*Onderstepoort J. vet. Res.* 44 (3), 157–168 (1977)

# THE FINE STRUCTURE OF INTRA-ERYTHROCYTIC STAGES OF *BABESIA BIGEMINA*

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ABSTRACT

POTGIETER, F. T. & ELS, H. J., 1976. The fine structure of intraerythrocytic stages of *Babesia bigemina*. *Onderstepoort Journal of Veterinary Research* 44 (3), 157–168 (1977).

The electron microscope was used to study the structure of merozoites, merozoites in the process of transformation to trophozoites, trophozoites, and the method of multiplication of *B. bigemina*. The merozoites were piriform in shape and surrounded by 3 peripheral membranes of which the 2 inner ones often appeared as a single thick osmiophilic structure (inner membrane). Anterior and posterior polar rings, microtubules, micronemes, rhoptries and mitochondria with and without tubular cristae were discernible. A single large unidentified spherical body was present in most of the mature merozoites. After penetration of an erythrocyte, merozoites developed into trophozoites through a transformation process which involved the loss of the inner membrane of the pellicle, rhoptries, most of the micronemes and the spherical body. The trophozoites were surrounded by a single membrane, were pleomorphic in shape and contained large inclusions of host cell cytoplasm, but no cytostomes or food vacuoles could be identified. Reproduction took place through a process resembling schizogony resulting in the production of 2 merozoites, the cytoplasmic constituents of the original trophozoite (mother cell) being virtually entirely incorporated into the daughter cells in the process. None of the parasites were contained in parasitophorous vacuoles.

*Résumé*

*ULTRASTRUCTURE DES STADES INTRA-ÉRYTHROCYTAIRES DE* BABESIA BIGEMINA

*On a étudié au microscope électronique la structure des mérozoïtes, des mérozoïtes se transformant en trophozoïtes, des trophozoïtes et le mécanisme de multiplication chez* B. bigemina. *Les mérozoïtes étaient de contour piriforme et entourés de 3 membranes périphériques dont les 2 membranes internes présentaient souvent l'apparence d'une seule structure osmiophilique épaisse (membrane interne). On a pu discerner des anneaux polaires antérieurs et postérieurs, des microtubules, des micronèmes, des rhoptries et des mitochondries avec et sans crêtes tubulaires. Un grand corps sphérique isolé, non identifié, était présent dans la plupart des mérozoïtes murs. Après pénétration dans un érythrocyte, les mérozoïtes évoluaient en trophozoïtes par un processus de transformation caractérisé par la perte de la membrane interne de la pellicule, des rhoptries, de la plupart des micronèmes et du corps sphérique. Les trophozoïtes étaient entourés d'une membrane unique, leur contour était pléiomorphe, et ils contenaient du cytoplasme de la cellule-hôte dans de grandes inclusions; mais il n'a pas été possible d'identifier des cytostomes ou des vacuoles alimentaires. La reproduction s'effectuait par un mécanisme semblable à celui de la schizogonie, résultant dans la production de 2 mérozoïtes, les constituants cytoplasmiques du trophozoïte original (cellule-mère) subissant au cours de ce processus une incorporation virtuellement complète dans les cellules-filles. Aucun des parasites n'était contenu dans des vacuoles parasitophores.*

## INTRODUCTION

Reports on the fine structure of intra-erythrocytic stages of *Babesia bigemina* were published by Hoyte (1966), Friedhoff (1970) and Scholtyseck & Mehlhorn (1970). Of the reports of these authors, only the latter contained an illustration of the fine structure of a pair of merozoites. Another paper (Friedhoff & Scholtyseck, 1970, cited by Scholtyseck & Mehlhorn, 1970) in preparation at the time, could not be traced. The purpose of this report is to illustrate the morphology of the intra-erythrocytic merozoites of *B. bigemina* in greater detail, the transformation from merozoites to trophozoites and the multiplication of the parasite in the erythrocyte of the host.

## MATERIALS AND METHODS

During the course of an investigation on the life-cycle of *B. bigemina* in ticks (Potgieter, unpublished observations, 1976), a splenectomized ox became infected after infestation with *Boophilus decoloratus* larvae. At the height of the parasitaemia (8% of infected red blood cells), blood was collected for electron microscopy and for the preparation of a stabilate.

### *Electron microscopy*

Jugular blood was collected in Clay-Adams heparinized microhaematocrit capillary tubes half-filled with a cold (4 °C) 2,5% solution of glutaraldehyde, buffered with 0,05 M sodium-cacodylate (pH 7,2). One end of each tube was sealed with plasticine, and the tubes were immediately centrifuged* for 3 min. Each tube was then severed immediately above the packed cells and the pellet pushed out into fresh cold fixative by forcing the plasticine plug into the tube with a wire plunger.

The pellets of packed blood cells thus obtained were subsequently subdivided into 2 mm pieces. Further processing for electron microscopy took place as described by Potgieter, Els & Van Vuuren (1976). The sections were viewed in a Siemens 102 electron microscope.

### *Stabilate*

Jugular blood was collected in a solution consisting of equal volumes of dimethyl sulphoxide and a phosphate-buffered saline solution pH 7,15–7,2 containing 2% of the disodium salt of ethylene dinitrilotetra-acetic acid in a ratio of 4 volumes blood: 1 volume of the solution. The blood was subsequently transferred into 2 ml volumes to glass ampoules which were hermetically sealed and placed into the gas phase of a liquid nitrogen refrigerator.

For the purpose of this study one 2 ml ampoule of frozen blood was thawed rapidly at 37 °C and processed for electron microscopy as described above.

Received 25 April 1977—Editor

* Krist Microfuge model HC 100

## RESULTS

*B. bigemina* is well-known for its pleomorphism in thin blood smears. Although dividing forms, amoeboid parasites (= trophozoites) and pairs of piriform parasites (= merozoites) are, for example, readily distinguishable under the light microscope, the image is so small that not much information can be obtained about how they live and multiply. The electron microscope was therefore used quite extensively in this study to obtain as much information as possible about the structure and function of the various morphological types encountered. Morphologically-distinct intra-erythrocytic merozoites, transforming merozoites, trophozoites and reproducing trophozoites, were identified.

### *Merozoites*

The merozoites observed in this study had a characteristic piriform shape (Fig. 1, 8, 9 & 12). A small mass of vacuolated cytoplasm, attached to the posterior end of most parasites (Fig. 1 & 8), was apparently the only remnant of the original mother cell after the formation of the 2 daughter cells (Fig. 10 & 11) and was associated, in some cases, with an aggregation of fine, granular, intensely osmiophilic material (Fig. 1 & 8).

