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Studies on Dicyemid Mesozoa
I. The fine Structure of the Adult (The Nematogen and Rhombogen Stage)

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STUDIES ON DICYEMID MESOZOA
I. THE FINE STRUCTURE OF THE ADULT (THE NEMATOGEN AND RHOMBOGEN STAGE)

With plates XXI–XXVI

By

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Introduction.

The dicyemid mesozoa form a small and systematically isolated group. Its species all live in the renal organs of cephalopods.

VAN BENEDEN (1876), who was one of the first to study these animals, believed them to be a link between protozoa and metazoa, hence gave the name mesozoa to the group. The group Orthonectida, which was studied in some detail only later and which parasitise many invertebrate groups other than cephalopods is also included in the group.

After van Beneden, many authors notably Nouvel and Mcconaughey have studied the dicyemids and have elucidated the extremely complex life cycle and morphogenesis of the group. The pertinent litterature has most recently been reviewed by CZIHAK (1958), MCCONAUGHEY (1951, 1963) and NOUVEL (1947, 1948) where also all hitherto known details concerning the dicyemids can be found. The life cycle and morphology as revealed by the light microscope will here only be summarised shortly.

The newly hatched cephalopods are allways free from dicyemids. At a very young age however the cephalopods become infected, the source of infection still being unknown. In the kidneys of these young hosts a stage called stem nematogen is found.

A stem nematogen consists of three axial cells covered by a number of trunk cells of which the anterior are called calotte cells and the posterior uropolar cells. The number of trunk cells is constant within each species.

Within the axial cell germinal cells the so called axoblasts are found. The axoblasts divide mitotically forming multicellular embryos (vermiforme larvae) lying in the cytoplasma of the axial cell. When the embryos are mature they emerge from the parent and are now called the primary

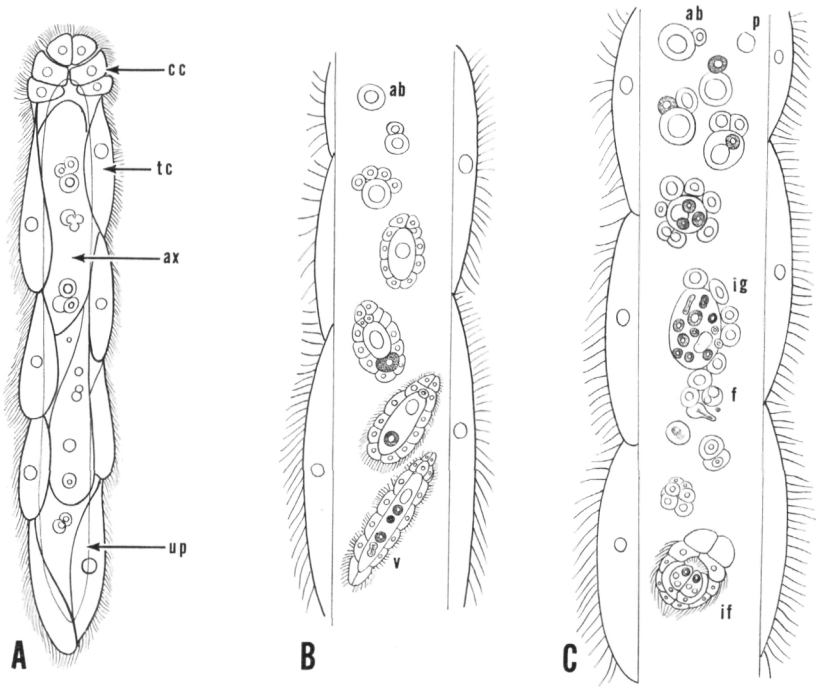

Fig. 1. A: rhombogen; B: development of a vermiform larva; C: development of a infusorigen and a infusoriform larva. ab: axoblast; ax: axial cell; cc: calotte cell; f: fertilization and emission of polar bodies; if: infusoriform larva; ig: infusorigen; p: paranucleus; tc: trunk cell; up: uropolar cell.

nematogens. This stage resembles the stem nematogen with the exeption that it possesses only one axial cell. Within the axial cell axoblasts are found which give rise to several generations of primary nematogens, colonising the renal organ of the host.

