+
DDD	+	–	++	–	–
DDD + thioglycollic acid	–	–	++	+++	–
Peracetic acid + aldehyde fuchsin	–	–	+	++	–
Aldehyde fuchsine	–	–	+	+	–
Luxol blue	+++	++	+	++++	+++
Chromatein + acid haematein	+++	+	+	+++	+++
Fettrot 7 B	–	–	–	++	–
Sudan black B	++	–	–	++	++ (blue)

res (Plate II, Fig. 4). The thickness of the connective tissue sheath on the suckers of *M. endothoracicus* is 3.2 μm. It forms invaginations among the muscle myofibrils (Plate II Fig. 3). The sheath covering the suckers of the larval *M. multiceps* is smooth, its thickness is 1.8—2.3 μm. However, this feature might have been influenced by a different degree of shrinkage during fixation. The granulated sarcoplasm of the deeper set nuclei forms evaginations among the myofibrils. The thickness of the sarcolemma on the surface of the myofibrils is 7 nm. The sarcoplasm of the myofibrils contains an appreciable quantity of beta glycogen and a small number of lipid bodies. Mitochondria can be seen below the sarcolemma. The myofibrils of the larval *M. multiceps* are longer and denser organized than those of the larva of *M. endothoracicus*, and between them there are large

fibrillar spaces of interstitial tissue containing beta particles of glycogen (Plate III, Fig. 4). The cell nuclei are oval with an accumulation of chromatin beneath their membrane. The perinuclear cytoplasm contains numerous ribosomes, vesicles and RES cisternae (see Inset-Plate IV, Fig. 1). The sarcoplasm contains glycogen (Plate IV, Fig. 1). The tegument of the suckers of the larva of *M. endothoracicus* is composed of a distal cytoplasm containing rod-shaped bodies; it is separated from the myofibrils by a thick membrane of connective tissue which forms invaginations into the distal cytoplasm. It is underlain by circular myofibrils (Plate IV, Fig. 2). The tegument of the suckers of the larva of *M. multiceps* is vacuolated and contains ovoid bodies. The connective tissue membrane beneath the distal cytoplasm is thin (Plate IV, Fig. 3). The myofibrils are densely organized and have numerous myoblast nuclei among them. The sarcoplasm contains mitochondria, ribosomes and vesicles of different sizes. The base of the microtriches on the sucker of a larval *M. endothoracicus* attains a thickness of up to 0.6 μm, the microthrix point is short and thick. The length of the base (2—3 μm) is in proportion to the length of the point (2.5—3 μm). A clearly visible treble membrane separates partly the point from the base. The points are slightly bent in direction to the base. Secretory vesicles are present among the microthrix points (Plate IV, Fig. 3). The microtriches on the suckers of *M. multiceps* are bigger and thicker than those of *M. endothoracicus*. Their base measures 0.8 μm in width at a height of 3.5 μm, the length of the points ranges from 3.5—4 μm. Secretory vesicles are present among the microtriches (Plate IV, Fig. 4).

DISCUSSION

Our study disclosed morphological differences in the scoleces of two larval species of the genus *Multiceps*. Morphological differences in the shape and size of the rostellum might evidently have been derived from differences in the number and the size of hooks of the two larval species as evidenced by their fine structure and their histochemistry. By contrast to *M. endothoracicus*, the hypertrophied tegument of the rostellum of the larval *M. multiceps* is more densely packed with larger tubes issuing a substance at the time of hook differentiation (Hulínská and Fedoseenko 1977). After the completion of hook differentiation, the tubes change in a system of vacuolated and membranous structures as this has been observed in larvae of *M. multiceps* but not in those of *M. endothoracicus*, in which these tubes become merely vacuolated. Globules evidently specialized for secretory activities have been demonstrated in the rostellar tegument of both species. According to Hyman (1951), secretory activities might not be a common feature of the rostellum of larval Taenioidea because glands are absent in it. Smyth (1964) and Jha and Smyth (1971) pointed out that secretory activities in the adult cestode were dependent on the existence of rostellar glands. Sometimes, the secretory substance released from the glands is typical in its nature of acid mucosubstances and protects worms penetrating into the intestinal mucosa. Šlais (1958) confirmed the existence of these glands for *Davainea proglottina*. The same author (Šlais 1961) demonstrated glands in *Hymenolepis parvula* and *Aploparaksis furcigera*, but the effect of the secretory substance released by them was rather lytic in nature and destructive to the mucosa. Smyth (1964) studied rostellar glands of *Echinococcus granulosus* important for the evaginated scolex in the attachment to the intestinal mucosa. The fine structure of the tegument and rostellar glands and their differentiation in the protoscolex has been reported by Jha and Smyth (1971). Shield et al. (1973) demonstrated large "secretory" cells in the cysticercus of *Taenia pisiformis* on day 6 of its development. Having traced changes in the glands and their location for 10 days, the authors concluded that they are of importance in the initial period of migration of the larvae in the liver.

