التركيب الدقيق للساركوست بلانشاردى دوفلين المعزولة من الجاموس المصرى

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الملخص

درس لأول مرة التركيب الدقيق للساركوست بلانشاردى دوفلين المعزولة من الجاموس المصرى . وقد وجد أن التركيب الدقيق لجدار الحويصلات والتروفوزيت وكذلك طريقة التكاثر تشبه الى وصفها سابقا لودفك ١٩٥٨ وميلهورن وشولتبسيك ١٩٧٣ فى الخراف وعلى هذا اقتراح أن تكون حويصلات بلانشاردى دوفلين ما هى الا اسم مقابل لحويصلات التينيلا

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FINE STRUCTURE OF *SARCOCYSTIS BLANCHARDI* DOFLEIN, 1901 FROM THE EGYPTIAN BUFFALO

(With 6 figures)

By

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SUMMARY

The fine structure of *Sarcocystis blanchardi* DOFLEIN, 1901 from the Egyptian buffalo, has for the first time been studied. The ultrastructure of the cyst wall and the trophozoites as well as the mode of multiplication has been found to be more or less identical with that described in *Sarcocystis tenella* of sheep, previously described by LUDVICK (1958) and MEHLHORN and SCHOLTYSECK (1973). Accordingly it is suggested that *S. blanchardi* DOFLEIN, 1901 is a synonym to *S. tenella* RAILLIET, 1886.

INTRODUCTION

More than fifty species of *Sarcocystis* have been reported from mammals including man, from birds, reptiles and fishes (MANDOUR, 1965). As a group they have a world wide distribution (SCOTT, 1943). The morphology of *Sarcocystis* has received more attention than any other part of the subject, in attempts to elucidate its exact taxonomic position and its life history.

Only a few species of *Sarcocystis* have so far been studied, by the aid of electron microscopes. Thus LUDVICK (1958), SENAUD and PUYTORAC (1961) and recently MEHLHORN and SCHOLTYSECK (1973), have described the fine structure of *S. tenella* of sheep. LUDVICK (1960) has studied the ultrastructure of *S. miescheriana* of pigs. MANDOUR (1971) described the fine structure of *S. kortei* of a rhesus monkey and later MANDOUR (1972) studied the electron microscopic structure of *Sarcocystis* sp. in the skeletal muscle of a harsh-furred rat (*Lophuromys flavopunctatus*).

The present work deals, for the first time, with the fine structure of *S. blanchardi DOFLEIN,* 1901 from the Egyptian buffalo.

MATERIAL AND METHODS

Macroscopic cysts were collected from oesophagi of aged buffaloes. The material was available, one or two hours after the animals were slaughtered in Assiut Abattoir. Giemsa-stained smears were prepared from squashed cysts. A few cysts were fixed in 10% formol-saline for histological examination. One cyst measuring about 2.5 mm. in length and about 1 mm. in width, was selected for electron microscopy. The cyst was fixed in 1% osmium tetroxide in RHODIN and ZETTERQVIST (R. and Z.) buffer at 4°C, and it was dehydrated in absolute alcohol. The material was stained with 1% phosphotungstic acid in absolute alcohol. Embedding was carried out in methacrylate. The block was sent to Dr. R.G. Bird, the head of the Electron microscope unit at London School of Hygiene and Tropical Medicine. Through his kind help, sections were cut on an ultramicrotome and they were viewed in a A.E.I.E.M. 6. microscope. Micrographs were taken on the Ilford 'Lantern' contrasty plates. Original copies of the photomicrographs were sent to the writer for description and comments.

