

**ULTRASTRUCTURAL STUDIES ON THE MEROGONY
OF *SHELLACKIA CF. AGAMAE*
(LANKESTERELLIDAE, APICOMPLEXA)
FROM THE STARRED LIZARD *AGAMA STELLIO***

K. OSTROVSKA*, I. PAPERNA*

SUMMARY. Meront stages of *Schellackia cf. agamae* (Laveran and Petit, 1909) were obtained from the anterior intestine mucosal epithelium of experimentally infected *Agama stellio* Hasselq. and Linn., 1757. Infection was recovered 5, 7, 10 and 15 days after feeding on blood and liver containing sporozoites from naturally infected *A. stellio*. The parasitophorous vacuole wall consisted of one bilaminar and one single unit membranes apposed at regular intervals. Following nuclear division merozoites differentiated by exogenous budding. The ultrastructure of the meronts, the merozoite and the merogony process conformed in all details with that of species of *Eimeria*.

Key-words: Ultrastructure. Merogony. *Schellackia cf. agamae*. Parasitic in *Agama stellio*.

Étude ultrastructurale de la mérogonie de *Schellackia cf. agamae* (Lankesterellidae, Apicomplexa) chez le Lézard *Agama stellio*.

RÉSUMÉ. Les stades mérogoniques de *Schellackia cf. agamae* sont étudiés dans l'épithélium de la partie antérieure de l'intestin d'*Agama stellio* infectés expérimentalement. Les différents stades ont été étudiés 5, 7, 10 et 15 jours après l'ingestion, par ces Lézards, de sang et de foie d'un *A. stellio* naturellement infecté et dont les organes contenaient des sporozoïtes. L'enveloppe de la vacuole parasitophore comprend une membrane unitaire et une membrane bi-laminée accolées à intervalles réguliers. Après la division des noyaux les mérozoïtes se différencient par bourgeonnement externe. L'ultrastructure des méronts, du mérozoïte et le déroulement de la mérogonie sont conformes dans tous les détails à ceux des autres espèces d'*Eimeria*.

Mots-clés : Ultrastructure. Mérogonie. *Schellackia cf. agamae*. Parasite d'*Agama stellio*.

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

Accepté le 26 mars 1987.

Introduction

Data on merogony in *Schellackia* are available from light microscopy studies of 7 species (Lainson, Shaw and Ward, 1976). Rogier (1979) described merogony stages, oocysts and sporozoites of *S. agamae* (Laveran and Petit, 1909) obtained by experimental infection of *Agama colonorum*. Infection was achieved by feeding on blood and tissues containing sporozoites, taken from naturally infected agama of the same species, collected in the Central African Republic.

S. cf. agamae was found to infect *Agama stellio* Hasselq. and Linn. in Israel. Only sporozoite stages of *S. cf. agamae*, in the blood and the liver, were found in naturally infected lizards (Ostrovskaja and Paperna, unpublished data). Merogony was studied in experimentally infected animals. This is the first electron microscopic study of merogony in a species of *Schellackia*.

Materials and methods

Juvenile sporozoite-free *Agama stellio* were inoculated with blood or force fed on blood and livers of co-specific adult lizards naturally infected with *Schellackia cf. agamae*. The lizards were kept in heated cages (24-29 C) and were autopsied 5, 7, 10 and 15 days after infection.

Presence of infection was verified by light microscopic (LM) examination of Giemsa stained blood, intestine and liver smears. For transmission electron microscopy (TEM), pieces from the anterior gut 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 ethanol and embedded in Epon. Thin sections cut with diamond knife, were stained on grid with uranyl acetate and lead citrate and examined with a Joel 100 CX TEM.

Results and discussion

Young meronts were recovered from smears and sections of the anterior intestine of lizards autopsied 5 and 7 days post infection (p. i.). Mature, dividing meronts and merozoites were recovered in lizards sacrificed 10 and 15 days p. i. In *S. agamae* from *Agama colonorum* merogony had terminated by the 9th day p. i. (Rogier, 1977); in *S. balli* merogony terminated also by the 9th day p. i. (Le Bail and Landau, 1974), while in *S. landauae* merogony was observed in animals sacrificed 23 days p. i. (Lainson *et al.*, 1976).

