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Ultrastructural Study of Developmental Stages of *Eimeria procera* in Partridges *(Perdix perdix)*

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ABSTRACT

In this paper we are first presenting the results of transmission electron microscopy study of endogenous development (merogony and gamogony) of Eimeria procera in partridges (*Perdix perdlx*) together with duration of merogony and gamogony.

INTRODUCTION

Coccidiosis is an important disease of chickens and other Galliformes world-wide, and is recognized as a problem associated with intensive rearing (Reid, 1990). There are some similarities, mainly in farm-breeding systems of wild birds e. g. partridges when in stress situations, but also during the introduction of farm-bred partridges to the wild, when coccidiosis may cause high losses. Intestinal lesions in caecal coccidiosis of chukar partridges has been described Ruff, 1986 as whitish caecal cores even though the caecal wall, which did not appear thickened or damaged. Little is known about the fine structure of the endogenous developmental stages of *Eimeria* species in partridges. This paper describes ultrastructural features of merogony and gamogony in the most pathogenic species *Eimeria procera* which cause caecal coccidiosis of partridges.

MATERIALS AND METHODS

One-day-old partridge chicks (*Perdix perdix*) were maintained in coccidia-free conditions in special cages, until the beginning of the experiment. Origin of the partridges chicks is from the Hungarian, Czech and Italian farms and kept on the University farm in Rozhanovce for 3 years. Partridges were fed an unmedicated corn-wheat ration throughout all experiments and had free access to water. Two-week-old partridges were experimentally infected with a suspension of

sporulated oocysts of *Eimeria procera* at a dose 35,000 oocysts per chicken via crop intubation.

By means of transmission electron microscope studies have been made of the intestinal wall of caeca in 12 hrs, 36 hrs, 48 hrs, 96 hrs, and 108 hrs after infection to observe the endogenous developmental stages (merogony and gamogony). Small peaces of caeca were fixed immediately after removal in a mixture of fixatives (2.5 paraformaldehyde and 2.0 M glutaraldehyde). Osmium tetroxide 0.2 M solution in distilled water diluted 1:1 with cacodylate buffer was used as postfixation solution at a concentration of 1.0%. The samples were processed using conventional techniques and embedded in Durcupan AMC. Semi-thin section 1 μm thick were cut on a Pyramitome LKB and ultrathin-sections on an LKB Nova ultramicrotome. Sections were collected on copper grids and stained with lead nitrate and uranyl acetate. Samples were observed using a Tesla 500 at 80 kV.

