

THE ULTRASTRUCTURE OF SOME STAGES OF *HAEMOGREGARINA*  
*TARENTANNULARI* INFECTING THE GECKO  
*TARENTOLA ANNULARIS*

BY

Nadia F. Ramadan, Shadia H. Mohammed and Samia M. Fawzi

Department of Zoology, Faculty of Science, University of Ain Shams, Cairo, Egypt

التركيب الدقيق لبعض مراحل هيموجريجارينا تارنتانولاري  
الذي يصيب البرص تارنتولا أنيولاريس  
نادية فريد رمضان ، شادية حسن محمد ، سامية محمود فوزي

يتناول هذا البحث دراسة التركيب الدقيق بالمجهر الإلكتروني لبعض مراحل الدم ومراحل التزاوج اللاجنسي لطفيلي الهيموجريجارينا تارنتانولاري في العائل الفقاري تارنتولا أنيولاريس . وقد أوضحت الدراسة تركيب بعض الأطوار داخل كريات الدم الحمراء وبعضها الآخر داخل الخلايا المبطنة للشعيرات الدموية في رئة العائل الفقاري (البرص) . ووجد أن التركيب الدقيق لأغشية كل من الميروزويتات والأطوار الأخرى بداخل كريات الدم الحمراء متشابهة تركيبياً وتحمل المواصفات القياسية لشعبية إبيكومبلكسا والتي تتكون من قمع وحلقة أمامين وميكرونيومات وروبيرات ، وكذلك الثقب الدقيق والأنابيب تحت الجدارية .

**Key Words :** Ultra structure, Blood and tissue stages, Haemogregarines, Geckos, Egypt

ABSTRACT

Certain blood and schizogonic stages of *Haemogregarina tarentannulari* - occurring in the vertebrate host *Tarentola annularis* - were studied by electron microscopy. Certain erythrocytic stages, as well as some schizogonic stages observed within the endothelial cells of the lung are described. The fine structure of both the blood stages and merozoites exhibited the general characteristics of the phylum Apicomplexa regarding the pellicle, conoid, polar ring, micropore, rhoptries, micronemes and subpellicular microtubules

INTRODUCTION

With the advent of the electron microscope, it was realized that most "Sporozoa" have an apical complex. Accordingly, the Apicomplexa became one of the largest phyla of the Protozoa. Although much has been achieved in the study of certain Apicomplexan groups, especially the coccidia due to their economic importance, much information is still lacking concerning some of the other groups, especially the haemogregarines .

Mackerras [1] was the first to describe the ultrastructure of *Haemogregarina sp.*, from the lizard *Heteronota binoci*. Stehbens and Johnston [ 2, 3 ] described for the first time the ultrastructure of a haemogregarine prevailing in both the erythrocytes and lungs of a heavily infected gecko, *Gehyra variegata* . Beyer [4] discussed the ultrastructural interrelationships between the lizard erythrocytes and haemogregarines. Bashtar *et al.* [5] presented an electron microscopic study on the erythrocytic stages and schizogony of

*Hepatozoon aegypti* in the snake *Spalerosophis diadima* in Egypt. Abdel Ghaffar [6] carried out both light and electron microscopic investigations on a haemogregarine infecting the poisonous snake *Cerastes vipera* in Egypt. In the same year, Nadler and Miller [7] used both transmission electron microscopy and scanning electron microscopy to investigate the fine structure of *Hepatozoon mocassini* gamonts and their effect on the infected erythrocyte plasmalemma.

El Wasila [8] found that *Tarentola annularis* - in the area around Khartoum (Sudan) - was infected with *Haemogregarina* sp. The present work presents an ultrastructural description of the merogonic stages of *Haemogregarina tarentannulari* [9] in the pulmonary endothelial cells and of gamonts in the erythrocytes of infected geckos.

#### MATERIAL AND METHODS

One hundred and sixty two *Tarentola annularis* were collected from Faiyum, Abu Rawash, Aswan, Ismailia and St-Catherine, in Egypt and examined for blood parasites. Small pieces were taken from the lungs and liver of highly infected geckos and were immediately fixed in 5% (v/v) glutaraldehyde buffered in 0.1 M sodium cacodylate (pH 7.3) for at least one day. Then they were post - fixed with 2% (w/v)  $O_5O_4$  in the same buffer.

After 4 - 5 washings in the cacodylate buffer for 10 - 15 minutes each, the specimens were dehydrated in graded series of ethanol, transferred to propylene oxide and finally embedded in Araldite. Semi - and ultra-thin sections were cut on a Reichert - Jung Ultracut microtome. Semi - thin sections were stained with methylene blue and Azur II, while ultra-thin sections were stained with a saturated alcoholic solution of uranyl-acetate for 20 minutes and alkaline lead citrate for 2 - 3 minutes. These sections were then examined and photographed with a Philips 400 Transmission Electron Microscope, operating at 25 KV at the E.M. Unit at Ain Shams Especialized Hospital, Cairo.