The development from axoblast to nematogen (fig. 1, B), a process which is entirely agametic initiates with a mitosis resulting in a big and a small cell. The small cell passes through a number of divisions and the resulting cells cover the big cell. These cells are the future trunk cells of the nematogen. In the genus *Dicyema* the big cell passes through only one mitosis resulting in an oblong anterior cell, the future axial cell and a smaller posterior germinal cell. The latter is engulfed by the former. Inside the axial cell it undergoes a number of mitotic divisions forming axoblasts.

In the genus *Dicyemennea* the large central cell of the embryo passes through two divisions resulting in two axial cells and one germinal cell. Usually however, one of the axial cells is later absorbed.

It is a characteristic and peculiar feature in the dicyemids that embryos develop intracellularly and that several generations are formed inside each other. Thus the axial cell of a mature nematogen contains multicellular embryos the axial cell of which contains axoblasts representing the third generation.

When the cephalopod host becomes sexually mature the nematogens are transformed into rhombogens (fig. 1, A) or axoblasts may develop directly into rhombogens. These differ from the nematogens mainly in the number of axoblasts being reduced so that only a few and large ones remain in the axial cell. These axoblast may either develop new nematogens (secundary nematogens) or may develop sexual hermaphroditic individuals, the so called infusorigens which never leave the axial cell. In the latter case (fig. 1, C) the development is initiated by a nuclear division inside the axoblast and one of the resulting nuclei, the paranucleus is rejected into the cytoplasma of the axial cell. The axoblast then undergoes two divisions resulting in three cells of unequal size. The smallest cell is engulfed by the largest. When inside the large cell, the small cell passes through a number of mitotic divisions. The resulting cells then undergo spermatogenesis and form small tailless sperm.

The third cell of intermediate size also undergoes a number of divisions forming egg cells. These lie on the surface of the large cell containing the sperm cells. The latter move to the surface of the infusorigen and attach to the egg cells which are then dispersed into the axial cell of the parent. Here the sperm cell penetrates the egg which then gives off polar bodies, and the first cleavage is initiated.

The zygotes develop into small ciliated ovoid larvae, the infusoriforms. These are among others characteristised in possessing a ciliated cavity, the urn, which contains four binucleate cells each containing a small (germinal?) cell. When mature, the infusoriforms emerge from the parent and escape to the surrounding water with the urine of the host.

The fate of the dicyemids from leaving their cephalopod host as infusoriforms until they reappear in young cephalopods as stem nematogens is not known. It is however believed that there is an intermediate host since it has not been possible to infect young uninfected cephalopods with free swimming infusoriforms.

It was found of considerable interest to study the dicyemids with the aid of modern cytological techniques such as the electron microscope and the histochemical methods. Such studies may improve our understanding of the remarkable morphogenetical processes and the biology of the dicyemids such as the food uptake from the kidneys of the host and the

food uptake of the embryos from the axial cell. Also it was found possible that knowledge of the fine structure may give a clue concerning the systematical position held by the mesozoa.

Hence the present paper is the first in a series dealing with the dicyemid mesozoa. In the present paper the fine structure of the adult stages viz. the nematogen and rhombogen is described. Following papers will describe the development of the embryos with the aid of the electron microscope and the histochemistry of the dicyemids.

The work was performed at the Institute of General Zoology, University of Copenhagen. Our gratitude is due to Dr. K. J. PEDERSEN and Mag. scient. E. J. FJERDINGSTAD for advice concerning electron microscopical technique. One of us who collected part of the material at Stazione Zoologica, Naples was supported by a grant from "Den danske komité for samarbejde med Stazione Zoologica Napoli" for which we here express our gratitude. This material will be treated in following papers.

Material and methods.

The material described in the present paper was collected at the Zoological Station of Kristineberg, Gullmarfjord, West coast of Sweden. The dicyemid *Dicyema truncatum* Whitman was obtained from *Rossia macrosoma* (delle Chiaje) and fixed in Palade. Methacrylate was used as imbedding medium. 300–800 Å thick sections were cut by an LKB microtome and stained in uranyl acetate or lead hydroxide. Potassium permanganate followed by citric acid as described by ANDRÉ (1961) was also used. The sections were studied with a Siemens electron microscope.

