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Ultrastructural Study of Characteristic Organelles (Paired Organelles, Micronemes, Micropores) of Sporozoa and Related Organisms*

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Summary. By means of electron microscopy a study has been made of different developmental stages of *Eimeria callospermophili*, *E. falciformis*, *Toxoplasma gondii*, *Frenkelia* spec. (=M-organism), *Babesia bigemina*, and *B. ovis*. Major emphasis was given to the analysis of some characteristic organelles of the motile stages of the sporozoans. These organelles were the paired organelle, the micronemes and the micropores.

During the last ten years studies on the fine structure of sporozoa and related groups have revealed a great number of new similar structures. Some of these were given different names, but may be considered to be homologous structures. Predominantly, they can be found in motile stages, i.e. in sporozoites, merozoites and ookinetes. Most important among these structures are the paired organelles (Garnham *et al.*, 1960), the micronemes (=toxonemes), and the micropores. At the IIIrd International Congress on Protozoology (Leningrad, 1969) attempts were made to agree upon a general terminology in this field of research (Garnham, 1969a and b; Levine, 1969a; Scholtyseck, 1969; Sénaud, 1969). Considering similarities of certain fine structures in gregarines, coccidians, haemosporidians, babesians, theilerians, *Toxoplasma*, *Besnoitia*, *Sarcocystis* and M-organism (*Frenkelia* spec.), Levine (1969b) proposed a revised classification of the subphylum Sporozoa (=Polannulifera). In a previous study we have discussed this classification starting with a comparative analysis of the fine structure of the conoid, the polar ring and the pellicle (Scholtyseck, Mehlhorn and Friedhoff, 1970). The present study deals with the ultrastructure and the function of the paired organelles, the micronemes and the micropores.

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Material and Methods

1. *Eimeria callospermophili*

The methods of handling the material for the electron microscope studies of this species, a parasite of the Uinta ground squirrel (*Citellus armatus* syn. *Spermophilus armatus*), were the same as those described previously (Scholtyseck, Hammond, Todd, 1968; Todd, Hammond, 1968). The infected tissue was fixed with glutaraldehyde and with OsO_4, dehydrated with acetone and embedded in Vestopal W. Ultrathin sections were cut with an LKB microtome and the electron micrographs were made with a Zeiss electron microscope EM 9A.

2. *Eimeria falciformis*

The material used for this study originated from laboratory infections of white mice (*Mus musculus*). Small infected pieces of the intestine were removed, fixed, and embedded as for *E. callospermophili*. Further details will be published elsewhere (Scholtyseck, Haberkorn, 1970).

3. *Toxoplasma gondii*

The material used for the electron microscope study originated from infected chorio-allantois membranes of chicken embryos. The methods were described in a previous paper (van der Zypen, Piekarski, 1967). Small pieces of infected tissue were removed, fixed with glutaraldehyde, and postfixed with OsO_4. After dehydrating in acetone the material was embedded in Vestopal W. The plates were then examined with a compound microscope and the infected host tissues or the individual parasite stages identified and marked. These were then sawed out and sectioned with an LKB microtome. The electron micrographs were made with a Zeiss electron microscope EM 9A.

4. M-Organism (*Frenkelia* spec.)

The material used for the electron microscope study of this species, parasitic in the brain of the bank vole (*Clethrionomys glareolus*), originated from wild voles. The methods of handling the voles were the same as those described previously (Scholtyseck, Kepka, Piekarski, 1970).

5. *Babesia bigemina*

Material and methods used in this study were the same as those described in a previous publication (Scholtyseck, Mehlhorn, Friedhoff, 1970). Further details will be published elsewhere (Friedhoff, Scholtyseck, 1970).

6. *Babesia ovis*

The described stages of this species were located in the ovary tissue of the tick *Rhipicephalus bursa*. The methods of handling the ticks were the same as those described previously (Friedhoff, Scholtyseck, 1968).

Results

a) *Toxoplasma gondii*

In transverse sections through zoites of this species we found about 8 large, somewhat tortuous rhoptries. A closer examination of the interior

reveals distinctly different characteristics in different specimens (Figs. 1 a, b, *PO*, *AT*). In some instances the interior is completely homogeneous and electron dense, in others it is of alveolar or sponge-like structure, and at least occasionally, it is partly empty. Obviously these organelles are covered by a membranous layer as shown in Fig. 1 a. The homogeneous structures are more spherical and their diameter reaches a maximum of 2,500 Å. The empty or alveolar structures are only 1,200 Å in diameter; it is possible that any previously present contents had been discharged. In Figs. 1 a and b micronemes are not to be seen, because they do not extend to the prenuclear zone, which has been sectioned in an oblique way.

b) *Eimeria callospermophili*

In cross-sections through merozoites of this species, parasitic in the Uinta ground squirrel (*Citellus armatus*, syn. *Spermophilus armatus*), we found more than two rhoptries, contrary to the situation in other species of *Eimeria* (Fig. 1 c, *PO*, *AT*). Their shape varies; structures of elongated club-shaped forms appear as well as those of a rounded shape. In some cases, the interior had a slightly alveolar appearance, as that in *Toxoplasma gondii*. In diameter, these structures varied between 1,500 and 4,000 Å. A few micronemes were scattered randomly mostly in the marginal area; they measured about 600 Å in diameter. Further details were published elsewhere (Scholtyseck, Mehlhorn, Friedhoff, 1970).

The study at a high magnification has revealed some interesting details on the fine structure of the pellicle. The outer membrane was clearly demonstrated to be a unit membrane (Fig. 1 d, *OM*). The inner membranous layer (*IM*) seemed to be composed of two unit membranes (Fig. 1 d, arrow), normally closely attached to one another.

c) *Eimeria falciformis*

In cross-sections through the anterior half of the merozoite, numerous micronemes of a typically tortuous shape can be seen (Fig. 3 a, *MN*). In some cases they seem to be empty, and to have a covering membrane (Fig. 3 a, *M*). In the same region of the parasite cell we found a micropore (Fig. 3 a, *MP*). Its inner diameter measured about 1,200 Å, whereas the outer diameter, formed by a cylindrical thickened invagination of the inner pellicle membrane, was about 2,400 Å.

d) M-Organism (*Frenkelia* spec.)

In cross-sections through zoites of this species, parasitic in the brain of the bank vole (*Clethrionomys glareolus*) we could detect 5—8 rhoptries of about 2,500 Å diameter (Fig. 3 d, *PO*). Part of their interior is

Abbreviations of all Figures

A	Anterior pole
AC	Apical cup
AP	Alkaline phosphatase
AT	Alveolar aspects of the paired organelles
C	Conoid
CA	Canals
CLR	Bulbous end of the club-shaped rhoptries (= PO)
CO	Connections between the micronemes
CS	Cyst stage
E	Erythrocyte
EEM	Exo-erythrocytic merozoite
EK	Bulbous end (mostly very electron dense)
EM	Erythrocytic merozoite
EN	Empty micronemes
G	Glycogen
HT	Electron dense posterior end of the PO
IM	Inner membranous layer of the pellicle
IIM	Interruption of the IM
LU	Empty interior of the PO
M	Membrane
MC	Mucous cover
ME	Merozoite
MF	Microfibrils
MG	Microgametocytes
MH	Middle membrane of the pellicle
MI	Mitochondrion
MKG	Macrogametes
MN	Micronemes
MP	Micropore
MT	Microtubules lying under the pellicle
MV	Foldings of the outer membrane
N	Nucleus
NM	Nuclear membrane
NP	Nuclear pore
O	Ookinete
OM	Outer membrane
PO	Paired organelles (= Rhoptries)
P	Polar ring
RIM	Remaining part of the inner membrane
RS	Rod-shaped tubules
S	Surface
SB	Spherical body
SS	Inner membranous layer of the pellicle (stratum sous-pelliculaire)
ST	Sponge-like aspect of the PO
TA	Tangential striping
TR	Trophozoite
U	Underlying cylindrical structure
V	Vacuole
WMN	Wall forming micronemes (?)
Z	Zoite
$\_/^-$	not mentioned

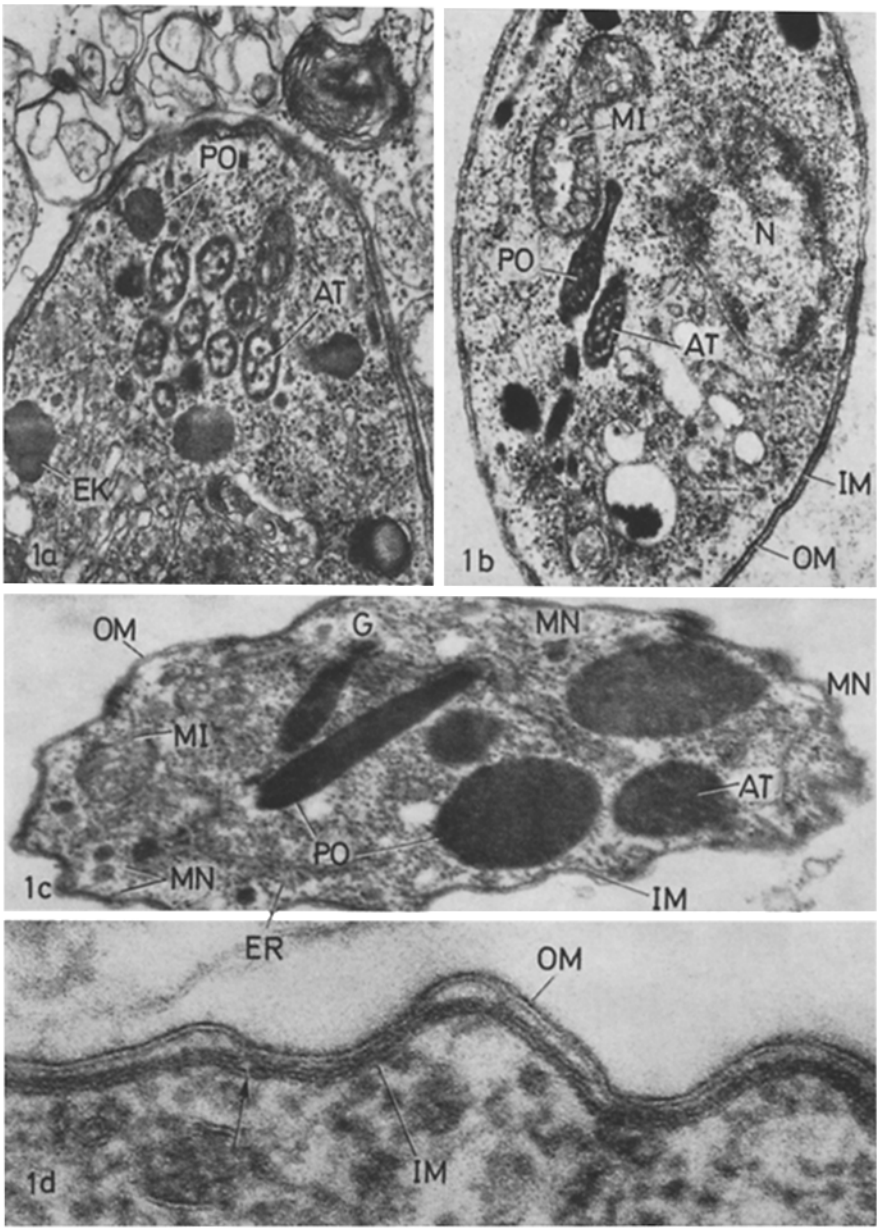

Fig. 1a—d. Electron micrographs. a and b *Toxoplasma gondii*. Cross-sections through the anterior half of a zoite. ×34,000. c *Eimeria callospermophili*. Cross-section through a merozoite. ×43,000. d *E. callospermophili*. The pellicle of the merozoite. Note that the thick inner membrane is composed of two unit membranes (arrow). ×220,000