RESULTS

The macroscopic cysts are fusiform, whitish, worm-like tubes, measuring about 2.5 mm. to 1 cm in length and 1.0-1.5 mm in breadth. Haematoxylin and eosin-stained sections showed that the cysts are surrounded by a conspicuous wall which is provided with cyst wall-processes (cytophanères) of various thickness and shape. In general, the outer surface of the cyst wall appears to be basophilic and rough. On some occasions, this layer is surrounded by oesophageal muscle cells. The cyst is divided into numerous chambers by septa which project from the inner surfcae of the cyst wall inwards. Such chambers contain crescentic organisms (trophozoites), being crowded peripherally while the centre of the cyst contain degenerating trophozoites. In Giemsa-stained smears, the trophozoites are banana-like withone end broader than the other. They measure 15-20 μ in length and 4-5 μ in breadth. The body of the trophozoite could be divided into three segments. The anterior segment appears to be homogeneous and is pinkish in colour. The middle segment contains about twenty rounded granules which are aggregated near the junction between the anterior and middle segments. The granules are deeply stained, while the cytoplasm of this segment is blue. The posterior segment contains a large vesicular nucleus. The space between the nucleus and the posterior rim of the trophozoite is blue in colour.

Electron microscopic appearance of the cyst and trophozoites : The cyst wall is about 2000-3000 m μ. thick and it consists of an outer spongy and inner granular layers (Fig. 1). On higher magnifications, ramified villi were found projecting towards the host tissue. The terminal ramifications lie very close to those originating from the adjacent villi, thus the outer layer of the cyst wall appears spongy (Fig. 2). Tangential sections of the villi showed bundles of fine fibrils, which could not be traced in the inner layer of the cyst wall. Scattered electron dense bodies (Fig. 2) could be detected within the stroma of the villi and the substance of the inner layer of cyst wall. It is worthwhile mentinoing here that no cellular body reaction was observed around the cyst wall. The outer surface of the villi is provided with minute papillae. The inner layer of the cyst wall (Fig. 2) shows minute lacunae containing a small number of developing trophozoites. Larger chambers usually harbour mature trophozoites which appear to multiply by endodyogeny (Fig. 3).

The mature trophozoite is covered with a pellicle which consists of two layers of ultra thin membranes. On one occasion, the continuity of the pellicle is broken at one point (in the anterior zone of the trophozoite), forming a concave pit commonly known as 'micropyle' (Fig. 4). The body of the trophozoite could be divided into three zones : 1. The anterior fibrillar zone. which contains fine convoluted tubules, previously known as 'sarconemes'. This zone is limited anteriorly by the conoid (Fig. 5) and posteriorly by the vesicular layer of the middle zone. A mitochondrion could be seen lying within the fibrillar zone. 2) The middle zone could be divided into two layers (Fig. 6). A vesicular layer lying in close contact with the fibrillar zone and a lipoid layer occupying the rest of the middle zone. Within the middle zone, lie ribbon-like or loop-like mitochondria. In addition, the middle zone showed portions of the paired organelle (Fig. 6). 3) The posterior zone extends from the anterior border of the nucleus till the posterior end of the trophozoite. No Golgi appartus was detected in the present material.

The ultra-structure of *Sarcocystis blanchardi* of buffaloes has for the first time been studied in Egypt. It was found that this species appears to be identical with the parasite of sheep previously described by LUDVICK (1958) and MEHLHORN and SCHOLTYSECK (1973). However, it could not be concluded that both parasites are related to one species on the basis of morphological characters, unless their biological characters and life history are studied. The structure of the cyst wall appears to be one of the important criteria to differentiate species of *Sarcocystis*. This suggestion is based on

the fact that, the cyst wall of the present parasite differs from that of *S. miescheriana* of pigs described by LUDVICK (1960), *S. kortei* of monkeys and *S.* sp. of rodents described by MANDOUR (1971, 1972). The absence of body reaction around the cyst wall may lead to the belief that *Sarcocystis* is an innocent parasite. However, it has been shown by MANDOUR (1965) that when the cysts rupture, they become surrounded by cellular reaction, which may be attributed to the toxic substance 'sarcocystin' produced by the parastie. EL-AKKAD and MANDOUR (1969) studied the pharmacological and toxicological effects of 'sarcocystin' prepared from the parasite of Egyptian buffaloes.