Results.

The outer surface of the trunk cells was found to be covered with a cell membrane which is folded to form irregular ridges with some anterior-posterior orientation (plate XXI, 1, 2; plate XXII, 3, 4). The height of the ridges is approximately 0.5 μ, they can be seen in stained sections in the light microscope as a fine "striated border". The ridges are strongly folded and convoluted so that it often appears as though several surface membranes are present in one cross section. In tangential sections near the surface the ridges have a regularly longitudinal orientation, at a greater distance from the surface the ridges are so folded and convoluted that they seem to be quite irregular (plate XXII, 4). The distance between the ridges is about 0.5 μ. In the ridges as well as in the cytoplasma beneath the surface, a great number of pinocytosis vesicles were observed, often arranged in chains (plate XXI, 2; plate XXII, 3; plate XXIV, 6).

The trunk cells are densely ciliated. The cilia are arranged in somewhat irregular longitudinal rows. In cross sections the cilia show the "nine plus two" structure with arms on the outer fibrilles (plate XXI, 2). In the basal granule the two central fibrilles are missing, and the outer fibrilles show a skew orientation. The so called "cart wheel" structure with spokes radiating from the center was also seen. These structures have been described from a variety of organismes (see GRIMSTONE, 1962).

The basal granules are connected somewhat asymetrically to ciliary rootlets (plate XXIV, 6–8) which run posteriad and parallel to the surface of the trunk cell. The ciliary rootlets are about 0.2 μ thick and cross striated with a periodicity of about 500 Å. We have no evidence that the ciliary rootlets connect with each other, they seem to end 1–2 μ behind the basal granule from which they originate passing laterally to the following basal granule.

Numerous vacuoles and mitochondria (plate XXIV, 6) were found in the cytoplasma of the trunk cells. The mitochondria were usually spheric and with short cristae. In the center of the mitochondria "parasitic mitochondria", as described by RUDZINSKA and TRAGER (1959) and by PAPPAS and BRANDT (1959), were often found. The presence of small cristae may have connection with the fact that the dicyemids live under anaerobic conditions. The infusoriform larvae, living part of their time in the free have larger cristae (plate XXIII, 5). In addition to mitochondria so called laminar bodies were found.

Nouvel described large crystals as well as other inclusions in the trunk cells. These were never observed by us in the material from the Gullmarfjord, neither in living or stained specimens nor in the electron microscope.

Between adjacent trunk cells cisterns often containing an electron dense substance were found (plate XXI, 1). Desmosomes were never observed.

The inner membrane of the trunk cells and the membrane of the axial cell form complicated intercellular spaces at several places (plate XXIII, 5).

The axial cell is nearly free from cytoplasmic structures, specially in individuals containing mature embryos. Some vacuoles and mitochondria are however always present. In sections of the axial cell an irregular network of lines composed of small electron dense particles was observed (plate XXI, 1). These lines were also pictured in earlier light microscopic accounts. We have no suggestions as to the nature of these structures.

As far as we could observe the axial cell does not form a membrane towards axoblasts and embryos (plate XXIII, 5; plate XXV, 9; plate