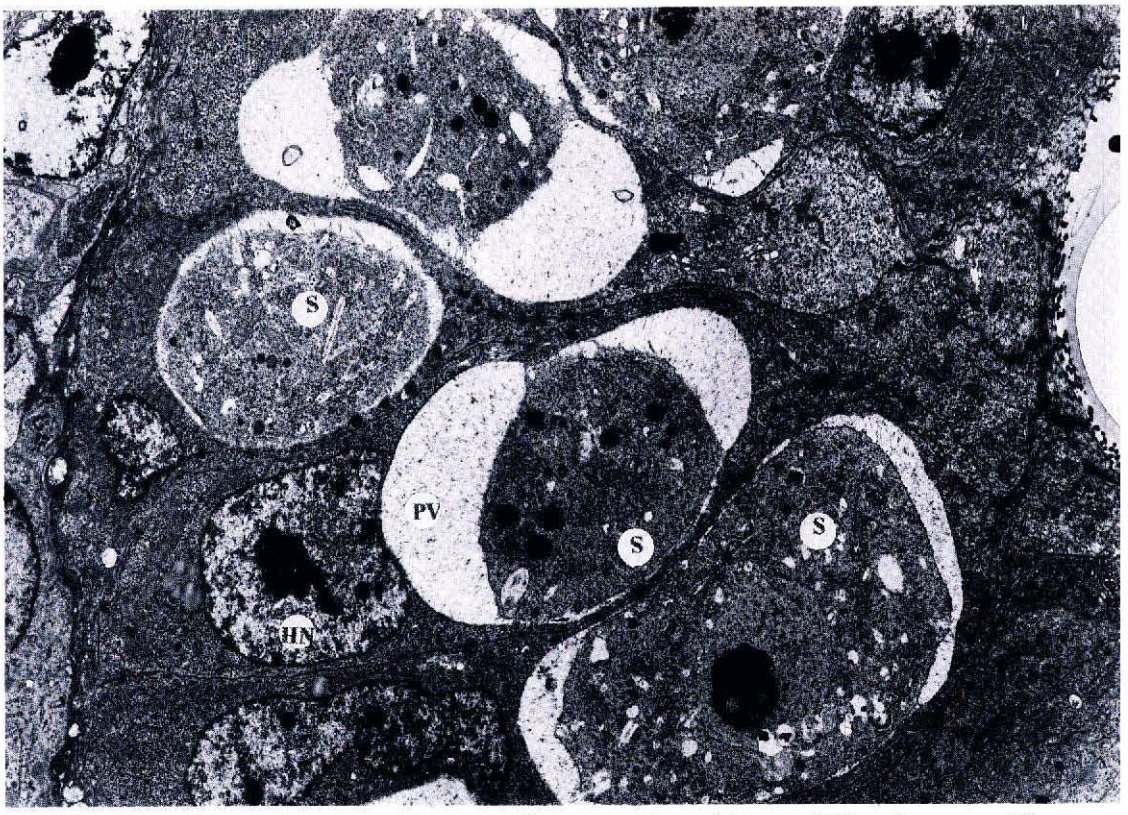

Fig.1 Electron micrograph of immature first-generation schizonts of *Eimeria procera* 12 hrs post infection. Schizonts (S) are enclosed in the parasitophorous vacuoles (PV) and located close to the host cell nuclei (HN) near microvillous border of caecal epithel. 2.400 x.

RESULTS

We observed the first coccidian stages 12 hrs post-infection. Numerous immature first-generation schizonts occurred in the epithelial cells of caecal crypts within the parasitophorous vacuole which was limited by a single membrane (Fig. 1). Thirty-six hours post-infection this first-generation of schizonts had matured with micronemes, rhoptry, and inner and outer membranes of merozoites were clearly visible (Figs. 2 and 3). Young second-generation schizonts were observed in the lamina propria of caecal crypts, 48 hrs post-infection (Fig. 4). Maturation of third-generation schizonts occurred 96 hours post-infection (Fig. 5), during which ektomerogony occurred (Fig. 6). In the early stages of gamogony - 108 hrs post-infection - numerous young macrogamonts were first found, in a single host cell (Fig. 7). Young macrogamonts enclosed in parasitophorous vacuole appeared to be in contact with the outer surface of the plasmalemma by intravacuolar vesicles. Within the parasite cytoplasm were Golgi complexes and food vacuoles (Fig. 8).

