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STUDIES ON GLOBIDIAL SCHIZONTS IN THE ABOMASUM OF NORWEGIAN SHEEP

THE FINE STRUCTURE OF ONE OF THE FOUR MEROZOITE FORMS INVESTIGATED*

By

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HILALI, MOSAAD: *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. — Norwegian sheep were investigated for globidial schizont infection in the abomasum. The frequency of infection was found to be 78.2 %. Light microscope studies of the various mature schizonts revealed the existence of four morphologically different merozoites, small A, small B, intermediate and long forms. Each globidial schizont was found to contain only one form of these merozoites. However, these four schizont types occurred in the same abomasum.

The intermediate form of globidial schizont merozoites was investigated by the aid of an electron microscope, with the aim of comparing its internal morphology with that previously published for Eimeria species. A striking resemblance was observed between the fine structures of the intermediate merozoite and that of Eimeria species, particularly the first generation merozoites described in giant schizonts of Eimeria bovis.

The present status of globidial schizonts infecting the abomasum of sheep was discussed. It was concluded that these four forms of merozoites could represent different generations of one Eimeria species or different E. species producing giant schizonts in the abomasum.

Due to the practical difficulties in studying the life histories of the different Eimeria species infecting sheep, it was proposed that the in vitro propagation of the individual species in cultured cells may shed some light on the corresponding asexual, as well as the sexual, stages. This would offer a new approach to the study of the ultrastructure of the developing parasite.

Coccidia; Eimeria; globidium; merozoites; schizonts; sheep; ultrastructure.

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One of the ovine enteric protozoal infections which has long been recognized is globidiosis. The parasite is manifested by the occurrence of whitish spheroidal, pin head bodies or nodules in the internal lining of the abomasum but rarely in the small intestine.

Since the early description of Globidium (Gastrocystis) in the abomasum of sheep, by *Maske* (1893), which was erroneously identified as a gregarine, the definition of globidia has remained, and still is, rather confused. It was later observed by *Gilruth* (1910) and studied in detail by *Chatton* (1910), who named it Gastrocystis gilruthi. These findings were confirmed by *Triffitt* (1925, 1928) and *Wenyon* (1926). The parasite was observed in sheep in a number of countries as Globidium gilruthi by *Alicata* (1930), *Canham* (1931), *Marsh & Tunnicliff* (1941), *Sarwar* (1951), *Guralp & Urman* (1957), *Soliman* (1958, 1960) and *Rac & Willson* (1959). However, *Reichenow & Carini* (1937) and, later, *Reichenow* (1940, 1953) have suppressed the genus Globidium and included it as a subgenus in the genus Eimeria. Later, *Pellérdy* (1960) called for a clearer taxonomy of the so-called Globidium group in order to eliminate overcrowding of the genus Eimeria. More recently, *Levine* (1961) and *Soulsby* (1968) considered that these globidial schizonts belonged to Eimeria gilruthi, while *Rakoveč et al.* (1970) related them to Eimeria intricata. However, both conclusions were just suggestions which lack any experimental confirmation.

The aim of the present work was to assess the incidence of such globidial nodules on the internal lining of sheep abomasum during a natural infection and to investigate the detailed structures of these schizonts. As the fine structure of Eimeria species merozoites infecting several hosts has been described by several authors, an electron microscope study of one form of these globidial merozoites was undertaken with the aim of comparing its internal morphology with that recorded for Eimeria species. This study was also initiated to provide information concerning the present status of globidial schizonts infecting the abomasum of sheep as there has been a great deal of confusion in connection with their nature and identity.

MATERIALS AND METHODS

The materials used in the present investigation were obtained from the abomasum of naturally infected sheep supplied from

slaughtered experimental animals at Wøyen farm near Oslo, as well as from Oslo and Stavanger slaughter houses, during the period October 1970—May 1971. Each abomasum was opened longitudinally with a pair of bowel scissors, its contents removed and the mucosal surface examined carefully, after cleaning, for the presence of nodules.

Preparation of histological sections from the suspected nodules

As the macroscopic appearance of the globidial schizonts may be confused with nodules caused by nematode larvae, histological sections were usually prepared from the suspected nodules in each case. In addition, accurate measurements of the sectioned schizonts were also taken.

Preparation of the merozoites for light microscope studies

Fine smears were usually prepared on cover slips from the crushed contents of each fresh cyst. The cover slips were dropped, surface down, in Schaudinn's fixative. For staining the merozoites, Mallory's technique as advocated by *Haiba* (1953) was successfully adopted, in which the specimens were left in the stain at 37°C overnight.

Preparation of the tissues for studying the ultrastructure of the merozoites

The suspected nodules were dissected from the abomasum of freshly slaughtered sheep and fixed immediately using 4 % paraformaldehyde in *Millonig*'s buffer (1961) at pH 7.2—7.4 for one hr. The cysts were postfixed in 1 % osmium tetraoxide in Millonig's buffer at 4°C for one hr. and then dehydrated in increasing concentrations of acetone. The material was embedded in araldite according to the method of *Luft* (1961). Sections were cut with glass knives using LKB ultramicrotome, collected on copper grids and stained with 5 % uranyl acetate (*Pease* 1964) and lead citrate (*Reynolds* 1963). The sections were observed with Siemens IA electron microscope operated at 80 kv using an instrumental magnification of 2300 to 60,000.

Sections, 0.5—2 μ, of the araldite blocks were cut and stained with aqueous toluidine (pH 11.1) to give an identical light microscope evaluation of the sections examined with the electron microscope (*Trump et al.* 1961).

The present study has been illustrated by camera lucida drawings, and whereever possible by photomicrographs and statistical tables.

RESULTS

Incidence of globidial schizonts

A thorough inspection of 124 lamb and adult sheep abomasa, selected at random, showed that 97 harboured globidial schizonts (78.2 %).

The number of these schizonts in each abomasum usually varied from four to numerous (1—5 in every cm^2). The globidial nodules were observed more frequently in the fundic portion of the abomasum than in the pyloric portion.

Macroscopic examination

The first globidial schizont was encountered in an abomasum while carrying out a routine post-mortem examination of a sheep that was the subject of a coccidiosis investigation (*Helle & Hilali* 1973). Positive cases revealed the existence of a number of whitish, rounded or ovoid, pin head nodules, or cysts, bulging from the mucosal surface of the abomasum. By exerting slight pressure on these cysts, particularly mature ones, a milky white inspissate could be extruded. Some of these cysts were ruptured and resembled small crater like eminences marking the position of the former cysts.

Light microscope observations

Microscopic examination of crushed, Mallory stained cysts led to the finding, in positive cases, of four morphologically different merozoites: small A, small B, intermediate and long forms. Each globidial schizont was found to contain only one form of these merozoites. However, all four schizonts occurred in the same abomasum.

Of 100 globidial schizonts collected at random from 10 abomasa (10 from each abomasum), 49 were immature while the remaining contained mature merozoites. In the mature schizonts (Fig. 1), the four forms of merozoites had the following distribution: 10, 11, 17 and 13, for small A (Plate 1 A and Fig. 2), small B (Plate 1 B and Fig. 3), intermediate (Plate 2 A and Fig. 4) and long forms (Plate 2 B and Fig. 5) respectively, i. e. 19.6 %, 21.5 %, 33.3 % and 25.4 %. The average dimensions of

Table 1. Dimensions of globidial schizonts containing the different merozoites.