At the time of scolex differentiation of *M. endothoracicus*, we found globules in rostellar cells which were positive for tyrosine and PAS. They were present in evaginations of the cytoplasm arising above the surface of the rostellum, but not in the distal cytoplasm. The globules were highly electron-dense and similar to those in subtegumental cells observed in an earlier study (Hulínská and Fedoseenko 1977). Shield et al. (1973) pointed out that even the tegument might be an important secretory organ. The development and proliferation of PAS-positive globules inside the cells was associated with functional changes in the tegument. The presence of globules in lobate microtriches of a 25 day-old *C. pisiformis* was suggestive of secretory activities. Also in our case, the presence of globules might be construed as indicative of secretory activities. In the larva of *M. multiceps*, globules were present in the hypertrophied tegument of the rostellum and not in the tegument of the prebulb and in the cells. A substance containing acid mucosubstances was demonstrated even in the basal part of the spiral canal and above the prebulb. Featherston (1975) maintained that microtriches on the hook organ and on the surface of the rostellum were connected transversely in several cysticerci, and that these microtriches might sometimes be found on the suckers (Featherston 1972). Similar microtriches were not observed by Morseth (1966). Jha and Smyth (1971) recorded branched microtriches forming a network on the surface of the rostellum of *Echinococcus granulosus*. The microtriches on the surface of the rostellum of our two larval species were slender and long, their point was not sharp, but rather obtuse. The suckers of the two species differed in the organization and size of the myofibrils which were denser and longer in the larva of *M. multiceps*. Waitz (1963), Erasmus (1957a, b) and Hulínská et al. (1976) demonstrated with histochemical tests enzymatic activities of proteins in the suckers. The suckers of both larval species gave a positive reaction for tyrosine, a less marked reaction for tryptophane and arginine; they contained phospholipids and a large quantity of glycogen. Proteins of the SH and SS groups were demonstrated in the connective tissue sheath of the suckers and in the tegument. Lumsden and Byram (1967) reported the presence of rosette-shaped structures of alpha glycogen in addition to beta particles of glycogen in the acetabulum of adult cestodes. We found beta particles of glycogen in the sarcoplasm of the myofibrils of the suckers of *M. multiceps*, and in the large fibrillar spaces between them. The quantity of glycogen was lower in the larva of *M. endothoracicus* which might be ascribed to a variability in the contents of the fine, fibrillar, interstitial tissue composing the stroma of the muscle fibres. The tegument of the suckers of the two species differed in the character of the distal cytoplasm and in the size of microtriches. The distal cytoplasm of the larval *M. multiceps* was vacuolated and its structure resembled that of the tegument of *M. serialis* (Race et al. 1965). The distal cytoplasm of the suckers and the rostellum of the larval *M. endothoracicus* contained rod-shaped bodies, it was not vacuolated and was similar to the tegument of cysticerci (Lumsden 1966a, b, Baron 1968). The suckers of both species contained secretory vesicles and bleb-like structures as these have been recorded for various parts of the body of larval cestodes by Threadgold (1962, 1965), Ubelaker et al. (1970), Featherston (1972). In our opinion, secretory activities might be part of the defensive mechanism of the larva in its reaction to host cells entering the invaginated canal.

СРАВНЕНИЕ ГИСТОХИМИИ И ТОНКОЙ СТРУКТУРЫ ПРИСОСОК И ХОБОТКА ЛИЧИНОК *MULTICEPS MULTICEPS* LESKE, 1780 И *MULTICEPS ENDOTHORACICUS* KIRSCHENBLATT, 1948

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Резюме. Обнаружены вариации в гистохимии и тонкой структуре хоботка и присосок зрелых сколексов двух полицефальных личинок. В ободке микротрихий тегумента хоботка

и в клетках хоботка личинки *M. endothoracicus* обнаружены сферические образования, содержащие белки и микополисахариды. Эти образования не обнаружены в тегументе хоботка личинки *M. multiceps* содержащем вакуоли. Тегумент личинки *M. endothoracius* не содержит вакуолей, а главным образом палочковидные тельца. Тегумент хоботка этих двух видов личинок отличается по густоте миофибрилл, по содержанию гликогена и по размерам микротрихий. В тегументе присосок и хоботка выявлена секреторная активность.

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Received 2 December 1977.