#### RESULTS

##### 1 - Gametocytes

Electron microscopic examination showed that the gametocytes or gamonts of *Haemogregarina tarentannulari* were elongate in shape, having a narrow end and a broad posterior one; they were surrounded by wide electron - lucent parasitophorous vacuoles (Fig. 1) Each parasite was covered with a typical coccidian pellicle of 0.06  $\mu\text{m}$  thickness (Fig. 2) The pellicle was trimembranous, consisting of an outer unit membrane and an inner complex structure composed of two unit membranes. The outer membrane of the pellicle covered the whole parasite without interruption, but occasionally it had short evaginations or folds on the surface near the apical end of the parasite (Fig. 1). The inner membrane complex was discontinuous showing interruptions at both the apical pole and at the micropore located anterior to the nucleus (Fig. 1). The micropore was a punctate invagination of the outer membrane together with the inner membranous layer.

The anterior end of the parasite had an apical complex formed of a typical conoid, measuring about 0.11  $\mu\text{m}$  in length and paired organelles or rhoptries located mainly at the anterior region of the parasite (Fig. 3). The rhoptries were seen as typical electron-dense, drop - shaped structures at the posterior extremity, but they tapered gradually toward the neck portions.

The narrow neck portions of the rhoptries-ductules of rhoptries appeared to arise from inside the conoid. Apart from the rhoptries, the parasite seemed to have a large number of micronemes, which were dense osmiophilic structures found on both sides of the nucleus (Fig. 3). These structures (rhoptries and micronemes) appeared to be similar in their electron density, only differing in their diameter and length.

The parasite had a large nucleus, occupying more than 3/4 of the width of the parasite (Fig. 1) Its material was not uniform; peripherally arranged chromatin with a prominent nucleolus were often seen. The nucleus was bound by a double - layered membrane with a perinuclear space in between the two membranes and with intervening areas of light electron density, identified as nucleopores.

The cytoplasm of the parasite contained numerous inclusions formed of some electron lucent structures, found around the nucleus, presumably representing amylopectin granules and a few larger or smaller ovoid to spherical vesicles with electron-dense contents which were apparently lipid inclusions (Fig. 2.).

## 2 - Schizogony and Merozoites

Schizogony in this parasite occurred only in the lungs. The cells parasitized in such instances were mainly those of the capillary pulmonary endothelia. At the ultrastructural level, the parasite appeared to become more rounded or ovoid after invading the endothelial cells (Fig. 4). It then appeared to increase markedly in size, while the parasitophorous vacuole developed around it. These morphological changes were obviously accompanied by a gradual disappearance of the inner pellicular complex, subpellicular microtubules, as well as other organelles. At the same time, the frequency of nuclear divisions increased and the produced nuclei migrated to the periphery of the schizont directly beneath its border (Figs. 5, 6).

Merozoite formation in the schizont, started with the development of osmiophilic materials along the limiting membrane in areas overlying the nuclei. These areas began to elevate into conical protrusions and the merozoites continued to elongate and protrude into the parasitophorous vacuole.

The inner membrane complex of this young merozoite appeared to extend posteriorly and the nucleus was incorporated into it, and seemed to contain tubular structures at the apical pole indicating the formation of the conoid (Figs. 7, 8). Moreover, the developing merozoite contained subpellicular microtubules, as well as rhoptries, micronemes and mitochondria. The infolding of the surface layer separated the merozoites from each other and from the mother schizont. The cytoplasm remaining after the separation of merozoites formed the residual body.

The cross section of the mature schizont in lung sections showed merozoites formed inside the schizont enclosed in a parasitophorous vacuole. Each merozoite

contained the nucleus, micronemes and amylopectin granules.

The number of merozoites produced varied according to the type of schizont, whether macro - or micro - schizonts. The resulting merozoites varied in size, being either macro - or micromerozoites yet they had nearly the same structure as depicted in Figures 11 & 12.

## DISCUSSION

The fine structure of the parasite described in the present work agrees generally with that of other representatives of the phylum Apicomplexa. The apicomplexan parasites are characterized by a number of specific organelles, including the trimembranous pellicle, polar rings, subpellicular microtubules, micropores, rhoptries, micronemes and conoid, in addition to the nucleus and other common cellular organelles such as the mitochondria and amylopectins [10].

The ultrastructure of the pellicle of *Haemogregarina tarentannulari* is in agreement with the previous findings obtained from other species of the same genus [8, 11]. Similar observations were also recorded from other genera of haemogregarines eg. *Hepatozoon* [12] and *Karyolysus* [13, 14]. The presence of a three - layered pellicle is in agreement with Viver *et al.* [15] who stated that the pellicular complex consists of a triple layer, composed of three individual unit membranes, which is characteristic for all motile and infectuous stages of Apicomplexa (merozoites and sporozoites). The description of only two unit membranes may be due to imperfect fixation or insufficient enlargement [6, 16].