Form of merozoite	Number of globidial schizonts investigated	Dimensions		
		min.	max.	mean
small A	10	342 × 212 µ	701 × 527 µ	481±49 × 352±51 µ
small B	11	279 × 189 µ	645 × 439 µ	436±32 × 327±31 µ
inter-mediate	17	394 × 291 µ	621 × 493 µ	502±15 × 373±15 µ
long	13	422 × 341 µ	711 × 623 µ	584±29 × 459±32 µ

these schizonts were 481 × 352 µ, 436 × 327 µ, 502 × 373 µ and 584 × 459 µ, respectively (Table 1).

The morphology of the investigated forms have the following points of interest.

The small form A merozoites (Plate 1 A and Fig. 2) were spindle shaped, pointed at both ends and contained a dense, homogenous, finely granular cytoplasm. The dimensions were 4—5 µ × 0.93—1.25 µ (average 4.46 × 1.07 µ, Table 2). The nuclei were oval, measuring 1.09—1.7 × 0.62—1.25 µ (average 1.33 × 0.89 µ), and located approximately in the centre of the organism, while, in few specimens, they were somewhat close to one of the extremities. Each nucleus was enclosed by a thin membrane, inside which chromatin granules of varying sizes and distribution could be observed. In most specimens the granules were arrangcd as fine particles on the inner surface of the nuclear membrane leaving an eccentrically positioned small karyosome. In others, they were fused into a single mass on one side, while on the opposite side they were arranged as fine particles. The karyosome was observed as a compact mass without a perikaryosomal halo.

The second small form B merozoites (Plate 1 B and Fig. 3) were lanceolate in shape, relatively broad and stumpy, with pointed ends, and measuring 4.7—5.6 µ × 1.25—1.87 µ (average 5.12 × 1.71 µ, Table 2). The cytoplasm was homogenous and finely granular, but less dense than the form mentioned above. The nuclei were globular or subglobular, varying in size from 1.4—2.1 µ × 1.25—1.87 µ (average 1.81 × 1.39 µ) and located at one of the extremities. The nuclear membrane was very thin,

Table 2. Average dimensions (in microns) of 50 merozoites. From each of the four forms investigated.

Form of merozoite	Total length of merozoites			Width of merozoites		
	min.	max.	mean	min.	max.	mean
small A	4	5	4.46±0.04	0.93	1.25	1.074±0.020
small B	4.7	5.6	5.12±0.041	1.25	1.87	1.712±0.028
inter-mediate	6.8	8.4	7.65±0.054	1.25	1.7	1.37 ±0.017
long	7.1	9.6	8.58±0.073	0.78	1.5	1.12 ±0.030

Table 2 (continued).

Form of merozoite	Nuclear length			Nuclear width		
	min.	max.	mean	min.	max.	mean
small A	1.09	1.7	1.33 ±0.02	0.62	1.25	0.89±0.014
small B	1.4	2.1	1.81 ±0.026	1.25	1.87	1.39±0.017
inter-mediate	1.5	2.5	1.94 ±0.028	0.93	1.25	1.12±0.02
long	1.5	2.6	2.010±0.033	0.62	1.09	0.89±0.014

while the nuclear granules were relatively large and of varying sizes, shapes and distribution, but without any tendency to fuse together. The karyosome was a dense mass situated eccentrically without any perikaryosomal halo.

The intermediate merozoites were elongated fusiform in shape, pointed at both ends, and varying in size from 6.8—8.4 μ × 1.25—1.7 μ (average 7.65 × 1.37 μ) with their maximum breadth near the middle (Plate 2 A and Fig. 4). The cytoplasm was finely granular and contained one or two oval vacuoles. The nucleus was oval in shape, measuring 1.5—2.5 μ × 0.93—1.25 μ (average 1.94 × 1.12 μ), and usually situated in the middle of the organism, although in some specimens it was closer to one of the extremities. The nuclear granules were irregularly shaped coarse masses, distributed on the inner surface of the very thin nuclear membrane. The karyosome was a dense spherical mass situated eccentrically without any surrounding halo.

The long form merozoites (Plate 2 B and Fig. 5) had an elongated banana shape, with pointed ends and measured 7.1—

9.6 μ × 0.78—1.5 μ (average 8.58 × 1.12 μ). The cytoplasm was finely granular and contained a relatively large vacuole close to the nucleus.

The oval shaped nucleus was located at one of the extremities, and its dimensions varied within 1.5—2.6 μ × 0.62—1.09 μ (average 2.01 × 0.89 μ). The nuclear membrane was thin, and the chromatin granules were irregularly shaped, elongated masses, mostly fused together, while the eccentric karyosome was not surrounded by a halo.

Fine structure of the intermediate merozoites

From the electron micrographs, the longest intermediate merozoite observed was fusiform in shape, measuring 7.6 × 1.2 μ (Fig. 6). Each merozoite was enclosed by a pellicle formed of two membranes. The outer membrane was triple layered, 110 Å thick (Fig. 7) with the outer and inner layers denser than the intermediate one. The inner membrane of the pellicle was single layered, 166 Å thick and separated from the outer membrane by a variable distance due to the uneven appearance of the latter membrane (Figs. 7, 8 and 9).

The outer membrane enclosed the whole merozoite, whereas the inner membrane was continuous except at the two poles of the cell. The inner membrane had a circular opening at the anterior end about 0.26 μ in diameter (Fig. 8). At the edge of this opening the inner membrane was thicker forming a so-called polar ring.

The subpellicular fibrils were usually 22 in number, each having a diameter of 0.027 μ. They were uniformly distributed around the periphery of the merozoite just inside the inner membrane (Figs. 9 and 10), and were probably attached to the polar ring anteriorly. They could also be traced to the posterior end of the merozoites.

The dense cylindrical, or truncate cone-like structure, the conoid, (Figs. 7 and 8) extended from the level of the polar ring inwards. Its position was never found to vary from that described above. The conoid measured 0.25 μ in length, and its base and top had diameters of about 0.27 μ and 0.20 μ, respectively. In most of the examined specimens the wall of the conoid consisted of a dense solid structure measuring 300 Å in thickness. In very few of the specimens, especially those which were sectioned tangentially, zones of strong and weak density could

be observed. Two rings were always observed near the anterior end of the conoid in longitudinal and tangential sections of the organism. They were linked to the conoid and with each other by a delicate membrane. A vesicle-like structure was observed near the end of this membrane in tangential sections (Fig. 10). This vesicle was globular in shape, measuring 0.077 μ in diameter, and was situated between the anterior limiting membrane and the termination of the rhoptries.

The two rhoptries (*Senaud* 1967, *Levine* 1969a and *Scholtyseck & Mehlhorn* 1970) measured 2.24 μ in length and extended from near the anterior end toward the centre of the organism for approx. one third of its total length (Figs. 7 and 11). Each one was club-shaped having a narrow neck, 0.05 μ, and increased to a diameter of about 0.3 μ at the posterior end.

In longitudinal and cross sections of the anterior end (Figs. 8 and 9), a rod-shaped body was observed between the necks of the rhoptries. Its longitudinal axis was parallel to those of the rhoptries, and it usually had a slightly larger diameter.