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EXPLANATION TO PLATES

A — microtriches
B — plasmic membrane
C — distal cytoplasm
D — rod-shaped bodies
E — vacuoles
F — oval bodies
G — electron-dense secretory globules
H — extensions of the distal cytoplasm
I — tubes
J — membraneous structures
K — ribosomes
L — hook
M — myofibrils
N — glycogen in the interstitial spaces
O — beta glycogen
P — mitochondria
Q — connective tissue sheath
R — vesicles
S — nucleus
T — cisternae RES
U — lipids
V — rostellar bulb
X — suckers
Y — hooks
Z — tegument of the spiral canal

FOLIA PARASITOLOGICA (PRAHA) 25: 255—256, 1978.

EXPERIMENTAL INFECTION OF CHICKENS WITH LEDNICE (YABA 1) VIRUS

The Lednice (Yaba 1) virus — now M'Poko (see the 1976 Annual Report on the Catalogue of Arthropod-borne and Selected Vertebrate Viruses of the World) has a significant relation to birds (Kolman J. et al., Folia parasit. (Praha) 23: 251—255, 1976, Danielová V., Málková D., Folia parasit. (Praha) 23: 367 to 372, 1976, Málková D., Danielová V., Folia parasit. (Praha) 24: 382—384, 1977). In previous reports it was already ascertained that chickens do not perish either after scut or ic infection with virus (Málková D. et al., Folia parasit. (Praha) 21: 363—373, 1974), but the death of the chicken embryos was observed both after inoculation into the yolk sac and the chorionallantois (Málková D., Kolman J., Čs. epidemiol. mikrobiol. imunol. 24: 225—230, 1975). The present paper completes the findings about capability of viremia and antibody formation in chickens and elucidates thus their role in the circulation of virus in nature.

One-day-old chickens were inoculated scut into the occipital part of the head with virus strain 6118, which underwent two mouse passages. The first group obtained a dose of virus 3.83 log mouse ic LD_{50} and the second one 1.83 log mouse ic LD_{50} per one chicken. There were 5 chickens in each group. Viremia was investigated at 24 hour's intervals since the last till the 5th day, then the 7th and 10th day p.i. Blood was collected from the tibial vein into heparin. Pools of blood from 5 birds of the respective group were immediately inoculated ic to litters of 1—2 day-old suckling mice in the amount of 0.01 ml. Inoculated mice were observed in experiment for 14 days. — Neutralizing antibody production was ascertained 3 and 6 weeks p.i., individually in each bird. Antigen in 10-fold dilutions was added to the plasm diluted by phosphate buffered saline in ratio 1 : 2. After incubation of the mixture "plasm + antigen" for 60 minutes at 37 °C, each dilution was inoculated to 1 litter of 1—2-day-old suckling mice ic per ,0.01 ml. Observation time of mice in experiments was 14 days. Virus titres were calculated according to Reed and Muench.

As may be seen from Table 1, viremia occurred in chickens after both doses of virus. In case of animals inoculated by a larger dose of virus (3.83 log LD_{50}), the first detection of viremia was 48 hours p.i., in case of inoculation by a smaller dose of virus (1.83 log LD_{50}) not until 72 hours p.i. The peak of viremia in both cases was the 5th day p.i. Virus titres were relatively low and did not exceed 1.5 log LD_{50}/0.01 ml.

As for neutralizing antibody production, it was ascertained that a significant antibody formation occurred after both doses of virus as early as the 3rd week p.i.

From development of virus titres in blood it may be concluded that the virus replicates in the organism, though to low titres only. With regard to the fact that it is not yet clear, whether the virus titres in the blood of the host amount-