Lainson *et al.* (1976) reported dividing merozoites of *S. landauae* in the liver; such extra-intestinal merozoites were not found in the liver or any other organs in either naturally or experimentally infected lizards presently studied.

1 — EARLY MERONTS (*fig. 1-4*)*

Stages of the merogony were located within the cells of the anterior gut epithelium. Infected cells contained 1-4 meronts (*fig. 1*). The wall of the parasitophorous vacuole consisted of two membranes apposed at regular intervals (*fig. 2*). The outer membrane was bilaminar and the inner one consisted of a single unit. Same type of parasitophorous vacuole wall occurs also in host cells infected with gamonts of *S. cf. agamae* Ostrovska and Paperna, unpublished). Multimembranous structure of the wall of the parasitophorous vacuole was described gamont stage infections of *Toxoplasma gondii* (Pelster and Piekarski, 1971), *Isospora rivolta* (Pelster, 1973), *I. felis* (Ferguson, Birch-Andersen, Hutchinson and Siims, 1980) and *Sarcocystis* spp. (Scholtyssek and Hilali, 1978, Entzeroth, Chobotar and Scholtyssek, 1985).

The wall of the parasitophorous vacuole consisted of a single membrane in sporozoite stage infections of *S. cf. agamae* (Ostrovska and Paperna, unpublished), of other species of *Schellackia* and of *Lankesterella* (Stehbens, 1966, Heller, 1974, Sinden and Moore, 1974, Bikeung, Barta and Desser, 1986). Single membrane parasitophorous vacuole wall is found in merogony and gamogony stage infections of *Eimeria* spp. (Scholtyssek, 1979).

The parasitophorous vacuole contained a very dilute flocculant substance. Early meronts with a single nucleus (*fig. 1*), with dividing nucleus (*fig. 3*) or already with two nuclei (*fig. 4*) were bound by a single unit membrane and a thicker discontinuous subpellicular membrane (*fig. 3*). The areas with double membrane may represent the future budding sites of the merozoites similar to those reported from meronts of *Eimeria* spp. (Kelly and Hammond, 1973, Dubremetz, 1975). Nuclei had prominent nucleoli (*fig. 1-4*). The cytoplasm contained one to several mitochondria (*fig. 4*), numerous ribosomes, smooth, and rough endoplasmic reticulum, a variable number of food vacuoles and electron lucent vacuoles, apparently lipid vacuoles exhausted of their content (*fig. 1-4*).

* Abbreviation to figures: A: Apical complex; C: centrocone; ED: electron dense vesicle; er: endoplasmic reticulum; F: food vacuole; H: host cell; L: lipid vacuole; M: mitochondria; Mz: merozoites; mn: micronemes; N: nucleus; pM: merozoite primordium; Pv: Parasitophorous vacuole; R: rhoptries; RB: residual body; S: multilaminated inclusions.