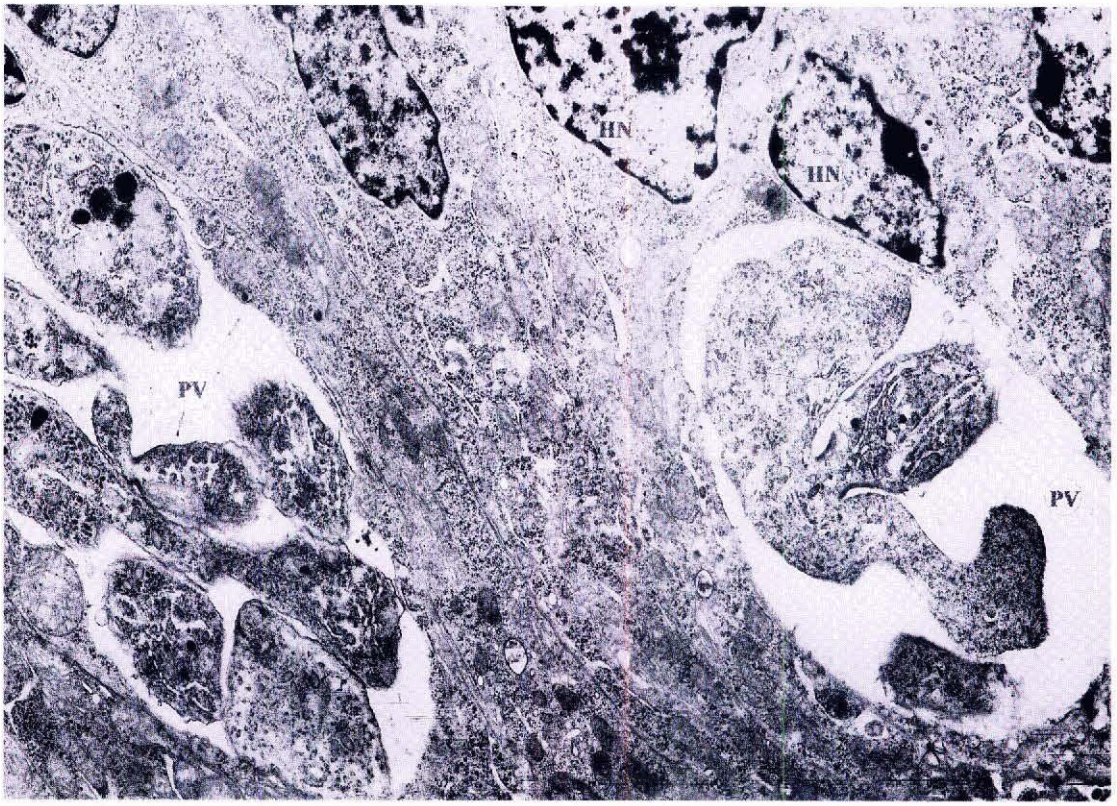

Fig. 2 Electron micrograph of *Eimeria procera* 36 hrs post infection. Section through two mature first-generation schizonts which are situated proximal to the host cell nuclei (HN). Parasites are enclosed in parasitophorous vacuoles (PV). 3.000 x.

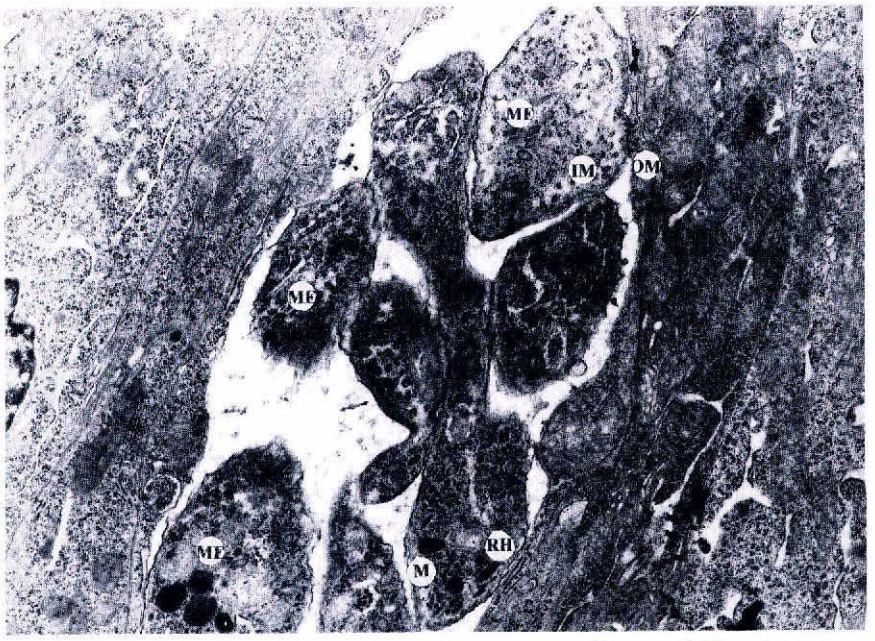

Fig. 3 Higher magnification of first-generation mature *schizont* of *Eimeria procera* 36 hrs post infection. Cross section through anterior part of merozoit (ME). Micronemes (M), rhoptry (RH), inner (IM). and outer (OM) membrane are apparent. 6,000 x.