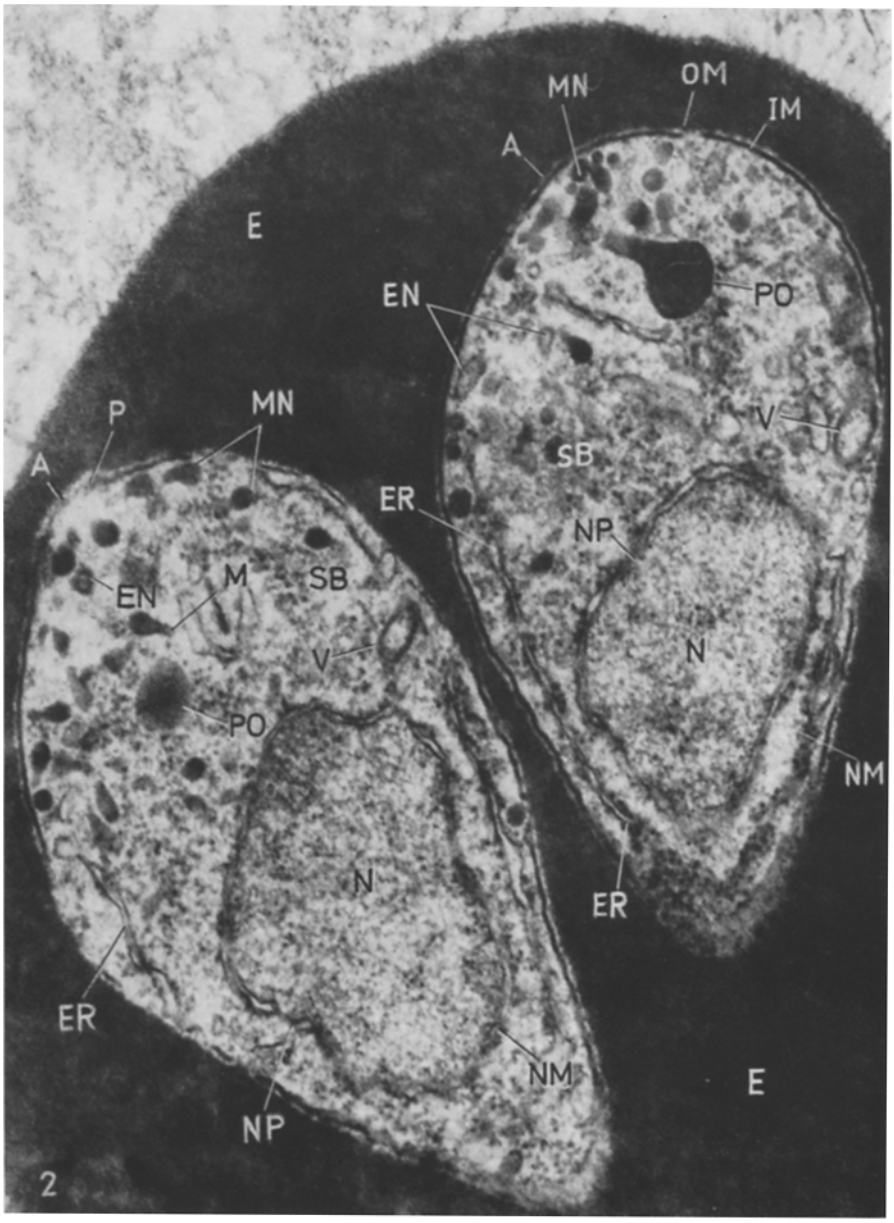

Fig. 2. *Babesia bigemina*. Electron micrograph of two erythrocytic merozoites cut longitudinally. ×56,000

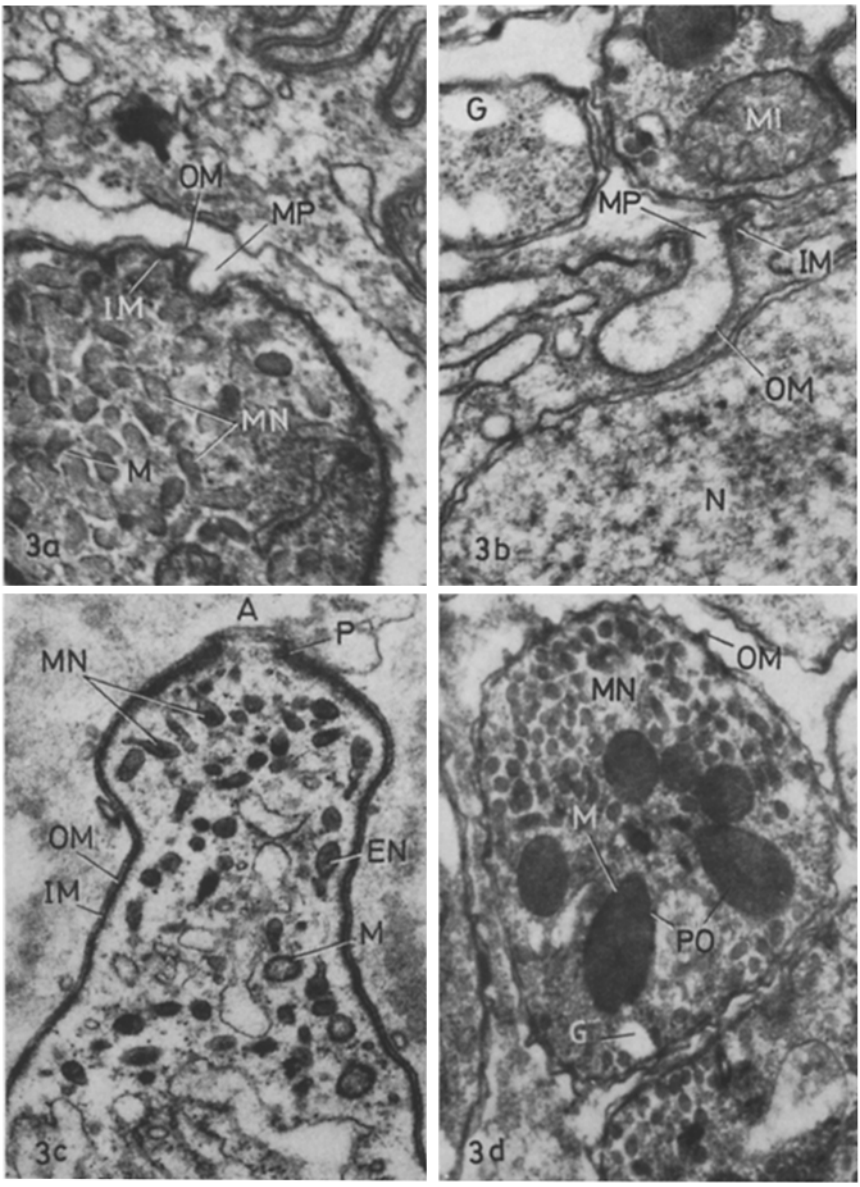

Fig. 3a—d. Electron micrographs. a *Eimeria falciformis*. Cross-section through a merozoite showing a typical micropore. ×41,000. b M-organism (*Frenkelia*). Micropore sectioned longitudinally in a metrocyte. ×41,000. c *Babesia ovis*. Longitudinal section through an ovary stage. ×27,000. d M-organism (*Frenkelia*). Cross-section through the anterior third of the zoite. ×36,000

partly homogeneous, and a covering membranous layer may be seen as well (Fig. 3d, *M*). Numerous micronemes (*MN*) are widely scattered, showing the characteristic structure and diameter of about 600—900 Å A micropore of typical structure was always observed in the anterior third of zoites (Fig. 3b, *MP*). Measurements and details are given in Figs. 7 and 8. In metrocytes, usually three micropores were seen in one section. Further details are reported by Scholtyseck, Kepka, Piekarski (1970), and Kepka, Scholtyseck (1970).

e) *Babesia bigemina*

The pear-shaped erythrocytic merozoites of *Babesia bigemina* are covered by a pellicle consisting of two layers, an outer unit membrane and a thick inner layer of another structure. Two drop-shaped, uniform osmiophilic structures represent the rhoptries. Their narrow ductules extend to the opening of the polar ring (Fig. 2, *P*). Numerous micronemes are scattered around the rhoptries. A spherical body, detected by Friedhoff and Scholtyseck (1970), which is usually in the anterior part of the merozoite, very close to the nucleus, could not be found in the stages studied here. Possibly this body may have changed into other structures (Fig. 2, *SB*). A detailed description of these *Babesia* stages will be published in the near future (Friedhoff, Scholtyseck, 1970).

f) *Babesia ovis*

The two developmental stages of *Babesia ovis* shown in Fig. 4a and b represent an early developmental stage of the transformation of so-called spherical or stationary forms into motile ones (Friedhoff and Scholtyseck, 1968). This is shown by the absence of the typical pellicle known to be present in all motile forms studied. On both pictures, the cell boundary of these *Babesia* stages from the ovary tissues of *Rhipicephalus bursa* is represented by a unit membrane only. The developing inner membrane of the typical pellicle of the motile stages was observed underlying the unit membrane in some places; in others it had not yet developed. Many microneme-like structures are present; it is likely that these structures give rise to the inner layer of the pellicle, which consists of two unit membranes. Some of these micronemes are in clear association with microtubules as shown in Fig. 4a and b (arrow).