The elongated, and nearly central, nucleus was surrounded by a very thin nuclear envelope whose outer surface was studded with numerous ribosomes. The chromatin was in the form of irregular dense clumps located on the inner surface of the nuclear envelope and mostly fused together (Fig. 12). The nucleolus was observed as an eccentric, large, irregularly spheroidal and less electron dense mass, measuring 0.5 μ in diameter. The nucleoplasm had the same density as the merozoite's cytoplasm but it was usually studded with spheroidal globules of the same density as the chromatin.

A globular prenuclear body, 0.7 μ in diameter, was observed in nearly all of the examined merozoites (Fig. 11). This body was surrounded by a very thin, single layered membrane which had a globular or oval invagination, 0.16 μ in diameter, just in front of the nucleus. The prenuclear body was nearly full of coarse, moderately dense granules. Characteristically, the prenuclear body in most of the examined material contained a central, or eccentric, electron dense globule measuring 0.2 μ in diameter (Fig. 11).

The anterior portion of the merozoite, from the prenuclear vacuole to the anterior tip, was usually packed with numerous oval bodies varying in number from 20 to 50. In longitudinal sections, they presented profiles varying from short to long oval

(length from 0.15 to 0.3 μ) (Figs. 7, 10 and 11). In cross sections the profiles were predominantly circular measuring about 0.06—0.08 μ in diameter. These bodies appeared as completely homogenous, electron dense structures without any limiting membrane. Their arrangement and structure suggested that they were long and tortuous. Many ribosomes were scattered between these bodies.

Some of the examined merozoites showed an invagination of the outer and inner membranes at the level of the anterior end of the nucleus. This invagination extended to the cytoplasm for a distance about 0.14 μ without having any openings, its diameter being about 0.06 μ (Fig. 12). The outer membrane limited the invagination at its base, whereas the inner membrane was interrupted and followed the outer membrane only on the lateral sides of the invagination. The invaginated inner membrane was often thickened by an accumulation of electron dense material.

The area posterior to the nucleus was occupied by several cisternae of rough-surface endoplasmic reticulum, free ribosomes and a small number of oval bodies measuring approx. 0.14 × 0.06 μ (Fig. 13). Some of these bodies had homogenous electron dense contents while others contained a less electron dense central zone and a dark outer zone.

The mitochondria were of the common protozoan type having tubular and vesicular cristae and usually situated at the posterior end of the organism (Fig. 14 a, b). In the centre of cross sectioned tubular cristae a dark spot was often seen (Fig. 14 b). In contrast to the immature schizonts, relatively indistinct mitochondrial walls and cristae membranes were observed in the mature merozoites.

Development of merozoites

The present investigation also gave some information about the cytological events occurring during the development of the merozoites. At the earliest stage the nuclei were divided into thousands (Fig. 15), and this was followed by the division of the cytoplasm into many lobes or spheroidal blastophores (Fig. 16), each one containing many nuclei particularly at the periphery. As development proceeded the blastophore membrane was extended as a finger-like bud (Fig. 17). This bud, the developing merozoite, contained the precursors of all the cytoplasmic constituents, derived from the blastophore. The attachment of the

merozoite then broke off resulting in the free merozoite and a residual body.

DISCUSSION

Incidence and light microscope observations

As the infection frequency of globidial schizonts in the abomasum of sheep was recorded as 78.2 %, it seemed to indicate that the occurrence of this parasite was relatively common in Norway. The protozoan was recorded by *Triffitt* (1925) and *Alicata* (1930) in 92 and 11 % of investigated sheep in England and the United States, respectively. *Sarwar* (1951) found it in 34—94 % of examined sheep and goats in the different parts of the Indo-Pakistan subcontinent, while *Soliman* (1958, 1960) recorded the infection in 18 and 32 % of the inspected sheep in Egypt and Sudan, respectively.

The data presented in Table 3 indicate that there is a great deal of confusion in the literature regarding the morphology and dimensions of the investigated globidial schizont merozoites. Meanwhile, most of the previous workers have recorded only one form of merozoites from globidial schizonts. These conflicting results could be accounted for by the fact that four morphologically distinct merozoites, small A, small B, intermediate and long forms, were encountered in the present study. The small form A merozoites have not been recorded previously in abomasal schizonts, and it is possible that this form represents a developing stage of the intermediate form described in this work.

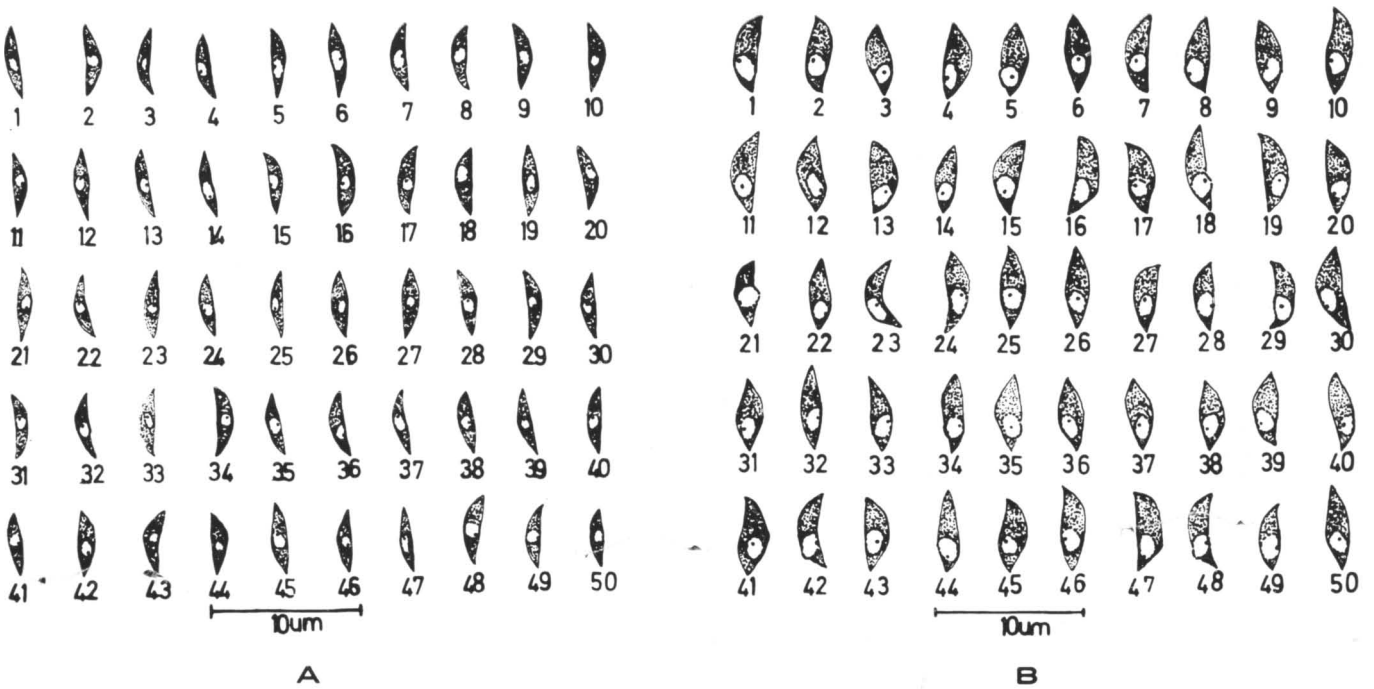
The dimensions and position of the nucleus in small form B merozoites were almost the same as those described by *Soliman* (1958) and *Levine* (1961). However, the general shape and pointed ends of the small form B merozoites do not conform to this previous description.