The outer membrane of the pellicle could not be identified in sections prepared from intact blood. Only a thin, electronlucid band was seen between the cytoplasm of the erythrocyte and the thick osmiophilic inner membrane. This membrane was clearly visible, however, as a very thin membranous envelope in parasites in the sections prepared from thawed blood (Fig. 15 & 16) in which lysis of the host erythrocyte invariably occurred. Owing to the osmiophilic nature of the inner membrane it was difficult to determine its detailed structure, but in some sections it appeared to be composed of a double membrane (Fig. 8, 11 & 13). Anterior and posterior polar rings were present in the merozoites and appeared as thickenings of the inner membrane (Fig. 8). Rhoptries and micronemes were concentrated in the anterior polar region and a few scattered microtubules were frequently seen directly below the inner layer of the pellicle in cross-sections through this region.

A large spherical body of approximately the same size and electron density as the nucleus was usually present in the anterior end of the merozoite (Fig. 1, 8 & 9). A few merozoites, however, were seen in which this organelle was absent (Fig. 12).

The nuclei could be distinguished from the spherical bodies by the presence of a small perinuclear space surrounding the nucleus (Fig. 8 & 9). This perinuclear area is continuous with the membranous endoplasmic reticulum that forms the nuclear envelope, but could not be clearly illustrated in these sections.

Mitochondria could be identified and appeared as diversely-folded, double-membraned organelles. In some sections tubular cristae surrounded by a very fine granular substance could be demonstrated (Fig. 1). Mitochondria without distinguishable cristae (Fig. 10) were, however, seen more regularly than those with cristae.

No food vacuoles, micropores or pellicular invaginations were encountered in any of the merozoites.

### *Transforming merozoites (Intermediate stage between merozoite and trophozoite)*

The transformation of merozoites to trophozoites was associated with the disappearance of the inner membrane of the pellicle, usually near the anterior polar ring, and the outpocketing of the cytoplasm in this region (Fig. 2 & 3). During transformation the inner membrane stained less osmiophilic and a double membrane could be clearly demonstrated (Fig. 2 & 3). The anterior and posterior polar rings disappeared and the micronemes became reduced in number and scattered throughout the cytoplasm. The rhoptries became less electron-dense (Fig. 2) than those in the merozoites (Fig. 1) and eventually disappeared (Fig. 3). The fate of the spherical bodies remains unknown as they were not observed in any of the intermediate stages.

### *Trophozoites*

Trophozoites did not have a characteristic shape and showed no evidence of the inner membrane of the pellicle, rhoptries or the large spherical bodies seen in the merozoites (Fig. 4). Occassionally a few scattered electron-dense bodies, which resembled micronemes, were observed in the cytoplasm.

Some of the trophozoites appeared to have engulfed large portions of the host cell cytoplasm through invagination of the plasma membrane (Fig. 4). In certain sections the engulfed host cell cytoplasm appeared to be continuous with the rest of the host cell cytoplasm through what could be called a cytostome, although it could not be structurally identified as such (Fig. 4 & 5). In the sections of the trophozoites, actual inclusions of host-cell cytoplasm or so-called pseudo-food vacuoles (Rudzinska, 1976), were observed (Fig. 10, 13 & 14). These inclusions stained as intensely as the rest of the erythrocytic cytoplasm and no indications of digestion were observed. Mitochondria and numerous ribosomes were seen, but typical rough, endoplasmic reticulum could not be demonstrated.

### *Multiplication*

At the onset of asexual reproduction, a short segment of the inner membrane reappeared below the limiting membrane of the trophozoite (Fig. 5). Rhoptries and micronemes appeared simultaneously in these areas and subsequently 2 protuberances, or merozoite anlagen, developed, overlying the nucleus (Fig. 5), which gave evidence of commencing division into 2 (Fig. 5 & 6). The 2 bud-like anlagen subsequently increased in size with the progressive incorporation of cytoplasm and nuclear material (Fig. 7 & 13). At this stage an anterior polar ring, which was continuous with the prominent osmiophilic inner membrane and involved the developing daughter cells only, was clearly visible (Fig. 7). In some sections, the inner membrane of the developing daughter cell (merozoite) stained less intensely and appeared as a double membrane (Fig. 13). Nuclear division was still incomplete at this stage, despite the fairly advanced state of cytoplasmic differentiation (Fig. 7 & 13). The stage at which final nuclear divison was achieved could not be determined in this study.

With the enlargement of the 2 merozoites, the mother cell decreased in size (Fig. 10) until only a small vacuolated residual mass of cytoplasm remained (Fig. 11). Subsequently the 2 merozoites presumably became separated. The structural detail of the posterior polar ring area of the merozoites after detachment was not observed.