It is the first time as well, to observe a 'micropyle' in this species of *Sarcocystis*. According to GARNHAM, BAKER and BIRD (1962), a protozoan parasite which possesses a 'micropyle' is related to the Sporozoa. For this reason *S. blanchardi* is considered a sporozoan parasite, which appears to be related to the family Eimeriidae (a coccidian parsite similar to the allied protozoan parasite, *Toxoplasma gondii*).

The sporoblasts observed herein are given a new name 'metrocytes' by MEHLHORN and SCHOLTYSECK (1973), which means 'mother' cells. Thus the 'metrocytes' seen in the present work are more or less similar to those in *S. tenella* described by the latter authors. It differs morphologically but not bascially from those observed in the parasite of monkeys described by MANDOUR (1971). Accordingly, the development of trophozoites begins from the peripheral part of the cyst inwards. A second type of multiplication is in the form of internal budding (Endodyogeny) which has also been observed in many species of *Sarcocystis*.

It could be concluded that all the species of *Sarcocystis* are essentially identical, with the exception of the structure of the cyst wall, the fine strucure of which could be used as one of the important criteria to identify the species of *Sarcocystis*. Moreover, *Sarcocystis blanchardi* DOFLEIN, 1901 could be a synonym to *S. tenella* RAILLIET, 1886, since their fine structure is identical.

C = Conoid
CW_2 = Cyst wall (inner layer)
F = Fibrills
Mp = Micropyle
N = Nucleus
P = Pellicle
Pp = Papilla
V = Vesicle
CW_1 = Cyst wall (outer layer)
Ed = Electron dense bodies
Mc = Mitochondrion
Mt = Microtubules
OM = Oesophageal muscles
PO = Paired organelle
Sp = Sporoblast
Vi = Villi

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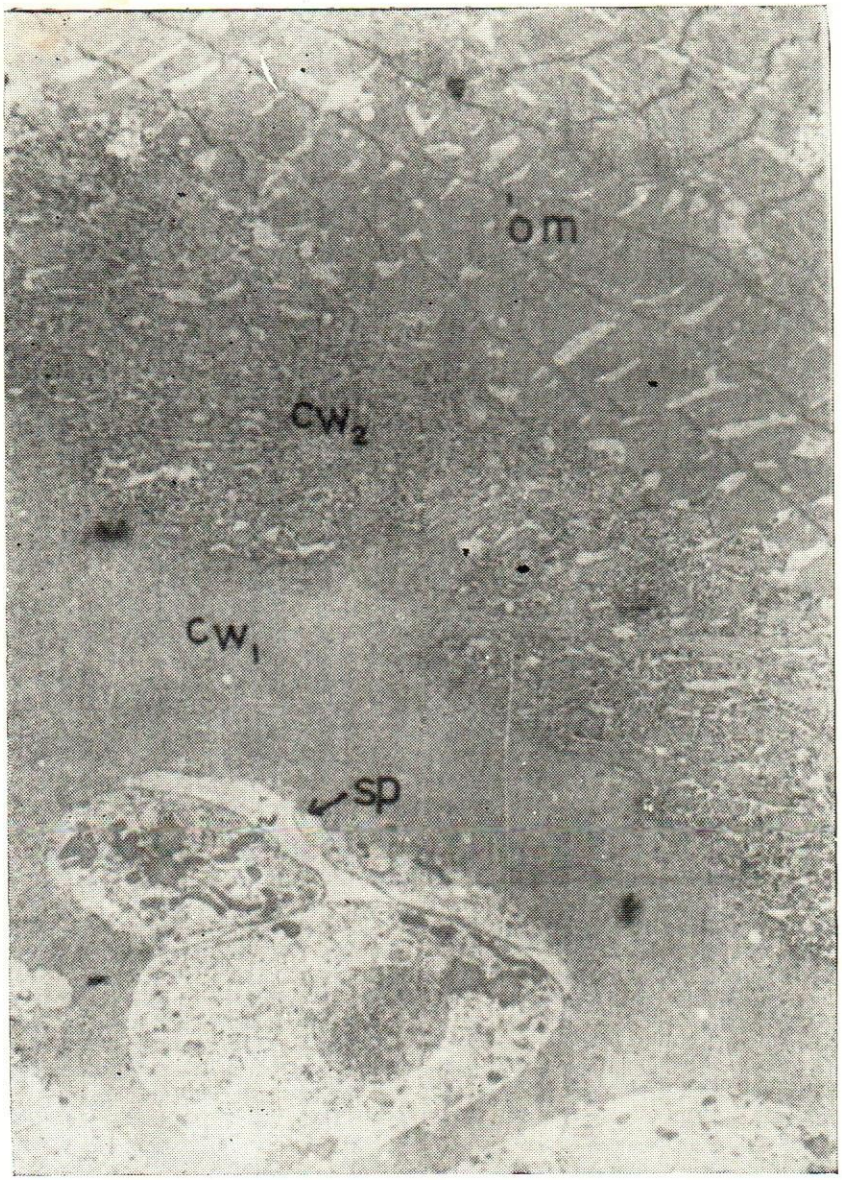