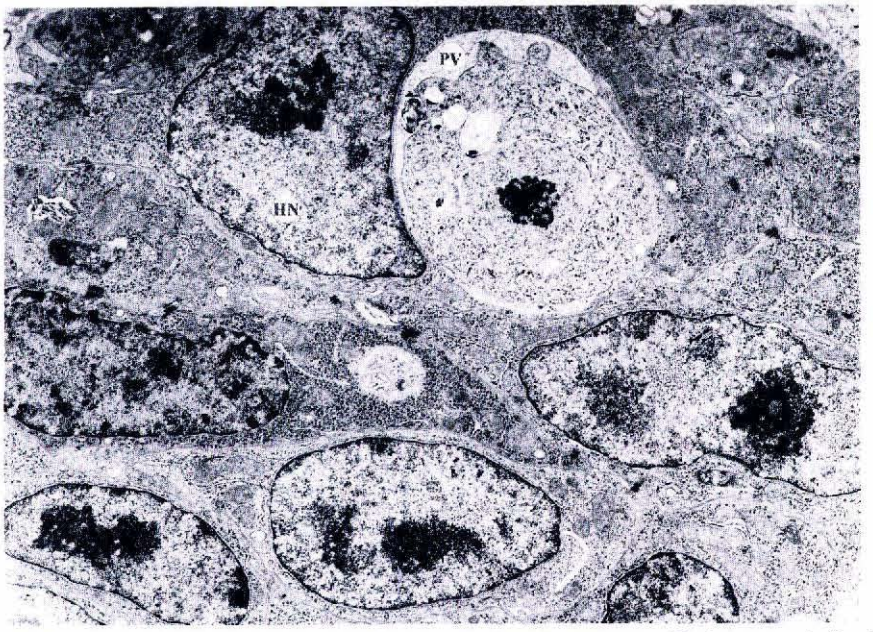

Fig. 4 Young second-generation schizont of *Eimeria procera* 48 hrs post infection. Schizonts are adjacent to the host cell nucleus (HN) and enclosed to the parasitophorous vacuole (PV). 3,400 x.

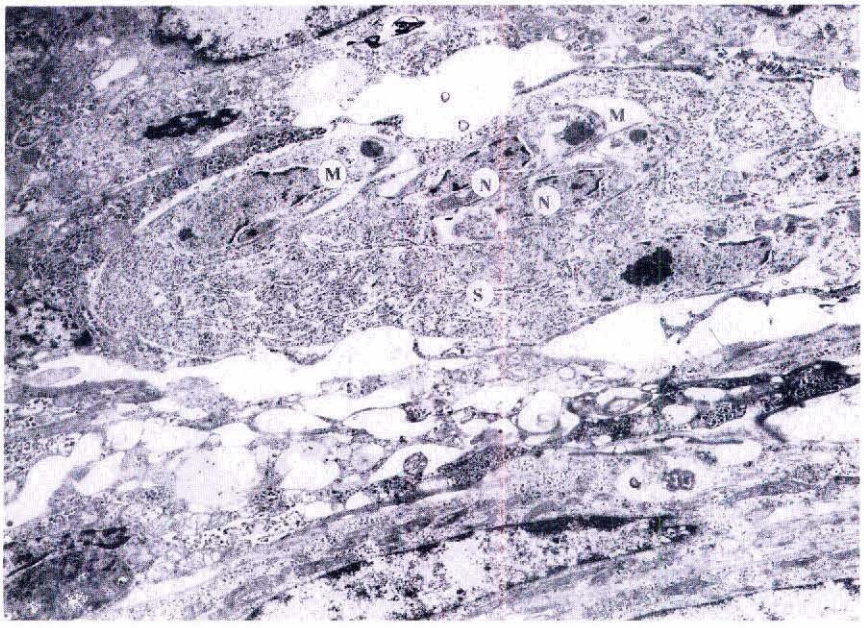

Fig. 5 Section is through mature third-generation schizont (S) of Eimeria procera 96 hrs infection. Merozoites (M) are located on the periphery of the schizont nuclei (N) of merozoites. 3,000 x.

