

ULTRASTRUCTURE OF *EIMERIA* (*S. L.*) SP. INFECTING THE MICROVILLAR ZONE OF THE INTESTINAL EPITHELIUM OF GECKOES

I. PAPERNA

SUMMARY. *Eimeria s. l.* sp. infecting gut epithelium of the geckoes *Hemidactylus turcicus* from Israel and *H. mabouia* from South Africa developed at the microvillar border of gut epithelial cell. Infected portion of the host cell, containing the parasite enclosed in a parasitophorous envelope, bulged above the epithelial layer into the intestinal lumen. The envelope was formed by merging of the host cell wall and the parasitophorous vacuole membrane. Meront and gamont stages conformed ultrastructurally with those of eimerian coccidia of mammalian and avian hosts. In the process of wall formation appeared five membranes derived from the oocyst wall, which supported the formation of an outer and inner oocyst wall.

Key-words: Ultrastructure. *Eimeria*. Geckoes. *Hemidactylus*. Intestine. Microvillar zone.

Ultrastructure de *Eimeria s. l.* sp. infestant la zone des microvillosités de l'épithélium intestinal de geckos.

RÉSUMÉ. *Eimeria s. l.* sp. infestant l'épithélium intestinal des geckos *Hemidactylus turcicus* d'Israël et *H. mabouia* d'Afrique du Sud se développe au niveau des microvillosités des cellules épithéliales de l'intestin. Les portions infestées de la cellule hôte, contenant le parasite inclus dans une enveloppe parasitophore, font saillies hors de la couche épithéliale, dans la lumière intestinale. L'enveloppe est formée par la jonction de la paroi de la cellule hôte et la membrane de la vésicule parasitophore. L'ultrastructure des stades de mérontes et de gamontes se conforme à celle des coccidies de type *Eimeria* des hôtes mammifères et oiseaux. Au cours du processus de formation de la paroi apparaissent cinq membranes dérivées de la paroi de l'oocyte, qui supportent la formation des parois externe et interne de l'oocyte.

Mots-clés : Ultrastructure. *Eimeria*. Gecko. *Hemidactylus*. Intestin. Zone des microvillosités.

Introduction

House geckoes, *Hemidactylus turcicus* L. in Israel and *H. mabouia* (Moreau de Jonnes) in Southern Africa were found infected by *Eimeria* (*s. l.*) sp. with endo-

Department of Animal Sciences, Faculty of Agriculture, Hebrew University of Jerusalem, Rehovot, 76-100, Israel.

Accepté le 6 juillet 1988.

genous stages developing at the microvillar zone of the gut epithelium. The infected microvillar portion of the host cell was characteristically bulging above the surface of the mucosal epithelium.

The eimerians from the Israeli and South Africa geckoes appear to be conspecific and a new species. Taxonomic communication on this eimerian is in preparation for publication elsewhere. In this communication we present the ultrastructural affinities of these eimerians and their peculiar relationship with the host cell.

Materials and methods

Infection in geckoes was confirmed by stools examination for oocysts. Pieces from the anterior gut of infected geckoes were fixed in Karnowski's for 24 hours at 4° C, rinsed repeatedly in Cacodylate buffer, 0.1 M, pH 7.4 and post-fixed in 1 % Osmium tetroxide, in the same buffer, for 1 hour. After rinsing in the buffer, the material was dehydrated in ascending ethanols and embedded in Epon. Thin sections cut by a LKB III ultratome with diamond knife, were stained on grid with uranyl acetate and lead citrate and examined with a Jeol 100 CX TEM.

Results

No fine structural differences could be demonstrated between endogenous stages from israeli and south african geckoes. Endogenous development was restricted to the anterior intestine (duodenum).

Entry into and establishment in the host cell

Young merozoites ($6.6 \times 3.0 \mu\text{m}$) were seen attached at the microvillar border of the host cell (*Fig. 1*) and were becoming enclosed by an envelope derived from the host cell boundary membrane and microvilli. The envelope forming around the parasite presented in *figure 1*, was incomplete, leaving the apex uncovered. In the end of the penetration process the parasite remains located at the microvillar end of the host cell, completely enclosed by an envelope — a double membrane, derived from the parasitophorous vacuole (PV) limiting membrane apposed by a boundary membrane of the host cell (*Figs. 3, 8, 16*). With the increase in size, the parasites enclosed in the host cell envelope progressively bulged above the

Abbreviation to figures: A: Amylopectin granules; b: electron dense band; bz: membranous enclave; C: canaliculi; c: granular-fibrillar coat; dN: differentiating nuclei into dense and granular portions; dg: Electron dense granules; E: Projection with micronemes; er: Endoplasmic reticulum; fd: food vacuole-like inclusion; H: Host cell; hw: Host cell wall; iw: Inner oocyst wall; ll: lamellar aggregates; M 1-5: Oocyst wall membranes; m: mitochondrion; N: Nucleus; n: nucleolus; Oo: Oocyst; ow: Outer oocyst wall; P/p: parasite; pe: parasitophorous envelope; pv: PV; RM: Residual body of the meront; r: rhoptries; t: tubuli-like structures; V: Lipid vacuoles; WF1: Wall forming bodies type 1; WF2: Wall forming bodies type 2; w: parasite cell wall; wpv: PV wall membrane; x: merging of host cell wall and PV; yg: young microgamete; Z: Merozoite.