Fig. 1.—Electron micrograph of *Sacrocystis blanchardi* showing the spongy appearance of the cyst wall, the fine structure of the sporoblasts and oesophageal muscles. (× 5000).

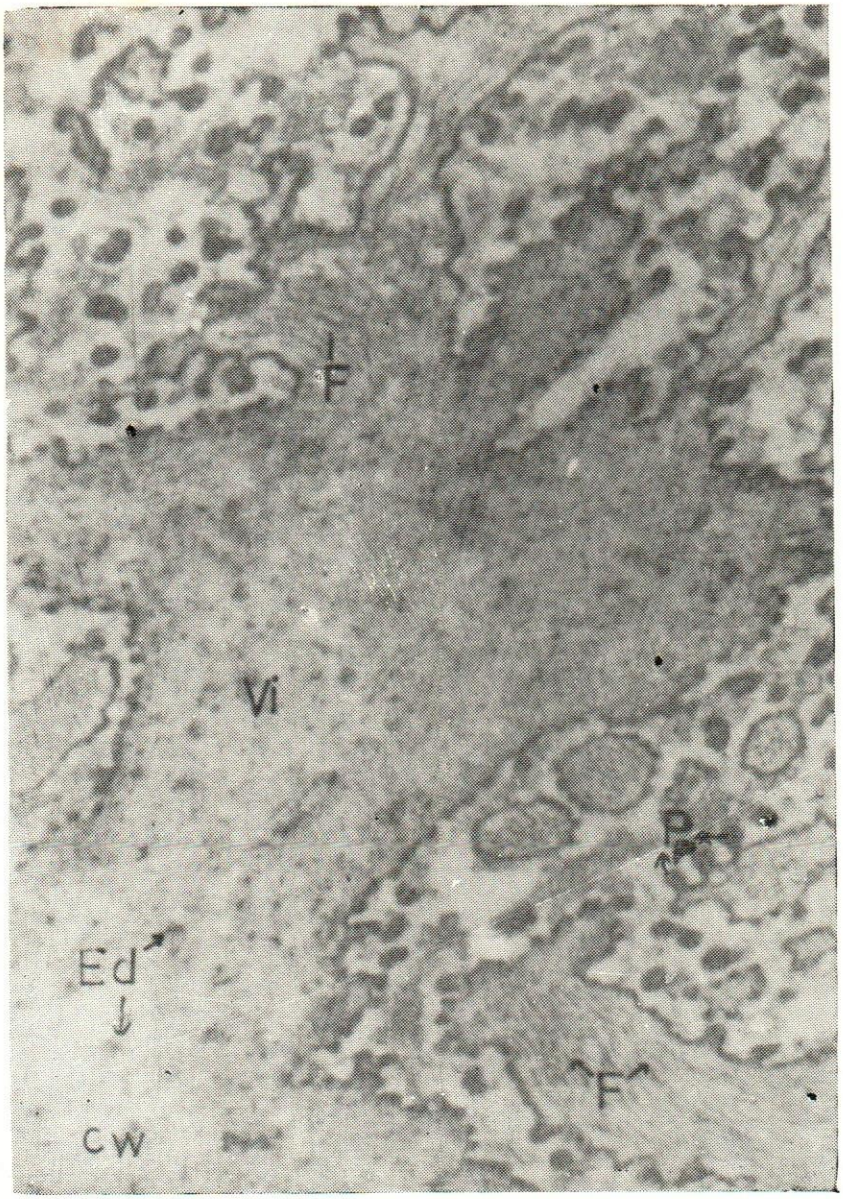

Fig. 2.—A highly magnified villus is projecting from the outer layer of cyst wall. The core of the villus shows fine fibrills, electron dense bodies and the **rough outer surface of the cyst wall. Electron micrograph** (× 42,000).