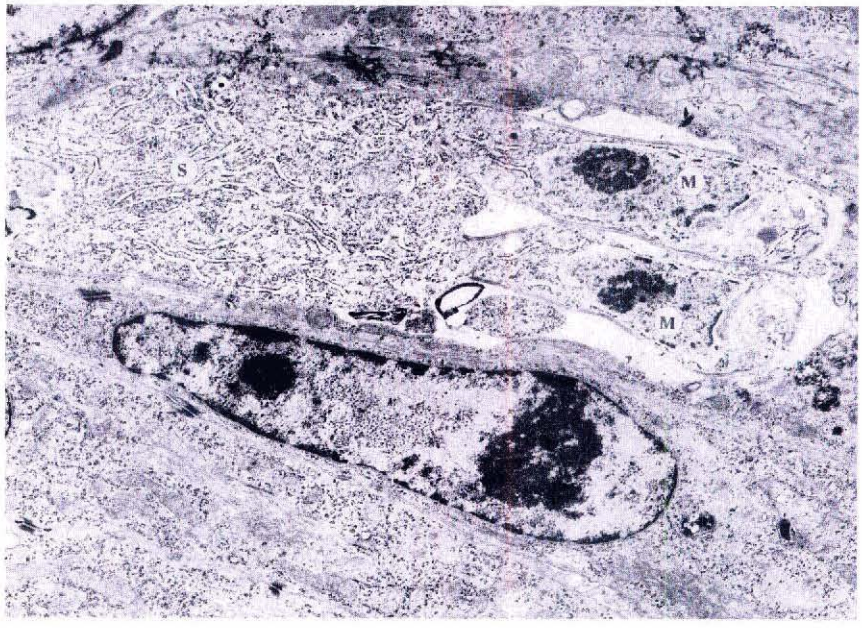

Fig. 6 Electron micrograph of ektomerogony of Eimeria procera 96 hrs post infection. Longitudinal section through third-generation schizont (S), merozoites are completely developed (M). 3,800 x.

DISCUSSION

Since the endogenous development of *Eimeria procera* has not yet been studied, this is a first report. Merogony begins 4-12 hrs post-infection and the first three generations schizonts of Eimeria procera occurred in the epithelial cells of caecal crypts. Ninety-six hours post infection we found mature third-generation schizont with merozoites located on the periphery of the schizont (Fig. 5). In the majority of *Eimeria* species in other hosts which have been studied thus far, merozoites originate at the surface of schizonts. Chobotar et al. (1969) described the formation of invaginations or infoldings in a schizont as a "ektomerogony".

The macrogametes of *Eimeria* species are relatively uniform in their fine structure (Todd et al., 1968, Gajadhar and Stockdale, 1986). They develop in an electron-lucent parasitophorous vacuole, which is bounded by a unit membrane of the host cell. This membrane undergoes folding, resulting in many intravacuolar folds. The cell boundary of the macrogamete of *Eimeria procera*, as well as other

