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ULTRASTRUCTURAL STUDY OF GLOBIDIAN PARASITES INFECTING THE ABOMASUM OF SHEEP IN GERMANY

By

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HILALI, M. and E. SCHOLTYSECK: *Ultrastructural study of globidian parasites infecting the abomasum of sheep in Germany.* Acta vet. scand. 1979, *20*, 321—328. — Globidian parasites infecting the abomasum of sheep in Germany were investigated by means of electron microscopy. The frequency of infection was found to be 93 %. The globidian cyst-like bodies contained multinucleate schizonts, developing merozoites or fully developed merozoites. Among the latter there were two different types, namely short and long forms. The process of merozoite formation was described in detail. The giant schizonts were subdivided into multinucleate cell portions of irregular size and shape. Their nuclei were then arranged at the periphery of the cell portions and underwent their last division which was combined with the differentiation of merozoites. The long form merozoites were elongated cylindrical in shape with terminal nucleus. They measured 7.7 μm in length and 1.0 μm in width. The merozoites of the short type were spindle-shaped with a central nucleus. They were 5.0 μm long and 1.0 μm wide. The globidian parasites were located in a parasitophorous vacuole of an intact host cell.

globidian parasites; sheep; schizonts; merozoites; ultrastructure.

Globidian developmental stages (schizonts and merozoites) occur in the abomasum of a great number of sheep in many countries. They appear as cyst-like bodies in the mucosa of the abomasum. Previous investigations revealed the existence of four morphologically different globidian merozoites in Norwegian sheep (*Hilali* 1973). Fine structural studies of one of these merozoite types showed a great similarity to those of Eimeria species.

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Mehlhorn & Heydorn (1976) described the fine structure of two different types of globidian merozoites infecting the abomasum of sheep in Germany. They concluded that these merozoites were ultrastructurally different from those of eimeria and sarcocystis species.

The present study deals with the fine structure of globidian schizonts and merozoites that have not been yet described from the abomasum of sheep in Germany. Furthermore, this study emphasizes the developmental mechanism of these coccidian parasites during merozoite formation.

MATERIAL AND METHODS

Globidian schizonts and merozoites were obtained from the abomasum of freshly slaughtered sheep at different farms in the surrounding of Bonn. Each abomasum was opened longitudinally, its contents were removed and the mucosal surface was examined carefully for the presence of globidian parasites. These were immediately dissected from the abomasum and fixed after *Karnovsky* (1965). The material was then washed in cacodylate buffer, post-fixed in 1 % OsO_4 for 4 h and dehydrated in increasing concentrations of acetone. It was poststained with uranyl acetate and phosphotungstic acid in 70 % acetone and finally embedded in Vestopal W. Ultrathin sections of the embedded material were cut with glass knives using an LKB ultramicrotome, mounted on copper grids and then examined with a Zeiss EM 9 S-2 electron microscope.

RESULTS

The globidian parasites appeared within cyst-like bodies and contained either giant schizonts or merozoites. These were located within a parasitophorous vacuole and the cytoplasm of the host cell was alive and intact. However, as they looked as cysts we use the term "globidian cyst".

Occurrence of globidian cysts

Examination of the abomasum of 30 lambs selected at random showed that 28 harboured globidian parasites (93 %). The number of these cysts in each abomasum was numerous (2—3 in every cm^2). The macroscopic appearance of the parasites was similar to that previously described (*Hilali* 1973).

Light microscope observation

Light microscopic examination of Giemsa-stained smears prepared from the contents of each individual cyst showed multinucleate giant schizonts, developing or fully developed merozoites. Among the merozoites there were two different types: long forms and short forms. Both types did not occur in the same cyst. However, they were present in the same host animal. The long form merozoites were cylindrical in shape (measuring 7.7×1.0 μm) and having a nucleus near the posterior end. The short form merozoites were spindle-shaped (5.0×1.0 μm) with a central nucleus.

Out of 40 globidian parasites collected at random from 10 abomasa (four from each abomasum) 22 were multinucleate schizonts, 10 contained long form merozoites and eight having short form merozoites. The dimensions of the multinucleate schizonts varied from 239×200 μm to 394×259 μm. The measurements of the cysts containing the long form merozoites ranged from 352×240 μm to 543×432 μm and those containing the short form varied from 256×212 μm to 423×351 μm.