The present findings concerning the intermediate merozoites were almost in agreement with *Matta & Pande* (1966) in their description of the small slender merozoites from globidial schizonts. However, the sizes observed in this work (6.8—8.4 μ, average 7.65 μ) were somewhat greater. This may be due to the fact that the dimensions given by these authors were based on sectioned material and not on crushed cysts as in the present work, and in addition, a different type of fixative was used in the preparation of the material.

Table 3. Morphological descriptions, dimensions and references pertaining to globidial schizont merozoites, compared with the present findings.

Morphology	Dimensions	References
Fusiform in shape and pointed at both extremities	4—6 μ × 0.5 μ	*Gilruth* (1910)
Fusiform in shape and pointed at both extremities, but the nucleus-containing end less pointed	10 × 1.5 μ	*Chatton* (1910)
Elongated, slightly curved bodies rounded at one end and slightly tapering at the other	12 × 2.5 μ nucleus 3 × 2 μ	*Triffitt* (1925)
Crescent-shaped	5.5—7.5 μ × 1.5—2 μ nucleus 2 × 1 μ	*Alicata* (1930)
Cigar-shaped with tapered ends and an oval nucleus. A large number of granules near the nucleus, but the extremity not containing the granules was more pointed	10 μ	*Canham* (1931)
Blunt at one end, pointed at the other with the nucleus at the blunt end	10 × 1.5 μ	*Sarwar* (1951)
Banana-shaped with the nucleus sometimes near the broad end, in others at the narrow end	4.5—7 μ × 1.2—1.4 μ	*Soliman* (1958)
Sickle-shaped with one end blunt, and the other pointed	6—9 μ × 1.8—1.8 μ	*Soliman* (1960)
Crescent-shaped merozoites, one end rounded and the other pointed	4.5—7.5 μ × 1.2—2 μ	*Levine* (1961)
Three forms were investigated:		
1. small and stumpy merozoites with one end pointed and the other tapering	4.5—5 μ × 1.8—2 μ	*Matta & Pande* (1966)
2. small and slender in shape tapering at both ends, while the nucleus was almost central	6.1 × 1.5 μ	„ „ „
3. large merozoites, pointed at both extremities, and the nucleus was situated at one of them	6.9 × 7.7 μ × 1.3—1.5 μ	„ „ „
Four forms were investigated:		
1. small A	4—5 μ	Present work
2. small B	4.7—5.6 μ	
3. intermediate	6.8—8.4 μ	
4. long	7.1—9.6 μ	

Mosaad Hilali:
Studies on globidial schizonts in the abomasum of Norwegian sheep.



Plates 1 and 2. Camera lucida drawings of the various forms of merozoites found in globidial schizonts in the abomasum of sheep.

Plate 1 A: Small form A. Plate 1 B: Small form B.

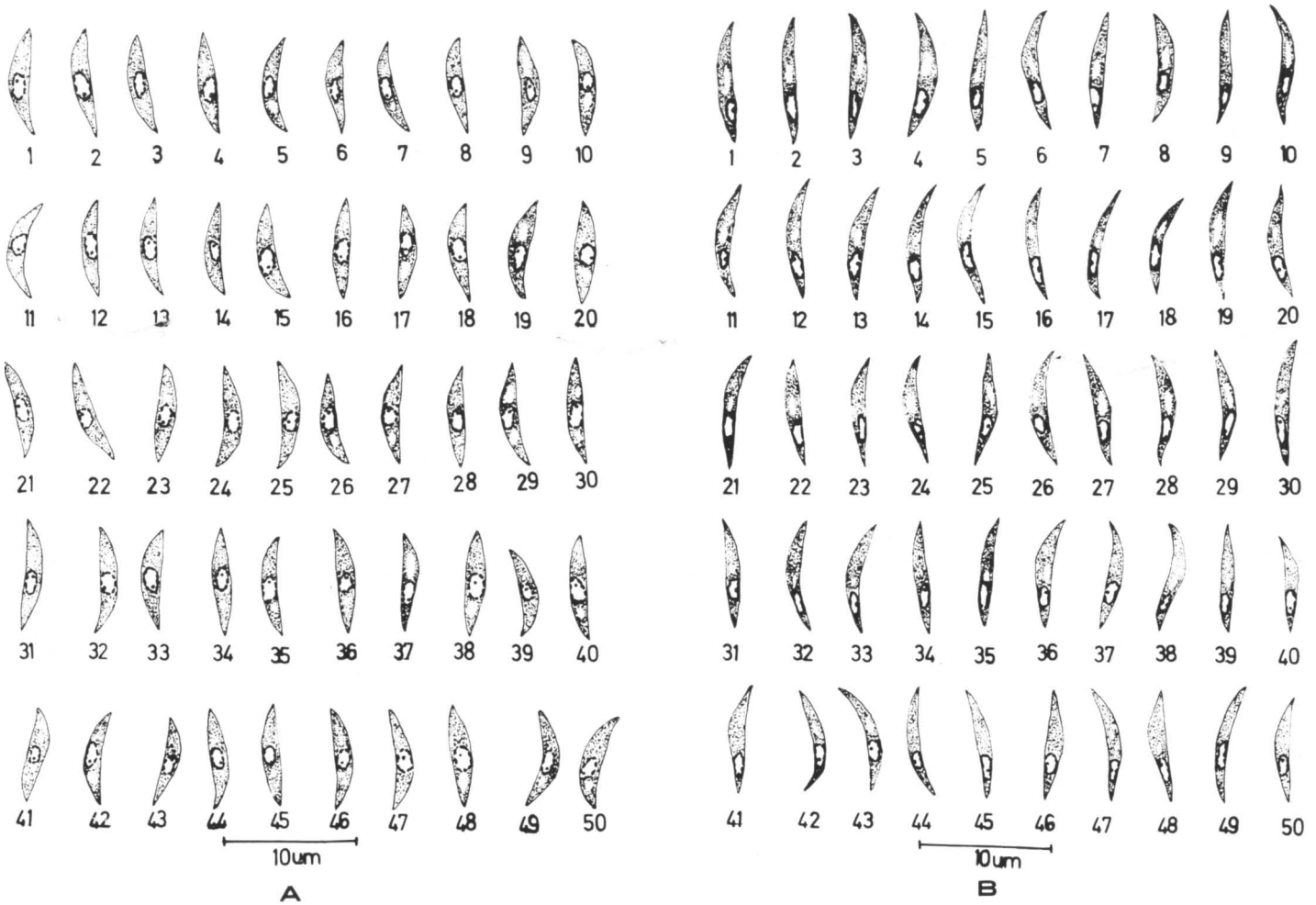


Plate 2 A: Intermediate form. Plate 2 B: Long form.