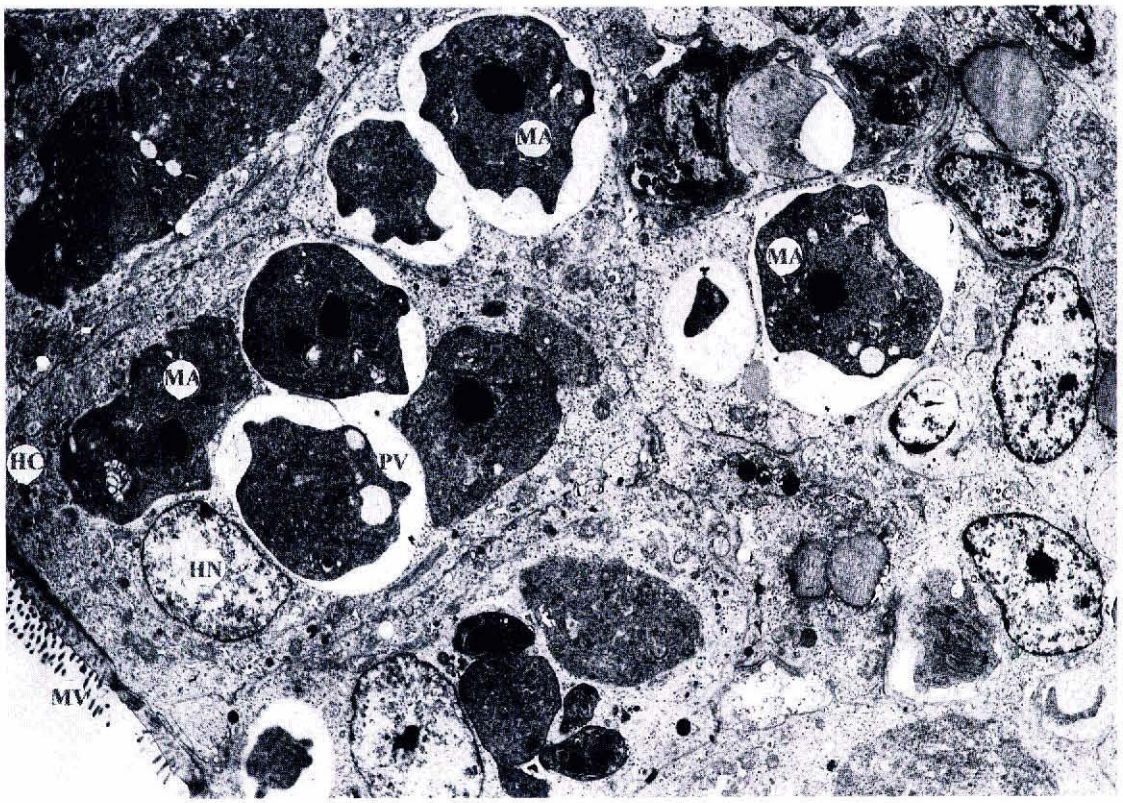

Fig. 7 Numerous young macrogamonts (MA), located in one host cell (HC). Host cell nucleus (HN) is adjanced to parasitophorous vacuole (PV) with macrogamonts, and located near the microvillous region (MV) of caecal epithel. 2,000 x.