Electron microscope description

1. Development of merozoites

The process of merozoites formation started by repeated divisions of the giant schizont cytoplasm into numerous multinucleate cell portions of irregular shape and size. These cell portions were named blastophores in Eimeria bovis schizonts by *Sheffield & Hammond* (1967). The cell portions (Fig. 1) were limited by a unit membrane and varied in the number of nuclei (N) considerably (from 6 to 50). They were filled with a well developed endoplasmic reticulum (ER), mitochondrial sections (MI) and lipid droplets (L). One or more micropores were usually observed at the surface of the blastophores.

The nuclei then began to be arranged at the periphery of the cell portions. Sometimes two nuclei were found in the same nuclear envelope indicating that they were undergoing nuclear division. Later on protrusion of daughter merozoites (DCA) were observed at the surface of the blastophores. They were covered by a three-membranous pellicle characteristic of the motile infective stages of the Apicomplexa (*Scholtyseck* 1978) and showed the anlagen of the anterior portion of the merozoites. Mostly a

nuclear protrusion with a centrocone extended into this differentiating merozoite. In some cases the differentiation of two merozoites started in the close vicinity of a dividing nucleus (N) the centrocones (CC) of which extended into the two daughter cells (Figs. 2 and 3). The young developing daughter cells (DCA) had a thick-walled vesicle (DV), a prominent dark body (DB) and conoid (C). As development proceeds the daughter cell anlagen became elongated into finger-like buds. In the last phase of merozoite formation the outer membrane (plasmalemma) of the newly formed daughter cells was still attached to the membrane of the blastophore. The differentiated merozoites were limited by a three-membranous pellicle.

2. The merozoites

The long form merozoites (Fig. 4) were elongated, cylindrical and pointed at both ends. They varied in length from 7.1 to 8.8 µm and in width from 0.93 to 1.17 µm. Each merozoite was limited by a typical coccidian pellicle (*Scholtyseck* 1978). The subpellicular microtubules were 22 in number. A conoid of normal structure was situated at the anterior end. In most cases two rhoptries (RH) were present. The anterior half of the merozoite contained numerous micronemes (MN). A micropore was observed just posterior to the area of the micronemes. A large ovoidal body (OB), 1.1 µm in length, was located posterior to the micronemes. This body was surrounded by a unit membrane. It contained many granules and a central or excentric relatively large and dense globule. Often one or two dark bodies (Fig. 6, DB) were situated posterior to this ovoidal body. The dark bodies also appeared to be surrounded by a membrane and were smaller (0.6—0.7 µm) than the ovoidal body. The nucleus (N) was oval in shape and situated nearly in the posterior third of the merozoite. The nuclear material was in the form of irregular dense clumps located mostly on the periphery. The nucleoplasm also contained smaller granules. Several cross sections of the mitochondrion (Fig. 6, MI) were seen just posterior to the micronemes and extending to the posterior end of the merozoite. Amylopectin granules (Fig. 4, A) were irregularly distributed in the merozoite cytoplasm.

The short form merozoites (Fig. 5) were spindle-shaped and pointed at both ends. They varied in length from 4.7 to 5.9 µm

Abbreviations of all figures

A	Amylopectin
C	Conoid
CC	Centrocone
DB	Dark body
DCA	Daughter cell anlagen
DV	Thick-walled vesicle
ER	Endoplasmic reticulum
HC	Host cell
L	Lipid inclusion
MI	Mitochondrion
MIH	Host cell mitochondrion
MN	Micronemes
MV	Microvilli
N	Nucleus
OB	Ovoidal body
PV	Parasitophorous vacuole
RBO	Residual body
RH	Rhoptries
T	Tubules

Figure 1. Globidian multinucleate schizont; notice the multinucleate cell portions with some daughter cell anlagen (DCA). 2829 ×.

Figure 2. Part of a globidian multinucleate schizont. Two daughter cell anlagen (DCA) in front of a dividing nucleus (N) showing centrocones (CC). 23,000 ×.

Figure 3. Part of a globidian multinucleate schizont; two daughter cell anlagen (DCA) in front of a dividing nucleus (N); each anlage showed a three-membranous pellicle, conoid (C), a dark body (DB) and a thick-walled vesicle (DV). 23,000 ×.