Interpretation and Discussion

A. Rhoptries and Micronemes

In 1954 Gustafson, Agar and Cramer found a certain number of electron-dense bodies at the anterior pole of *Toxoplasma gondii* and called them "toxonemes". Garnham, Baker, Bird (1960) introduced a

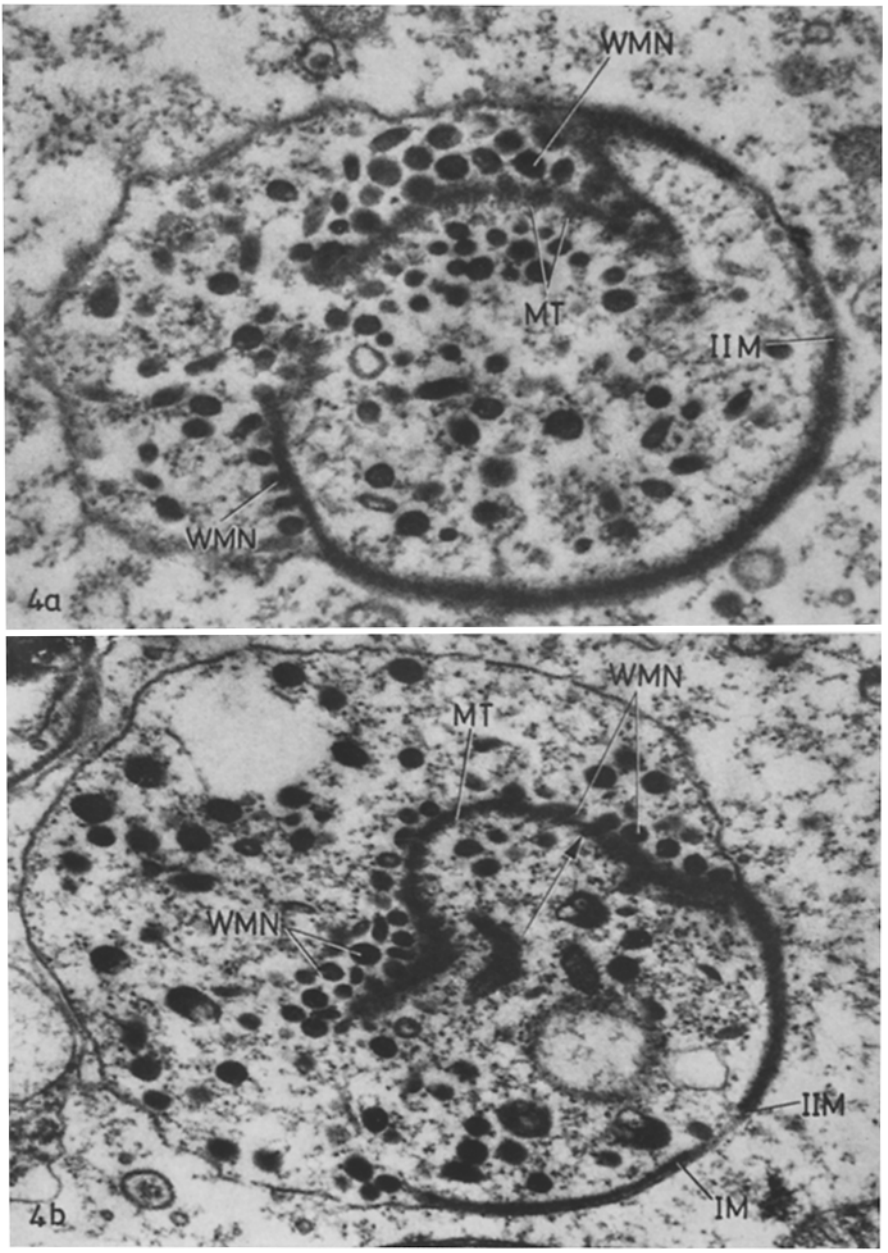

Fig. 4a—b. Electron micrographs. Sections through developmental stages of merozoites of *Babesia ovis* in the ovary of the tick *Rhipicephalus bursa*. ×40,000

complementary term, the "paired organelles". They had observed that in the sporozoite of *Haemamoeba* (= *Plasmodium*) *gallinacea*, among all the tortuous, electron-dense structures at the anterior pole, two were particularly striking because of their large size. These results were confirmed in all Sporozoa, according to Levine's classification, which were studied by means of electron microscopy (Tables 1, 2). These elements can be observed only in merozoites, ookinetes and sporozoites (= motile stages). They may still be found, however, in the next stage of development and then, in some species, their number is even higher. Tables 1 and 2 show the great variety of terms describing these structures and demonstrate that it is necessary to agree upon a general terminology. One should distinguish between two types of structures: 1. Large forms, 2. Small forms.

1. *The large forms* (Figs. 1 a—d; 5; Table 1) are much less numerous than the small ones in the various species. More than eight of these structures have not been observed in one individual until now. In *Eimeria* and *Plasmodium*, only two such structures occur; this would be a "paired organelle" in the true sense of the word. Contrary to the small forms, the ductules of the large forms always extend through the conoid (if a genuine conoid is present towards the outer pellicle membrane. Correspondingly, cross-sections through this region show only a small number of ductules. In typical cases the large forms may clearly be distinguished from the smaller ones only because of their size. In some species of parasites, such clear differences between the larger and the smaller forms have not been observed. Thus, only a number of structures of about the same size has been found in the merozoites of *Haemogregarina* spec. (Stehbens, Johnston, 1967), *Aggregata eberthi* (Heller, 1969), in the sporozoites of *Eucoccidium dinophili* (Bardele, 1966), *Eimeria nieschulzi* (Colley, 1967), and in the proliferative form of *Besnoitia jellisoni* (Sheffield, 1966; Table 2). Despite this resemblance at first sight there must be functional differences, for in cross-sections through the region of the conoid, only two or a few ductules can be found. Therefore, the extension to the very tip of the cell is characteristic for all these large structures.

Generally they appear in two shapes in the different species of parasites (Fig. 5). In *Plasmodium* they are very compact and are mostly described as "teardrop-like", whereas all others are club-shaped and some have a sort of bulbous end.

The ductules (Table 1) in the different species vary between 250 and 650 Å in diameter. Therefore, these structures are about 5—10 times narrower here than at their bulbous ends. There they are about 2,000 to 4,000 Å in diameter. Variations within the single species may be due to different functional phases. These organelles seldom extend beyond

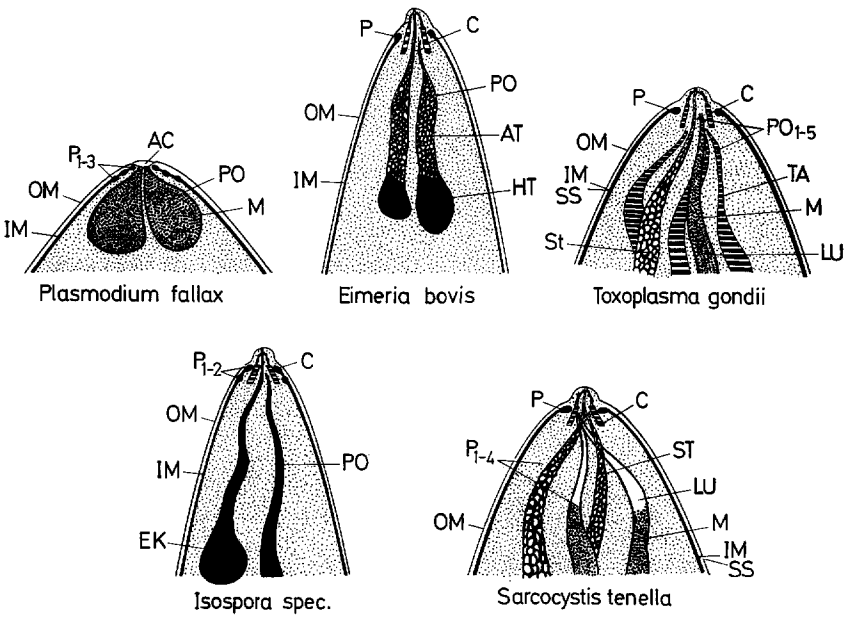

Fig. 5. Diagrammatic representation of some types of paired organelles (rhoptries) and their aspects

the middle of the cell towards the posterior pole (Figs. 2, 5; Table 1). The length of only 0.45 μ in the genus *Plasmodium* must not be underrated, for it reaches about $^1/_6$—$^1/_8$ of the length of this parasite. Thus, the size of these organelles corresponds to that in *Eimeria tenella* and *Isospora* spec. (Fig. 5; Table 1). The large structures are not completely straight, but are slightly tortuous. Consequently, authors studying this problem found only segments which were much larger than those of the smaller forms when cut longitudinally. There is good evidence that the smaller forms are much more tortuous than the large ones.