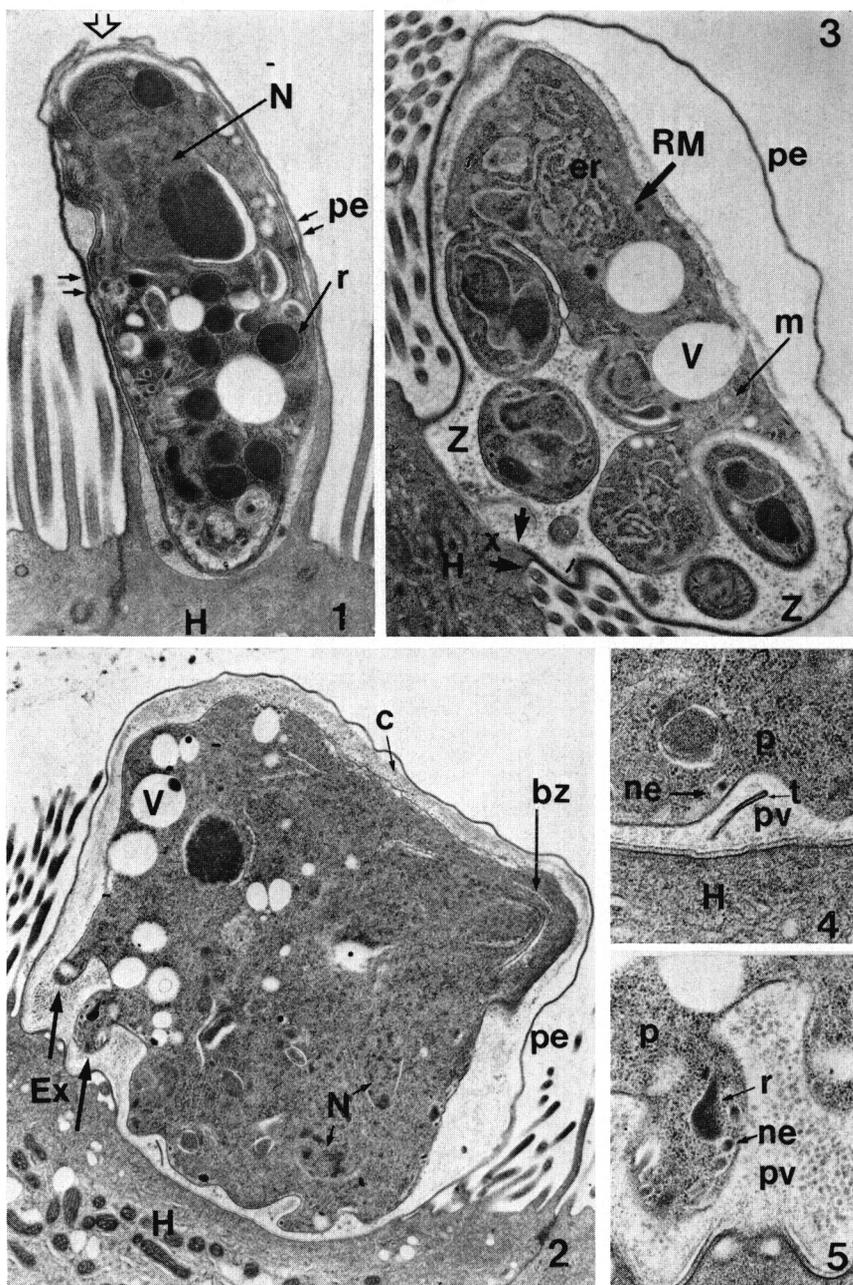


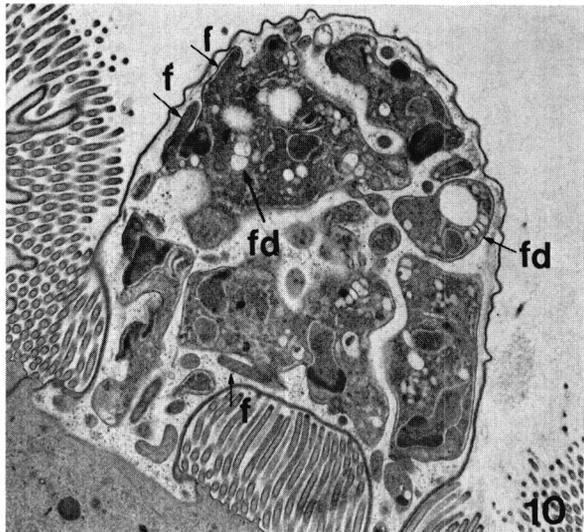
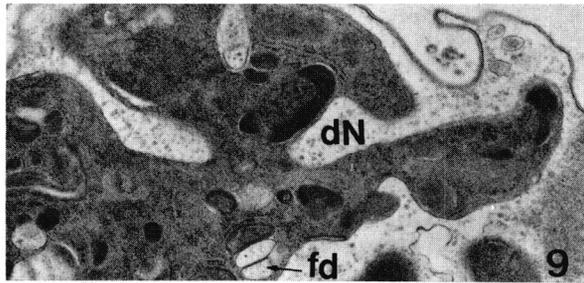
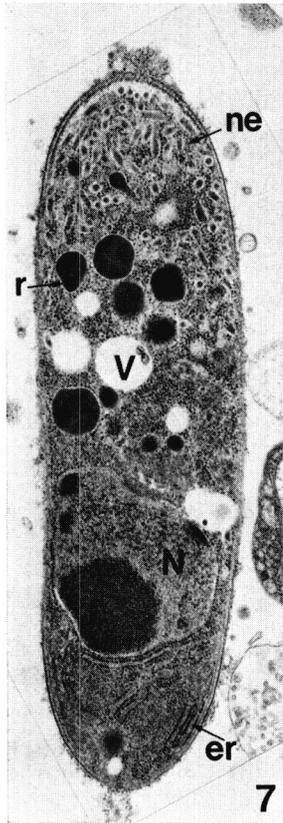
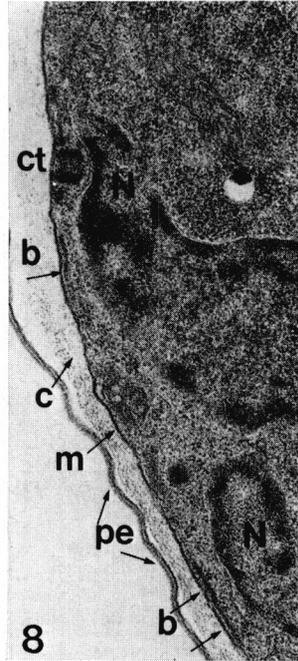
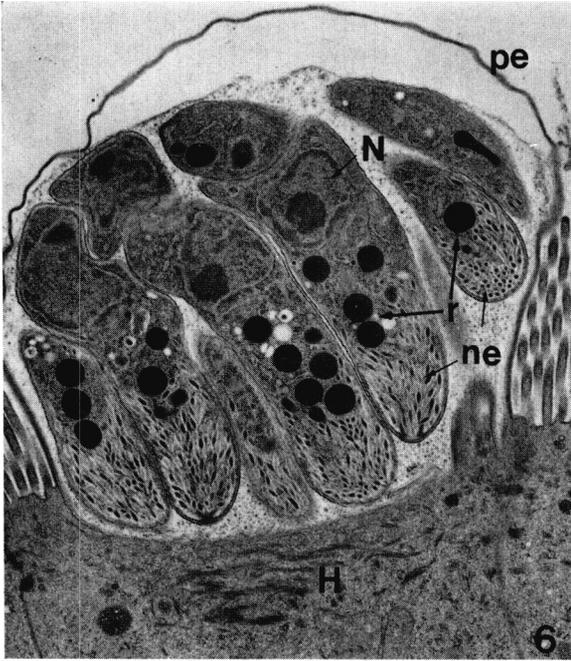
Fig. 1. — Young newly attached merozoite, parasitophorous envelope still incomplete, leaving apex uncovered (arrow) [Ex *H. mabouia*; $\times 11,300$].

Fig. 2. — Meront with several nuclei [Ex *H. mabouia*; $\times 8,200$].

Fig. 3. — Merozoites budding from a meront (x-arrows: merging point of parasitophorous vacuole and host cell wall membranes) (Ex *H. mabouia*; $\times 11,300$).

Fig. 4. — Enlarged sector of fig. 2: parasite's and host cell's bordering membranes [$\times 24,600$].

Fig. 5. — Enlarged sector of fig. 2: projections from the meront surface containing micronemes and a rhoptry-like structure [$\times 24,600$].



epithelial layer into the intestinal lumen (*Fig. 2*). The junction zone between the PV and the cytoplasmic (proximal) end of the host cell consisted of a single unit membrane (*Figs. 4, 5, 16, 18*). In macrogamonts, less frequently in other stages, the PV membrane, particularly at the junction zone was overlaid by one or several fragmented or continuous lamellae (*Figs. 16, 18*). The parasite within the vacuole was coated by a layer of microfibrils (*Fig. 13*) and fine granules, usually with a demarkated boundary (*Figs. 2, 3, 6, 16, 18, 20*). Some PVs contained tubuli-like structures (*Figs. 4, 16*). In cells infected with macrogamonts and in some