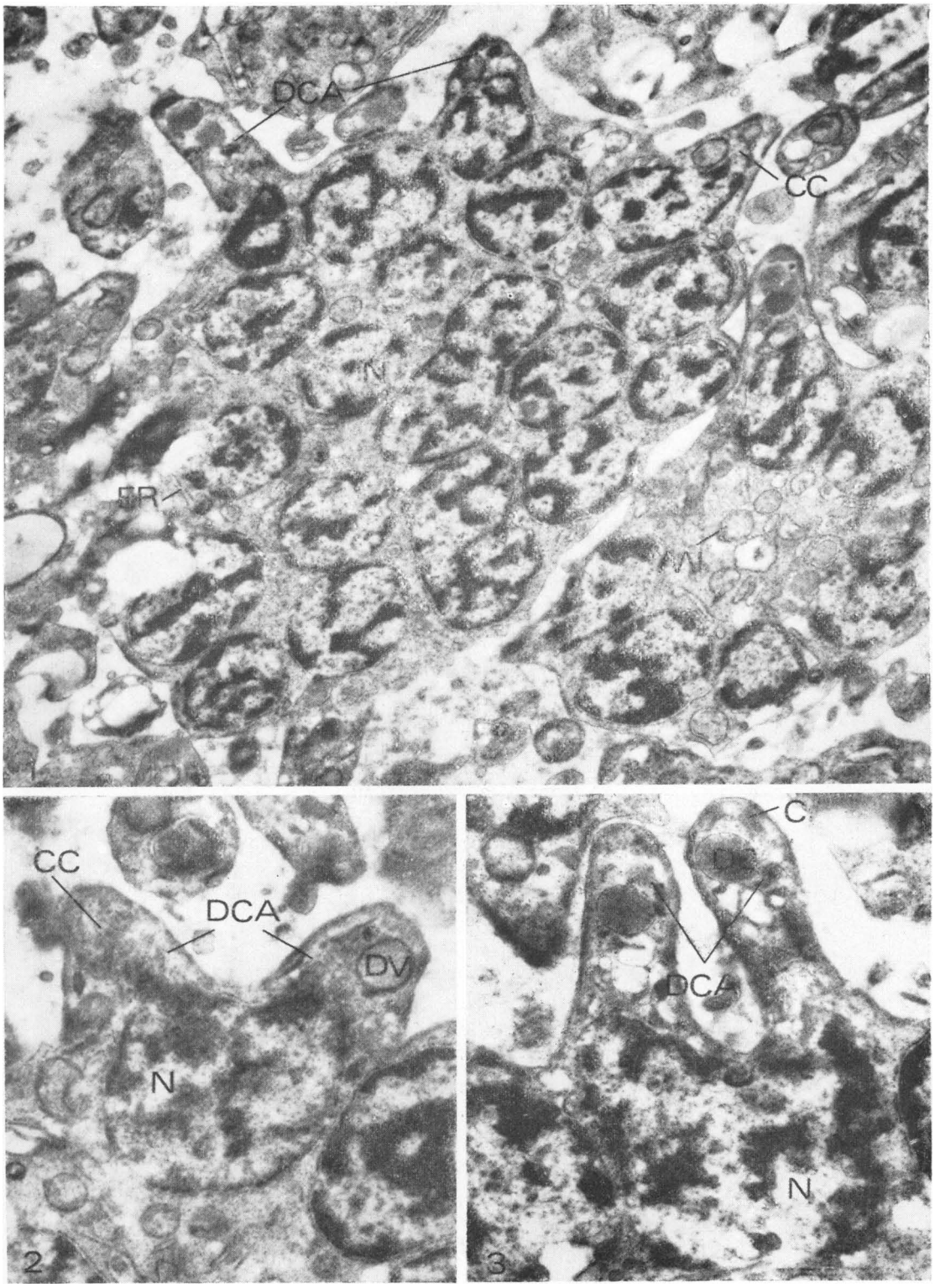
For these and the following figures reference is also made to "Abbreviations".

Figure 4. Globidian merozoites; long forms in transversal and longitudinal sections. 23,000 ×.

Figure 5. Globidian merozoites; short forms in transversal and longitudinal sections. 23,000 ×.

Figure 6. Globidian merozoites: transversal sections of long form merozoites showing dark bodies (DB). 23,000 ×.

Figure 7. Host cell cytoplasm surrounding the globidian parasites. 13,000 ×.