Abbreviations of all figures

C	Conoid	NCH	Nuclear chromatin
ER	Endoplasmic reticulum	NE	Nuclear envelope
F	Subpellicular fibrils	NH	Necks of the two rhoptries
G	Coarse granules		
GC	Golgi complex	OB	Oval bodies
GL	Globule	OM	Outer membrane
H	Rhoptries	OV	Oval invagination
IM	Inner membrane	P	Polar ring
LM	Limiting membrane	PNB	Prenuclear body
MN	Micronemes	R_1 and R_2	Rings belonging to the conoid
MP	Micropore		
MR	Median rod	R	Ribosomes
MT	Mitochondria	RB	Residual body
N	Nucleus	V	Vesicle
NC	Nucleolus		

Figure 1. Histological section of a mature globidial schizont in the abomasum of a sheep. (Light microscope photograph × 100).

Figures 2—5. The merozoite forms found in the various globidial schizonts. (Light microscope photographs × 1500).

Fig. 2. Small A. Fig. 3. Small B. Fig. 4. Intermediate. Fig. 5. Long.

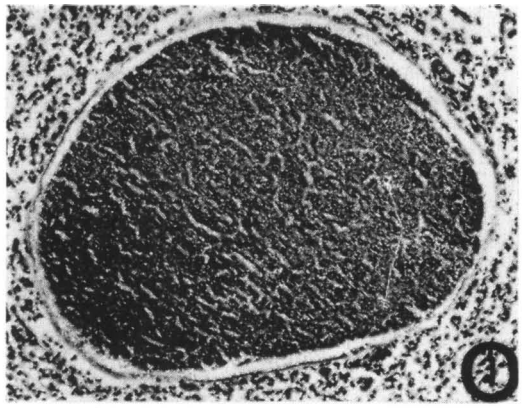
Figures 6—9. Electron micrographs of the intermediate form of globidial schizont merozoites.

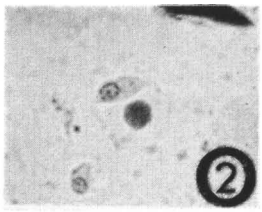
Fig. 6. Section showing numerous randomly orientated intermediate merozoites in a globidial schizont. × 7500.

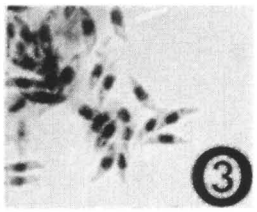
Fig. 7. Anterior end of a merozoite. Note the outer and inner membranes, the two rhoptries, the conoid and the micronemes. × 30,000.

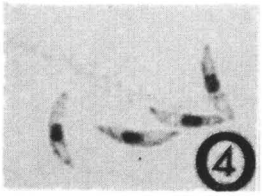
Fig. 8. Longitudinal section of the anterior end of a merozoite showing the median rod between the necks of the two rhoptries, the conoid and the two rings in front of the conoid. × 60,000.

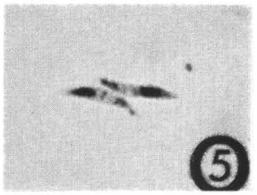
Fig. 9. Cross section of a merozoite at its anterior end. Note the two rhoptries and the median rod within the conoid, the subpellicular fibrils apparently attached to the polar ring and the two membranes (arrows). × 60,000.

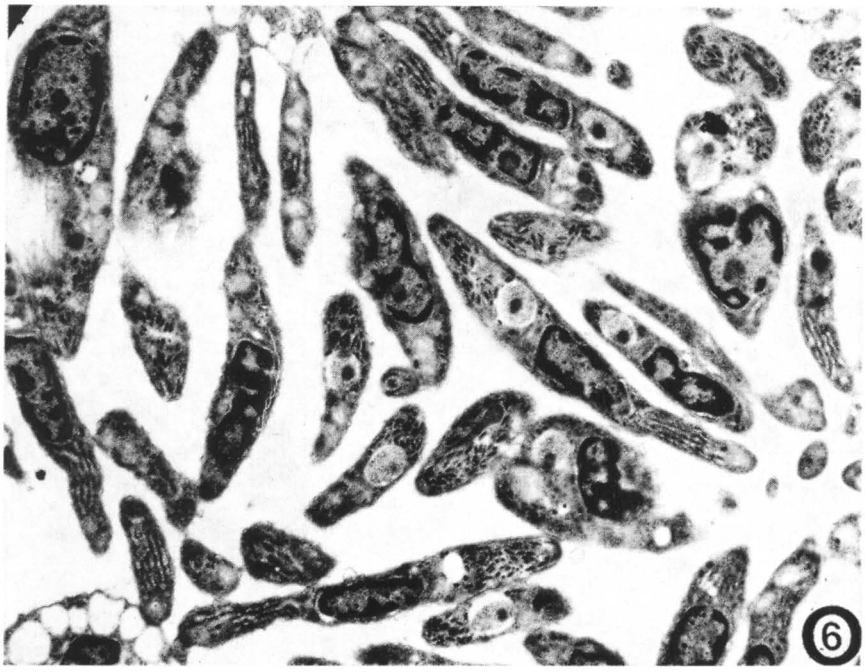


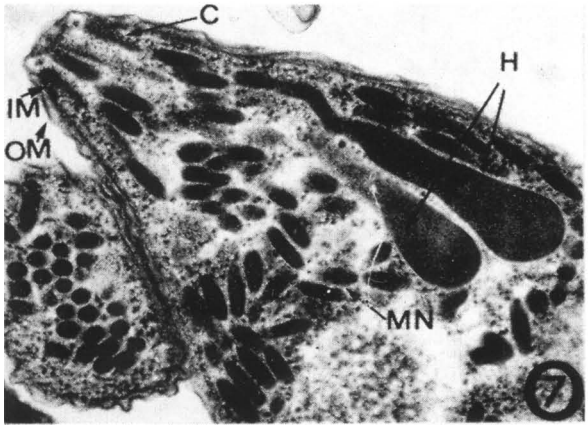








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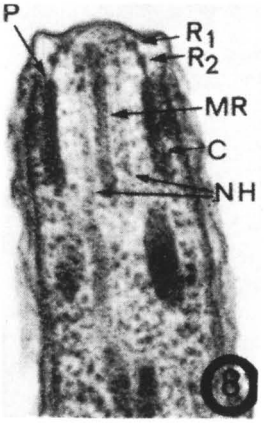
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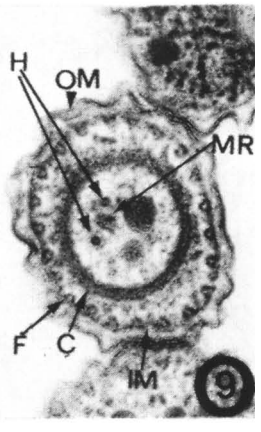
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Figures 10—14. Electron micrographs of the intermediate form of globidial schizont merozoites.