Until recently these organelles were thought to be completely homogeneous; because of their great electron density, study of the fine structure is difficult. Only very recently certain differentiations have been recognized. A single limiting membrane, which could be clearly distinguished from the less dense interior, has hitherto been demonstrated in *Eimeria intestinalis* (Cheissin, Snigirevskaya, 1965), *Plasmodium fallax, P. lophurae, Pl. cathemerium* (Aikawa, 1966), *Toxoplasma gondii, Sarcocystis tenella* (Sénaud, 1967), *Eimeria tenella* (McLaren, Paget, 1968), *E. miyairii* (Andreassen, Behnke, 1968), M-organism (Scholtyseck, Kepka, Piekarski, 1970), and *Aggregata eberthi* (Heller, 1969). An artifact due to preparation must be considered unlikely since Sénaud (1967) always

Table 1. *Measurements, details and references pertaining to the paired organelles*

Parasites	Number	Greatest diameter (Å)	Diameter of the ductules (Å)	Length (μ)
Isospora spec. (M)	2	2,100	650	2.4—0.1
Lankesterella garnhami (TR)	2	2,000	400	1.8
Aggregata eberthi (M)	some	1,500	_/‾	anterior half
Eimeria stiedae (M)	2	3,000	700	anterior half
Eimeria bovis (M)	2	1,400	300	_/‾
Eimeria tenella (M)	2	2,500	500	$^1/_8$ of the cell
Eimeria miyairii (M)	2	1,500	500	3—5
Eimeria pragensis (M)	2	2,000—4,000	_/‾	5—6
Eimeria magna (M)	2	2,000—3,000	500	2.5—3
Toxoplasma gondii (TR)	8	a) 800—1,200 b) 4,000	_/‾	−7
Toxoplasma gondii (TR)	4—8	1,600	250	_/‾
Sarcocystis tenella (TR)	8	a) 800—1,200 b) 4,000	_/‾	to the nucleus
Besnoitia jellisoni (CS)	Some	1,600	350	to the central region
M-Organism (Z)	5—8	−3,800	600	anterior half
Plasmodium fallax (EEM)	2	2,500	330	0.47
Plasmodium floridense (EM)	2	2,800	300	0.5
Babesia bigemina (EM)	2—3	1,700	425	0.6

(rhoptries) in the different groups of parasites studied by means of electron microscopy

Shape	Characteristics	References
club-shaped	slightly tortuous, bulbous	Schmidt *et al.* (1967)
sausage-shaped	Ductules seem to be hollow	Garnham *et al.* (1962)
club-shaped	covered by a single membrane; 7—8 stripes with a periodicity of 200 Å	Heller (1969)
club-shaped	covered by a membrane-like layer; partly hollow; partly alveolar	Scholtyseck *et al.* (1965, 1970)
club-shaped	partly alveolar	Sheffield and Hammond (1966)
bulbous end	partly hollow, partly alveolar;	McLaren and Paget (1968)
	changing diameters	Scholtyseck *et al.* (1969)
club-shaped, bulbous	membrane-bound	Andreassen and Behnke (1968)
vermiform	covered by a cortical layer; partly alveolar	Sénaud et Černa (1968)
vermiform	tortuous; partly alveolar; membrane-bound	Sénaud et Černa (1969)
a) club-shaped b) pendulum-shaped	a) membrane-bound b) sponge-like shape	Sénaud (1967)
⌐	stripes with a periodicity of 500 Å	Sheffield and Melton (1968)
a) club-shaped b) pendulum-shaped	a) membrane-bound b) sponge-like shape	Sénaud (1967)
club-shaped	partly of moderate electron density	Sheffield (1968)
club-shaped bulbous	covered by a membrane-like layer; partly alveolar; partly hollow	Scholtyseck, Kepka and Piekarski (1970)
tear-drop-shaped	covered by a membranous layer	Aikawa (1966)
tear-drop-shaped	covered by a membrane	Aikawa and Jordan (1968)
club-shaped bulbous	covered by a membranous layer; partly alveolar; ductules often hollow;	Scholtyseck, Mehlhorn, Friedhoff (1970)

Table 2. *Measurements, details and references pertaining*

Parasites	Number	Diameter (Å)	Name	Length
Haemogregarina spec.	many	650—2,000	Toxonemes	posterior to the nucleus
Selenidium hollandei (TR)	many	a) 2,300 b) 480	Corps denses	anterior half
Eucoccidium dinophili				
a) young trophozoites	a) 15—20	1,300—1,500	Toxonema	2 μ
b) old trophozoites	b) 40—50			
Isospora spec. (M)	many	600—900	Toxonemes	anterior half
Lankesterella garnhami (TR)	many	_/¯	Lankesterel-lonemes	anterior half
Lankesterella hylae (S)	many	400—500	Lankesterel-lonemes	throughout the cell
Aggregata eberthi (M)	many	—1,500	Toxonema	anterior half
Eimeria stiedae (M)	many	400—600	Cytoplasma-stränge	throughout the cell
Eimeria bovis (M)	many	—1,400	Tortuous structures	anterior to spherical body
Eimeria nieschulzi (S)	numerous	600—3,500	Toxonemes	throughout the cell
Eimeria miyairii (M)	—150	600—700	rod-shaped granules	anterior to the nucleus
Eimeria magna (M)	many	400—500	Sarconemes	anterior pole
Eimeria tenella (M)	24	800	Toxonema	anterior pole
Toxoplasma gondii				
a) virulent-in Proliferation	a) few	a) 600—1,600	Toxonema	a) in the first third of the cell
b) avirulent-in cysts	b) numerous	b) 400—2,200		b) throughout
c) avirulent-in proliferation	c) numerous	c) 600—1,600		c) throughout
Toxoplasma gondii (TR)	±8	250	Micronemes	anterior pole
Sarcocystis fusiformis (TR)	numerous	500	Sarconemes	first third of the cell
Sarcocystis tenella (TR)	±500	500—600	Sarconemes	first third of the cell

to micronemes in the different groups of parasites[a]

Shape	Characteristics	References
club-shaped	membrane-bound; changing diameter and density	Stehbens and Johnston (1967)
a) club-shaped b) tortuous	membrane-bound; a dense central zone	Schrevel (1968)
tube-like	membrane-bound; connections to the cristalloid ?	Bardele (1966)
tortuous	covered by a single membrane; granular;	Schmidt *et al.* (1967)
tortuous	arranged in bundles;	Garnham *et al.* (1962)
cylindrical-tortuous	arranged in bundles, light cortex	Stehbens (1966)
cylindrical-tortuous	membrane-bound; central core;	Heller (1969)
tortuous	arranged in bundles; partly alveolar	Scholtyseck und Piekarski (1965)
tortuous	many ribosomes are scattered among these bodies	Sheffield and Hammond (1966)
club-shaped	2 in the conoid; branches; not solid; connections with the paranucl. body	Colley (1967)
rod-shaped	membrane-bound; intensely stained central core	Andreassen and Behnke (1968)
tube-like	tortuous; in bundles arranged	Sénaud et Černa (1969)
_/‾	tortuous; often absent	McLaren and Paget (1968)
clubshaped	a) honeycomb-like structures	Van der Zypen und Piekarski
	b) rarely lightly stained	(1967)
	c) honeycomb-like structures	
elongate-tortuous	connections with the paired org.	Sheffield and Melton (1968)
_/‾	covered by an amorphous sheath; arranged in bundles;	Simpson (1966)
cylindrical	connections with each other; amorphous sheath; arranged in bundles	Sénaud (1967)

[a] The larger organelles, however, must be considered as rhoptries, although the cited authors don't distinguish between the terms.

Table 2

Parasites	Number	Diameter (Å)	Name	Length
M-Organism (Z)	many	600—900	Mikronema	first third of the cell
Besnoitia jellisoni (TR)	30	650—2,600	Toxonemes	anterior to the nucleus
Besnoitia jellisoni (CS)	many	500—600	Sarconemes	first third of the cell
Plasmodium fallax (EM)	some	_/‾	dense bodies	_/‾
Plasmodium floridense (EM)	some	600	dense bodies	anterior pole
Plasmodium berghei (O)	many	—1,000	convoluted tubules	_/‾
Theileria parva (EEM)	6—10	900—1,300	dichte Körperchen	0.2 µ
Babesia caballi	some	_/‾	food vacuoles	anterior pole
Babesia ovis (EEM)	numerous	800	Mikronema	anterior pole
Babesia bigemina (EM)	many	500	Mikronema	anterior to the nucleus

detected this membrane when the rhoptries (= paired organelle) did not exceed 800—1,200 Å in diameter. In organelles with diameters of about 4,000 Å he observed a different, sponge-like fine structure. Probably, in these the limiting membrane is no longer visible because it is masked by the more voluminous and, consequently, more dense interior. Possibly it has different contents because it is fully developed. A similar sponge-like or alveolar interior was also observed in *Eimeria bovis* (Sheffield, Hammond, 1966), *E. tenella*, *E. magna* (Sénaud, Černa, 1969), *E. pragensis* (Sénaud, Černa, 1968), *E. tenella* (Scholtyseck, Strout, Haberkorn, 1969), *E. nieschulzi* (Colley, 1967, 1968), and *Toxoplasma gondii* (van der Zypen, Piekarski, 1967), Fig. 1a, b. In merozoites of *E. callospermophili* we found longitudinally stretched and bulbous structures in the same cell (Fig. 1c). This observation is another indication that these two phenomena are the result of different stages of development.

In a merozoite of *E. tenella*, which had just suceeded in invading a host cell, McLaren, Paget (1968) found a completely empty paired organelle, which obviously had pressed out its filling. A similar appear-

(continued)

Shape	Characteristics	References
elongate-tortuous	arranged in rows, homogeneous	Scholtyseck *et al.* (1970)
club-shaped	2 or 3 in the conoid; occasionally posterior to the nucleus	Sheffield (1966)
cylindrical	arranged in bundles; tortueous	Sénaud (1969, 1970)
⌐	connections with the paired organelles	Aikawa (1966)
rounded in cross section	the same density as the PO	Aikawa and Jordan (1968)
ovoid in cross section	after penetration they disappear	Garnham *et al.* (1969)
pear-shaped	membrane-bound; 2—3 are greater; they reach the nucleus	Büttner (1967)
flask-shaped	same density as the host cytoplasm; covered by an amorphous sheath	Simpson *et al.* (1967)
tortuous	covered by a membranous layer; partly hollow with a central core	Friedhoff und Scholtyseck (1968)
elongate-tortuous	not homogenous; many within the spherical body	Scholtyseck *et al.* (1970)

ance was observed in the M-organism (Scholtyseck, Kepka, Piekarski, 1970), and again in *E. tenella* (Scholtyseck, Strout, Haberkorn, 1969). The disappearance of the paired organelle after penetration of the host cell reported in *Pl. fallax*, *Pl. lophurae*, *Pl. cathemerium* and *Pl. elongatum* (Aikawa, 1966; Aikawa *et al.*, 1967) also suggests that certain functions are performed during the process of penetration. Sheffield, Melton (1968) described another phenomenon in *Toxoplasma gondii*. In longitudinal sections of the paired organelle cut tangentially they noticed a clearly visible striping in a distance of about 500 Å. Heller (1969) discovered a similar striping in *Aggregata eberthi*. These results possibly indicate a fibrillar structure of the wall of the organelle. From this it might be tentatively concluded that these organelles are contractile, which would explain how the contents are discharged.