DCA
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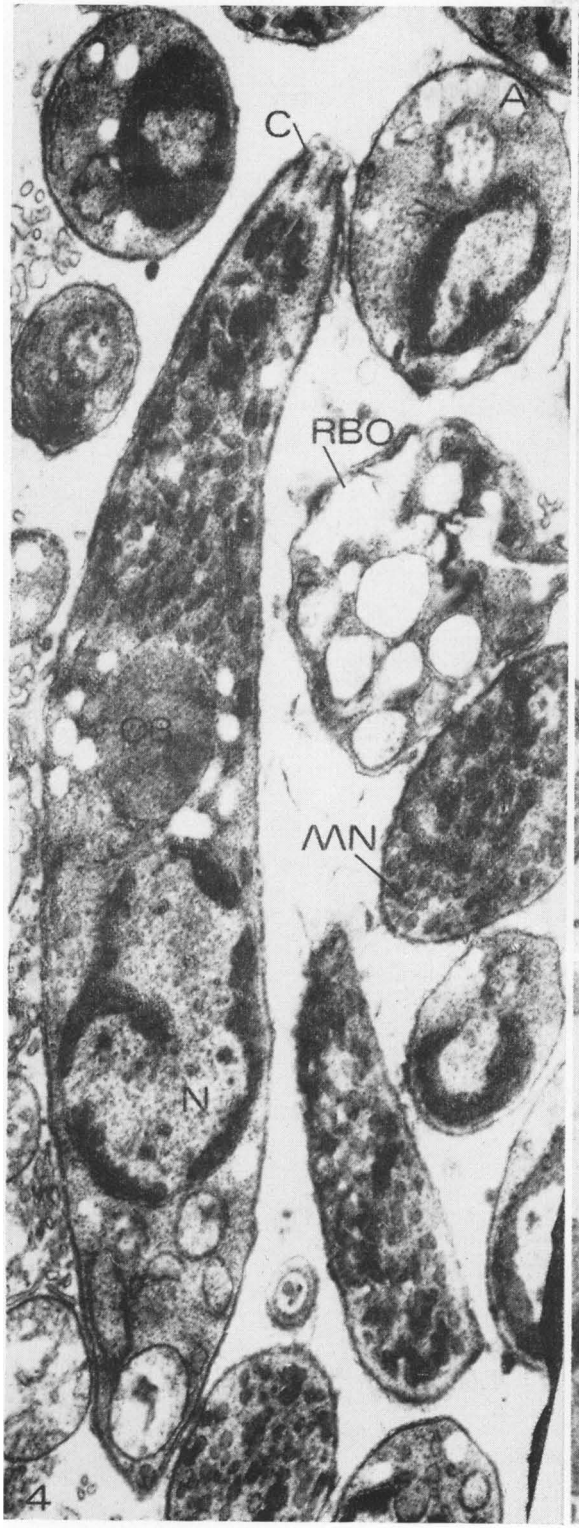
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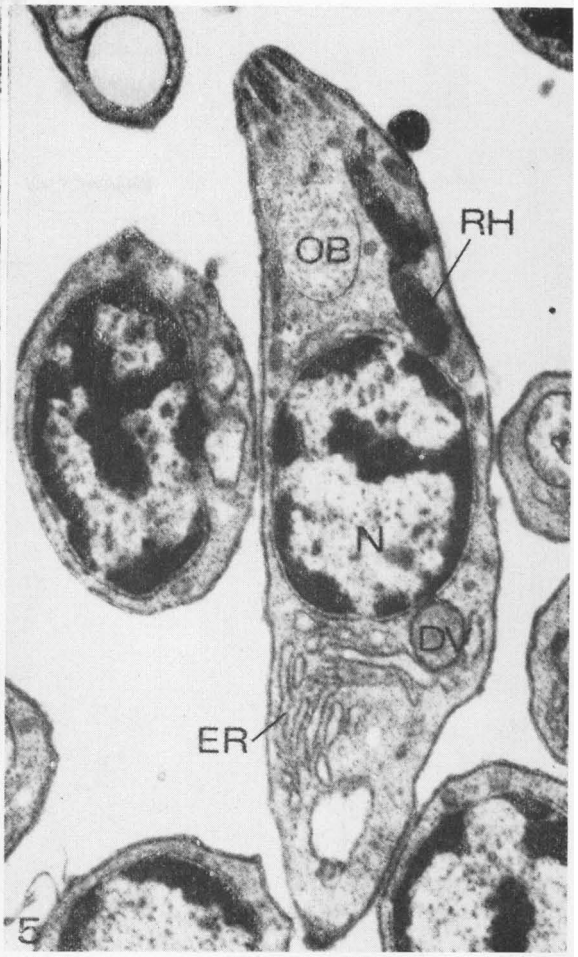