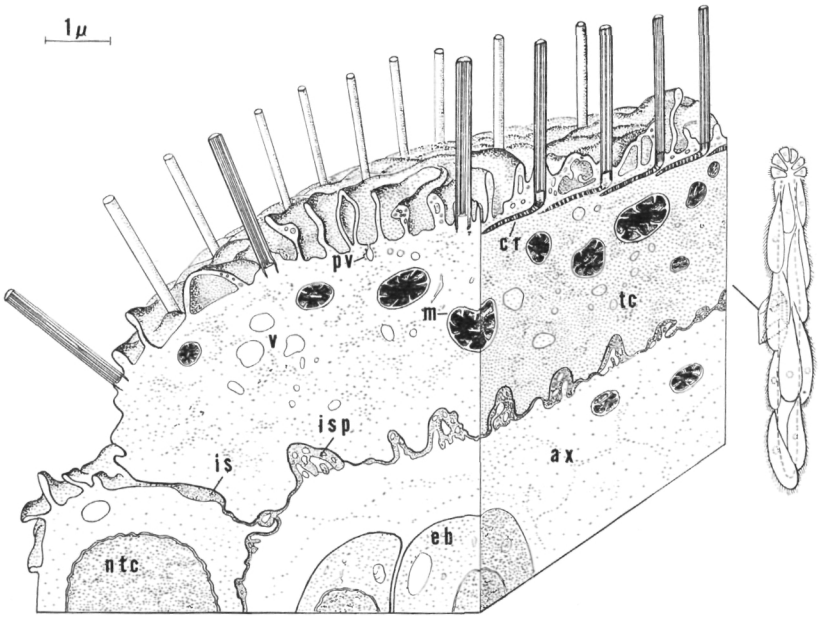

Fig. 2. Reconstruction of the morphology of a dicyemid as revealed by the electron microscope. ax: axial cell; cr: ciliary rootlet; eb: embryo in the axial cell; is: intercellular space between two adjacent trunk cells; isp: intercellular space between a trunk cell and the axial cell; m: mitochondria; ntc: nucleus of a trunk cell; pv: pinocytosis vesicles; v: vacuole.

XXVI, 10). The cytoplasma of the axial cell fits tightly against axoblasts and young embryos which have a smooth cell membrane (plate XXVI, 10). Embryos in a later stage of development possess cilia and complicated foldings of the cell membrane similar to those of the trunk cell of the adult, only in the embryos the foldings are much more dense (plate XXIII, 5; plate XXV, 9). These more mature embryos are found in a lumen in the cytoplasma of the axial cell. In living rhombogens the infusoriform larvae can be seen moving in this lumen.

Discussion.

Plasma membranes as those of the trunk cells with a greatly increased surface area as well as numerous pinocytosis vesicles have been described from a great variety of cells believed to have a high activity of absorption such as intestinal protozoa (for example *Opalina*, see NOIROT-THIMOTHÉE, 1958) and numerous cell types from metazoans. The surface differentiation of the trunk cells thus shows that the dicyemids are well adapted to live on the dissolved nutrients found in the urine of the host. Likewise

the enlarged surface area between the trunk cells and the axial cell suggests a great transport of substances to the latter.

The fact that the axial cell becomes less electron dense as the embryos become mature confirms the observation of Nouvel that the embryos utilise the content of the axial cell during growth. The extremely well developed foldings of the cell membrane of the mature embryos probably function as a placental organelle.

The submicroscopic structure of the adult individuals gives no clue concerning the systematic position held by the dicyemids. As mentioned above the type of surface differentiation found in the dicyemids is a property found in cells from a great variety of animal groups. The surface of turbellarians (see SKAER, 1961) cannot be compared to that of the dicyemids. The former have a real epithelium with a basal membrane which the latter do not possess. The trematods, which could be imagined as ancestors of the dicyemids have a very specialised body surface, among others being syncytial (BJÖRKMANN and THORSELL, 1964). Neither can this body surface be compared with that of the dicyemids.

The structure of the cilia of the dicyemids is of a nearly universal type and shows no particularity of systematical significance.

The ciliary rootlets of most metazoans run perpendicularly to the cell surface as opposed to what is found in the dicyemids. But in tunicates (OLSSON, 1962) the ciliary rootlets of the endostyle cilia run parallel to the cell surface. The fibrillar system combined with the basal granules of ciliates (kinetodesmata) cannot be compared with that of the dicyemids, since in ciliates the fibrilles from the basal granules in one ciliary row combine to form a bundle of fibrilles running parallel to each row and to the right of it (see GRIMSTONE, 1961). In any case it seems unreasonable to derive the dicyemids from any known protozoan group.

The intracellular development of multicellular embryos from a cell not being an egg cell remains an unique feature in the mesozoa.

An abstract of this paper was presented at the 1st International Congress of Parasitology in Rome, September 1964.