with microgamonts the junction between the PV and the host cell was alternating with enclaves of free microvillar margins of the host cell (*Figs. 10, 17, 18, 20*). From material examined by a scanning electron microscope (unpublished data) it became apparent that the transition zone between the uninfected and parasitophorous portion of the host cell was deeply infolded.

Merogony

Developed meronts ($7.5-11.5 \times 6.8-9.6 \mu\text{m}$) with already several nuclei (*Fig. 2*) contained some lipid vacuoles, many small mitochondria in the peripheral cytoplasm, inclusions of variable sizes and electron densities of undetermined nature and had also few small projections which contained micronemes and a larger electron dense vesicle reminiscent of a rhoptry (*Figs. 2, 5*). Enclaves with membranes marked sites of emerging merozoites (*Fig. 2*), while developed merozoites were seen budding from the surface of the meront (*Fig. 3*). The meront's residual body contained dense endoplasmic reticulum (ER), mitochondria and few large lipid vacuoles (*Fig. 3*). Merozoites ($5.4 \times 1.8 \mu\text{m}$) still in a $13.6-15.0 \times 7.6-9.0 \mu\text{m}$ PV (*Fig. 6*) or free, $5.5-6.6 \times 1.5-1.6 \mu\text{m}$ in size, in the intestinal lumen (*Fig. 7*) had their large rhoptries (about 7) and the micronemes restricted to the anterior prenuclear end. The nucleous contain large nucleolus and the cytoplasm contains few amylopectin granules, lipid vacuoles, mitochondria (at least two) and very pronounced rough endoplasmic reticulum.