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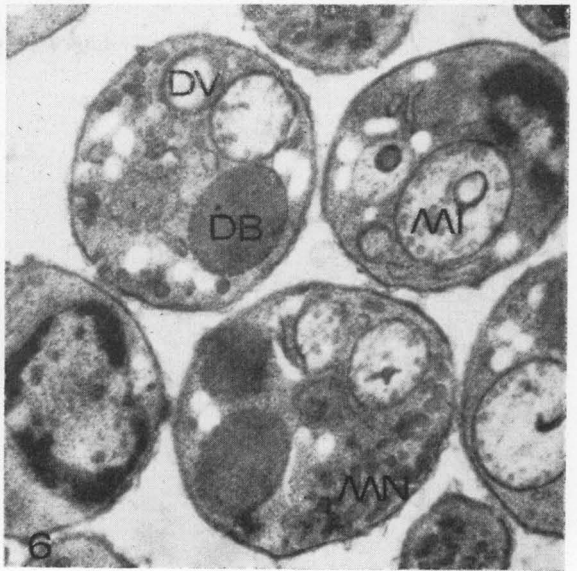
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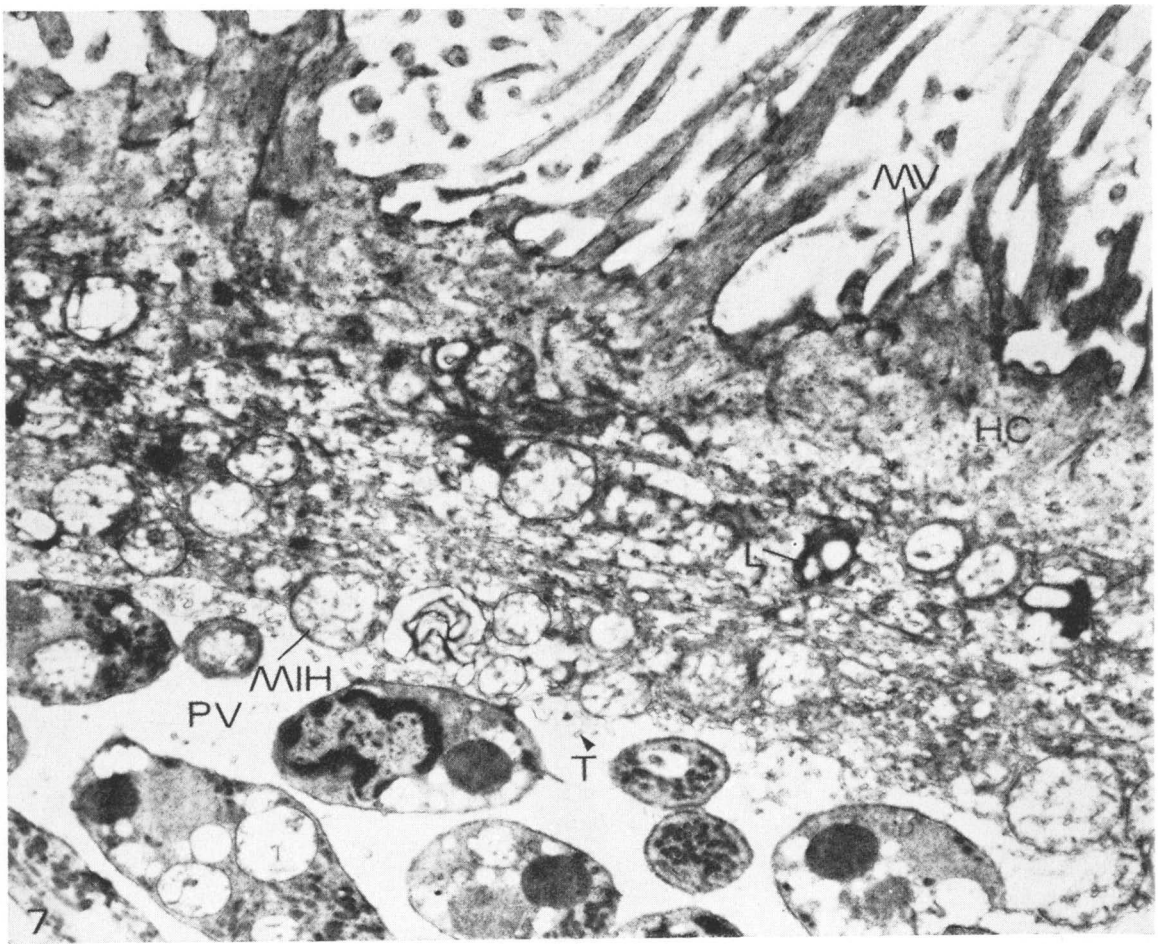
MIV
HC
L
MIH
PV
T
7