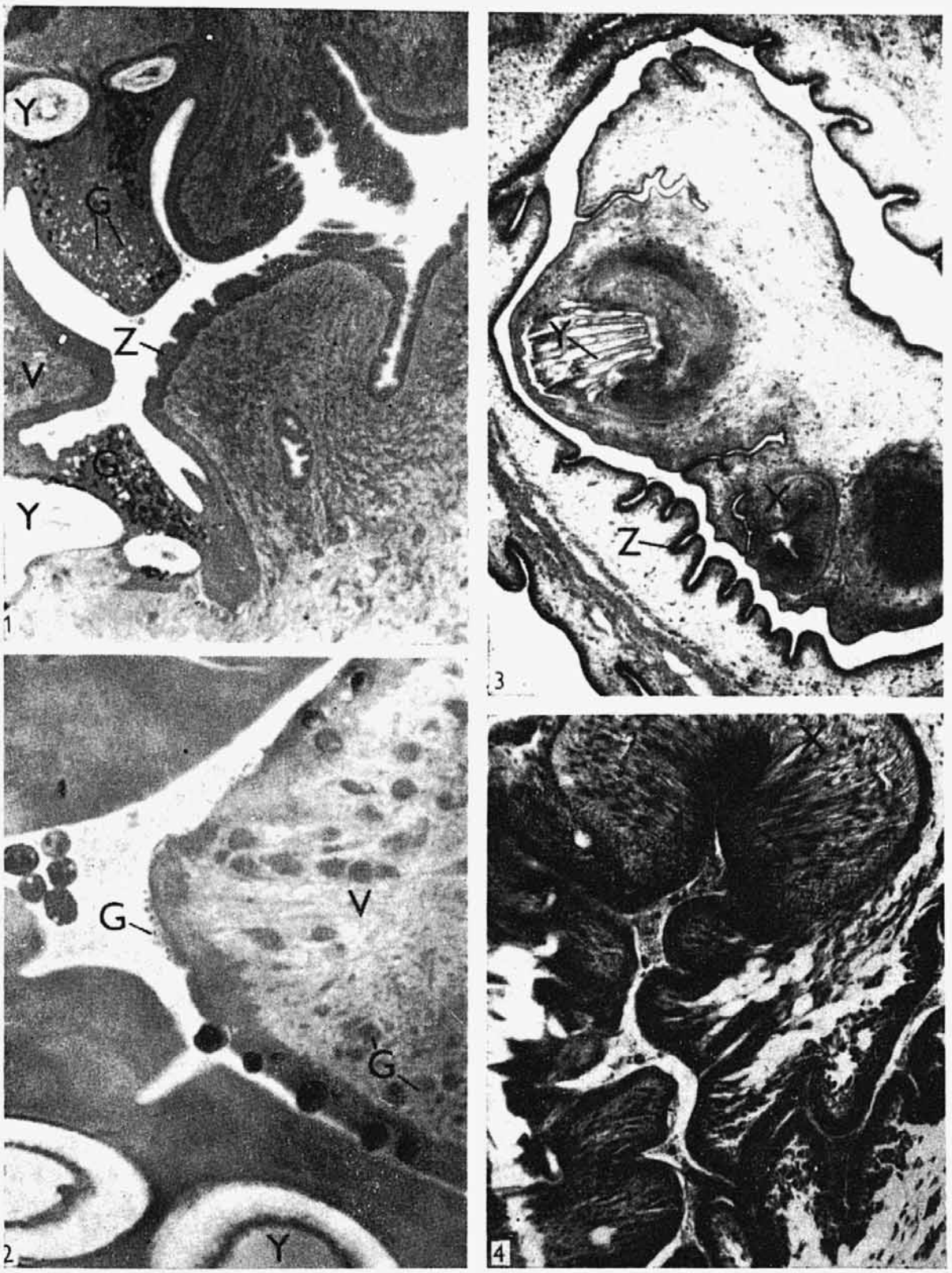
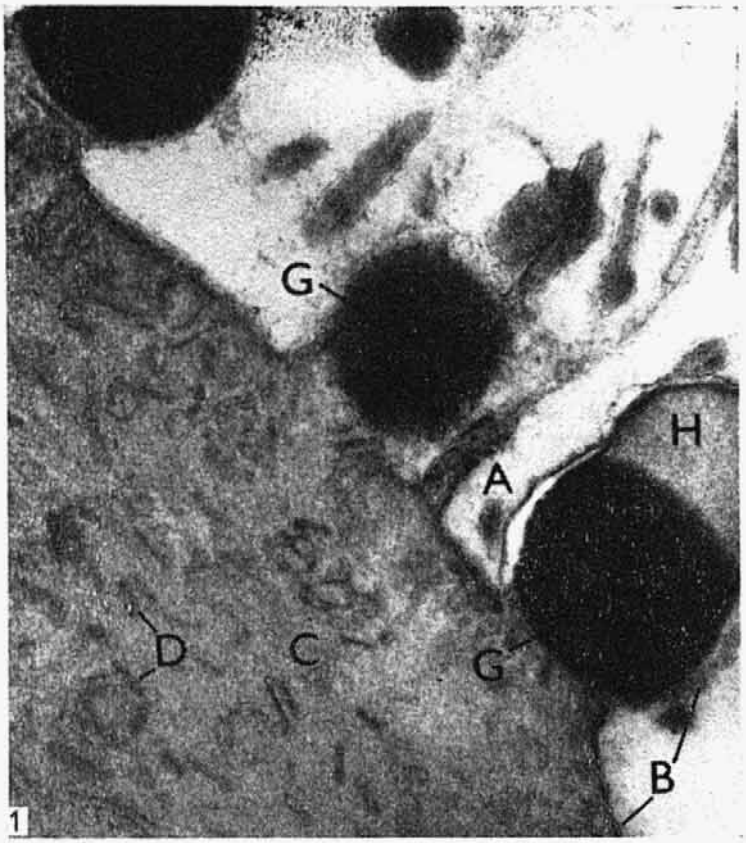