In some species of *Eimeria* a short rod-shaped body at the very tip of the cell has been seen between the two bottle-shaped narrow necks of the paired organelle. Such an organelle was found in *E. bovis* (Sheffield, Hammond, 1966), *E. nieschulzi* (Colley, 1968), *E. pragensis*, *E. tenella*, *E. magna* (Sénaud, Černa, 1968, 1969). Possibly this body

serves to prevent the ductules from being moved out of place. Its diameter is about the same as that of the paired organelle in this region.

2. *The small forms*, which have been given various names (Table 2), are much more numerous than the large ones and they never extend to the very tip of the cell. They are much more tortuous than the larger forms, as indicated by their oval appearance in longitudinal sections. In cross-sections they appear to be circular. Their average diameter is about the same as that of the paired organelle (= rhoptry) at its neck (Table 1, 2) and measures about 600—900 Å. All reports concerning the longitudinal extent of these structures must be considered with discretion because in many cases these structures were found to extend throughout the whole cell, contrary to earlier observations. Possibly, these organelles vary in length and diameter in accordance with different stages of development and activity.

In most cases these tortuous structures appeared to be completely homogeneous. In some species of parasites, however, differences in the fine structure could be described. In *Isospora* spec. (Schmidt *et al.*, 1967), *Haemogregarina* spec. (Stehbens, Johnston, 1967), *Babesia gibsoni* (Büttner, 1968b), *B. canis* (Simpson *et al.*, 1967), *Theileria parva* (Büttner, 1967), and *Selenidium hollandei* (Schrevel, 1968) they are limited by a membranous cover. Sometimes they are surrounded by an electron lucid space; this is the case in *Sarcocystis tenella* (Sénaud, 1967), *S. miescheriana* (Ludvik, 1960), *S. fusiformis* (Simpson, 1966), *Besnoitia jellisoni* (Sheffield, 1968), *Lankesterella hylae* (Stehbens, 1966), *Babesia caballi* (Simpson *et al.*, 1967), and *E. pragensis* (Sénaud, Černa, 1968). In *Toxoplasma gondii* (van der Zypen, Piekarski, 1967), *Sarcocystis tenella* (Sénaud, 1967), *E. bovis* (Sheffield, Hammond, 1966), *E. nieschulzi* (Colley, 1967, 1968), *Haemogregarina* (Stehbens, Johnston, 1967) and *Babesia bigemina* (Friedhoff, Scholtyseck, 1969) these structures were found to be partly empty.

In some species, these organelles are so crowded together that typical bundles are formed. This phenomenon and the fact that these tortuous elements are branched and linked with one another in *Sarcocystis tenella* (Sénaud, 1967), *Haemogregarina* spec. (Stehbens, Johnston, 1967), *E. nieschulzi* (Colley, 1967), and some avian malarial parasites (Aikawa *et al.*, 1966, 1969) suggest that the large and small structures take part in the same function. Moreover, Aikawa (1966) showed that connections with the paired organelles exist.

In merozoites of *Babesia ovis* we have detected structures which appear similar in electron micrographs, but the function of which is, we believe, confined to the forming of the inner membrane of the pellicle and the subpellicular microtubules (Figs. 4a, b). Within the spherical body of *Babesia bigemina* (Scholtyseck, Mehlhorn, Friedhoff,

1970) we described another type of similar structures, the function of which has not been understood as yet.

These similarities support the theory that the large as well as the small structures in the anterior half of the parasite cell might be functional stages of the same complex of organelles (Sheffield, Melton, 1968). The large elements might be only excretory ductules of a greater tubular system. This is even more probable because in several cases branches and connections between the single structures have been observed. The thin elements may have no openings of their own and may empty into the large organelles by means of these connections. As a result the larger organelles may become enlarged (Figs. 1c, 3d, 5, 6). Because of the tapering of the parasites at their anterior pole it is not possible for each tube to extend through the conoid or the polar ring to the outer membrane. Therefore such a functional arrangement would not be surprising. The greater internal pressure of this tubular system would also explain the formation of spherical rounded forms. Consequently, the larger elements would be much less tortuous than the smaller ones, which is in accordance with observations. The fibrillar basic structure of the wall would enable the parasite to discharge the contents (Fig. 6).

This hypothesis just mentioned is based upon the presupposition that this organelle has secretory functions. This concept has not been seriously doubted ever since Ludvik (1958) published it for the first time. On the other hand, no attempts have been made until now to indicate enzymes in these organelles. There are only the results of Schrevel (1968), who found acid phosphatase in the "rhoptries" of *Selenidium*. The fine structure and the obviously different fillings seem to point to the fact that there is such a function. The activity of these substances, however, cannot be confined to the process of penetration only, it must extend to the phase of life of the parasite thereafter. Otherwise, the observations that the number of toxonemes increases after the process of penetration (*Eucoccidium dinophili*, Bardele, 1966) and that they can be found even during endodyogeny in *Toxoplasma gondii*, *Sarcocystis tenella*, *Besnoitia jellisoni*, M-organism (authors Table 1, 2) cannot be properly explained.

Therefore we may assume with good reason that the structures just mentioned may be considered as a complex of organelles which produces enzymes and discharges them at the apical pole by means of reservoirs or excretory ductules. The mechanical perforating activity of the conoid would be supported by these proteolytic enzymes. At the same time, the cytoplasm of the host cell might be affected in such a way as to produce an environment more suitable for the parasite.

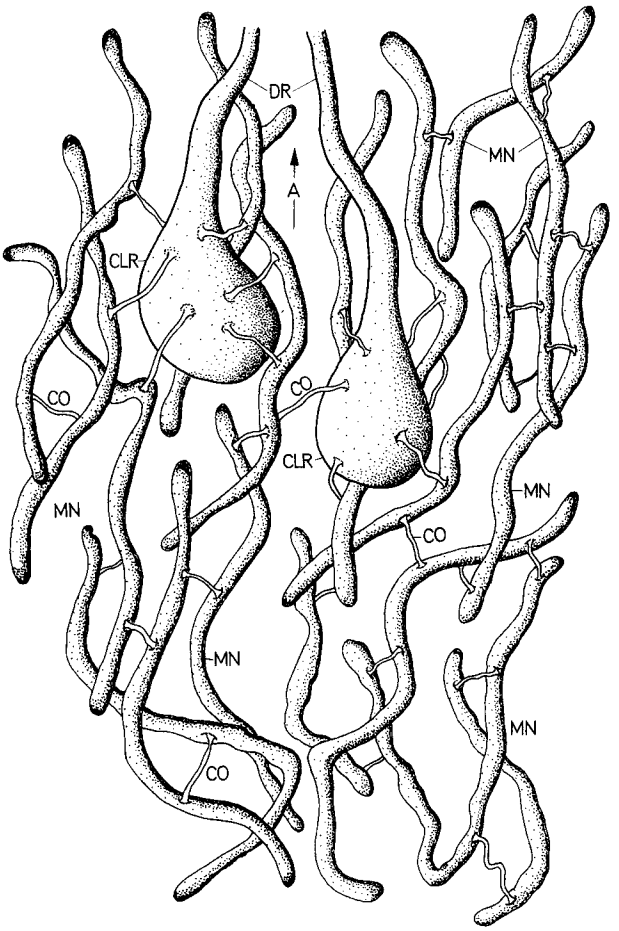

Fig. 6. Diagrammatic representation of the shape of the paired organelles as excretory ductules, proceeding from the hypothesis that the paired organelles and the micronemes in the anterior half of the parasite must be considered as a single functional complex

Some of these organelles, however, obviously do not have this function only, but also produce substances for the formation of the inner membrane and the subpellicular fibrils (Fig. 4).

Since the structure and the function of all these organelles have been found to be so complex, a general term should be introduced. The term "paired organelles" suggested by Garnham *et al.* (1960) for the large elements is perhaps no longer appropriate, since it has been shown that different groups of parasites have several of these structures (Table 1).

Sénaud (1967) proposed the term "rhoptry", which was accepted by Levine (1969a), too. Jacobs (1968) coined the term "micronemes" to characterize the smaller structures, and this term has been used in the studies of many authors. The "micronemes", therefore, produce the substances, whereas the "rhoptries" discharge them. We think that both terms have the advantage of not bearing the burden of wrong or obsolete connotations, but that they can acquire their true meaning only by continuing research.

B. Micropore

This structure first was observed by Garnham *et al.* (1960) in sporozoites of avian malarial parasites. It was termed " micropyle" because the authors thought this organelle to be "the place through which the sporoplasm emerged." Cheissin, Snigirevskaya (1965) proved the same "double ring structure" in merozoites of *E. intestinalis*, but they realized that this organelle had to be an "ultracytostome". This result was confirmed by Aikawa *et al.* (1966a and b) in the genus *Plasmodium*. For the first time they succeeded in proving that the formation of pinocytotic vesicles takes place at this part of the pellicle. This place, therefore, has rightly been called "cytostome". Since then the presence of this structure and its function as an organelle ingesting cytoplasm of host cells, has been demonstrated in all Sporozoa (= Polannulifera) studied by means of electron microscopy (Figs. 7, 8).