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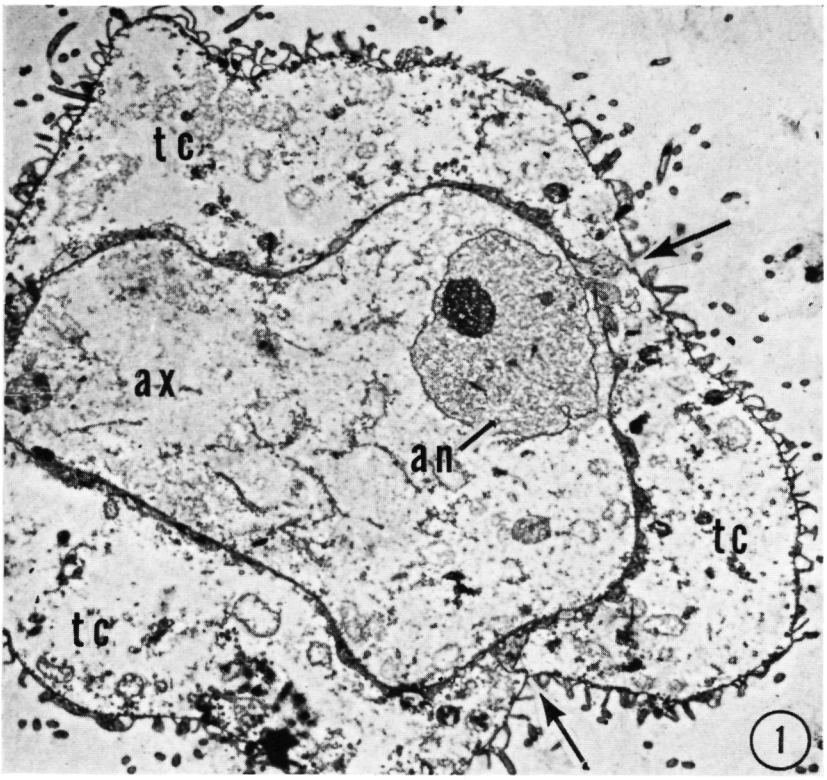
(Særtrykkene udkommet den 25. oktober 1965)

PLATES

Plate XXI.

1. Cross section of a rhombogen; 4800 ×. Three trunk cells are present in the section. The arrows point at where two adjacent trunk cells meet.
2. Cross section of the surface of a trunk cell; 56000 ×, (ultrathin section).

an: nucleus of the axial cell.
ax: axial cell.
tc: trunk cell.
vc: chain of pinocytosis vesicles.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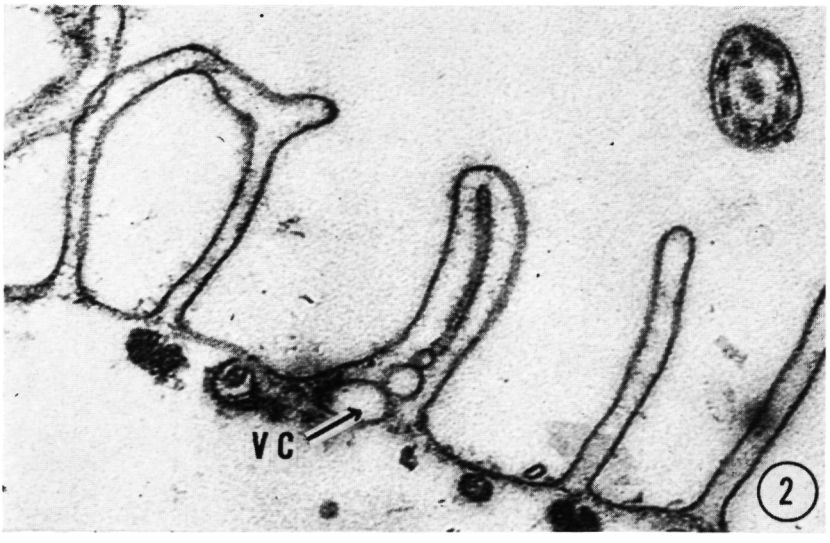
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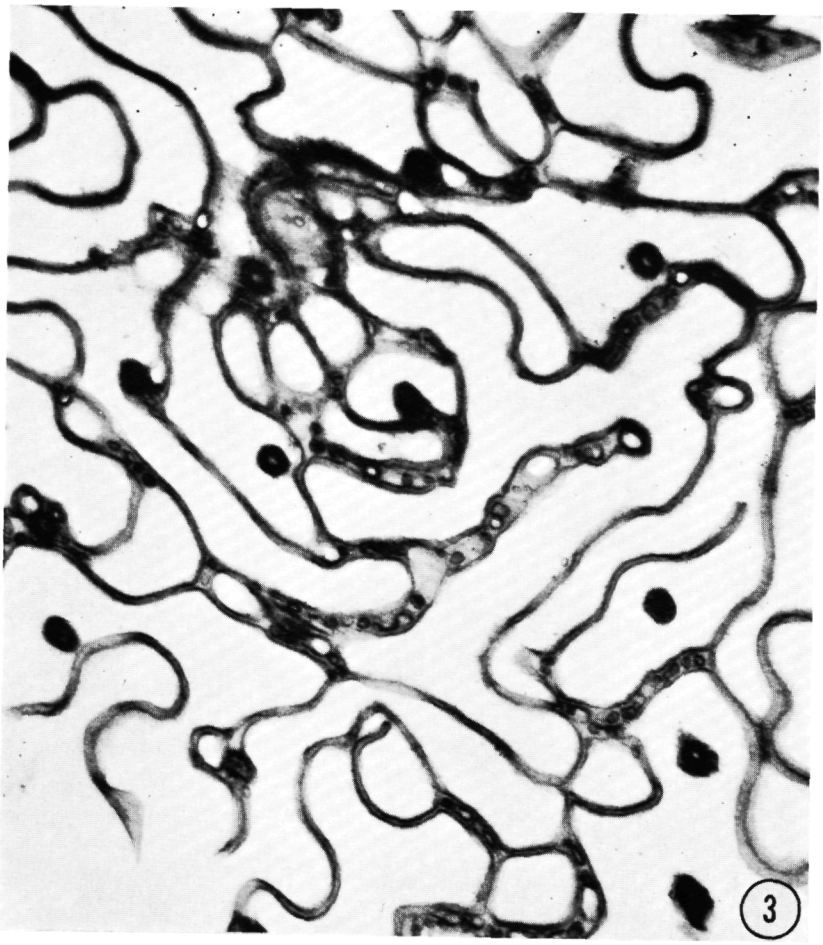
PLATE XXII.