Microgamonts and Microgametogenesis:

Immature microgamonts, $9.8-10.6 \times 5.6-9.0 \mu\text{m}$, contained numerous peripherally arranged nuclei accompanied by mitochondria and centrioles (only one of the presumed two could be seen in the ultrathin section plane) (*Fig. 8*). The plasma membrane next to the centriole was accompanied beneath by an electron dense band (the perforatorium anlage of Furguson *et al.* 1977) (*Fig. 8*).

Fig. 6. — Meront segmented into merozoites [Ex *H. turcicus*; $\times 7,650$].

Fig. 7. — Free merozoite from the intestinal lumen [Ex *H. mabouia*; $\times 16,600$].

Fig. 8. — A sector from young microgamont showing nuclei associated with a mitochondrion and centrioles [Ex *H. turcicus*; $\times 23,600$].

Fig. 9. — Sector of infolded microgamont with differentiating nuclei [Ex *H. mabouia*; $\times 15,500$].

Fig. 10. — Infolded microgamont already demonstrating early outlines of the microgametes [Ex *H. turcicus*; $\times 5,600$].

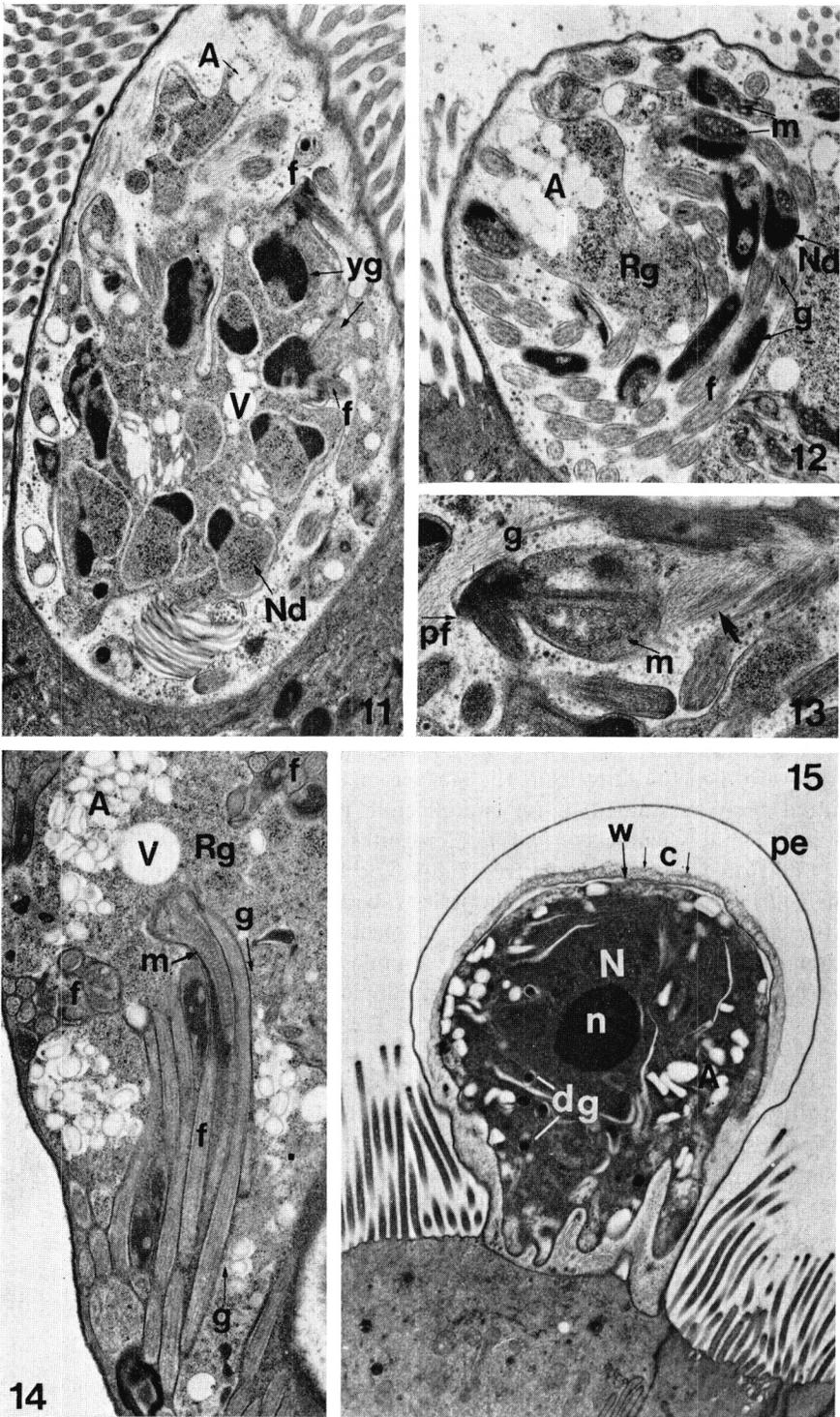


Fig. 11. — Microgamont with differentiating microgametes [Ex *H. mabouia*; $\times 10,100$].