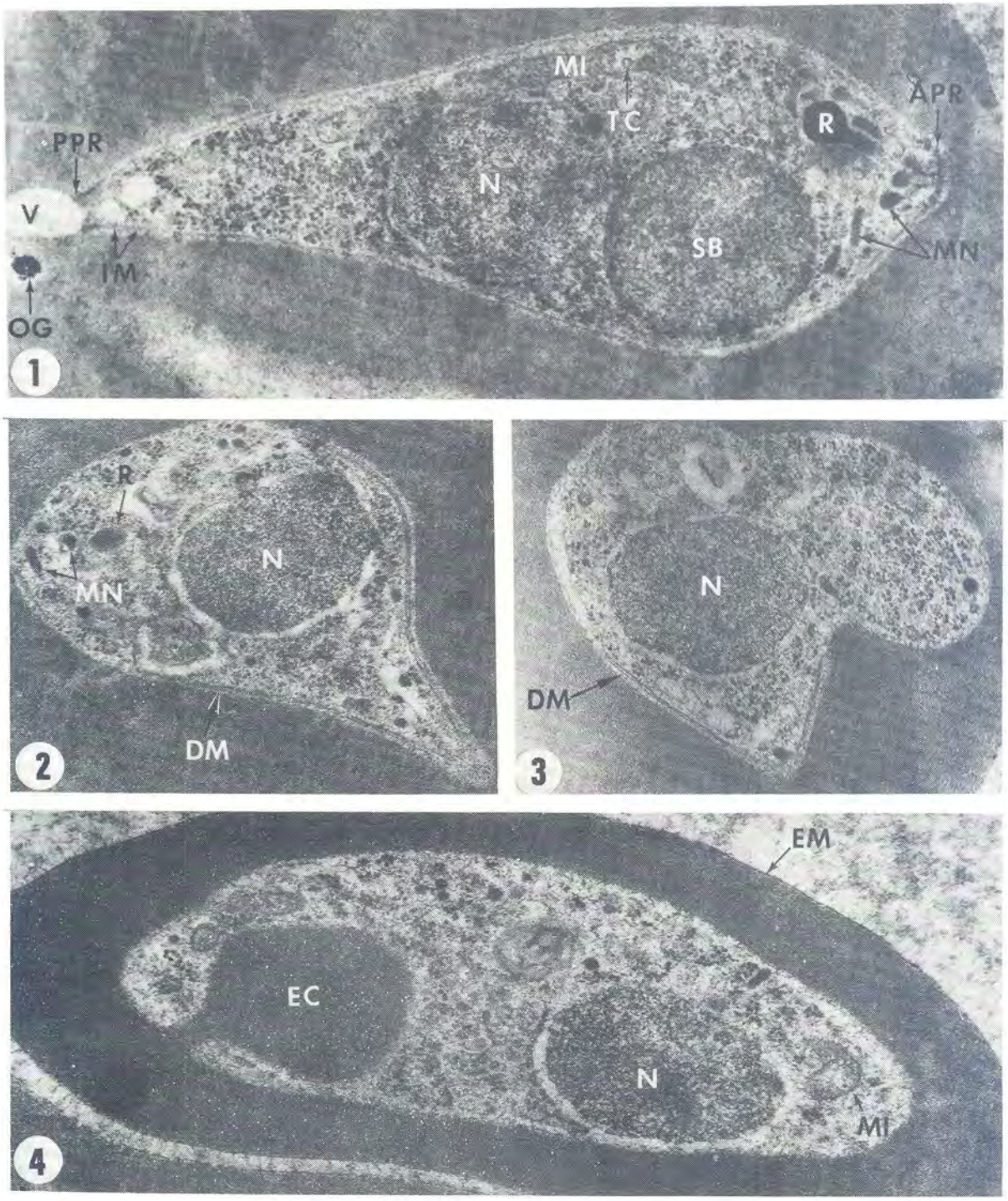



FIG. 1–16 Electron micrographs of intra-erythrocytic forms of *B. bigemina*

FIG. 1 Longitudinal section through a typical piriform merozoite. Note tubular cristae of mitochondria and dark staining aggregation of granular osmiophilic material (= remnants of mother cell after completion of division). ×37 500

FIG. 2 Section through a merozoite undergoing transformation to a trophozoite (intermediate form). Note double membrane of disappearing inner membrane of the parasite pellicle. ×37 500

FIG. 3 Section through an intermediate form. Note disappearance of double membrane, rhoptries and micronemes as the parasite becomes amoeboid. ×37 500

FIG. 4 Longitudinal section through a typical elongated trophozoite. Note complete absence of inner membrane and the apparent engulfment of a large portion of erythrocyte cytoplasm by the parasite. ×37 500

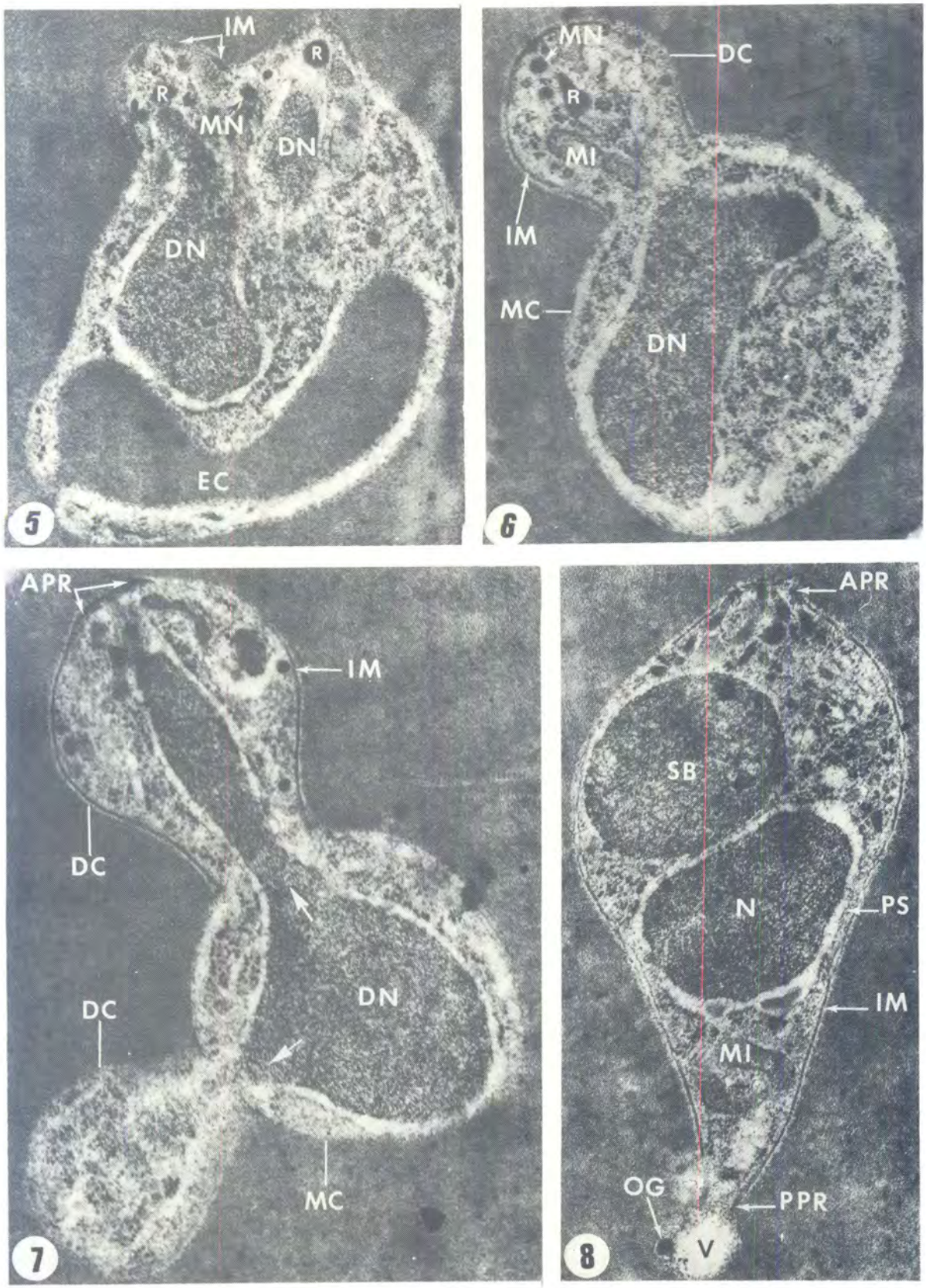



FIG. 5 Section through dividing trophozoite showing merozoite anlagen. Note appearance of rhoptries and micronemes at these sites as well as commencement of nuclear division. ×37 500