 PLANCHE I

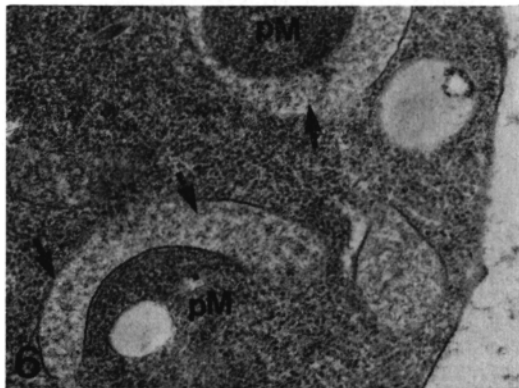
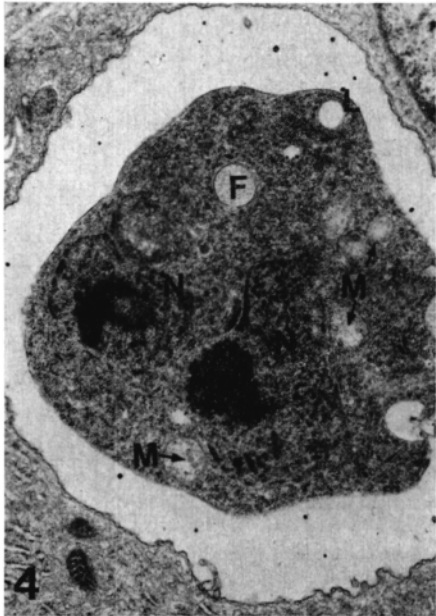
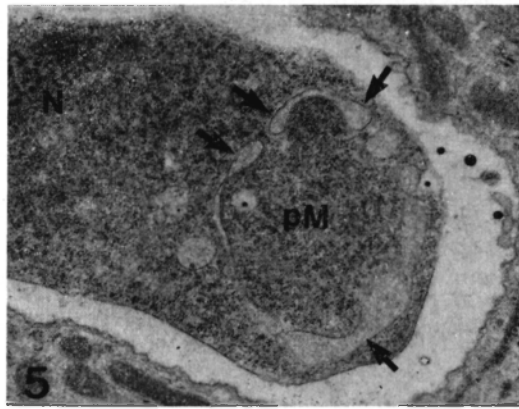
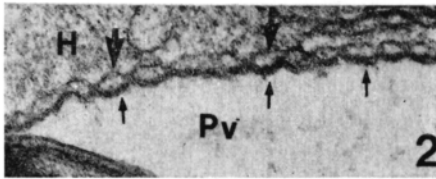
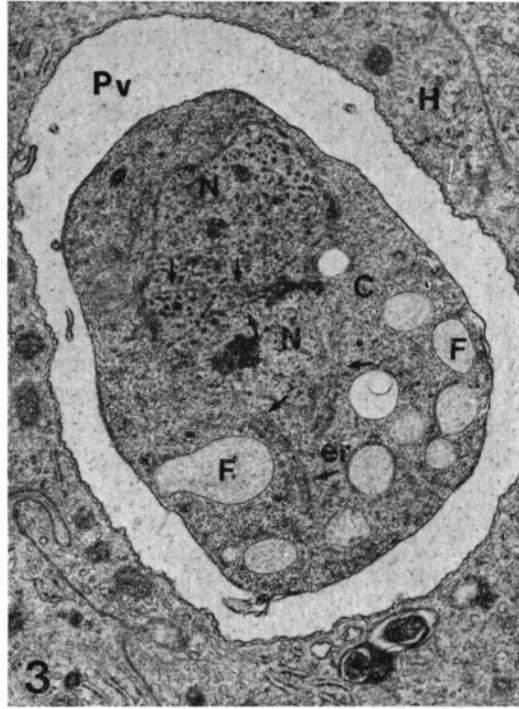
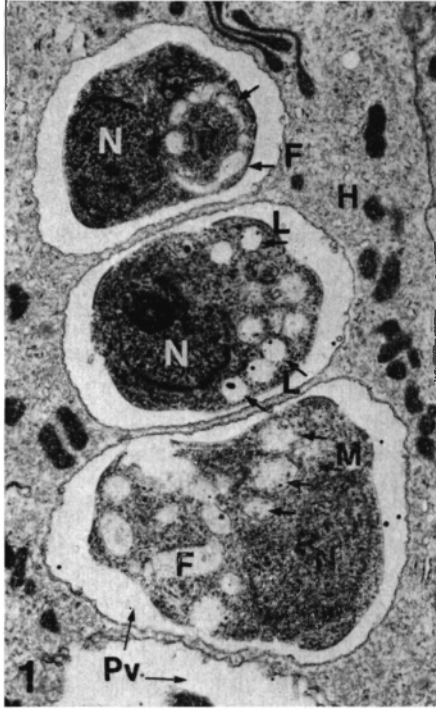
Fig. 1. — Mucosal epithelial cell infected by 4 young meronts ($\times 7,500$).

Fig. 2. — Wall of the parasitophorous vacuole ($\times 32,000$).

Fig. 3. — Meront with dividing nucleus, showing spindle (arrows) and one centrocone ($\times 9,900$).

Fig. 4. — Meront with two nuclei ($\times 9,900$).

Fig. 5 and 6. — Merozoites budding into a subpellicular inclusion (arrows) ($\times 14,850$ and $\times 22,700$).