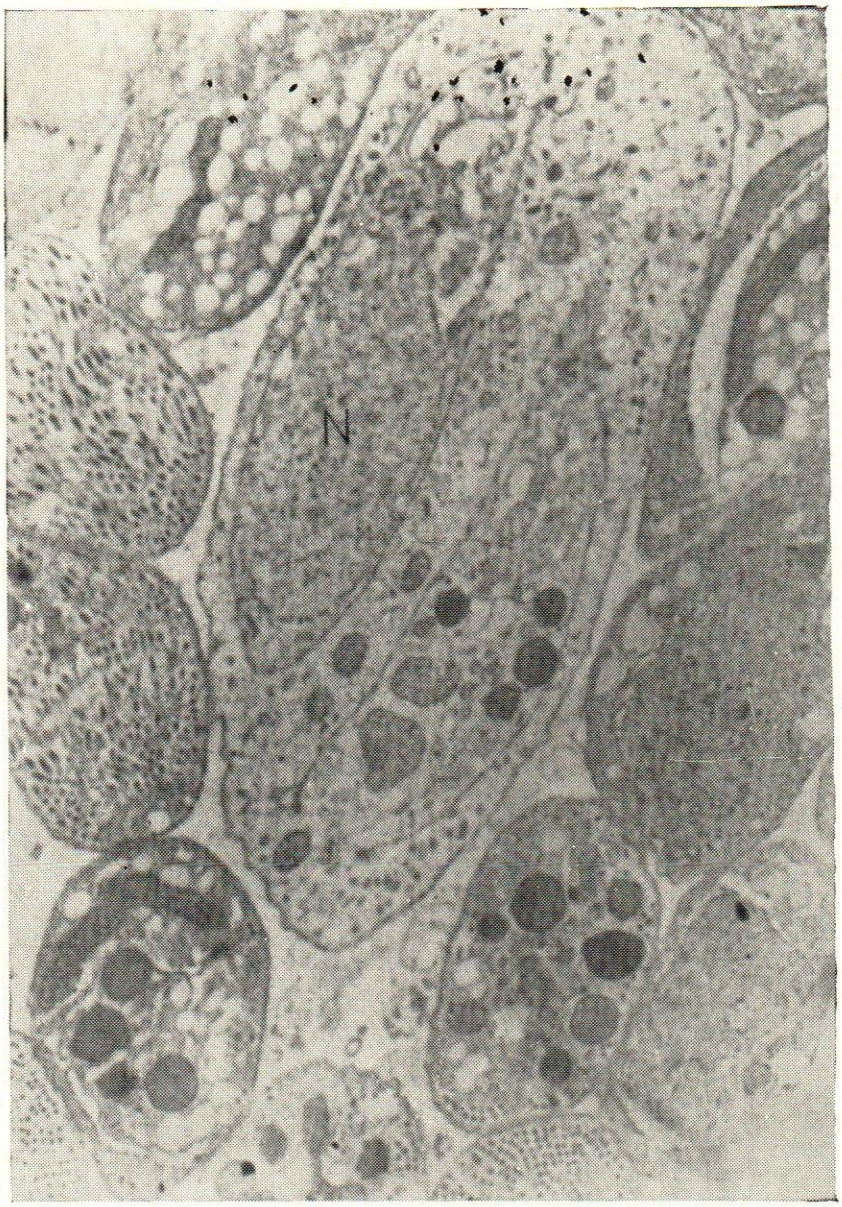

Fig. 3.—Electron micrograph of *S. blanchardi* showing a mother cell containing two trophozoites, indicating meultiplication by endodyogeny.(× 12,600)