This structure is, according to its particular function, not confined to the motile stages as is the conoid, but it is present in almost every stage of the parasite's development and always has the same fine structure. In all cases it is an invagination of the two-layered pellicle. The outer membrane limits the invagination at its base, whereas the inner membrane is interrupted and follows the outer membrane only for a short distance vertically into the interior. Often these membranes are laterally thickened by an accumulation of electron-dense material, so that in longitudinal sections the "cytostome" seems to be bounded by two darkly stained lineal segments, whereas its base is limited only by a single membrane. In sections close to the surface of the parasite, this organelle is composed of two darkly stained concentric rings, which correspond with the two darkly stained lineal segments of the cytostomal wall seen in the longitudinal sections (Figs. 3a, b; 7; 8). This fine structure can also be found in those stages which do not have an inner pellicle membrane as, for instance, the microgametocytes of *E. auburnensis* (Hammond, Scholtyseck, Chobotar, 1969), *E. intestinalis* (Snigirevskaya, 1968), *E. nieschulzi* (Colley, 1967), *E. perforans*, *E. stiedae*, *E. bovis*, *E. auburnensis* (Hammond, Scholtyseck, Miner, 1967) and the macrogametes of *E. perforans*, *E. stiedae*, *E. auburnensis*,

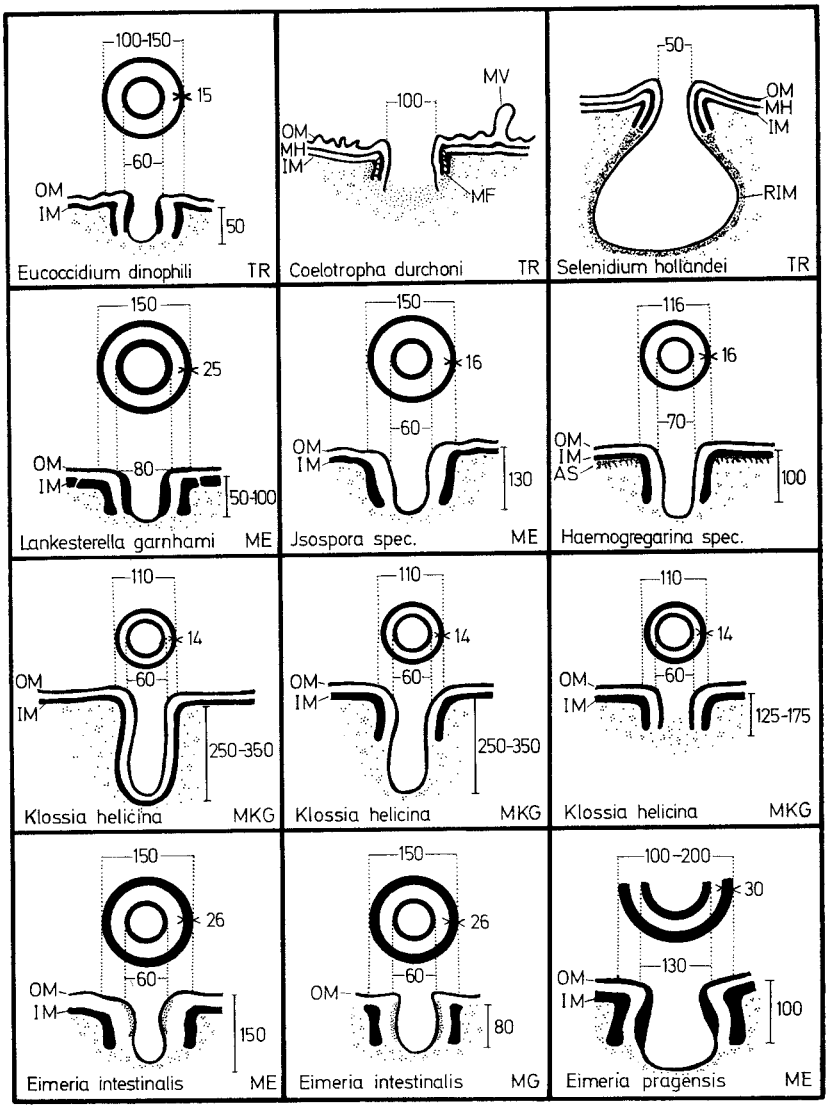

Fig. 7. Measurements and details of micropores in different groups of parasites. References cf. Table 1. Measurements in mμ

E. bovis (Scholtyseck, Hammond, Ernst, 1966). There a cylindrical structure is preserved, indicating the previous invagination of the inner membrane (Fig. 8).

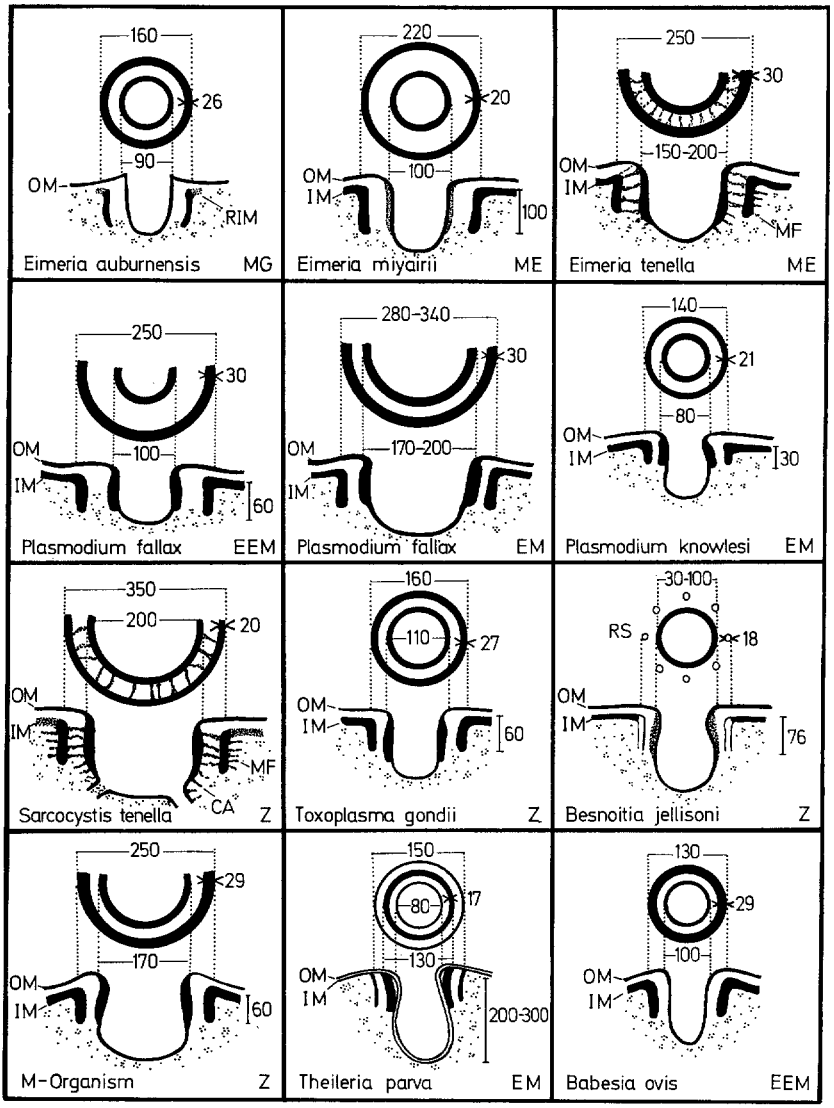

Fig. 8. Measurements and details of micropores in different groups of parasites. References cf. Table 1. Measurements in mμ

In the gregarine *Selenidium hollandei* (Schrevel, 1968, 1969) and in the protococcidian *Coelotropha durchoni* (Vivier, Henneré, 1965; Henneré, 1967) three pellicle membranes were observed, all invaginated, contri-

buting to the fine structure of this organelle. In *Coelotropha durchoni* (Vivier, Henneré, 1965; Henneré, 1967), *Eimeria tenella* (Sénaud, Černa, 1969) *Sarcocystis tenella* (Sénaud, 1967) and *E. auburnensis* (Hammond, Scholtyseck, Chobotar, 1969) specific additional differentiations could be found. In these parasites, microfibrils connect the invaginated thickened parts of the membrane and thus produce a further stabilization (Figs. 7—9). In the case of *Besnoitia jellisoni* (Sheffield, 1966, 1967) the outer, thickened cylindrical structure is replaced by eight rod-like tubules (Fig. 8).

The size reported for this organelle in the different groups of parasites are compiled in Figs. 7, 8. It is evident that, although the structure of this organelle is identical in all the parasites, the diameter of the invagination varies considerably, from 50—200 mμ. These variations must not be overrated, however, because they can be found even in a single genus (*Plasmodium*). For instance, the diameter of the "cytostome" of the erythrocytic merozoite in *Plasmodium fallax* is almost four times greater than in the erythrocytic merozoite of *Plasmodium knowlesi* (Aikawa *et al.*, 1966a and b), which is only about 50—80 mμ in diameter. An interesting phenomenon may be observed in *Plasmodium fallax*. There the opening of the "cytostome" in erythrocytic merozoites is twice as big (170—200 mμ) as in exoerythrocytic merozoites (80 to 100 mμ), indicating a particular activity in the former phase of life (Fig. 8).

In most cases the organelle which has the function of an ultracytostome, is limited at its base by the outer pellicle membrane in form of a unit membrane (Figs. 3a, b). This is true also in *Sarcocystis tenella*, where Sénaud (1967) observed two to three side canals branching off from the main invagination. In some parasites, however, variations of this basic structure were described. The invaginations in *Coelotropha durchoni* (Vivier, Henneré, 1965; Henneré, 1967), *Klossia helicina* (Volkmann, 1967), and *Eimeria intestinalis* (Snigirevskaya, 1968) were not bounded by a unit membrane at their bases, but were open, i.e. a direct contact existed between the environment and the interior of the cell. Contrary to this finding, Volkmann (1967), Schulte (1969) and Sheffield, Hammond (1966) demonstrated a third form of appearance in *Klossia helicina* and *Eimeria bovis*. In electron micrographs an invagination of corresponding size was noted, but the inner membrane was not interrupted (Figs. 7, 10). Possibly — as discussed by Hammond *et al.*, (1967a) — these three forms of appearance represent three stages of development as well. Undoubtedly, this typical fine structure is the result of an invagination of the two pellicle membranes. At the base of the invagination, the inner membrane may be dissolved and the material used to thicken the remaining parts. At this stage, the

micropore does not undergo any further development, however, because in practically all cases, the formation of vesicles then begins. These vesicles, which were always found near micropores, were surrounded by a unit membrane and had the same electron density as the parasitophorous vacuole (Hammond *et al.*, 1967b). The openings observed at the base of the invaginations must be considered as unusual occurrences, possibly artifacts.

In the development, this organelle is formed very early. Thus, Aikawa (1966), Sénaud, Černa (1969), Sénaud (1967), Snigirevskaya (1968), Sheffield, Hammond (1967), and Heller (unpublished) detected it even in immature merozoites and zoites, specifically in *Pl. fallax*, *E. magna*, *E. tenella*, *T. gondii*, *S. tenella*, *E. intestinalis*, *E. bovis*, and *E. stiedae*.