3–4. Tangential section through the surface of a trunk cell (3 is an enlargement of 4). 3:25000×, 4:4500×.
The straight line in the left hand corner of 4 indicates the anterior-posterior axis of the animal.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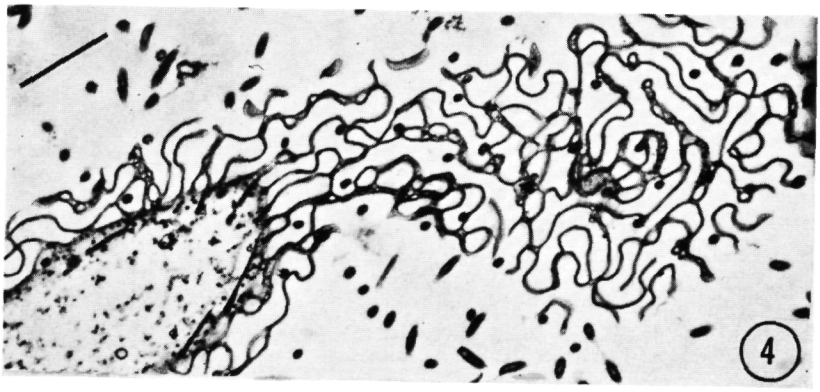
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PLATE XXIII.

5. Part of an infusoriform larva in the axial cell; 26000×, (enlargement of fig. 9).

ax: axial cell.
bg: basal granule.
cr: ciliary rootlet.
ebm: embryo.
isp: intercellular space.
l: lumen in the axial cell in which the infusoriform is situated.
lb: laminar body.
m: mitochondria.
tc: trunk cell.