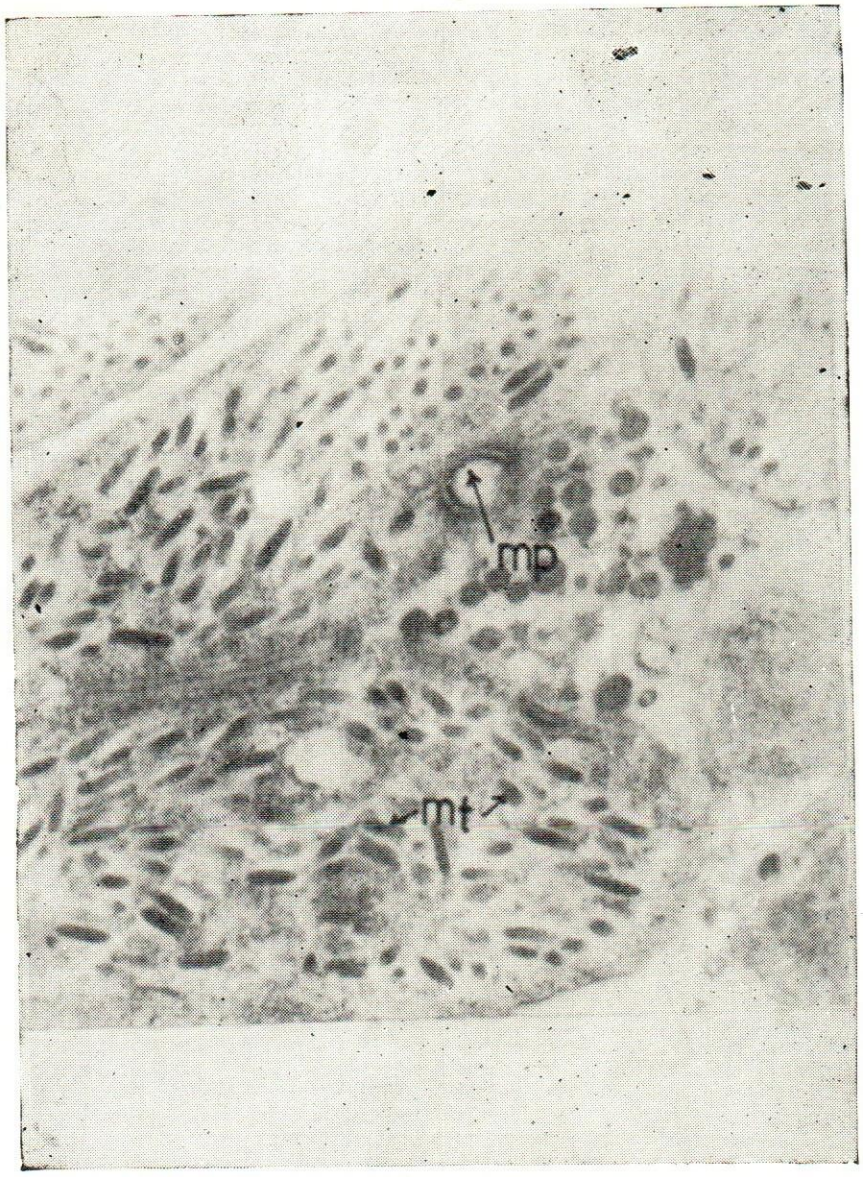

Fig. 4.—Electron micrograph showing the micropyle lying at the fibrillar zone of the trophozoite. (× 32,000).