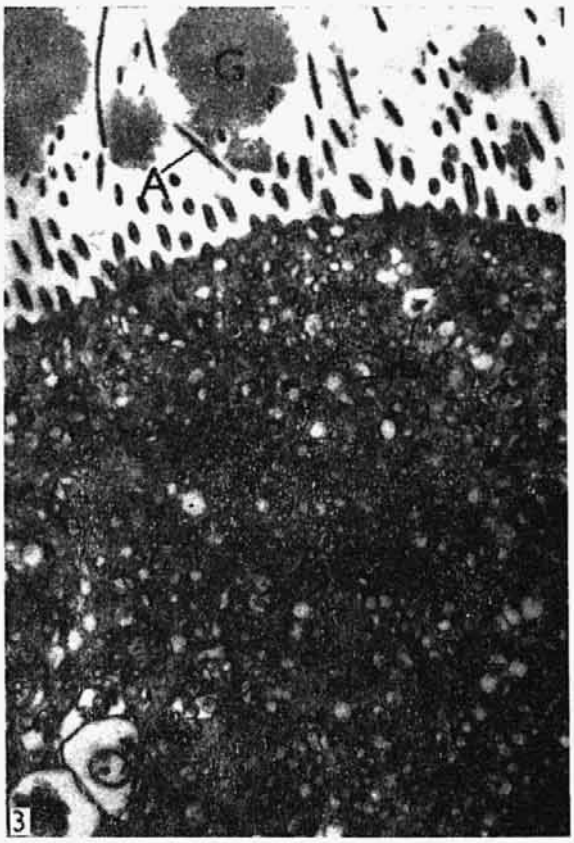
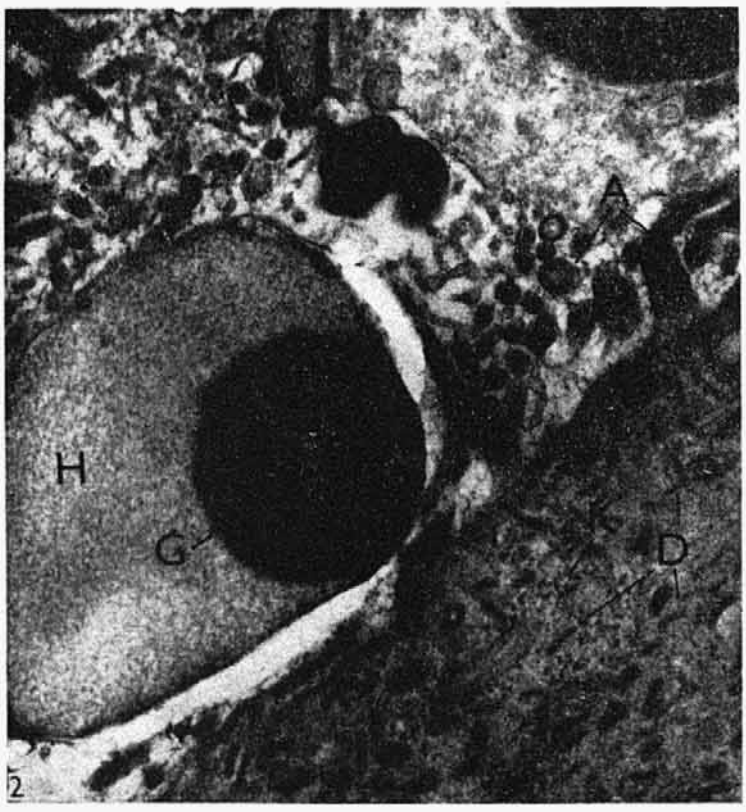