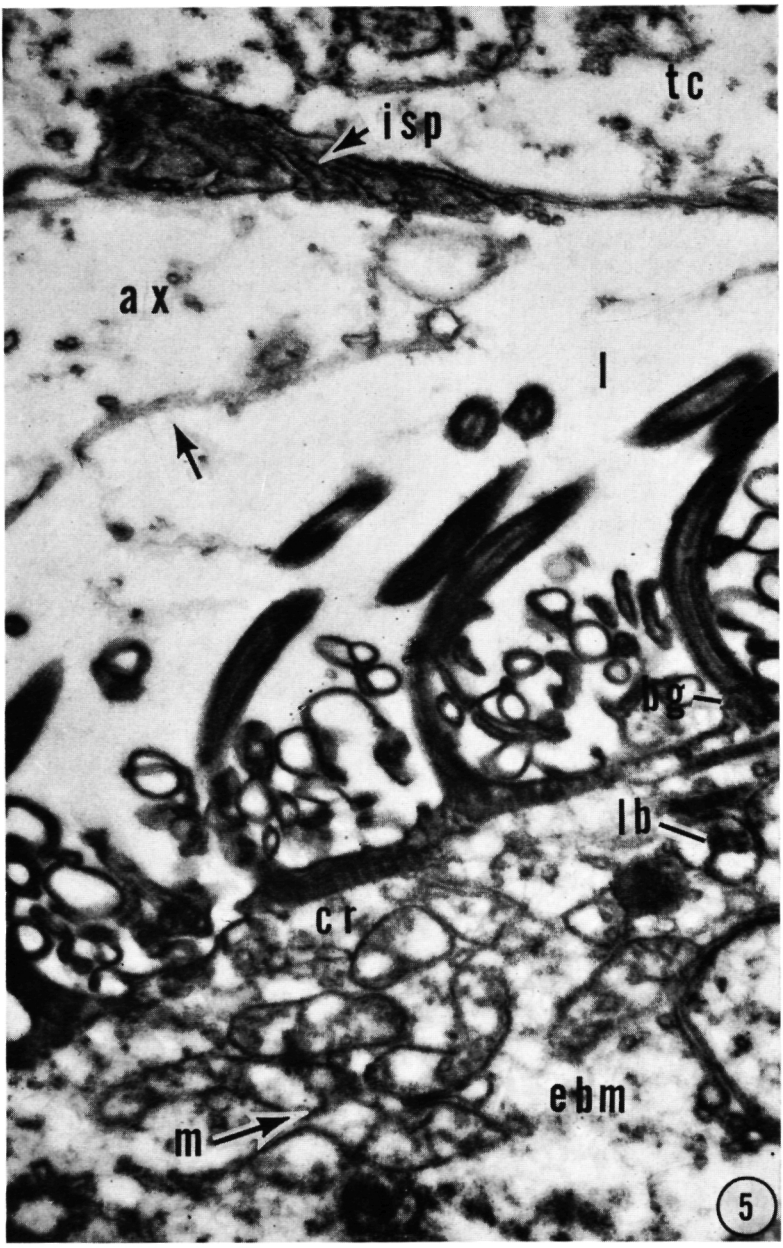
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PLATE XXIV.

6. Cross section of a trunk cell; 25000 ×.
7. Tangential section of a trunk cell showing ciliary rootlets.
8. Longitudinal section of a trunk cell showing a ciliary rootlet; 54000 ×. Remark the membranes of the ridges.

cr: ciliary rootlet.
m: mitochondria.
p: "parasitic mitochondria".
v: pinocytosis vesicles.

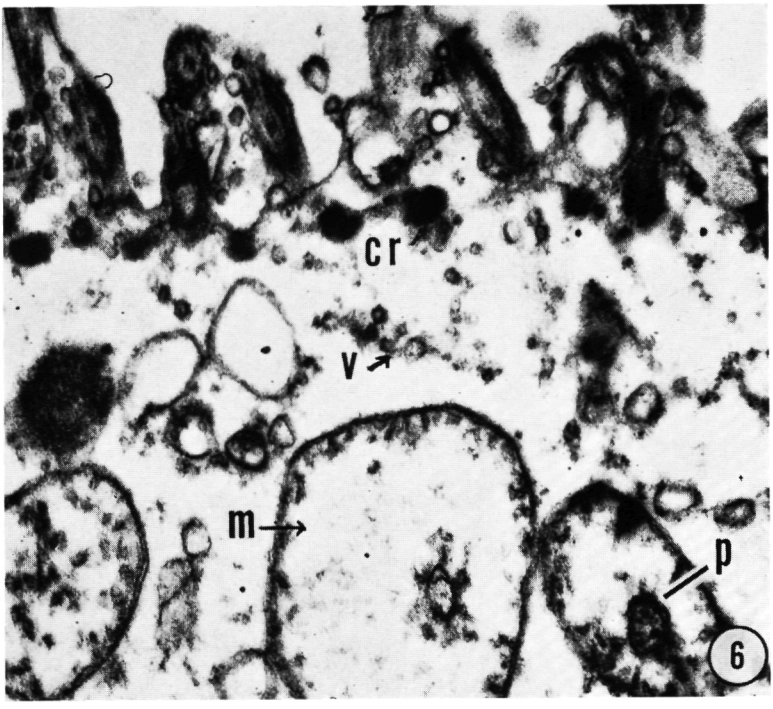
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PLATE XXV.