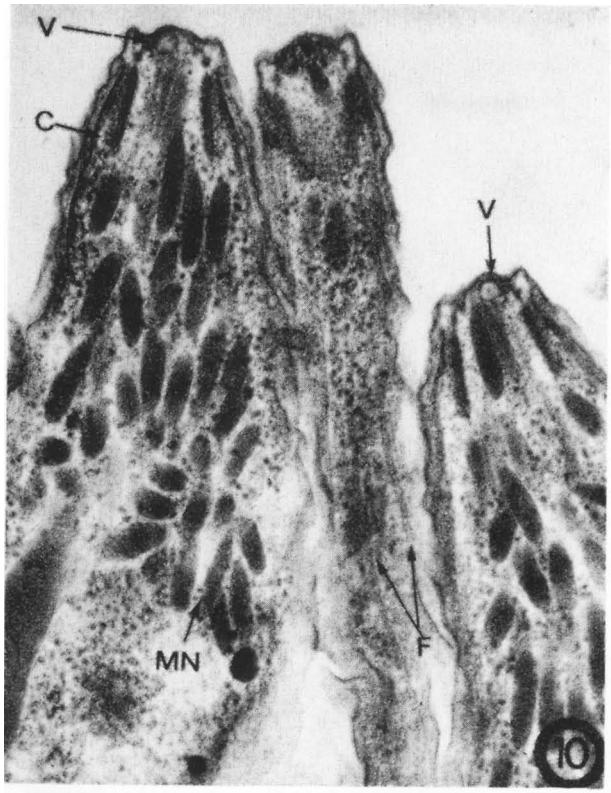
Fig. 10. Longitudinal section of three merozoites showing subpellicular fibrils in one and the anterior vesicle in the other two. × 45,000.

Fig. 11. Longitudinal section of the anterior end of a merozoite showing the prenuclear body with an oval invagination in front of the nucleus. × 45,000. Note its limiting membrane and the contents, which consist of numerous coarse, moderately dense granules and a relatively large, electron dense globule.

Fig. 12. Longitudinal section of the micropore and the nucleus. × 45,000.

Fig. 13. Longitudinal section of the posterior end of two merozoites. Note the cisternae of rough-surface endoplasmic reticulum, free ribosomes and the oval bodies. × 24,000.

Figure 14 a and b. Cross section through the mitochondria showing tubular and vesicular cristae. Note a dark dot in the centre of the tubular cristae (b). × 20,000.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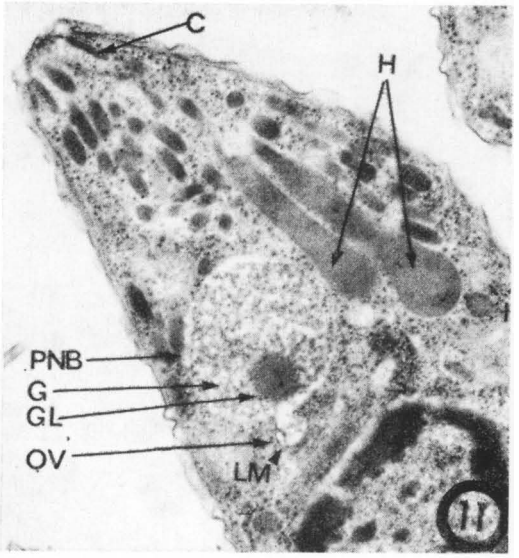
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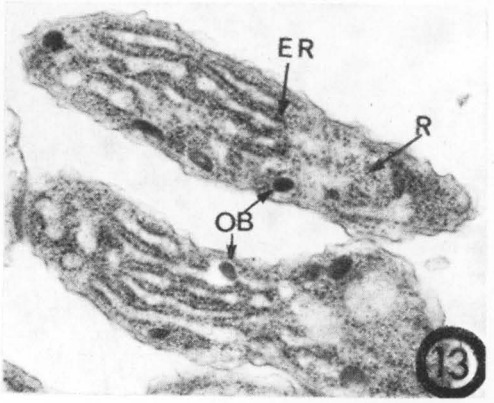
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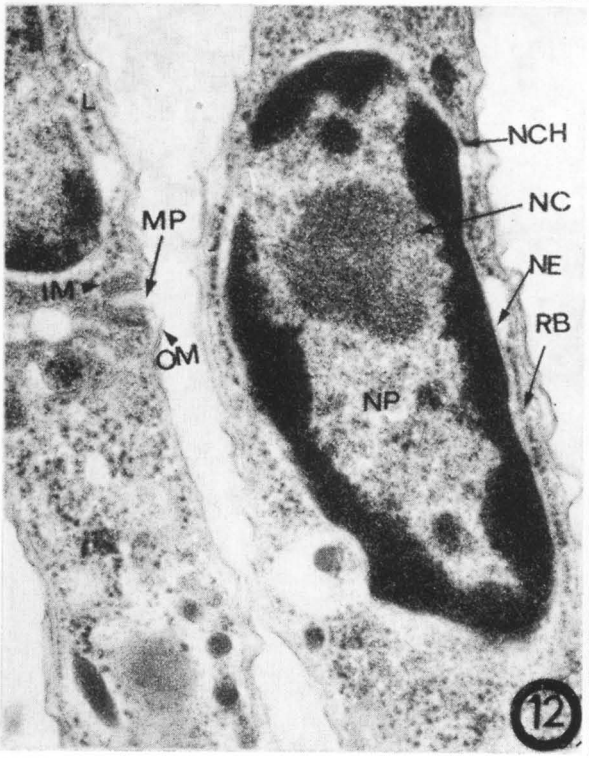
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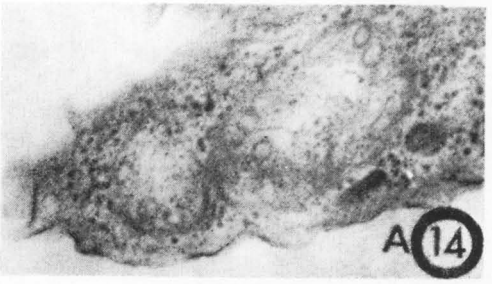
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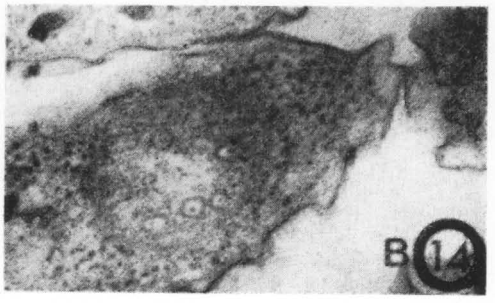
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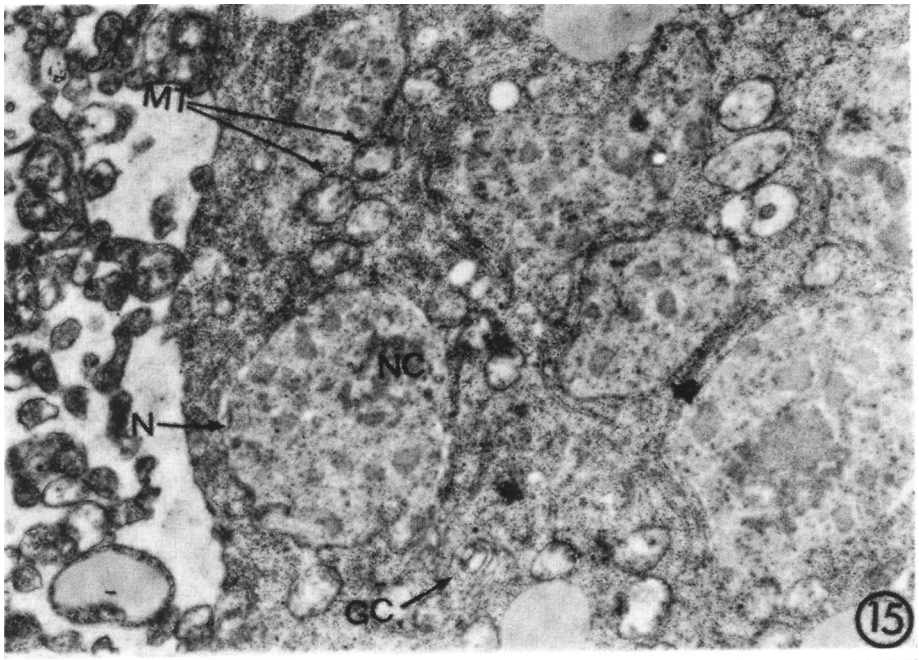
Figures 15—17. Sections through immature globidial schizont.