In merozoites and in the proliferative stages of *Besnoitia*, *Toxoplasma*, *Sarcocystis* and M-organism, the number of these organelles is limited. In most cases, only one such structure can be detected (Andreassen, Behnke, 1968; Scholtyseck, Mehlhorn, Friedhoff, 1970). In *Lankesterella garnhami* (Büttner, 1968), *Haemogregarina* spec. (Stehbens, Johnston, 1967), and *Besnoitia jellisoni* (Sheffield, 1966, 1967, 1968; Sénaud, 1969), however, two of these organelles were demonstrated; in *Pl. gallinaceum* (Aikawa *et al.*, 1968) also two were found, but immediately beside each other. These results point to the fact that, possibly, these motile stages have more than one such organelle; altogether, however, their number is limited.

As soon as the phase of nutrition and growth begins, the number of these structures increases. Scholtyseck *et al.* (1966), in macrogametes of *E. perforans*, *E. stiedae*, *E. bovis*, and *E. auburnensis*, and Hammond *et al.* (1967), in the microgametocytes of the same species, detected a great number of such organelles. Bardele (1966) found four in a space of 2.5 μ^2 in *Eucoccidium dinophili*. The studies of Snigirevskaya (1968) in *E. intestinalis* yielded similar results. She demonstrated three of these structures lying closely together. Schrevel (1968) found a great number too, in the trophozoites of the gregarine *Selenidium hollandei*. By far the greatest number of these organelles was detected by Hammond, Scholtyseck and Chobotar (1969) in macrogametocytes of *E. auburnensis* (Fig. 9). Not less than nine such structures were observed in close proximity to each other. It seems that these organelles are formed in great numbers in direct response to suddenly arising needs.

The situation is different in merozoites and zoites. In these stages, such structures obviously are formed at particular places. Some parasites have one of these organelles each; it may be found midway between the anterior and the posterior end of the cell in erythrocytic and exoerythrocytic merozoites of *Pl. fallax*, *Pl. lophurae*, *Pl. cathemerium* (avian malarial parasites), *Besnoitia jellisoni* (Sheffield, 1968), *Toxo-*

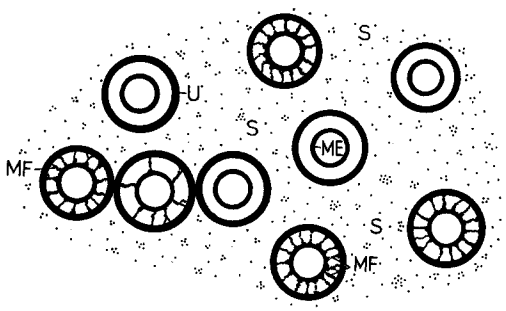

Fig. 9. Diagrammatic representation of 9 cross-sectioned micropores in a macrogamete of *Eimeria auburnensis* (*ME* membrane)

plasma gondii (Sénaud, 1967), M-organism (Scholtyseck, Kepka, Piekarski, 1969), *Eimeria pragensis* (Sénaud, Černa, 1968), *E. magna* and *E. tenella* (Sénaud, Černa, 1969; Ryley, 1969), and *Theileria parva* (Büttner, 1967). In *E. miyairii* (Andreassen, Behnke, 1968), *Sarcocystis tenella* (Sénaud, 1967) and *Babesia ovis* (Friedhoff, Scholtyseck, 1968), it may be found at the middle third of the cell. In *E. bovis* (Sheffield, Hammond, 1966), *Isospora* spec. (Schmidt *et al.*, 1967) and *E. intestinalis* (Snigirevskaya, 1968) this organelle can be seen near the level of the anterior end of the nucleus.

The experiments by Schulte (1969) throw some light on the function of this structure. He demonstrated the presence of alkaline phosphatase in microgametocytes and macrogametes of *Klossia helicina* (Fig. 10a) within the invagination and (Fig. 10b) in vesicles obviously pinched off from this. This observation is in line with the morphological results of other authors. They described a clearly visible formation of vacuoles, in which the digestion of cytoplasm of the host cell takes place, or from which even smaller vesicles are pinched off (Aikawa *et al.*, 1966a and b; Rudzinska, Vickerman, 1968). Such vacuoles have a wall consisting of a single unit membrane. In some parasites, there are, in addition, other vesicles with several limiting membranes. This occurs in *Lankesterella garnhami* (Büttner, 1968), *Selenidium hollandei* (Schrevel, 1968), *Klossia helicina* (Volkmann, 1967; Schulte, 1969), *Babesia ovis* (Friedhoff, Scholtyseck, 1968) and *Babesia bigemina* (Scholtyseck, Mehlhorn, Friedhoff, 1970). The origin of these additional 2—3 membranes is unknown.

Considering the morphological and functional identity, some authors have created a common name for these structures and called them "micropores" (Vivier, Henneré, 1965; Hammond *et al.*, 1967). This term,

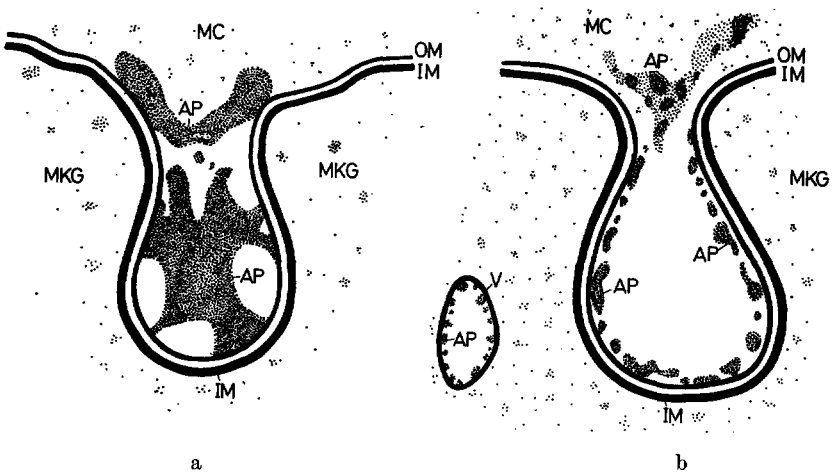

Fig. 10. Diagrammatic representation of two micropores in a macrogamete of *Klossia helicina*. Schulte (1969) demonstrated the presence of alkaline phosphatase in a sort of micropore (the inner membrane is not interrupted)

which has been adopted by Levine (1969a and b) and seems to adequately denote this specific organelle. "Cytostomes" and "micropyle" appear in different forms in other protozoa, therefore the term "micropore" is less misleading.

C. Taxonomic Questions

This comparative structural analysis of the "rhoptries", "micronemes" and "micropores" has demonstrated that there are striking similarities — as indicated also by our study of the conoid, the pellicle and the microtubules — which cannot be explained by convergence only, but which must be the expression of a close relationship, particularly as similar structures have not been observed in other intracellular protozoa (Scholtyseck *et al.*, 1970). Levine (1969b), drawing conclusions from all these studies, revised the system of Sporozoa on the basis of ultrastructural criteria. We agree with this system in principle; in our opinion, however, the organisms listed under the term "Toxoplasmea" are more related to the coccidians than to the "Piroplasmea". As in our previous study (Scholtyseck, Mehlhorn, Friedhoff, 1970) we propose the introduction of a third order besides "Protococcida" and "Eucoccida". "Endodyococcida", we believe, is a useful term relating to the typical way of proliferation of these parasites. We shall submit a detailed classification in the near future.

Referecnes

Aikawa, M.: The fine structure of the erythocytic stages of three avian malarial parasites, *Plasmodium fallax*, *Pl. lophurae*, and *P. cathemerium*. Amer. J. Trop. Med. Hyg. **15**, 449—471 (1966).

— Ultrastructure of the pellicular complex of *Plasmodium fallax*, J. Cell Biol. **35**, 103—113 (1967).

— Hepler, P. K., Huff, C. G., Sprinz, H.: The feeding mechanism of avian malarial parasites. J. Cell Biol. **28**, 355—373 (1966b).

— Huff, C. G., Sprinz, H.: Comparative feeding mechanisms of avian and primate malarial parasites. Milit. Med. **131**, No 9 Suppl. (1966a).

— — — The fine structure of the asexual stages of *Plasmodium elongatum*. J. Cell Biol. **34**, 229—249 (1967).

— — — Exoerythrocytic stages of *Plasmodium gallinaceum* in chick-embryo liver as observed electronmicroscopically. Amer. J. trop. Med. Hyg. **17**, 156—169 (1968).

— — — Comparative fine structure study of the gametocytes of avian, reptilian malarial parasites. J. Ultrastruct. Res. **26**, 316—331 (1969).

— Jordan, H. B.: Fine structure of a reptilian malarial parasite. J. Parasit. **54**, 1023—1033 (1968).

Andreassen, J., Behnke, O.: Fine structure of merozoites of a rat coccidian *Eimeria miyairii* with a comparison of the fine structure of other sporozoa. J. Parasit. **54**, 150—163 (1968).

Bardele, C. F.: Elektronenmikroskopische Untersuchungen an dem Sporozoon *Eucoccidium dinophili* Grell. Z. Zellforsch. **74**, 559—595 (1966).

Büttner, D. W.: Die Feinstruktur der Merozoiten von *Theileria parva*. Z. Tropenmed. Parasit. **18**, 224—244 (1967).

— Das Cytostom von *Lankesterella garnhami*. Z. Zellforsch. **188**, 126—137 (1968a).

— Vergleichende Untersuchungen der Feinstruktur von *Babesia gibsoni* und *Babesia canis*. Z. Tropenmed. Parasit. **19**, 330—342 (1968b).

Cheissin, E. M., Snigirevskaya, E. S.: Some new data on the fine structure of the merozoites of *Eimeria intestinalis* (Sporozoa, Eimeriidea). Protistologica **1**, 121—128 (1965).

Colley, F. C.: Fine structure of microgametocytes and macrogemetes of *Eimeria nieschulzi*. J. Protozool. **14** (4), 663—674 (1967).

— Fine structure of sporozoites of *Eimeria nieschulzi*. J. Protozool. **14** (2), 217—220 (1967).

— Fine structure of schizonts and merozoites of *E. nieschulzi*. J. Protozool. **15** (2), 374—382 (1968).

Friedhoff, K., Scholtyseck, E.: Feinstrukturen von *Babesia ovis* (Piroplasmidea) in *Rhipicephalus bursa* (Ixodoidea): Transformation sphäroider Formen zu Vermiculaformen. Z. Parasitenk. **30**, 347—359 (1968).

— — Feinstrukturen der Merozoiten von *Babesia bigemina* im Ovar von *Boophilus microplus* und *Boophilus decoloratus*. Z. Parasitenk. **32**, 266—283 (1969).