FIG. 6 Section through a more advanced dividing stage. Note development of the osmiophilic inner membrane of the pellicle of the developing merozoite. ×37 500

FIG. 7 Section through a dividing form, showing the development of 2 daughter cells. Note incomplete division of nucleus and fairly advanced stage of cytoplasmic differentiation in the one merozoite. ×37 500

FIG. 8 Longitudinal section through a single merozoite. Note perinuclear space and incompletely differentiated posterior end which marks area of final attachment to exhaused mother cell. ×37 500

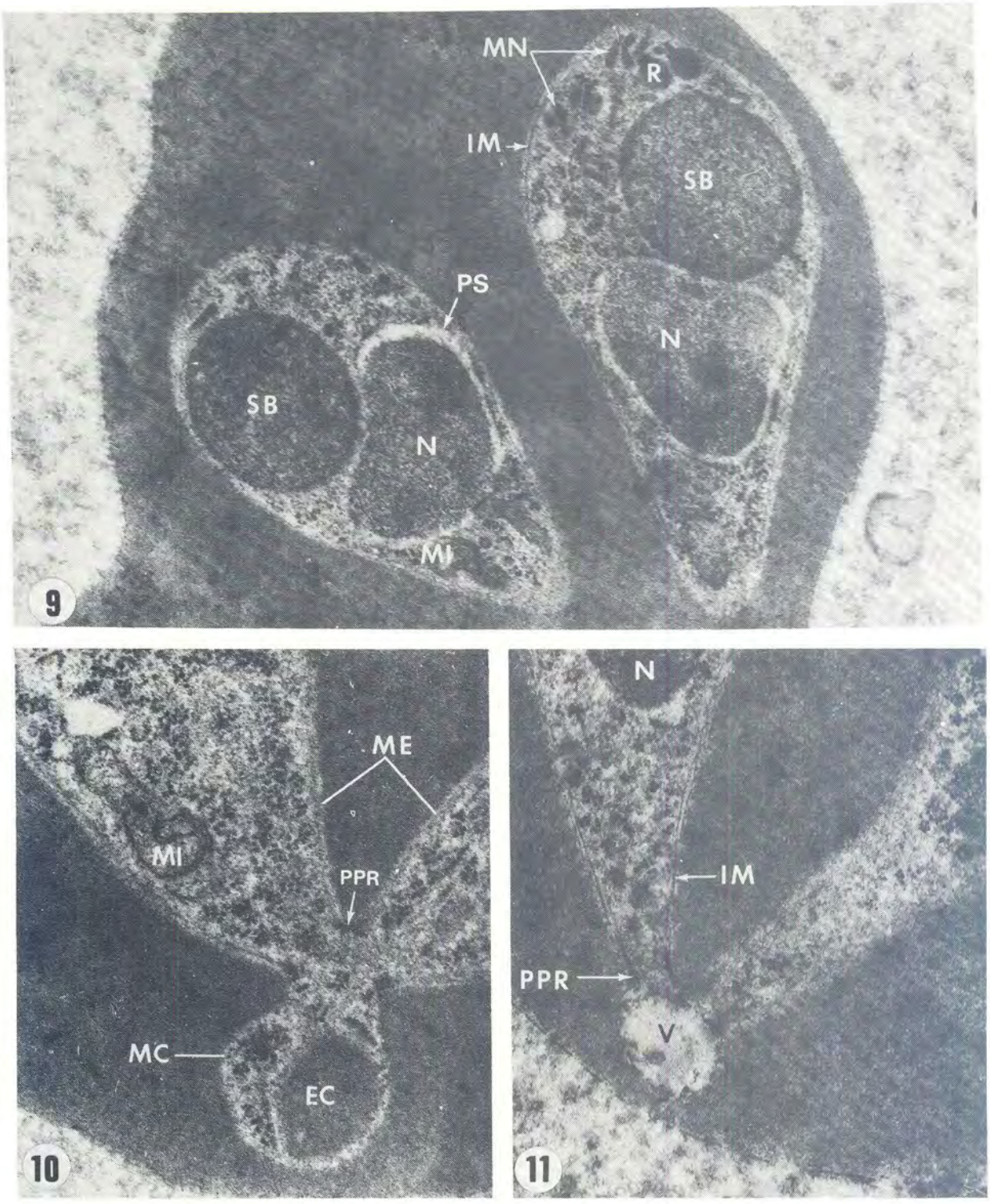
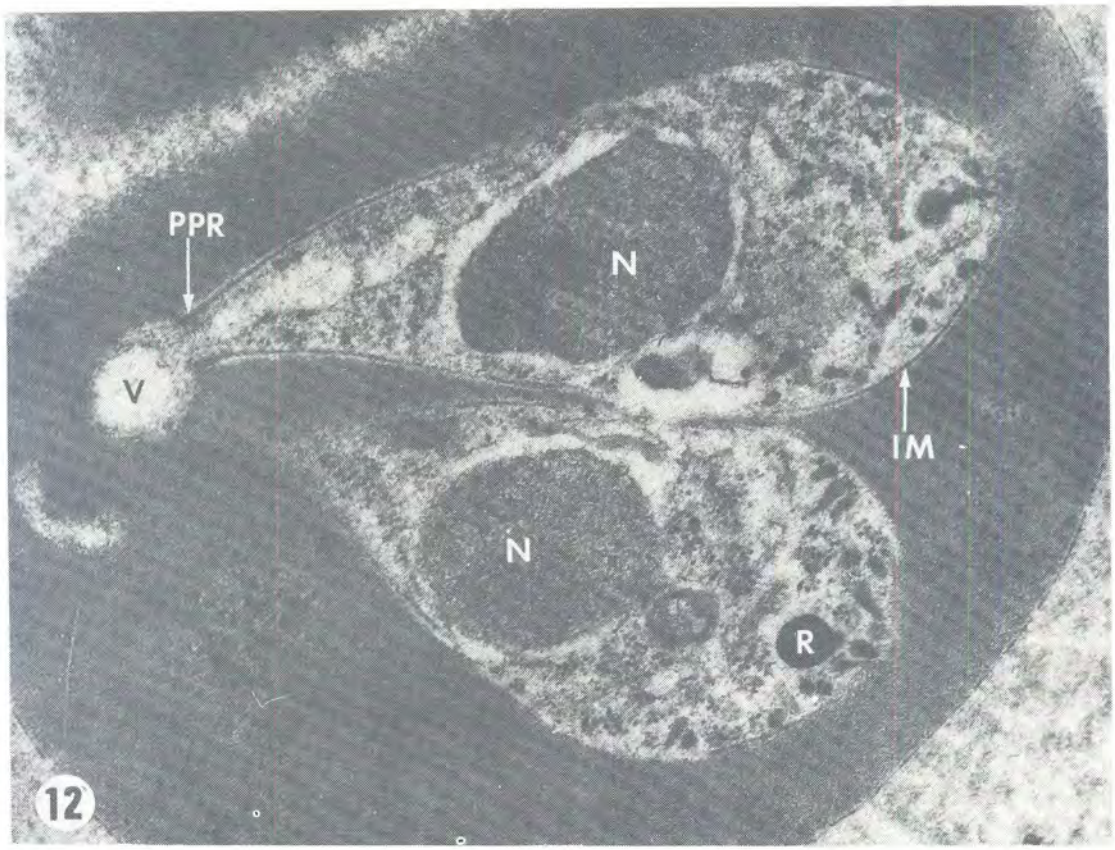
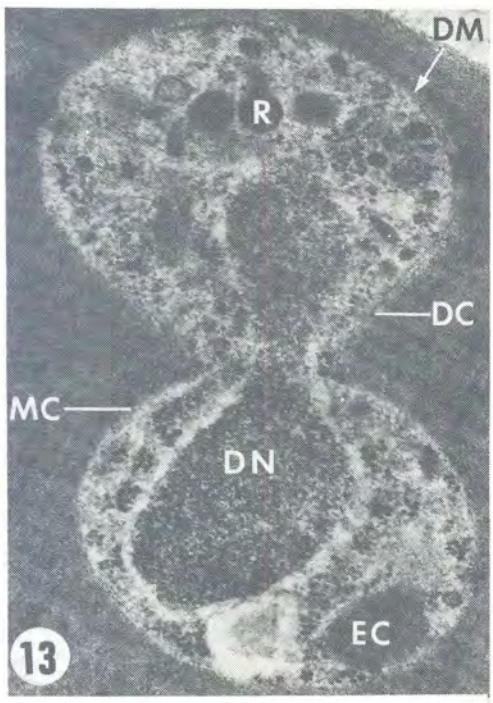