Fig. 12. — Microgamont with mature microgametes [Ex *H. mabouia*; $\times 14,800$].

Fig. 13. — Enlarged view of microgamete showing perforatorium, flagellae and mitochondrion, note (arrow) microfibrillar content of the parasitophorous lumen [Ex *H. mabouia*; $\times 16,300$].

Fig. 14. — Mature microgamont revealing mitochondria and flagellae of microgametes [*H. turcicus*; $\times 8,925$].

Fig. 15. — Young macrogamont [Ex *H. mabouia*; $\times 7,500$].

At a further development, the surface of the microgamont became deeply infolded (*Figs. 9, 10*). The nuclei located in the peripheral cytoplasm developed an electron dense portion (*Fig. 9*) which was gradually separating from the granular, residual portion (*Figs. 9, 10, 11*). At this stage microgamont's cytoplasm contained numerous food vacuole-like inclusions (*Figs. 9, 10*). The flagella appear on the surface (*Fig. 10*) prior to the microgametes differentiation (*Fig. 10*). Nuclei of emerging young microgametes still contained granular portions (*Fig. 11*). Mature, 8-9 μm long microgametes (*Figs. 12, 13, 14*) contained elongate mitochondrion, with its posterior portion wrapped in the electron dense nucleus (*Figs. 12, 14*) and appeared to have three flagella (*Fig. 14*) connected to basal bodies behind the perforatorium (*Fig. 13*). The microgamont's residual body, 10.0-18.0 \times 9 μm , contained aggregates of amylopectin granules, few lipid vacuoles and residual nuclei (*Fig. 14*).

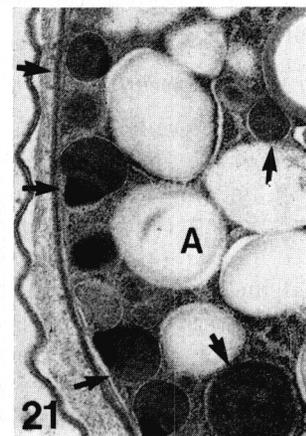
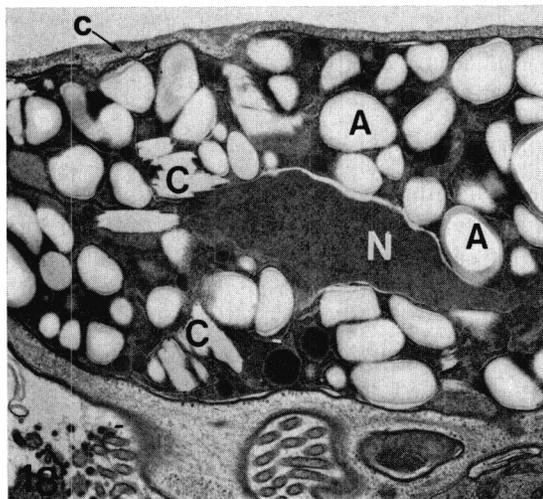
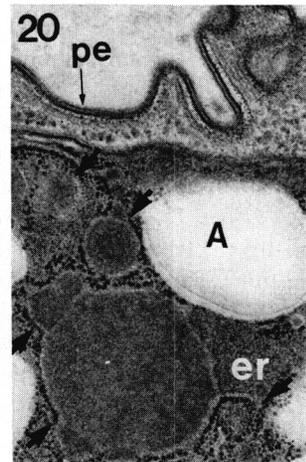
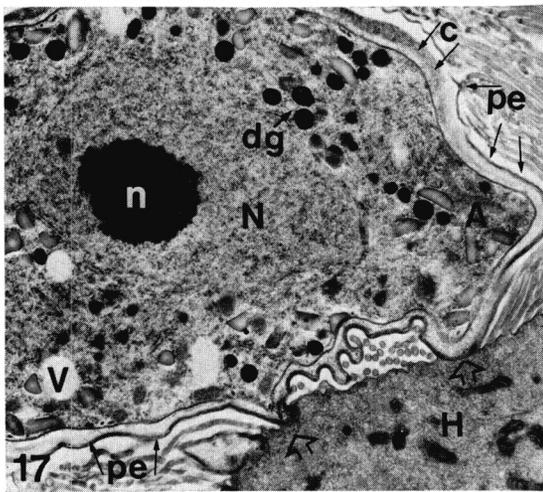
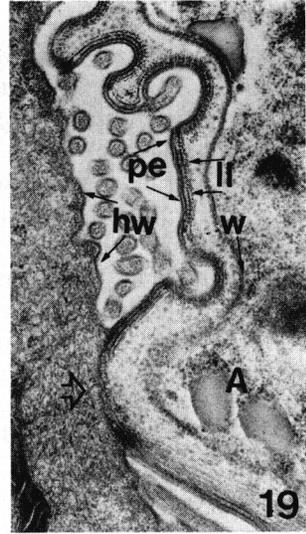
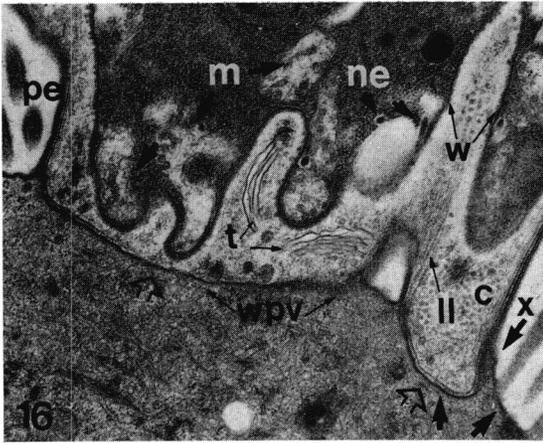
Macrogamonts:

The macrogamonts (*Figs. 15, 17, 18, 22*) were limited by a one unit membrane (*Figs. 16, 19, 20, 21*), micropores were not found. The nucleus contained large nucleolus and the mitochondria occurred in the peripheral cytoplasm (*Fig. 16*). Few micronemes could be still found in young macrogamont (*Fig. 16*). Cytoplasm of young, 6.3-13.4 \times 6.8-7.8 μm macrogamonts (*Figs. 15, 17*), contained only few amylopectin granules and small electron dense granules — probably analogs of wall forming bodies. In mature 12.1-18.0 \times 6.0-14.0 μm macrogamonts and zygotes (*Figs. 18, 22*), the cytoplasm was loaded by amylopectin granules, accompanied by electron — lucent clefts similar to canaliculi, but void of limiting membrane (processing damage?) and electron dense wall forming bodies. Wall forming bodies type 1 (WF1) were of variable sizes (*Fig. 20*) but considerably smaller than the wall forming bodies type 2 (WF2). The latter were located within a rough endoplasmic reticulum cisternae (*Fig. 22*). In the presumed zygote, WF1's electron dense content was partly disaggregated into a granular substance of somewhat lower density (*Fig. 21*).

Detachment from the host cell occurred either at late macrogamogony or during oogony.

Early stages in oocyst wall formation:

Zygotes were bound by a single membrane (*Fig. 21*). The young oocyst was enclosed first by one (*Fig. 23*) and later by two envelopes (*Figs. 24, 25*) formed between limit membranes. The outer envelope (OW) consisted of a broad layer of electron lucent reticulate substance with a thin exterior (M2) and considerably thicker interior (M3) limit membranes. M2 was superimposed by a stratum of several fine, sloughing off lamellae (M1, or a veil), often accompanied with or adhering to a superficial layer of debris. The inner envelope (IW), a thin layer of reticulate electron lucent substance was limited externally by a membrane (M4) of similar quality to M3 and was closely apposed to the oocyst plasma limiting membrane (M5). membranes M4 and M5. Wall material of IW was similar in consis-



tency to that of OW. M5 was the limiting membrane of the zygote cytoplasm (Fig. 24). In further developed oocysts M2 appeared as a very defined electron lucent membrane, there was an increase in the density of OW which became widely separated from IW which remained apposed to the oocyst body (Fig. 25).

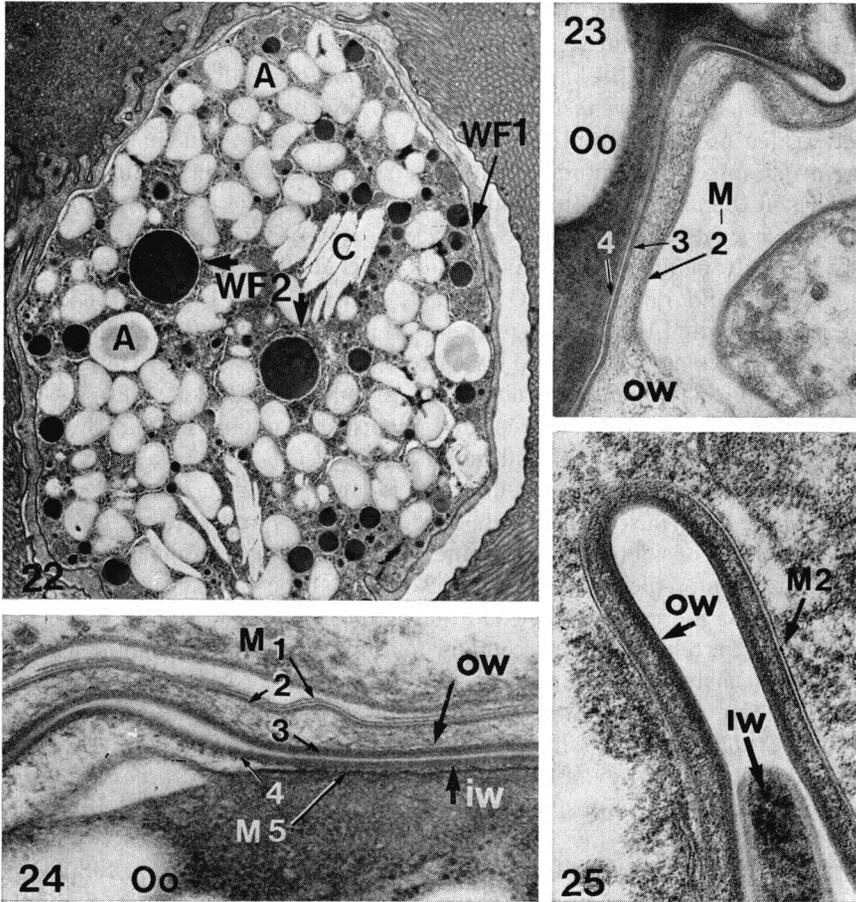


Fig. 22. — Mature macrogamont/Zygote [Ex *H. turcicus*; $\times 6,400$].