Fig. 1. Part of the small rostellar prebulb of a larval *M. multiceps* and the prominent hypertrophied tegument with hooks overlaying lateral extensions from the wall of the basal part of the spiral canal. Distinct globules with metachromasia in the hypertrophied tegument. Globular structures on the surface of the tegument and in the lumen of the canal (Toluidine blue, 165×).
Fig. 2. Part of the large rostellar prebulb of a larval *M. endothoracicus* with a thick tegument and globular structures on its surface. Host cells are seen in the lumen of the slitlike cavity occupied by the hook organ. (Coupled tetrazonium reaction, 625×). **Fig. 3.** Phospholipids in the tegument of the spiral canal and in that of the outgrowing scolex of *M. endothoracicus*. The opening of the canal into the suckers is situated close to the ovoid rostellar bulb (Luxol blue, 125×). **Fig. 4.** A granular and fibrillar secretory substance is seen in the lumen of the spiral canal of a larval *M. multiceps*. The canal opens into the suckers. The nuclei of the suckers are positioned in the direction of the course of the myofibrils. Of the two layers distinguished in the tegument of the suckers, the inner layer is more positive for tyrosine (Coupled tetrazonium reaction, 125×).







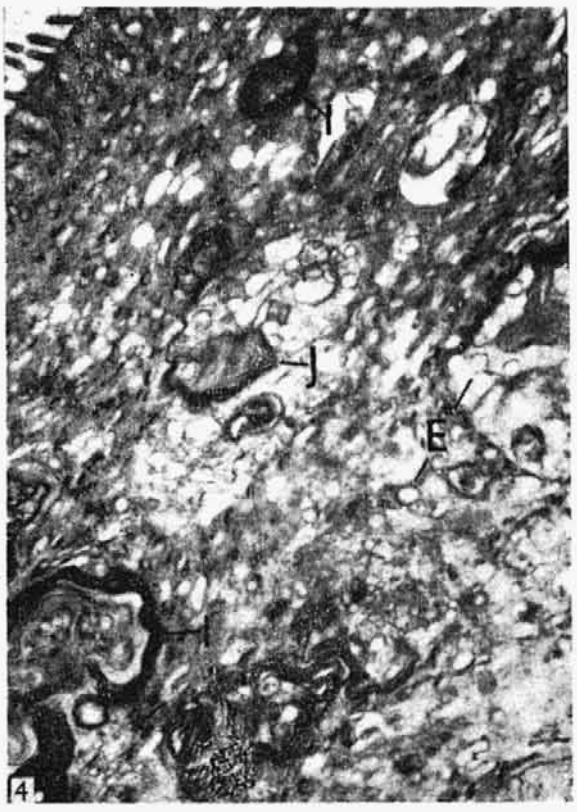
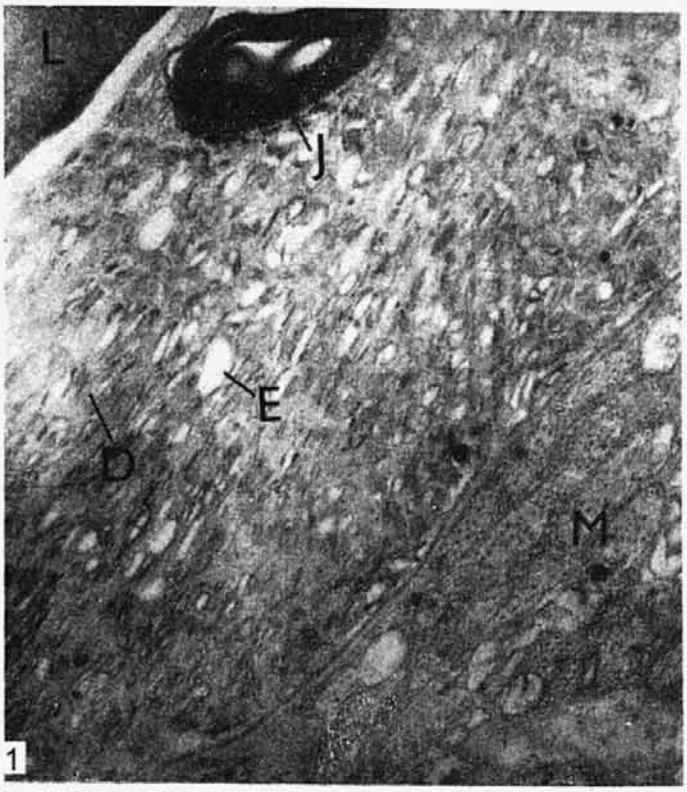
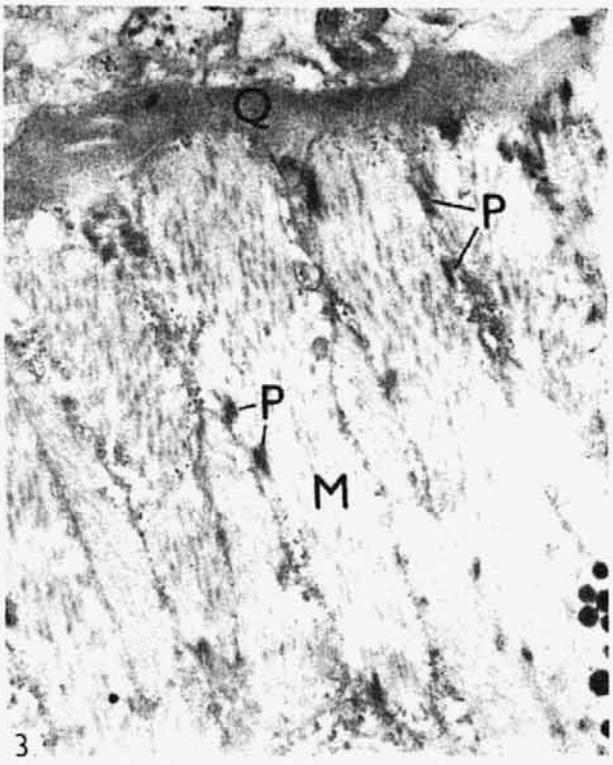