FIG. 9 Section through a fully developed pair of merozoites containing large spherical bodies. Note perinuclear space. ×37 500
FIG. 10 Longitudinal section through the posterior ends of 2 merozoites near the end of the division process of multiplication. Note inclusion of host cell cytoplasm in the remnant of the mother cell. ×41 000
FIG. 11 Longitudinal section through posterior ends of 2 newly formed merozoites. Note complete disappearance of mother cell leaving a vacuolated area in the host cell cytoplasm. ×37 500



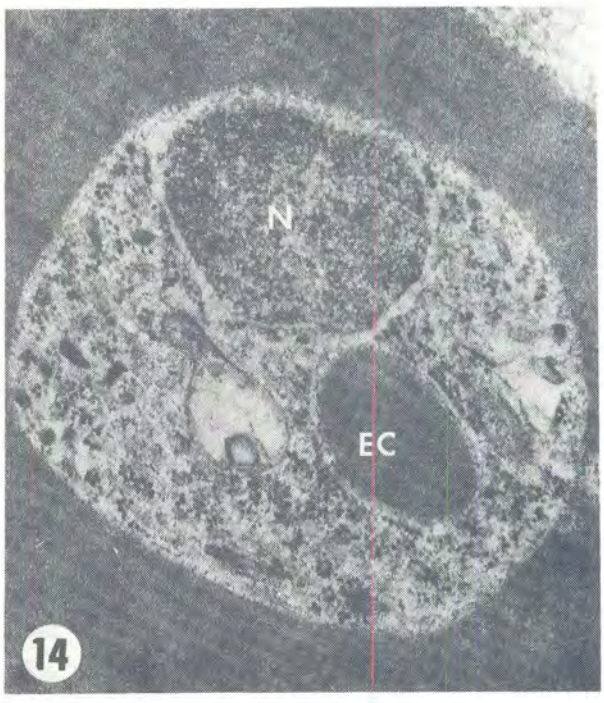
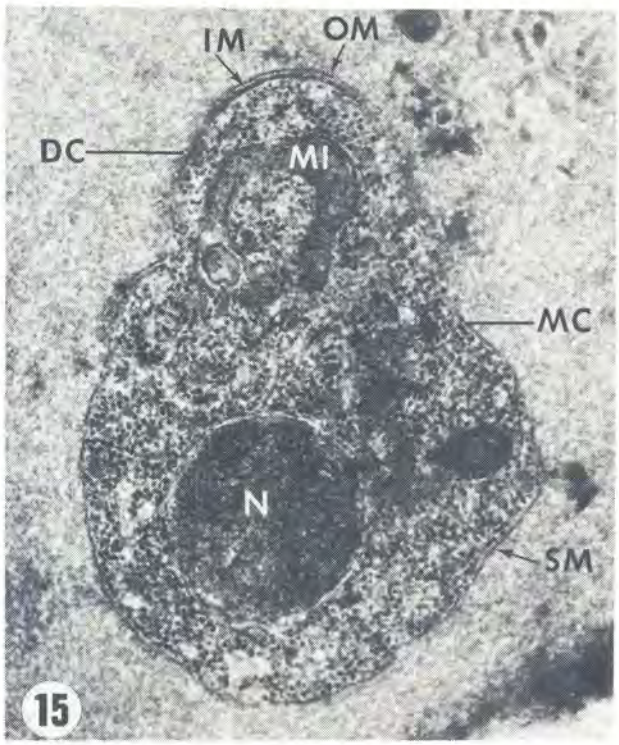




FIG. 12 Longitudinal section through a pair of merozoites. Note absence of the large spherical bodies in both merozoites. ×37 500
FIG. 13 Section through a dividing form. Note inclusion consisting of host cell cytoplasm in mother cell (below) and the double membrane structure of the inner pellicular layer of the developing daughter cell (above). ×37 500
FIG. 14 Section through a trophozoite. Note large inclusion of host cell cytoplasm (= pseudo-food vacuole). ×37 500