Fig. 23-25. — Stages in oocyst wall formation [Ex *H. turcicus*; $\times 23,800$, $\times 46,000$, $\times 61,900$].

Fig. 16. — Junction zone between host cell and PV containing young macrogamont [Detail of fig. 15; $\times 13,600$].

Fig. 17. — Premature macrogamont [Ex *H. turcicus*; $\times 6,732$].

Fig. 18. — Mature macrogamont [Ex *H. turcicus*; $\times 7,850$].

Fig. 19. — Junction zone between host cell and PV containing premature macrogamont [Details of 17; $\times 18,500$].

Fig. 20. — Parasitophorous envelope and peripheral zone of mature macrogamont containing WF1s [Ex *H. turcicus*; $\times 18,600$].

Fig. 21. — Peripheral zone of a zygote containing disaggregating WF1s [Ex *H. turcicus*; $\times 18,600$].

Discussion

Eimerian endogenous stages, meronts and gamonts, infecting the microvillar zone of the gut epithelium of the geckoes, establish themselves in the host cell in a pattern identical to that seen in TEM studies of the piscine coccidia *Epieimeria anguillae* (Molnar and Baska, 1986), *E. isabellae* (Daoudi, Marques and Bouix, 1985) and *Eimeria s. l. vanasi* (Paperna and Landsberg, 1987). In all other ultra-structural features these reptilian coccidia closely conform in fine structure with *Eimeria* spp. infecting mammalian and avian hosts, notably in having wall forming organelles in the macrogamonts and oogony leading to the deposition of hard wall around the oocyst (Chobotar and Scholtyseck, 1982). Piscine coccidia most conspicuously differ from mammalian and avian coccidia in having soft fragile oocyst wall (Dykova and Lom, 1981, Overstreet, Hawkins and Furnie, 1984, Paperna and Cross, 1985) and the absence of true wall forming bodies (Paterson and Desser 1981, 1984, Hawkins, Solangi and Overstreet, 1983, Morrison and Hawkins, 1984, Paperna, Landsberg and Feinstein, 1986, Molnar and Baska, 1986).

Microvillar localization as reported in piscine and in reptilian hosts so far have not been found in avian and mammalian hosts.

Among reptiles, a microvillar infection is suggested from the light microscopic description of *Eimeria sceloporis* from lizards by Bovee and Telford, 1965. Dykova and Lom (1981) considered the terrapine coccidium *E. mitraria* (Laveran and Mesnil, 1902) to be an «epieimerian» species. Microvillar localization of coccidians may either represent more primitive condition of host-parasite relationship characteristic to lower vertebrates, or a secondary feature occurring independantly in piscine and reptilian hosts.

The piscine *Epieimeria* spp. and the now described coccidium from geckoes resembles *Cryptosporidium* which also becomes established in the microvillar zone of the gut epithelium. Levine (1984) placed *Epieimeria* and *Cryptosporidium* into a common family — Cryptosporiidae Leger, 1911. This apparently cannot be justified in light of existing fine structural data. Epieimerians and microvillar eimerians remain free in the parasitophorous vacuole (Molnar and Baska, 1986, Paperna and Landsberg, 1987 and present study), while *Cryptosporidium* forms elaborate attachment through specialised structures and organelles to the parasitophorous vacuole wall at its proximal (cytoplasmic) end (Vetterling, Takeuchi and Madden, 1971, Landsberg and Paperna, 1986). Species of *Cryptosporidium* moreover differ from all emeriorine coccidia (suborder: Eimeriorina Leger, 1911), in their peculiar, non flagellated microgamete (Vetterling *et al.*, 1971, Iseki, 1979, Landsberg and Paperna, 1986).

Oocyst wall formation in the presently studied eimerian resembles that described in coccidia of avian and mammalian hosts (Sibert and Speer, 1980, Chobotar, Senaud, Ernst and Scholtyseck, 1980). The membrane supporting the forming envelopes around the young oocysts OW and IW were given the same

designation of M1-M5 to emphasize their apparent homology with M1-5 membranes formed at the periphery of zygotes of *Eimeria* spp. from avian and mammalian hosts. OW limited by M2 and M3 and IW limited by M4 and M5 appear to develop into the outer and inner oocyst wall of the presently studied reptilian species.

ACKNOWLEDGEMENTS. — This study was supported by the S. A. Schonbrunn Research Endowment Fund. I wish to thank Mr. W. D. Haake, curator of reptiles in the Transvaal Museum, Pretoria, for the supply of *Hemidactylus mabua*.