Fig. 1. Globules are seen among the fine microtriches in evaginations of the distal cytoplasm of the rostellar tegument of *M. endothoracicus*. The distal cytoplasm contains rod-shaped bodies and an occasional mitochondrion (23 075×). **Fig. 2.** The micrograph shows a bleb-like structure with an electron-dense substance condensed into the shape of a globule. In its vicinity are vesicles and a dense substance accumulated between the microtriches. The distal cytoplasm contains mainly rod-shaped bodies and an occasional vesicle. (20 000×). **Fig. 3.** The tegument of the rostellar prebulb of a larval *M. multiceps*. The slender microtriches have fine, sharply elongate points. The substance between them is less electron-dense and condensed into unbordered structures. The vacuolated distal cytoplasm contains oval bodies and vesicles (11 540×). **Fig. 4.** The tegument of a larval *M. multiceps* after the differentiation of the hooks is vacuolated and contains numerous membranous structures (15 380×).





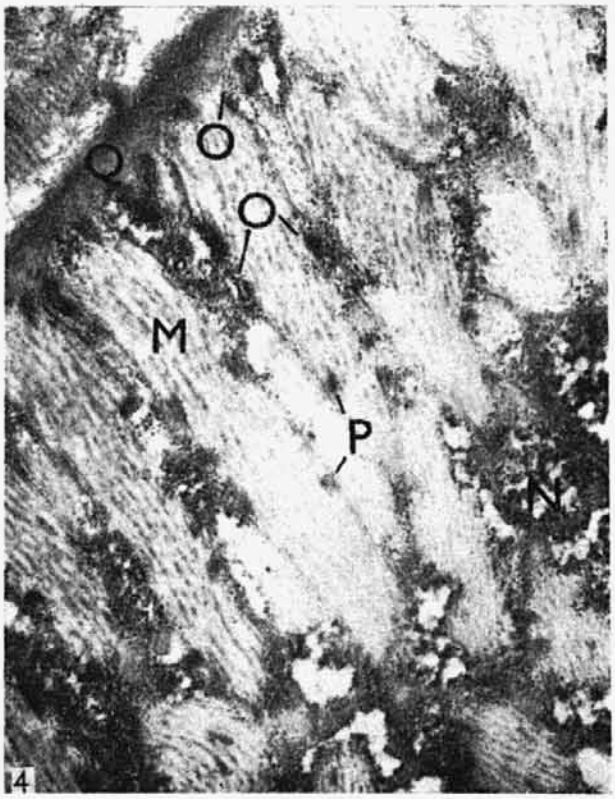


Fig. 1. The hypertrophied tegument of *M. endothoracicus* after the differentiation of the hooks. The tegument on the lateral extensions of the canal wall is separated from the muscles of the wall by a fine membrane. The cytoplasm contains vacuoles and rod-shaped bodies. A tube located in the vicinity of the hook is filled with an electron-dense substance (11 540×). **Fig. 2.** After the differentiation of the hooks, the hypertrophied tegument of *M. multiceps* contains vesicles and vacuoles. Close to the hook there are large tubular structures with an electron-dense substance: further away from the hook there are layered membranous structures only (11 540×). **Fig. 3.** The thick connective tissue sheath on the sucker of a larval *M. endothoracicus* produces invaginations among the muscle myofibrils. The myofibrils are composed of parallelly organized myofilaments. Beta particles of glycogen and lipid bodies are seen in the sarcoplasm (11 540×). **Fig. 4.** The sucker of a larval *M. multiceps* is made up of densely organized myofibrils; between them are extensions of the sarcoplasm containing beta glycogen. Also the larger fibrillar spaces are filled with glycogen (11 540×).