and in width from 0.88 to 1.77 μm. The organelles and structures of the apical pole (conoid, polar rings, rhoptries, RH) were similar to those of the long form merozoites. However, the micronemes were less in number. Also in this short merozoite type an ovoidal body (OB) was present; it was structurally similar to that of the long form merozoites but relatively smaller in size (0.6 μm in length) and located more anteriorly. Dark bodies (DB) observed in the vicinity of the ovoidal body in the long form merozoites were not present in the short merozoites. Contrary to the merozoites of the long type, the short forms possessed a centrally located nucleus. Apparently, in these merozoites a thick-walled vesicle (DV) was always present posterior to the nucleus. Strands of the endoplasmic reticulum (ER) were observed at the posterior third of the merozoite.

The merozoites of both types were lying in an electron-pale substance of the cysts. Sometimes a residual body (Fig. 4, RBO) was observed between the merozoites. This body represented the remains of the blastophores after merozoite formation.

3. The host cell of globidian parasites

Our fine structural study has confirmed that the host cell of globidian parasites was alive and intact (Fig. 7). The host cell cytoplasm enclosed a large parasitophorous vacuole containing a schizont or merozoites. The vacuole was bounded by a unit membrane from which arised several tubules (T) of about 90 nm in width and up to 1.7 μm in length. The outer surface of the host cell had numerous microvilli (MV). These contained numerous longitudinal fibrillar elements. In the cytoplasm of the host cell a large number of cross sections of mitochondria (MIH), bundles of fibrils, parts of the endoplasmic reticulum, lipid droplets (L) and dense granules of unknown function were present.

DISCUSSION

The present investigation showed that the examined sheep harboured globidian cyst-like bodies containing multinucleate schizonts or merozoites. Among the merozoites there were two different types namely long and short forms.

The description of the giant globidian schizonts was generally similar to that of Eimeria bovis (*Sheffield & Hammond* 1967).

The electron micrographs showing the merozoite development indicated that this process was connected with the last schizogonic nuclear division, so that two merozoites developed from one dividing nucleus and the surrounding cytoplasm. This merozoite differentiation was similar to the process of endodyogeny with all its characteristic phenomena (*Scholtyseck* 1973).

The study of the fine structure of globidian parasites is a relatively new one. Therefore, little knowledge has yet been obtained in this area. One type of globidian merozoites (intermediate form) from the abomasum of Norwegian sheep has been described previously by means of electron microscopy (*Hilali* 1973). The present ultrastructural study of two additional types, namely the long and short merozoite forms, showed that all three types have the characteristics of globidian merozoites: one ovoidal body and one or two dark bodies anterior to the nucleus. However, the function of these inclusions is not understood. The number of the subpellicular microtubules in most of the merozoites studied was 22 (*Hilali, Porchet-Henneré* 1976, *Mehlhorn et al.* 1976). This result seemed to be of most interest since the presence of 22 subpellicular microtubules has been considered to be characteristic of the merozoites of the cyst-forming coccidian genera sarcocystis, Besnoitia jellisoni, frenkelia and toxoplasma (*Scholtyseck et al.* 1974).

The fine structure of two other types of globidian merozoites from sheep have been described by *Mehlhorn & Heydorn* (1976). The authors believed that these merozoites differed from those of eimeria and sarcocystis because of the presence of the ovoidal body in front of the nucleus. According to our knowledge similar bodies designated as protein granules have been described previously in eimerian merozoites (*Cheissin & Snigirevskaya* 1965, *Scholtyseck & Piekarski* 1965).