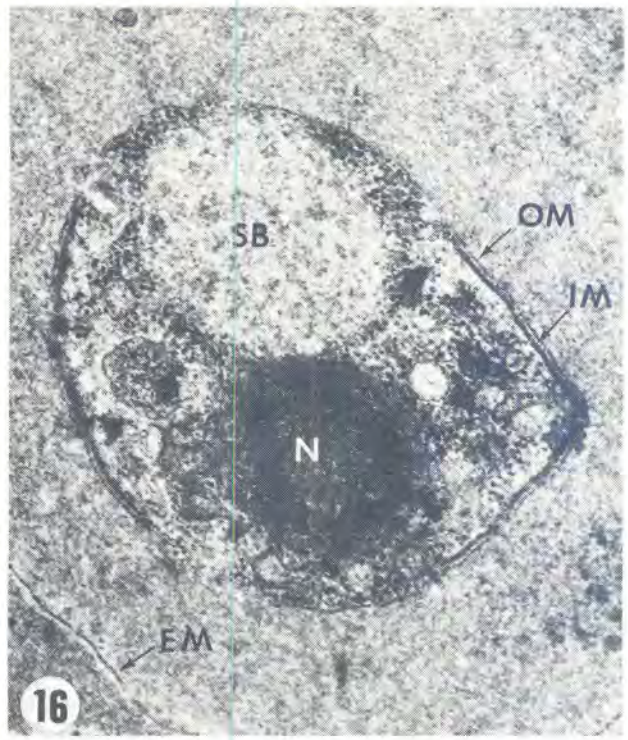




FIG. 15 Section through damaged dividing form in lysed erythrocyte. Note single membrane surrounding mother cell and outer membrane as well as the thick inner membrane (double membrane) of the developing daughter cell. ×37 500

FIG. 16 Section through damaged merozoite in lysed erythrocyte. Note clearly illustrated outer membrane of parasite pellicle. ×37 500

## Discussion

### *Developmental stages*

Only 2 developmental stages of the *Babesia* spp. have so far been recognized in the erythrocytes of domestic animals, namely, trophozoites and merozoites. The latter term has only recently been accepted.

In the case of *B. bigemina,* Friedhoff (1970) distinguished between trophozoites and piriform parasites. He believed that the latter form corresponded to the merozoites of mammalian *Plasmodium* spp., as regards their fine structure and development. Molyneux & Bafort (1970) recognized trophozoites and small piriform "merozoites" of *Babesia microti,* whereas Frerichs & Holbrook (1974) referred to the presence of a trophozoite stage and a mature piriform body in *Babesia equi.* Rudzindka & Trager (1974a) reported the formation of merozoites in *B. microti.* In subsequent publications the terms trophozoite and merozoite appear to have been generally accepted, e.g., in the case of *Babesia rodhaini* and *B. microti* (Kreier, Gravely, Seed, Smucker & Pfister, 1975) and *Babesia herpailuri* (Göbel & Dennig, 1976). These terms have also been applied to the developmental stages of *B. bigemina* observed during the course of this study and the intermediate stage between the merozoite and trophozoite was identified for the first time.

### *Resemblance to tick stages*

Friedhoff (1974) observed the resemblance between the merozoites of *B. ovis* in the salivary glands of the tick and the intra-erythrocytic forms. This resemblance also applies to the intra-erythrocytic merozoites and small merozoites of *B. bigemina* in the salivary glands of *B. decoloratus* and *B. microplus* nymphae and adults (Potgieter & Els, 1977) and provides strong evidence in favour of the concept that the small merozoites observed in the tick are in fact the infective form of the parasite.

### *Spherical body*

Friedhoff, Zwart & Brocklesby (1974) reported that a large spherical body, not seen in other members of the subphylum Apicomplexa, occurs in merozoites of certain *Babesia* spp. This organelle, which is approximately the same size as the nucleus, has been observed in the SM of *B. ovis* in the salivary glands of *Rhipicephalus bursa* (Friedhoff, Scholtyseck & Weber, 1972) as well as in the intra-erythrocytic merozoites of the same species (Friedhoff, 1974). Friedhoff (1970; 1974) also observed spherical bodies in the intra-erythrocytic stages of *B. bigemina,* but he did not specify the stage. In a recent study Göbel & Dennig (1976) recognized this spherical body in merozoites of *Babesia herpailuri* in the blood of a domestic cat. They were also observed in small merozoites of *B. bovis* in the salivary glands of *Boophilus microplus* larvae (Potgieter & Els, 1976) and were common in erythrocytic merozoites of *B. bigemina* in the present study. It would seem, therefore, that this organelle can be regarded as a characteristic feature of erythrocytic merozoites and their precursors in the salivary glands of many, if not all, *Babesia* spp.

The origin and function of this organelle is still unknown. In a cytochemical study on *B. ovis,* however, Weber & Friedhoff (1971) demonstrated the accumulation of extranuclear proteins in this body and suggested that it played a role, together with the so-called paired organelles (= rhoptries), in cell penetration.

Simpson, Kirkham & Kling (1967) described a large "digested food vacuole" in *Babesia caballi.* However, Friedhoff *et al.* (1972) suggested that this vacuole is probably identical with the spherical body found in *B. ovis.* Hoyte (1966) did not observe food vacuoles in the dividing or divided forms of *B. bigemina,* but described "stippled" vacuoles which he

believed contained advanced digested material. Since this report was not illustrated, there is a possibility that these vacuoles were actually spherical bodies.

In a light microscope study of *B. bigemina,* Riek (1964) described a "second nucleus" in the typical paired piriform organisms and found evidence of its extrusion from the parasite. This may explain the absence of the spherical body in some of the merozoites observed under the electron microscope in this study.

*Parasite membranes*

The recorded observations on the membranes which surround the parasites differ in some aspects. Merozoites with their typical piriform shape have a complex body wall, termed the pellicle, which is apparently characteristic for motile stages (Aikawa & Sterling, 1974). On the other hand, the trophozoites that remain in an intracellular position have no need for such a pellicle and are surrounded by a single membrane. The actual problem arises when an attempt is made to identify the origin of the various membranes surrounding the intracellular parasites and to determine the role the host cell membrane plays in cases where parasites are encountered in parasitophorous vacuoles. To avoid confusion, this discussion will be confined to reports in which a definite distinction was made between intra-erythrocytic trophozoites and merozoites of various *Babesia* spp.

Authors such as Büttner (1968), Friedhoff (1970), Kreier *et al.* (1975), Aikawa & Sterling (1974), Langreth (1976) and Rudzinska (1976) are of the opinion that trophozoites are surrounded by a single membrane, whereas the pellicle of the merozoites consists of a single outer membrane and a thick inner layer. Molyneux & Bafort (1970) found that the trophozoites of *B. microti* have 2 and the merozoites 3 unit membranes, whereas Kreier *et al.* (1975) observed a double membrane in some regions of the trophozoites of *B. rodhaini* and *B. microti.* Corresponding observations were reported by Rudzinska & Trager (1974a) and Rudzinska (1976) for trophozoites of *B. microti* approaching reproduction. Rudzinska & Trager (1974a) demonstrated that 2 segmented unit membranes formed the inner layer of the pellicle, whereas Rudzinska (1976) demonstrated that the body of the parasite is covered by a single plasma membrane. Below it, in tangential sections, the segmented membranes give the appearance of a single thick membrane, especially in electron micrographs of poor resolution or those prepared from inadequately fixed material. These membranes were observed in older trophozoites approaching reproduction as well as in merozoites.