Fig. 15. The earliest stage, showing numerous nuclei scattered in the undivided cytoplasm. Note also numerous mitochondria and the Golgi complex in the cytoplasm (only stained with uranyl acetate). × 10,000.

Fig. 16. The cytoplasm is divided into lobes, or spheroidal blastophores, each containing numerous nuclei particularly at the periphery. × 2,300.

Fig. 17. Final stage before the separation of the merozoites from the residual blastophore cytoplasm. Note that the blastophore membrane is extended as a finger-like bud. × 10,000.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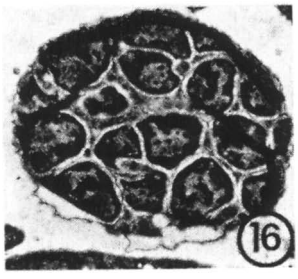
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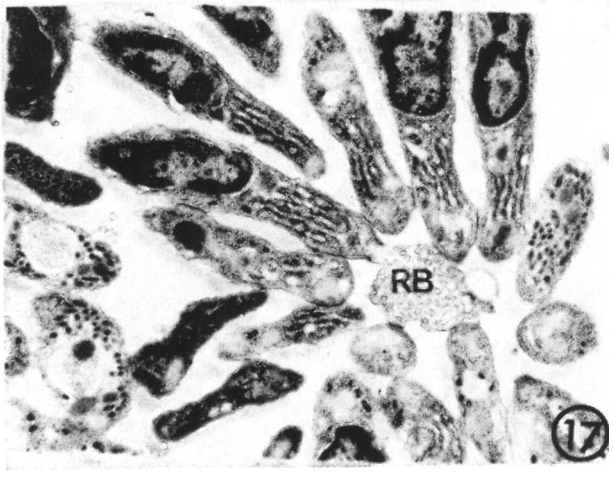
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The investigated long form compares favourably with the large sized merozoites described by *Matta & Pande.*

In contrast to *Chatton* (1910), *Triffitt* (1925), *Canham* (1931), *Sarvar, Soliman* (1958, 1960) and *Levine* (1961) and in agreement with the original description of *Gilruth* (1910) and *Matta & Pande* (for the small slender and large forms), all the investigated forms were equally pointed at both ends.

Electron microscopic observations

The superficial resemblance of the intermediate form of globidial schizont merozoites under the electron microscope to that of Eimeria species was quite striking.

In agreement with the previous studies on the fine structures of E. bovis and E. miyairii merozoites, the outer membrane of the pellicle was triple layered. *Cheissin & Snigirevskaya* (1965) and *Scholtyseck & Piekarski* (1965) thought that the inner membrane was a single triple layered membrane, whereas *Andreassen & Behnke* (1968) could observe it as a bi-layered membrane. The present description of the inner membrane was similar to the previous observations of *Sheffield & Hammond* (1966), where no differentiation of the inner membrane could be observed.

The subpellicular fibrils have been recorded in varying numbers in the different Eimeria species merozoites, 24—30 in E. intestinalis, 26 in E. miyairii and E. magna, and 24 in E. stiedae, E. perforans and E. tenella.

However, the present material consistently showed 22 subpellicular fibrils which is identical to the previous results of *Sheffield & Hammond* (1966) and *Scholtyseck et al.* (1970) for E. bovis and E. callospermophili, respectively. In contrast to the previous findings of *Cheissin & Snigirevskaya* and *Scholtyseck & Piekarski,* the subpellicular fibrils seemed to be connected with the polar ring and not the conoid.

The two rhoptries showed a strong resemblance to those seen in other E. species merozoites, Toxoplasma gondii and Plasmodium gallinaceum. The narrow neck-like anterior part, together with the occasional occurrence of a vesicle at the anterior end of the rhoptries, is suggestive of a glandular function, secreting a fluid to assist in the penetration of a new host cell as indicated by *Garnham et al.* (1960), *Sheffield & Hammond* (1966) and *Scholtyseck & Mehlhorn* (1970). However, the alveolar

appearance of the major portion of the rhoptries as described by *Sheffield & Hammond* (1966) was not observed in the present material. This may be due to the fact that the merozoites in our material were newly formed and nothing had been extruded from the rhoptries.

The rod-shaped body between the neck segments of the rhoptries has been reported before in the merozoites of E. bovis (*Sheffield & Hammond* 1966), E. nieschulzi (*Colley* 1968), E. pragensis, E. tenella and E. magna (*Sénaud & Černa* 1968, 1969). This body may serve to prevent the ductules from being displaced as was thought by *Scholtyseck & Mehlhorn*.

The numerous elongated oval bodies found in the anterior end of the merozoites were actually the micronemes (*Jacobs* 1968 and *Scholtyseck & Mehlhorn*). They were previously given different names in the different parasites, Sarconemes in Toxoplasma (*Ludvik* 1958); Lankesterellonemes in Lankesterella (*Garnham et al.* 1962); convoluted tubules in Plasmodium (*Garnham et al.* 1963, 1969); Cytoplasmastränge in E. stiedae (*Scholtyseck & Piekarski*); Toxonemes in E. intestinalis (*Cheissin & Snigirevskaya*) and Lankesterella (*Stehbens* 1966); Tortuous structures in E. bovis (*Sheffield & Hammond* 1966) and rod-shaped granules in E. miyairii (*Andreassen & Behnke*). *Scholtyseck & Mehlhorn* suggested that the micronemes secrete substances, whereas the rhoptries discharge them. In the present material, there was no evidence for a connection between the micronemes or between the rhoptries and micronemes. Further studies are essential in order to provide the necessary data for clarifying the function of the micronemes.

The striation of the conoid wall which has previously been observed in several protozoa investigated under the electron microscope (e. g. E. bovis, E. stiedae, E. callospermophili, Besnoitia jellisoni and Toxoplasma gondii) was not observed clearly in the merozoites in this work. In addition, the polar ring was always seen to enclose the conoid at its top. This may be explained by the hypothesis of *Scholtyseck et al.* that the conoid in the form mentioned above was always in a contracted state with its spiral elements being compressed so that they created the impression of solidity. It may be possible that the conoid serves as a penetration organelle as suggested by *Ludvik* (1963), *Cheissin & Snigirevskaya* and *Scholtyseck et al.*

The invagination found in the pellicle of some specimens cor-

responds to the structure described as a micropyle in sporozoites of Plasmodium species (*Garnham et al.* 1960), ultracytostome of E. intestinalis (*Cheissin & Snigirevskaya*), cytostome in plasmodium species (*Aikawa et al.* 1966a and b) and micropore in Coelotropha durchoni (*Vivier & Hennere* 1965) and some Eimeria species (*Hammond et al.* 1967). The latter name is recommended in the present work, and also by *Levine* (1969a, b) and *Scholtyseck et al.*, as it is more common and does not indicate any functional significance. *Garnham et al.* (1960) thought that this organelle was the place through which the sporoplasm emerged from the sporozoite of avian malaria. *Cheissin & Snigirevskaya* and *Aikawa et al.* (1966 a, b) believed that the micropore acts as ultracytostome in E. intestinalis and Plasmodium species respectively.