— — Fine structure of erythrocytic stages of *Babesia bigemina*. (In preparation, 1970).

Garnham, P. C. C.: The structure and the function of the cytostome (= micropyle) in the Sporozoa. In: Progress in protozoology, p. 8. Leningrad: Academy of Sciences of the USSR, Publishing House NAUKA 1969a.

— The significance of ultrastructure in the classification of the Toxoplasmea. In: Progress in protozoology, p. 365. Leningrad: Academy of Sciences of the USSR, Publishing House NAUKA 1969b.

Garnham, P. C. C., Baker, J. R., Bird, R. G.: The fine structure of *Lankesterella garnhami*. J. Protozool. **9** (1), 107—114 (1962).

— Bird, R. G., Baker, J. R.: Electron microscope study of motile stages of malarial parasites. 1. The fine structure of the sporozoites of *Haemamoeba* (= *Plasmodium*) *gallinacea*. Trans. roy. Soc. trop. Med. Hyg. **54**(3), 274—278 (1960).

— — — Desser, S. S., El-Nahal, H. M. S.: Electron microscope studies on motile stages of malaria parasites. VI. The ookinete of *Plasmodium berghei yoelii* and its transformation into the early oocyst. Trans. roy. Soc. trop. Med. Hyg. **63** (2), 187—194 (1969).

Gustafson, P. V., Agar, H. D., Cramer, D. J.: An electron microscope study of *Toxoplasma*. Amer. J. trop. Med. Hyg. **3**, 1008—1021 (1954).

Hammond, D. M., Scholtyseck, E., Miner, M. L.: The fine structure of microgametocytes of *Eimeria perforans*, *E. stiedae*, *E. bovis*, *E. auburnensis*. J. Parasit. **53** (2), 235—247 (1967).

— — Chobotar, B.: Fine structural study of the microgametogenesis of *Eimeria auburnensis*. Z. Parasitenk. **33**, 65—84 (1969).

Heller, G.: Elektronenmikroskopische Untersuchungen an *Aggregata eberthi* aus dem Spiraldarm von *Sepia officinalis* (Sporozoa, Coccidia), I. Die Feinstruktur der Merozoiten, Makrogamonten und Sporen. Z. Parasitenk. **33**, 44—64 (1969).

Jacobs, L.: Toxoplasmosis. In: Advances in Parasitology, vol. 5. New York: Academic Press 1968.

Kepka, O., Scholtyseck, E.: Die Endodyogenie beim M-Organismus (*Frenkelia*). (In press, 1970.)

Levine, N. D.: Uniform terminology for sporozoan protozoa. In: Progress in protozoology, p. 340. Leningrad: Academy of Sciences of the USSR, Publishing House NAUKA 1969a.

— Taxonomy of the Sporozoa. In: Progress in protozoology, p. 365—366. Leningrad: Academy of Sciences of the USSR, Publishing House NAUKA 1969b.

Ludvik, J.: *Toxoplasma* im elektronenmikroskopischen Bilde. Med. Bild *I*. 59—61 (1958).

— The electron microscopy of *Sarcocystis miescheriana*. (Kuhn 1865) J. Protozool. **7**(2) 128—135 (1960).

McLaren, D. J., Paget, G. E.: A fine structural study on the merozoites of *Eimeria tennella* with special reference to the conoid apparatus. Parasitology **58**, 561—571 (1968).

Porchet-Henneré, E.: Etude des premiers stades de développement de la coccidie *Coelotropha durchoni*. Z. Zellforsch. **80**, 556—569 (1967).

Rudzinska, M. A., Vickermann, K.: The fine structure. In: Infectious blood diseases of man and animals, ed. by Weinmann, D., and Ristic, M. New York: Academic Press 1968.

Ryley, J. F.: Ultrastructural studies on the sporozoite of *Eimeria tenella*. Parasitology **59**, 67—72 (1969).

Schmidt, K. M., Johnston, R. L., Stehbens, W. E.: Fine structure of the schizont and merozoite of *Isospora* spec. J. Protozool. **14**(4), 602—608, (1967).

Scholtyseck, E.: Neue Ergebnisse der Coccidienforschung. In: Progress in protozoology, p. 328—330. Leningrad: Academy of Sciences of the USSR, Publishing House NAUKA 1969.

— Haberkorn, A.: Die Feinstruktur von *Eimeria falciformis*. (In preparation, 1970.)

— Hammond, D. M., Ernst, J. V.: Fine structure of the macrogametes of *Eimeria perforans*, *E. stiedae*, *E. bovis* and *E. auburnensis*. J. Parasit. **52** (5), 975—987 (1966).

Scholtyseck E., Hammond, D. M., Todd, K. S.: Merozoite formation in *Eimeria callospermophili* from the Uinta ground squirrel *Spermophilus armatus*. J. Protozool., Suppl. (1968).

— Kepka, O., Piekarski, G.: Die Feinstruktur der Zoiten aus reifen Cysten des M-Organismus. (In press, 1970.)

— Mehlhorn, H., Friedhoff, K.: Ultrastructural study on the conoid of Sporozoa and related organisms. (In press, 1970.)

— Piekarski, G.: Elektronenmikroskopische Untersuchungen an Merozoiten von Eimerien und *Toxoplasma gondii*. Zur systematischen Stellung von *T. gondii*. Z. Parasitenk. **26**, 91—115 (1965).

— Strout, R. G., Haberkorn, A.: Schizonten und Merozoiten von *Eimeria tenella* in Macrophagen. Z. Parasitenk. **32**, 284—296 (1969).

Schrevel, J.: L'ultrastructure de la région antérieure de la grégarine *Selenidium* et son intérét por l'étude de la nutrition chez les sporozoaires. J. Microscopie **7** (3), 391—410 (1968).

— Étude de la nutrition chez les sporozoaires: cas des grégarines. In: Progress in protozoology, p. 69—70. Leningrad: Academy of Sciences of the USSR, Publishing House NAUKA 1969.

Schulte, E.: Cytochemische Untersuchungen an den Feinstrukturen von Klossia helicina (Coccidia, Adeleidea). Diss. Bonn 1969.

Sénaud, J.: Contribution à l'étude des sarcosporidies et des toxoplasmes (Toxoplasmea). Protistologica **3**, 170—232 (1967).

— Sur l'ultrastructure des kystes de *Besnoitia jellisoni* (Frenkel 1953) chez la souris (*Mus musculus*). C. R. Acad. Sci (Paris) **268**, 816—819 (1969).

— Ultrastructure des formations kystiques de *Besnoitia jellisoni* (Frenkel, 1953) Protozoaire, Toxoplasmea, parasite de la souris (*Mus musculus*). Protistologica **5** (3), 413—430 (1970).

— Černa, Z.: Etude en microscopie électronique des mérozoites et de la mérogonie chez *Eimeria pragensis* (Černa et Sénaud. 1968), coccidie parasite de l'intestin de la souris (*Mus musculus*). Ann. Stat. biol. Basse-en-Chandesse No **3**, 221—241 (1968).

— — Etude ultrastructurale des mérozoites et de la schizogonie des coccidies (Eimeriina): *E. magna* (Pérard 1925) de l'intestin des lapins et de *E. tenella* (Raillet et Lucet, 1891) des coecums des poulets. J. Protozool. **16** (1), 155—165 (1969).

Sheffield, H. H.: Electron microscope study of the proliferative form of *Besnoitia jellisoni*. J. Parasit. **52** (3), 583—594 (1966).

— The function of the micropyle in the cyst organisms of *Besnitia jellisoni*. J. Parasit. **53** (4), 888 (1967).

— Observations on the fine structure of the cyst stage of *Besnoitia jellisoni*. J. Protozool. **15** (4), 685—693 (1968).

— Hammond, D. M.: Fine structure of first generation merozoites of *E. bovis*. J. Parasit. **52** (3), 595—606 (1966).

— — Electron microscope observations on the development of first-generation merozoites of *E. bovis*. J. Parasit. **53** (4), 831—840 (1967).

— Melton, M. L.: The fine structure and reproduction of *Toxoplasma gondii*. J. Parasit. **54** (2), 209—226 (1968).

Simpson, Ch., F.: Electron microscopy of *Sarcocystis fusiformis*. J. Parasit. **52** (3), 607—613 (1966).

— Kirkham, W. W., Kling, J. M.: Comparative morphologic features of *Babesia caballi* and *B. equi*. Amer. J. vet. Res. **28** (127), 1693—1697 (1967).

Sjöstrand, F. S.: In: A. W. Pollister, Physical techniques in biol. research, vol. 3. New York: Academic Press Inc. 1956.

Snigirevskaya, E. S.: The occurrence of micropore in schizonts, microgametocytes, and macrogametes of *E. intestinalis*. Acta protzool. **5** (25), 381—396 (1968).

Stehbens, W. E.: The ultrastructure of *Lankesterella hylae*. J. Protozool. **13** (1), 63—73 (1966).

— Johnston, M. R. L.: The ultrastructure of a haemogregarine parasitic in *Gehyra variegata* (Dumeril and Bibron, 1836). Parasitology **57**, 251—261 (1967).

Todd, K. S., Hammond, D. M.: Life cycle and host specifity of *Eimeria callospermophili* (Henry 1932) from the Uinta ground squirrel *Spermophilus armatus*. J. Protozool. **15** (1), 1—8 (1968).

Volkmann, B.: Vergleichend elektronenmikroskopische und lichtmikroskopische Untersuchungen an verschiedenen Entwicklungsstadien von *Klossia helicina* (Coccidian, Adeleidea). Z. Parasitenk. **29**, 159—208 (1967).

Vivier, E., Henneré, E.: Ultrastructure des stades végetatifs de la coccidie *Coelotropha durchoni*. Protistologica **1**, 89—113 (1965).

Wohlfarth-Bottermann, K. E.: Die Kontrastierung tierischer Zellen und Gewebe im Rahmen ihrer Untersuchung an ultradünnen Schnitten. Naturwissenschaften **44**, 287—288 (1957).

Zypen, E. van der, Piekarski, G.: Ultrastrukturelle Unterschiede zwischen der sog. Proliferationsform (RH-Stamm, BK-Stamm und dem sog. Cystenstadium (DX-Stamm von *Toxoplasma gondii*. Zbl. Bakt., I. Abt. Orig. **203**, 495—517 (1967).

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