REFERENCES

- BOVEE E. C., TELFORD S. R. : *Eimeria sceloporis* and *Eimeria molochis* spp. n. from lizards. *J. Parasitol.*, 1965, *51*, 85-94.
- CHOBOTAR B., SENAUD J., ERNST J. V., SCHOLTYSECK E. : Ultrastructure of macrogametogenesis and formation of the oocyst wall of *Eimeria papillata* in *Mus musculus*. *Protistol.*, 1980, *16*, 115-124.
- CHOBOTAR B., SCHOLTYSECK E. : Ultrastructure. In: The Biology of the Coccidia (Long B. L. ed.). University Park Press, Edward Arnold Publishers, 1982, 101-165.
- DAOUDI F., MARQUES A., BOUX G. : Ultrastructure d'*Epieimeria isabellae* Lom and Dykova, 1982 Coccidie épiceplulaire parasite intestinal du poisson Téléostéen *Conger conger* Linné 1758. *Society of Protozoologists* (Abstracts), 1985, No. 114, p. 35.
- DYKOVA I., LOM J. : Fish coccidia: critical notes on life cycles, classification and pathogenicity. *J. Fish Dis.*, 1981, *4*, 487-505.
- HAWKINS W. E., SOLANGI M. A., OVERSTREET R. M. : Ultrastructure of the macrogamont of *Eimeria funduli*, a coccidian parasitizing killifishes. *J. Fish Dis.*, 1983, *6*, 33-43.
- ISEKI M. : *Cryptosporidium felis* sp. n. (Protozoa, Eimeriorina) from the domestic cat. *Jap. J. Parasitol.*, 1979, *28*, 285-307.
- LANDSBERG J. H., PAPERNA I. : Ultrastructural study of the coccidian *Cryptosporidium* sp. from stomachs of juvenile cichlid fish. *Dis. Aquat. Org.*, 1986, *2*, 13-20.
- LAVERAN A., MESSIL F. : Sur quelques protozoaires parasites d'une Tortue d'Asie (*Damonia reeversii*). *C. R. Acad. Sci. Paris*, 1902, *135*, 609-614.
- LEGER L., HOLLANDE G. C. : Coccidie de l'intestin de l'anguille. *C. R. Acad. Sci. (Paris)*, 1922, *168*, 904-906.
- LEVINE N. D. : The genera *Cryptosporidium* and *Epieimeria* in the coccidian family Cryptosporidiidae (Protozoa, Apicomplexa). *Trans. Am. Microsc. Soc.*, 1984, *103*, 205-206.
- MOLNAR K., BASKA F. : Light and electron microscopic studies on *Epieimeria anguillae* (Leger and Hollande, 1922), a coccidian parasitizing the european eel, *Anguilla anguilla* L. *J. Fish Dis.*, 1986, *9*, 99-110.
- MORRISON C. M., HAWKINS W. E. : Coccidians in the liver and testis of herring, *Clupea harengus*. *Can. J. Zool.*, 1984, *62*, 480-493.
- OVERSTREET R. M., HAWKINS W. E., FOURNIE J. W. : The coccidian genus *Calyptospora* n. gen. and the family Calyptosporidae n. fam. (Apicomplexa), with members infecting primarily fishes. *J. Protozool.*, 1984, *31*, 332-339.
- PAPERNA I., CROSS R. H. M. : Scanning electron microscopy of gamogony and sporogony stages of *Goussia cichlidarum* a coccidian parasite in the swimbladder of cichlid fishes. *Protistologica*, 1985, *21*, 473-479.
- PAPERNA I., LANDSBERG J. H. : Tubular formations extending from the parasitophorous vacuoles in gut epithelial cells of cichlid fish infected by *Eimeria* s. l. *vanasi*. *Dis. Aquat. Org.*, 1987, *2*, 239-242.
- PAPERNA I., LANDSBERG J. H., FEINSTEIN N. : Ultrastructure of the macrogamont of *Goussia cichlidarum* Landsberg dans Paperna, 1985, a coccidian parasite in the swimbladder of cichlid fish. *Ann. Parasitol. Hum. Comp.*, 1986, *61*, 511-520.
- PATERSON W. B., DESSER S. S. : Ultrastructure of macrogametogenesis, macrogametes and young oocysts of *Eimeria iroquoiana* Molnar and Fernando, 1974, in experimentally infected fathead minnows (*Pimephalus promelas*, cyprinidae). *J. Parasitol.*, 1981, *67*, 496-504.
- PATERSON W. B., DESSER S. S. : Ultrastructural observations on fertilization and sporulation in *Goussia iroquoiana* (Molnar and Fernando, 1974) in experimentally infected fathead minnows (*Pimephalus promelas*, Cyprinidae). *J. Parasitol.*, 1984, *70*, 703-711.
- SIBERT G. J., SPEER C. A. : Fine structure of zygotes and oocysts of *Eimeria neischulzi*. *J. Protozool.*, 1980, *27*, 374-379.
- VETTERLING J. M., TAKEUCHI A., MADDEN P. A. : Ultrastructure of *Cryptosporidium wrairi* from the guinea pig. *J. Protozool.*, 1971, *18*, 248-260.