2 — MEROGOGY AND MEROZOITES (*fig. 5-11*)

In the mature meront the numerous nuclei were arranged at the periphery as well as inside the cells. The nuclei, like those of the early stage meront, had prominent nucleoli (*fig. 7*) (and by this are distinguishable from nuclei of microgamonts which lack distinct nucleolus, Ostrovska and Paperna, unpublished). The general process of merozoite formation was by exogenesis, merozoites developed, like in most species of *Eimeria* (Hope, 1974, Dubremetz, 1975, Dubremetz and Elsner, 1979), at the exterior or infolded periphery of the meront (*fig. 7*). Primordia of merozoites were seen forming on the surface of the meront (*fig. 7*). Some primordia of merozoites, however, were seen budding into a subpellicular inclusion, as if they were developing by endogenesis (*fig. 5, 6*). Subpellicular budding concurrently with exogenous budding was reported in *E. tenella*, there, some of the subpellicular inclusions were in fact, deep invaginations of the meront surface (Hope, 1974). Merogony by endogenesis is rare among species of *Eimeria* (Roberts, Hammond, Anderson and Speer, 1970, Sampson and Hammond, 1972), it occurs in *Toxoplasma* (Vivier, 1970), in *Sarcocystis* (Cerna and Senaud, 1977), and in piscine eimerians (Paterson and Desser, 1981). Light microscopic studies revealed a progeny of up to 32 merozoites per meront, same number as reported by Rogier (1977) for *S. agamae*.

The budding merozoites were bound by two bilaminated membranes, while the residual body of the meront was bound by single bilaminated membrane and contained large one or two membrane bound electron dense bodies (*fig. 8, 9*). Similar organelles have been observed in immature merozoites of some species of *Eimeria* and were regarded as either rhoptry analgens (précurseur des rhoptries), or anterior refractile bodies (Sampson and Hammond, 1972, Danford and Hammond, 1972, Melborn, Senaud and Scholtyssek, 1973, Dubremetz, 1975). In more developed merozoites, rhoptries as well as micronemes appeared and the apical complex became distinct (*fig. 10, 11*). In some of these merozoites, however, the large round electron dense vesicle was still retained (*fig. 10*). The residual body of the meront contained some lipid vacuoles and multilaminated inclusions indicative of degenerative changes (*fig. 9*).