Aikawa & Sterling (1974) stated that the pellicle of *Babesia* merozoites is composed of 2 membranes, namely, an outer plasmalemma and a thick, interrupted, inner membrane. However, a merozoite of *B. rodhaini* that had just entered an erythrocyte and located in a parasitophorous vacuole showed the presence of an additional membrane, possibly originating from the host cell. They believed that 2 of the 3 membranes disappeared during merozoite dedifferentiation and found that the trophozoite was finally surrounded by a single membrane. Unlike *B. rodhaini,* the intra-erythrocytic stages of *B. bigemina, Babesia divergens* and *B. ovis* were not located in parasitophorous vacuoles (Friedhoff & Weber, 1974).

In a study on *B. microti,* Rudzinska, Trager, Lewengrub & Gubert (1975) found that, on invasion of a red blood cell, the parasite was surrounded by 2 membranes, namely, its own and that of the invaginated host cell. One of these membranes quickly disintegrated and the parasite was left with a single membrane. Presumably these authors referred only to the outer limiting membrane of the merozoite pellicle in this regard.

The dominant feature of the pellicle of the merozoites of *B. bigemina* observed in this study was the thick osmiophilic inner membrane. The actual composition could not be clearly defined in sections of mature merozoites, but in transforming merozoites it stained less intensely and consisted of 2 membranes. The outer membrane of the parasite could not be demonstrated in intact erythrocytes. In sections of previously frozen blood, however, an outer limiting membrane was clearly discernible. Microtubules occurred below the inner membrane and were seen in cross-sections through the apical region of the merozoite, but no indication could be obtained that they occurred in fixed numbers, as was the case with vermicules in *B. microplus* and *B. decoloratus* (Friedhoff & Scholtyseck, 1969).

*Mitochondria*

Uncertainty still exists regarding the identity of mitochondria in the babesias. Even the presence of these organelles appears to be in doubt, according to some reports. No mitochondria were, for instance, found in the erythrocytic stages of *B. canis* (Bayer & Dennig, 1961), *B. bigemina* (Hoyte, 1966), or *B. caballi* and *B. equi* (Simpson *et al.*, 1967).

Although Friedhoff & Weber (1974) reported that mitochondria had never been identified with certainty in any developmental stage of *Babesia* spp., some authors have used the term "mitochondrion" for certain types of membrane-bound structures in the cytoplasm of *Babesia* spp., viz., *Babesia felis* (Dennig & Hebel, 1969), *B. microti* (Molyneux & Bafort, 1970; Rudzinska & Trager, 1974b; Kreier *et al.,* 1975; Rudzinska, 1976), *B. herpailuri* (Göbel & Dennig, 1976) and *B. bigemina* (this study).

In other instances such structures have, for example been variously identified as: "mitochondria-like vesicles" in *B. bigemina* merozoites (Friedhoff, 1970) and *B. ovis* merozoites in the salivary glands of *R. bursa* (Friedhoff *et al.,* 1972; Friedhoff & Weber, 1974); "mitochondria-like concentric double-membraned organelles" in the trophozoites of *B. bigemina* (Friedhoff, 1970); "vacuoles with concentric membranes" in *B. bigemina* merozoites in the ovaries of *B. microplus* and *B. decoloratus* (Friedhoff & Scholtyseck, 1969); "concentric membraned structures" in *B. bovis* merozoites in the gut of *B. microplus* (Potgieter, Els & Van Vuuren, 1976); "double-membraned sausage-shaped structures with many granules and small vesicles" in intra-erythrocytic stages of *B. microti* (McMillan & Brocklesby, 1971); "double membrane encased organelles with cristae-like strands" in erythrocytic stages of *B. canis* and *B. caballi* (Simpson, Bild & Stoliker, 1963); "vacuoles with two membranes" in erythrocytic stages of *B. gibsoni* and *B. canis* (Büttner, 1968); "structures composed of concentric membranes" in *B. rodhaini* (Rudzinska & Trager, 1960; 1962); a "concentric double-membraned structure" in intra-erythrocytic stages of *B. microti* (Rudzinska & Trager, 1974a; Rudzinska, 1976).

Mitochondria with tubular cristae have been demonstrated in merozoites of *Plasmodium berghei* by Aikawa & Sterling (1974); who also remarked that "acristate mitochondria" predominated in the cytoplasm of trophozoites and some merozoites of mammalian *Plasmodium* spp., and appeared as "doubled membrane-bound structures". This corresponds to the observations of Molyneux & Bafort (1970) on *B. microti*.

Mitochondria with tubular cristae were also identified in erythrocytic merozoites of *B. bigemina* in this study. It must be added that it was difficult to obtain suitable sections for the demonstration of these tubular cristae. It seems very likely, therefore, that the double membrane-bound structures without tubular cristae described by some workers are actually mitochondria. The term "acristate mitochondrion" should, however, be cautiously applied as it would imply a different type of mitochondrion.

The relative scarcity and configuration of the cristae may account for the failures to demonstrate them in many cases. Terzakis, Vanderberg & Hutter (1974) came to a similar conclusion when studying the mitochondria of pre-erythrocytic stages of *P. berghei*. It is also known that cyclic changes occur in the morphology and activity of mitochondria during the lifecycle of *P. berghei* (Howells, 1970). It may not be too far-fetched, therefore, to suggest that this could be the reason why it is so difficult to demonstrate cristate mitochondria in the babesias.

*Feeding*

Only certain features concerning the feeding behaviour of these organisms can be detected by conventional fine structure studies. These include the detection of phagocytosis and pinocytosis, the formation of food vacuoles, the involvement of micropores and the detection of pigment resulting from incomplete digestion of ingested host cell material. Much work has been carried out on the intake of nutrients by the intra-erythrocytic stages of the babesias and has resulted in diverse observations and interpretations thereof.

Rudzinska & Trager (1960; 1962) observed the intake of large portions of erythrocytic cytoplasm by *B. rodhaini* through invaginations of the plasma membrane. This apparently led to the formation of food vacuoles and, as no pigment was detected, digestion was considered to be complete. Similar observations were made in the case of *B. microti* (Shortt & Blackie, 1965; McMillan & Brocklesby, 1971) and *B. felis* (Dennig & Hebel, 1969). Friedhoff (1970) reported the same process in the trophozoites of *B. bigemina, B. divergens* and *B. ovis*, but did not identify food vacuoles as such.