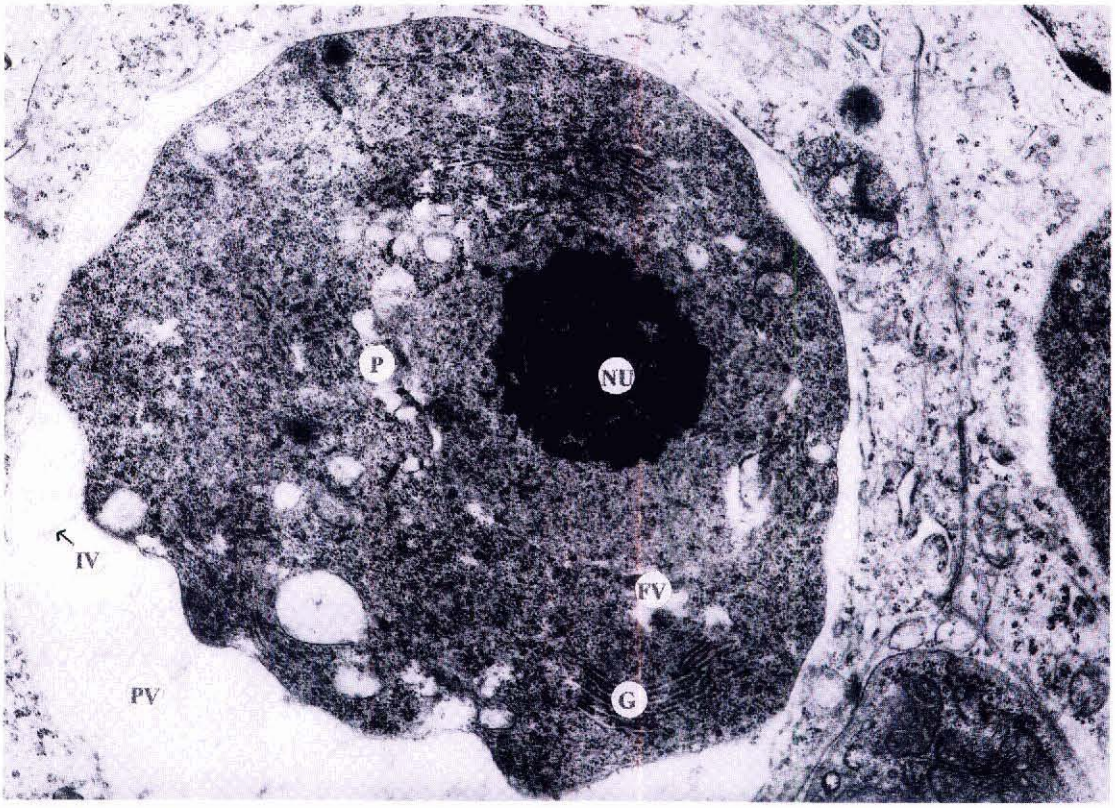

Fig. 8 Young macrogamont enclosed in parasitophorous vacuole (PV). Intravacuolar vesicles (IV) are scattered in parasitophorous vacuole. Nucleus (N) pore in nuclear membrane (P), nucleolus (NU), food vacuoles (FV) are situated near Golgi apparatus (G). 8,000 x.

species of the genus *Eimeria*, consists of a unit membrane with underlying osmiophylic material. Intravacuolar tubules are situated at the cell surface of the parasite and may be associated with its host cell. Scholtyseck and Schafer (1963) first describes these structures and suggested that the function of these intravacuolar tubules was transport of material between the parasite and its host cell. The developing macrogametes of *Eimeria procera* are characterized by a relatively large nucleus with a large compact nucleolus. The cytoplasm is granulated and contains lipid inclusions. The most prominent cytoplasmic inclusions are the wall-forming bodies of type 1 and 2 which, after fertilization, give rise to the outer and inner layers of the oocyst wall (Schotyseck, 1979). The wall-forming bodies in young macrogamonts of *Eimeria procera* originate 108 hours post-infection from large vesicles apparently derived from the Golgi apparatus and appear homogeneously osmiophylic (Figs. 7 and 8).

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REFERENCES

Chobotar, B., Hammond, D. M., Miner, M. L. 1969. Development of the first-generation schizonts of *E. auburnensis*. *J. Parasitol. 55:* 385-397.

Gajadhar, A. A., Stockdale, P. H. G. 1986. Ultrastructural studies of microgametogenesis and macrogametogenesis of *Eimeria truncata* of the Lesser snow goose. *J. Protozool., 33(3):* 345-351.

Reid, M.W. 1990. History of avian medicine in the United States. X. Control of coccidiosis. *Avian Dis. 34:* 509-525.

Ruff, M.D. 1986. Coccidiosis in Gallinaceous game birds and its control. *Res.* in *avian coccidiosis:* 107-115.

Scholtyseck, E., Schafer, D. 1963. Uber schlauchformige Ausstulpungen an der Zellmembran der Macrogametocyten von *Eimeria perforans* und seiner Wirtszelle. *Z. Zellforsch. 62:* 220-230.

Scholtyseck, E. 1979. Fine structure of parasitic Protozoa. Springer-Verlag Berlin Heidelberg New York. 206 pp.

Todd, K.S., Jr., Hammond D. M., Andreson, L. C. 1968. Observations on the life cycle of *Eimeria bilamellata* Henry,19323 in the Ground squirrel *Spermophilus armatus. J. Protozool., 15(4):* 732-740.