PLANCHE II

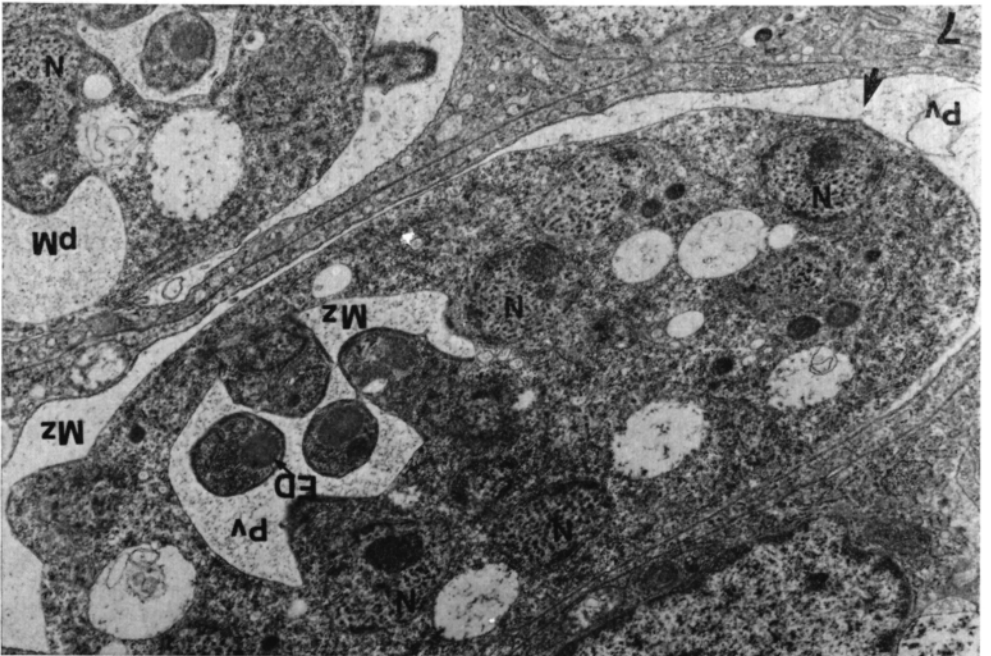
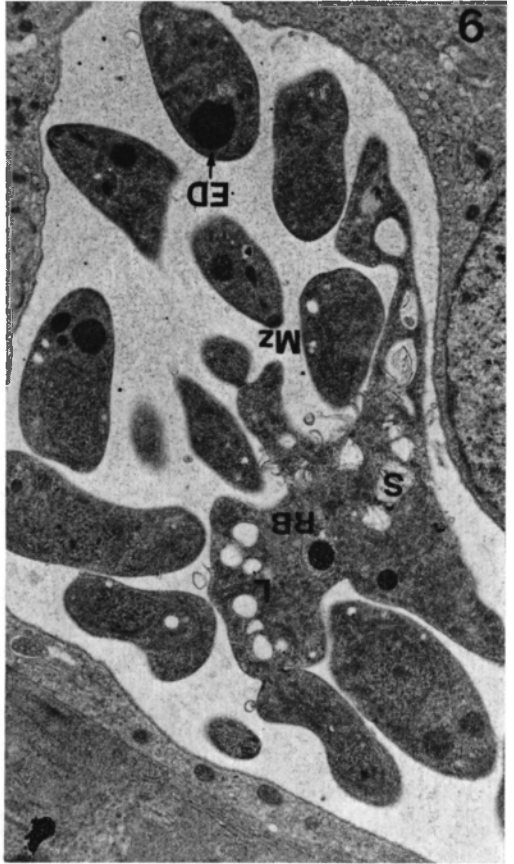
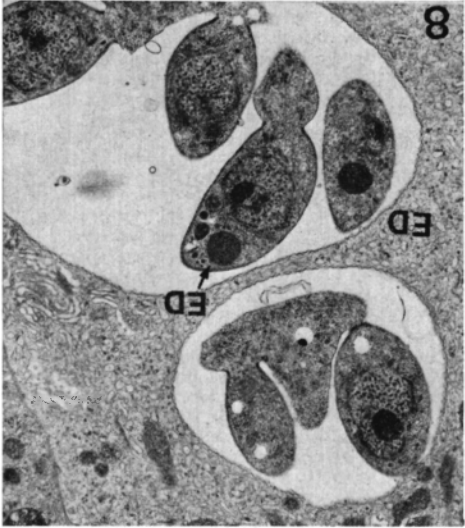
Fig. 7. — Mature meronts with merozoites budding by exogenesis, elevation with adjacent subpellicular membranes (arrow) marks vestige of forming merozoite ($\times 9,000$).

Fig. 8. — Premature merozoites still attached to the meront residual body ($\times 5,200$).

Fig. 9. — Merozoites in their final stage of differentiation bud off the meronts residual body ($\times 7,500$).

Fig. 10. — Anterior end of detached merozoite with apical complex and large electron dense vesicle ($\times 22,000$).

Fig. 11. — Anterior end of free merozoites with developed rhoptries ($\times 22,200$).



ACKNOWLEDGMENT. — This research was supported by the S. A. Schonbrunn research endowment fund.