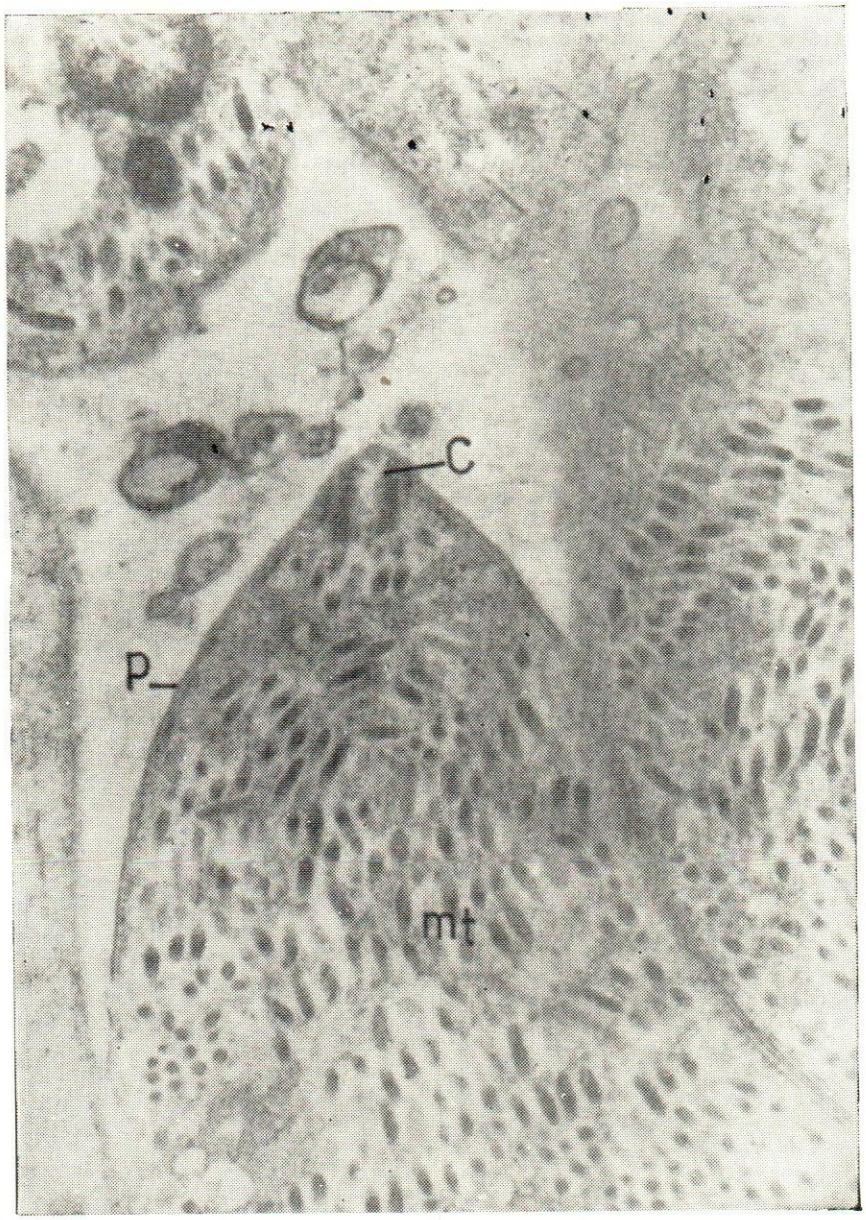

Fig. 5.—A highly magnified portion of *S. blanchardi* trophozoite showing the coinoid, pellicle and microtubules. Electron micrograph. (× 32,000).