9. Mature infusoriform larva in the axial cell: 7500 ×. The infusoriforme is cut in an obliquely tangential section.

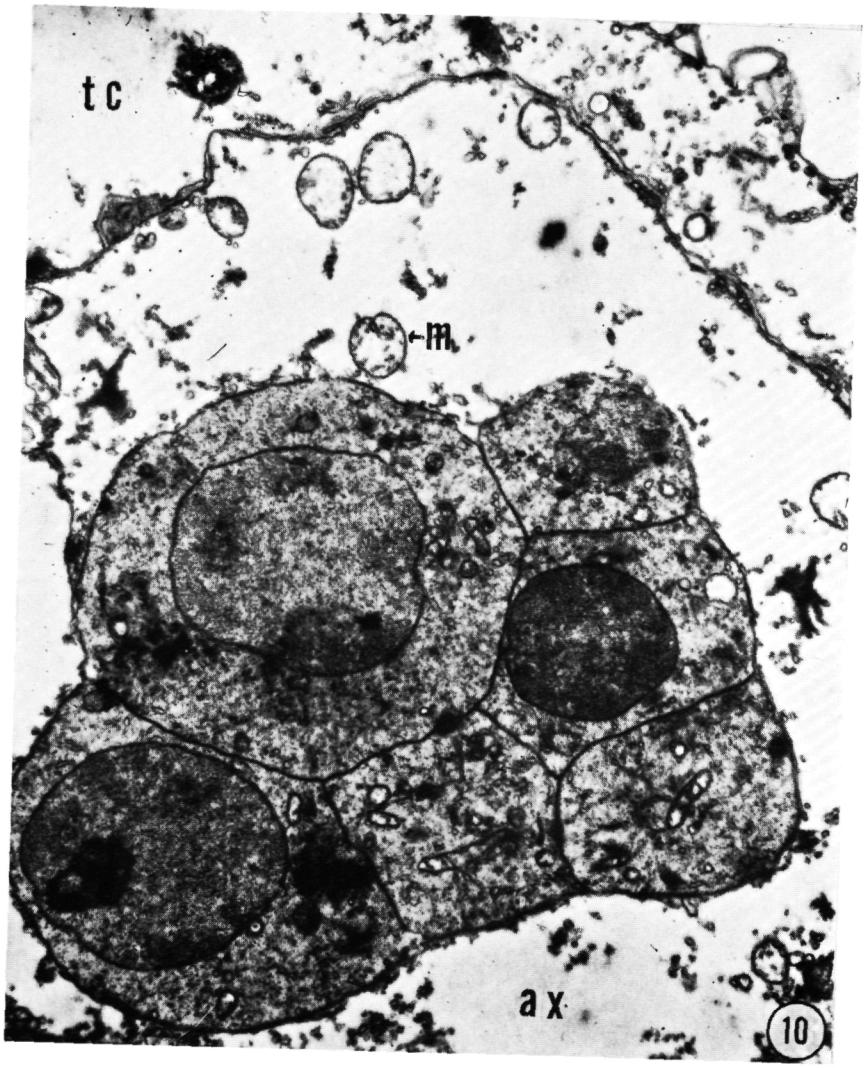
ax: axial cell.
tc: trunk cell.

ax
tc
tc
9

PLATE XXVI

10. Young embryo of an infusoriform; 3900 ×.

ax: axial cell.
m: mitochondria.
tc: trunk cell.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