Food vacuoles were also observed in both *B. canis* and *B. caballi* (Simpson *et al.*, 1963). A comparative morphological study of *B. caballi* and *B. equi* (Simpson *et al.*, 1967) revealed the presence of large "digested food vacuoles" surrounded by double membranes as well as smaller ones with the same density as the host cell cytoplasm. A 3rd type of vacuole that stained more intensely was identified in *B. caballi*. *B. equi* apparently had more abundant food vacuoles which appeared to be pinocytotic vesicles pinched off from the plasmalemma of the parasite. These vesicles are much smaller than the food vacuoles seen in *B. rodhaini* (Simpson, 1970) and may indicate that babesias of the *Nuttalia*-type have a different method of ingesting the cytoplasm of the host cell.

Hoyte (1966) reported 3 different types of food vacuoles in *B. bigemina:* vacuoles with a density similar to that of the host cell cytoplasm, vacuoles which showed progressive digestion (stippled vacuoles) and small dense vacuoles. In the present study only pseudo-food vacuoles were observed in the trophozoites of *B. bigemina*. They were similar to those described for *B. microti* (Rudzinska, 1976) and were in actual fact only invaginations of host cell cytoplasm, since no evidence of digestion was seen in them. The significance of this phenomenon is unknown, but they would certainly serve to increase the surface area of the parasite which is in direct contact with host cell cytoplasm, thereby facilitating the intake of nutrients.

In a detailed review of the ultrastructure of the micropore in the Sporozoa and related organisms, Scholtyseck & Mehlhorn (1970) suggested the use of the term "micropore" instead of "cytostome" or "micropyle". Levine (1973) defines a cytostome as a permanent mouth and a micropore as an organelle that may be used for ingestion of fluids or solids.

No micropores were identified in the intra-erythrocytic stages of *B. bigemina* in this study. This agrees with the observations made by Friedhoff & Weber (1974) on similar stages of *B. bigemina, B. divergens* and *B. ovis*. However, Theakston (cited by Molyneux & Bafort, 1970) observed cytostomes in *B. rodhaini*. This organelle has also been identified in other members of the Piroplasmea; for example, *Babesia musculi* and *Babesia galagolata* (Göbel & Dennig, 1974) as well as *Theileria mutans* (Büttner, 1966) and *Theileria parva* (Büttner, 1967a; 1967b). Aikawa & Sterling (1974) demonstrated the presence of a cytostome ingesting host cell cytoplasm in a trophozoite of *B. microti*. In more recent studies, however, Rudzinska (1976) as well as Langreth (1976) declared that there are no cytostomes in *B. microti*.

In a recent detailed review on the history, terminology, origin, morphology and function of the micropore of the Sporozoa, Sénaud, Chobotar & Scholtyseck (1976) reported that micropores are also used for the intake of nutrients from the parasitophorous vacuoles. These authors concluded that cytochemical and physiological methods must be utilized in attempts to determine the function of the micropore in the nutrition of these parasites. Such an attempt was made by Langreth (1976) when she studied the ferritin uptake of *B. microti* in an *in vitro* system. With the aid of haemoprotein cytochemistry she determined that the trophozoite of this parasite ingested small amounts of haemoglobin through pinocytosis over its entire surface. Rapid and complete digestion followed. The merozoite failed to take up tracer protein, probably on account of its complex pellicular structure.

In conclusion it must be clearly stated that ultrastructural evidence cannot serve as the sole source of information for unravelling the feeding mechanisms of these organisms and determining the role that certain organelles play in this respect. Alternative approaches to this interesting problem are essential.

*Multiplication*

Research workers hold divergent views on the method of reproduction of the intra-erythrocytic stages, even with regard to individual *Babesia* spp. In the case of *B. microti*, for example, Molyneux & Bafort (1970) described a process similar to schizogony of malaria parasites, whereas Rudzinska & Trager

(1974b) reported the formation of 4 or more merozoites by budding, but also accepted binary fission as a mode of reproduction. Kreier *et al.* (1975) observed a budding process in *B. microti* and *B. rodhaini* in which buds from the trophozoites developed into merozoites.

Friedhoff (1974) reported the formation of 2 merozoites in dividing *B. bigemina, B. divergens* and *B. ovis,* with complete incorporation of the cytoplasm of the original trophozoites into the merozoites. This agrees to a large extent with the observations made on *B. bigemina* and *B. bovis* (Potgieter, unpublished observations, 1976). This also agrees with the concept expounded by Aikawa & Sterling (1974) that merozoite-transformation in the Piroplasmea resembles schizogony in the Haemosporina in that the progeny are formed along the plasmalemma of the parasite. The conclusion can therefore be drawn that the erythrocytic stage of *B. bigemina* divides by a form of schizogony in which 2 daughter merozoites are usually formed. This form of reproduction is different from the schizogony process in *Plasmodium* described by Aikawa & Sterling (1974) in that the newly formed merozoites of *B. bigemina* are in direct contact with the host cell cytoplasm instead of occurring in a parasitophorous vacuole. Furthermore, the individual daughter nuclei do not migrate into the developing merozoites as in the case of *Plasmodium.* Instead, nuclear division is completed at a much later stage of the development of the merozoites. The latter observation is also supported by the findings of Rudzinska & Trager (1974b) on *B. microti.*

The fact that the merozoites are not formed in parasitophorous vacuoles is not exclusive to certain *Babesia* spp. Vivier & Petitprez (1969) reported similar observations in the case of merozoites of *Anthemosoma garnhami.*

Three basic methods of reproduction have been proposed for the intra-erythrocytic stages of the babesias, namely, binary fission, budding and schizogony. Levine (1973) believes binary fission to be the formation of 2 daughter cells, schizogony the formation of 4 daughter cells and regards the processes reported under the name "budding" as actually being binary fission. He defines budding as a type of asexual division where a small daughter individual is separated off from the side of the mother cell and then grows to full size. None of these definitions, however, applies to the form of multiplication seen in the investigation outlined above.

It is clear, therefore, that considerable confusion exists in this field. This is primarily due to the premature use of "new" terms based on too-limited observations (an inherent weakness of electron microscopy), as well as to the incorrect application of existing terms. It would seem prudent therefore to use greater caution in future if the creation of a serious semantic problem is to be avoided.

## List of Abbreviations Used in all Micrographs

| | |
|---|---|
| APR | Anterior polar ring |
| DC | Daughter cell |
| DM | Double membrane |
| DN | Dividing nucleus |
| EC | Erythrocyte cytoplasm |
| EM | Erythrocyte membrane |
| IM | Inner membrane |
| MC | Mother cell |
| ME | Merozoite |
| MI | Mitochondrion |
| MN | Micronemes |
| N | Nucleus |
| OG | Osmiophilic granules |
| OM | Outer membrane |
| PPR | Posterior polar ring |
| PS | Perinuclear space |
| R | Rhoptries |
| SB | Spherical body |
| SM | Single membrane |
| TC | Tubular cristae |
| V | Vacuole |

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