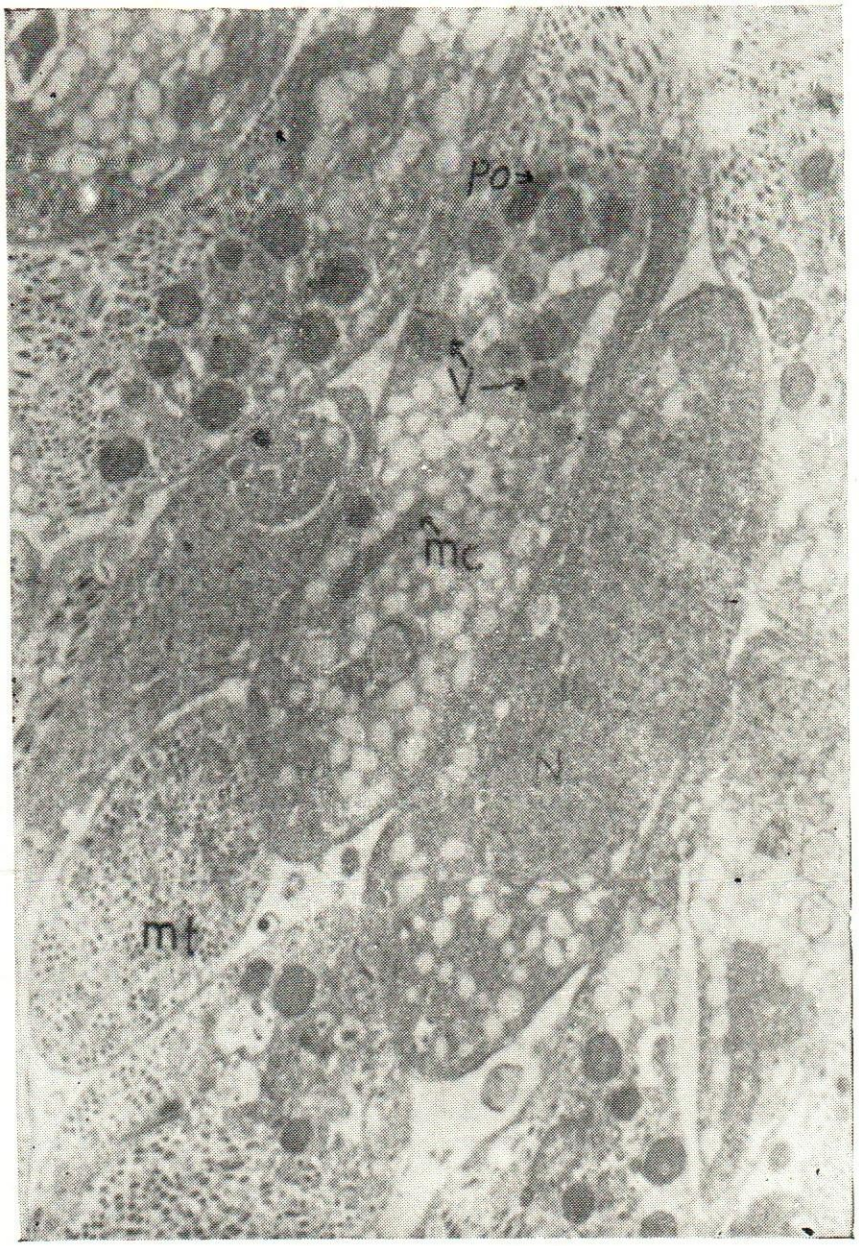

Fig. 6.—Electron micrograph showing *S. blanchardi* trophozoites cut at different levels. The internal anatomy of the trophozoite could be detected. (× 12,600).