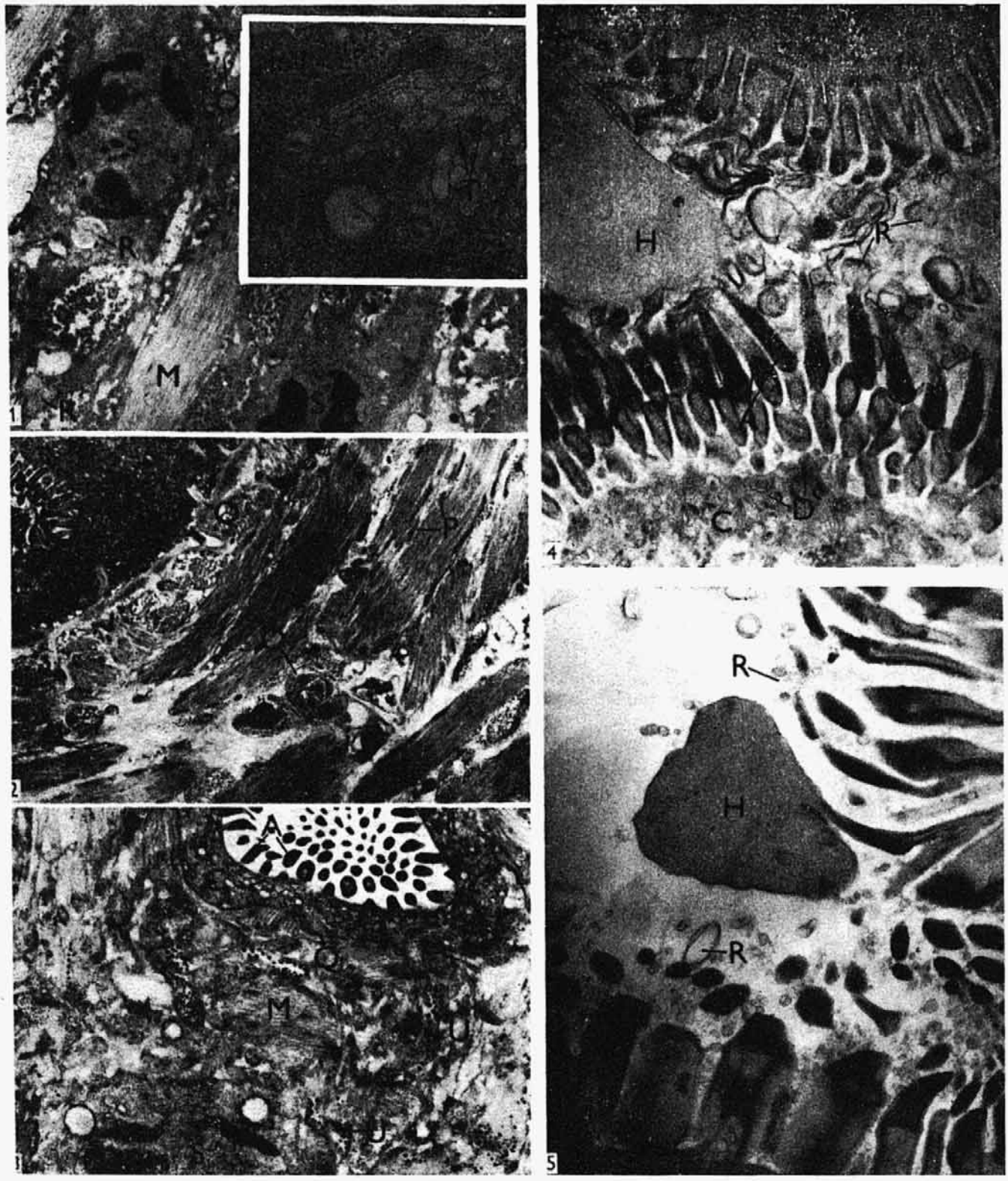


Fig. 1. The micrograph shows cells of the sucker of *M. multiceps*. The sarcoplasm of the cells enveloping vesicles, ribosomes and glycogen particles, forms extensions among the myofibrils. The cell nuclei contain chromatin aggregations beneath the nucleic membrane (11 540×). Inset: The granular, reticulo-endothelial system of muscle cells and the Golgi apparatus (23 075×). **Fig. 2.** The tegument of the sucker of *M. endothoracicus*. The distal cytoplasm is separated from the muscle layer by a thick layer of connective tissue which forms invaginations into the distal cytoplasm and among circular muscle fibrils. (7 700×). **Fig. 3.** The tegument of the sucker of *M. multiceps* is separated from the muscle layer by a fine membrane. Nuclei and the vacuolated sarcoplasm with glycogen particles can be seen among the myofibrils (7 700×). **Fig. 4.** Microtriches on the sucker of *M. endothoracicus* have a thick base and a thick point bending slightly in direction of the base. Numerous vesicles and bleb-like structures filled with a secretory substance are present among the points (11 540×). **Fig. 5.** Microtriches on the sucker of *M. multiceps* have a thick base and a short point. The partial separation of the point from the base by a tri-layered membrane is visible. Among the microthrix points are secretory veicles and bleb-like structures (11 540×).