REFERENCES

- CERNA Z., SENAUD J. : Sur un type nouveau de multiplication asexuée d'une Sarcosporidie, dans le foie de la souris. *C. R. Acad. Sci. (D) (Paris)*, 1977, 285, 347-349.
- DANFORTH H. D., HAMMOND D. M. : Stages of merogony in multinucleate merozoites of *Eimeria magna*. *J. Protozool.*, 1972, 19, 454-457.
- DUBREMETZ J. F. : La genèse des mérozoïtes chez la coccidie *Eimeria necatrix* : étude ultrastructurale. *J. Protozool.*, 1975, 22, 71-84.
- DUBREMETZ J. F., ELSNER Y. Y. : Ultrastructural study of schizogony of *Eimeria bovis* in cell cultures. *J. Protozool.*, 1979, 26, 367-376.
- ENTZEROTH R., CHOBOTAR B., SCHOLTYSECK E. : Electron microscopic study of gamogony of *Sarcocystis muris* (Protozoa, Apicomplexa) in the small intestine of cats (*Felis catus*). *Protistologica*, 1985, 21, 399-408.
- FERGUSON D. J. P., BIRCH-ANDERSEN A., HUTCHINSON W. M., SIM J. Chr. : Ultrastructural observations on microgametogenesis and the structure of the microgamete of *Isospora felis*. *Acta Pathol. Microbiol. Scand., Sect. B*, 1980, 88, 151-159.
- HELLER G. : The fine structure of *Lankesterella* sp. sporozoites parasitic in the frog *Rana pipiens*. *Acta Vet. Acad. Sci. Hungar.*, 1974, 24, 151-157.
- HOPPE G. : La formation des mérozoïtes chez la coccidie *Eimeria tenella* (Railliet et Lucert, 1891). Étude au microscope électronique. *Protistologica*, 1974, 10, 185-205.
- KELLY G. L., HAMMOND D. M. : Fine structural aspects of early development of *Eimeria ninkohlyakimovae* in cultured cells. *Z. Parasitenkd.*, 1972, 38, 271-284.
- LAINSON R., SHOW J. J., WARD R. D. : *Schellackia landauae* sp. nov. (Eimeriorina: Lankesterellidae) in the Brazilian lizard *Polychrus marmoratus* (Iguanidae): experimental transmission by *Culex pipiens fatigans*. *Parasitology*, 1976, 72, 225-243.
- LE BAIL O., LANDAU I. : Description et cycle biologique expérimental de *Schellackia balli* n. sp. (Lankesterellidae) parasite de Crapauds de Guyane. *Ann. Parasitol. Hum. Comp.*, 1974, 49, 663-668.
- MELHORN H., SENAUD J., SCHOLTYSECK E. : La schizogonie chez *Eimeria falciiformis* (Eimer, 1870) Coccidie, Eimeriidae parasite de l'épithélium intestinal de la souris (*Mus musculus*) : étude au microscope électronique des mérozoïtes et leur développement au cours d'infections expérimentales. *Protistologica*, 1973, 9, 269-271.
- PATERSON W. B., DESSER S. S. : An ultrastructural study of *Eimeria iroquoiana* Molnar & Fernando, 1974 in experimentally infected fathead minnows (*Pimephales promelas*, Cyprinidae). 3. Merogony. *J. Protozool.*, 1981, 28, 302-308.
- PELSTER B. : Vergleichende elektronenmikroskopische Untersuchungen an den Makrogameteten von *Isospora felis* und *I. rivolta*. *Z. Parasitenkd.*, 1973, 41, 29-46.
- PELSTER B., PIEKARSKI G. : Elektronenmikroskopische Analyse der Microgametenentwicklung bei *Toxoplasma gondii*. *Z. Parasitenkd.*, 1971, 37, 267-277.
- ROBERTS W. L., HAMMOND D. M., ANDERSON L. C., SPEER C. A. : Ultrastructural study of schizogony in *Eimeria callospermophilli*. *J. Protozool.*, 1970, 17, 584-592.
- ROGIER E. : Description et cycle biologique de *Schellackia agamae* (Laveran et Pettitt, 1909), Lankesterellidae parasite d'agames de République Centre-Africaine. *Protistologica*, 1977, 13, 9-13.
- SAMPSON J. R., HAMMOND D. M. : Fine structural aspects of development of *Eimeria alabamensis* schizonts in cell cultures. *J. Parasitol.*, 1972, 58, 311-322.
- SCHOLTYSECK E. : Fine structure of parasitic protozoa. Springer Verlag, Berlin, Heidelberg, New York, 1979, 206 p.
- SCHOLTYSECK E., HILALI M. : Ultrastructural study of the sexual stages of *Sarcocystis fusiformis* (Railliet, 1897) in domestic cat. *Z. Parasitenkd.*, 1978, 56, 205-209.
- SINDEN R. E., MOORE J. : Fine structure of the sporozoite of *Schellackia occidentalis*. *J. Parasitol.*, 1974, 60, 666-673.
- STEBBENS W. E. : The ultrastructure of *Lankesterella hylae*. *J. Protozool.*, 1966, 13, 63-73.
- TSE B., BARTA J. R., DESSER S. S. : Comparative ultrastructural features of the sporozoite of *Lankesterella minima* (Apicomplexa) in its anuran host and Leech vector. *Can. J. Zool.*, 1986, 64, 2344-2347.
- VIVIER E. : Observations nouvelles sur la reproduction asexuée de *Toxoplasma gondii* et considération sur la notion d'endogénèse. *C. R. Acad. Sci. (D) (Paris)*, 1970, 271, 2121-2126.