Our study has confirmed that the globidian parasites were located in a parasitophorous vacuole of an intact host cell. This vacuole was limited by a unit membrane. Therefore, a real "cyst wall" was not present. Similar host-parasite relationships have been observed in Besnoitia jellisoni (*Scholtyseck et al.*).

REFERENCES

Cheissin, E. M. & E. S. Snigirevskaya: Some new data on the fine structure of the merozoites of Eimeria intestinalis (Sporozoa, Eimeriidea). Protistologica 1965, *1*, 121—126.

Hilali, M.: Studies on globidial schizonts in the abomasum of Norwegian sheep. The fine structure of one of the four merozoite forms investigated. Acta vet. scand. 1973, *14*, 22—43.

Karnovsky, M. J.: A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 1965, *27*, 137—138.

Mehlhorn, H. & A. O. Heydorn: Licht- und elektronenmikroskopische Untersuchungen an zwei Typen von Riesenschizonten (sog. Globidien) aus dem Labmagen des Schafes. (Light and electron microscope studies on two types of giant schizonts (Globidial schizonts) in the abomasum of sheep). Zbl. Bakt., I. Abt. Orig. 1976, A 236, 386—406.

Mehlhorn, H., W. J. Hartley & A. O. Heydorn: A comparative ultrastructural study of the cyst wall of 13 Sarcocystis species. Protistologica 1976, *12*, 451—467.

Porchet-Henneré, E.: Structure du mérozoite de la coccidie Globidium gilruthi de la caillette du mouton (D'après l'étude de coupes fines et de colorations négatives). (Structure of the merozoite of the coccidium Globidium gilruthi of the abomasum of the sheep. (After a fine structural study using negative staining)). Protistologica 1976, *12*, 613—621.

Scholtyseck, E.: Die Deutung von Endodyogenie und Schizogonie bei Coccidien und anderen Sporozoen. (The significance of endodyogeny and schizogony in the Coccidia and other Sporozoa). Z. Parasitenk. 1973, *42*, 87—104.

Scholtyseck, E.: Fine Structure of Parasitic Protozoa. An Atlas of Micrographs, Drawings and Diagrams. Springer Verlag, Berlin, Heidelberg, New York 1978, 220 pp.

Scholtyseck, E. & G. Piekarski: Elektronenmikroskopische Untersuchungen an Merozoiten von Eimerien (E. perforans und E. stiedae) und Toxoplasma gondii. Zur systematischen Stellung von T. gondii. (Electron microscope studies of merozoites of Eimeria species (E. perforans and E. stiedae) and Toxoplasma gondii. A contribution to the systematic position of T. gondii). Z. Parasitenk. 1965, *26*, 91—115.

Scholtyseck, E., H. Mehlhorn & B. E. G. Müller: Feinstruktur der Cyste und Cystenwand von Sarcocystis tenella, Besnoitia jellisoni, Frenkelia sp. und Toxoplasma gondii. (Fine structure of the cyst and cyst wall of Sarcocystis tenella, Besnoitia jellisoni, Frenkelia sp. and Toxoplasma gondii). J. Protozool. 1974, *21*, 284—294.

Sheffield, H. H. & D. M. Hammond: Electron microscope observation on the development of first-generation merozoites of Eimeria bovis. J. Parasit. 1967, *53*, 831—840.

SAMMENDRAG

Studier av ultrastrukturen av Globidium-parasitter fra løpen hos sau i Tyskland.

Globidium-parasitter fra løpen hos sau i Tyskland ble undersøkt ved hjelp av elektronmikroskop. Infeksjonsfrekvensen var 93 %. Globidium-cystelignende dannelser inneholdt multinucleære schizonter, merozoitter under utvikling eller ferdig utviklede merozoitter. Blant de sistnevnte var det to typer, korte og lange former. Utviklingen av merozoitter ble beskrevet i detalj. Kjempeschizonter ble først delt opp i multinucleære celler av uregelmessig størrelse og form. De enkelte kjernene ble deretter arrangert perifert i cellene, og kjernene delte seg tilslutt i to med etterfølgende utdifferensiering av merozoitter. Den lange typen av merozoitter hadde en langstrakt, sylindrisk form med kjernen terminalt. Cellene var 7,7 μm lange og 1,0 μm i diameter. Merozoittene av den korte typen var spindelformet med kjernen sentralt. Lengden var 5,0 μm og diameteren 1,0 μm.

Globidium-parasittene var lokalisert i en „parasitophor vacuole" inne i en levende vertscelle.

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