The prenuclear body observed in the intermediate form of globidial schizont merozoites has not been described in any other organisms. It was not similar to the paranuclear bodies reported in the sporozoites of Lankesterella hylae (*Stebhens*), merozoites of Eimeria nieschulzi (*Colley* 1967, 1968) and sporozoites of E. tenella (*Ryley* 1969 and *Strout & Scholtyseck* 1970) in that a limiting membrane around the prenuclear body with an invagination in front of the nucleus was observed. In addition, the presence of a relatively large electron dense globule which occurred inside and outside the prenuclear body was often noted. This prenuclear body may be a food vacuole containing coarse, moderately dense granules, probably glycogen, while the large globule may consist of waste material to be disposed of outside the merozoite's body through the oval invagination of the limiting membrane to the micropore.

It is possible that the micropore in the intermediate form of the globidial schizont merozoites acts as an excretory pore associated with the disposal of unwanted substances. However, further investigations are essential before this may be stated with conviction.

Ovoid granules, with a dark outer zone and similar to those recorded in our material, have previously been described in merozoites of E. bovis (*Sheffield & Hammond* 1966) and in the different stages of E. perforans (*Scholtyseck* 1964). They considered that these granules consisted of glycogen. However, *Ryley et al.* (1968) showed that these bodies contained another polysaccharide, amylopectin, probably in association with protein.

The present status of globidial schizonts infecting the abomasum of sheep

There is a great deal of confusion in the literature regarding the nature and identity of globidial schizonts infecting the abomasum of sheep. These schizonts were previously recognized by several authors as Globidium (Gastrocystis) gilruthi, (*Chatton, Wenyon* 1926, *Triffitt* 1928, *Alicata, Canham, Marsh & Tunnicliff* 1941, *Sarwar, Guralp & Urman* 1957, *Soliman* 1958, 1960 and *Rac & Willson* 1959). All these authors were unaware of the fact that the sheep examined could be infected with coccidia.

Detailed studies on life histories of different Eimeria species infecting sheep have not been carried out. This has been due mainly to the difficulties of infecting the experimental animals with only one species. However, more or less complete data are available on the life cycles of E. arloingi (*Lotze* 1953), E. parva (*Kotlan et al.* 1951b) and E. ahsata (*Davis et al.* 1963), all producing globidial schizonts in the small intestine.

The abomasal nodules, or globidial bodies, were believed to be caused by E. gilruthi (*Levine* 1961, *Soulsby* 1968), for which details of the oocyst and gametogenic stages are still unknown. In the present study, globidial schizonts were first noticed at post-mortem inspections of sheep that had previously been examined weekly during the grazing period (six months) for the identification of the Eimeria species (*Helle & Hilali* 1973). Only eight Eimeria species, namely E. ahsata, E. arloingi, E. crandallis, E. faurei, E. intricata, E. ninakohlyakimovae, E. pallida and E. parva were encountered in this investigation. Consequently, it would not be correct to identify such globidial schizonts in the abomasum of sheep as belonging to E. gilruthi as published by *Levine* (1961) and *Soulsby,* because this species was recognized merely on its schizontic character.

In the present study, light microscope observations revealed the existence of four morphologically different merozoites from the different globidial schizonts. However, the fine structure of the intermediate form of globidial schizont merozoites showed a striking resemblance to that of Eimeria species, especially the first generation merozoites described in giant schizonts of E. bovis (*Sheffield & Hammond* 1966). The process of merozoite formation was also similar to that observed in E. bovis (*Sheffield & Hammond* 1967).

Structural features furnish almost conclusive evidence that

these abomasal schizonts, previously described as Globidium gilruthi and Eimeria gilruthi, represent the giant schizonts of one, or more, of the Eimeria species occurring in sheep, and currently known from their oocysts.

Reichenow (1940), *Becker* (1956) and *Rakovec et al.* (1970) considered that the giant schizonts described in sheep under the name E. gilruthi were those of E. intricata, without having any experimental or empirical evidence to support this view. *Kotlan et al.* (1951a) described the merozoites of E. intricata from globidial schizonts in the small intestine stating that they were about 16 μ long and bent like a hoe at one end.

In the present material, none of the four different forms of merozoites investigated corresponds to the previous description of E. intricata merozoites (*Kotlan et al.* 1951a). However, a relationship between the abomasal schizonts and E. intricata or other E. species, cannot be excluded without having any experimental confirmation.

The fact that four morphologically different merozoites were encountered in different abomasal schizonts in this work, may be due to different generations of merozoites of one of the sheep's Eimeria species, or to different E. species having giant schizonts in the abomasum.

In vitro cultivation of the asexual stages of coccidia in a variety of cells has been reported by several investigators (*Patton* 1965, *Strout et al.* 1965, 1969a, b, *Doran & Vetterling* 1967a, b, 1968, *Fayer & Hammond* 1967, *Hammond & Fayer* 1968, *Scholtyseck & Strout* 1968, *Matsuoka et al.* 1969 and *Strout & Scholtyseck*), although sexual stages in cell cultures have been observed less frequently (*Bedrnik* 1967, *Strout & Quellette* 1969).

In view of the practical difficulties in studying the life histories of the different E. species infecting sheep, it is suggested that the in vitro propagation of the individual species in cultured cells may shed some light on the corresponding asexual, as well as the sexual stages. Meanwhile, this work will offer a new approach to the study of the ultrastructure of the developing parasites.

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SAMMENDRAG

Studier av schizonter av Globidium i løpen hos norske sauer. Ultrastrukturen til en av de fire undersøkte merozoit-typene.

Forekomsten av schizonter av Globidium i løpen er undersøkt hos norske sauer. Infeksjonsfrekvensen var 78,2 %.

Ved undersøkelse med vanlig mikroskop ble det funnet 4 forskjellige typer av merozoitter, liten A, liten B, intermediære og lange former. I hver enkelt schizont var det bare 1 av disse formene. Alle 4 typene kunne imidlertid forekomme i den samme løpen.

Den intermediære form av merozoitter ble undersøkt ved elektronmikroskopi. Det ble funnet en påfallende likhet av de finere cellestrukturer med det som er funnet for merozoitter av Eimeria arter, spesielt for schizonter av Eimeria bovis.

Den systematiske posisjon av Globidium schizonter er diskutert. Det konkluderes med at de fire typene av merozoitter kan være forskjellige stadier av en av sauens Eimeria spp., eller representerer flere Eimeria spp. som har kjempeschizonter i løpen.

På grunn av praktiske vanskeligheter ved studiet av livscyklus til Eimeria spp., pekes det på mulighetene av å studere utviklingen av både ukjønnede og kjønnede stadier i cellekulturer. Dette ville kunne gi ytterligere opplysninger om ultrastrukturen av utviklingsstadiene av parasittene.

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