In the present study, the presence of rhoptries and micronemes at both sides of the nucleus, is a characteristic feature of other species of haemogregarines [13, 14, 17]. The structure and function of rhoptries have been the subject of a wide discussion. Ludvik proposed that rhoptries of *Toxoplasma gondii* might have secretory functions [18]. Other authors have offered similar suggestions for some Apicomplexa [19] and hence they concluded the possibility of their involvement in the penetration of host cells.

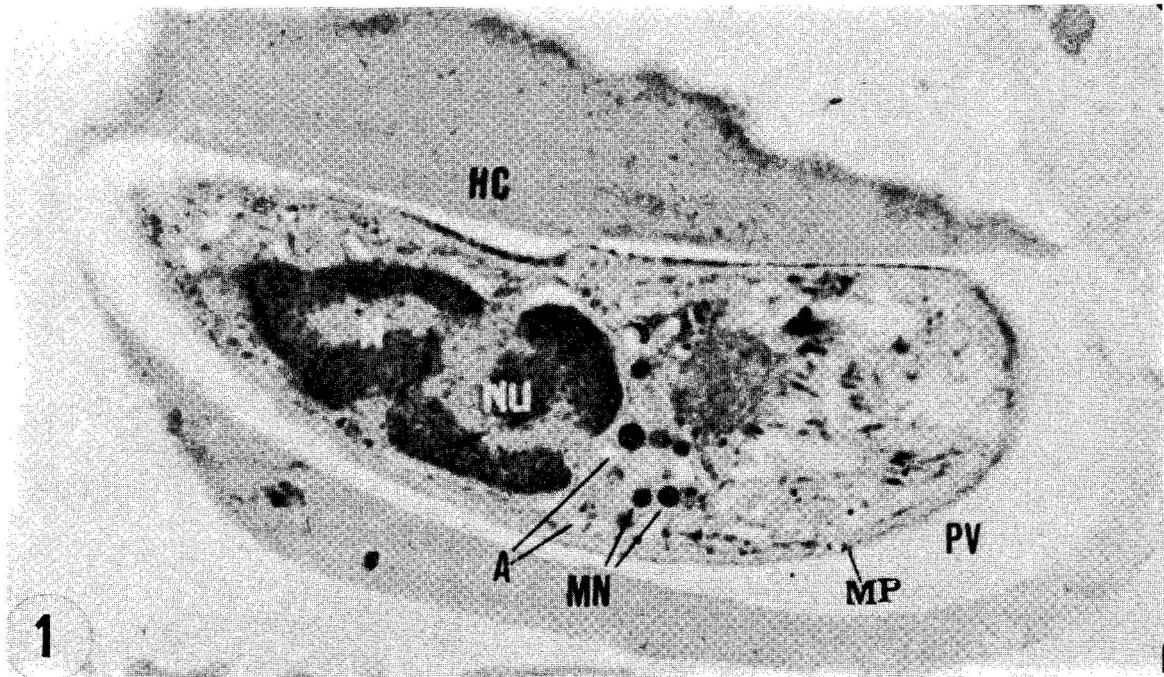


Fig. 1 : Longitudinal section of an intracellular parasite within the host erythrocyte (HC), lying within a parasitophorous vacuole (PV), and showing the distribution of the micronemes (MN) on both sides of the nucleus (N). The peripherally arranged chromatin and the nucleolus (Nu) are seen. A micropore (MP) lies anterior to the nucleus (N). Amylopectin granules (A) are also seen X10000

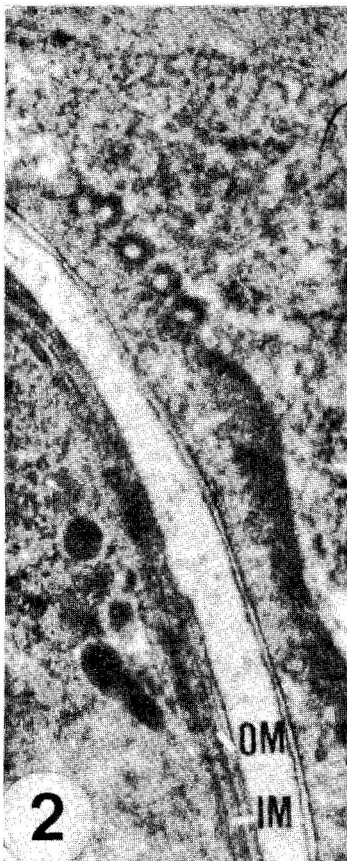


Fig. 2 : Longitudinal section of the pellicle, showing the outer unit membrane (OM) and the inner layer (IM) of the pellicle X 28000

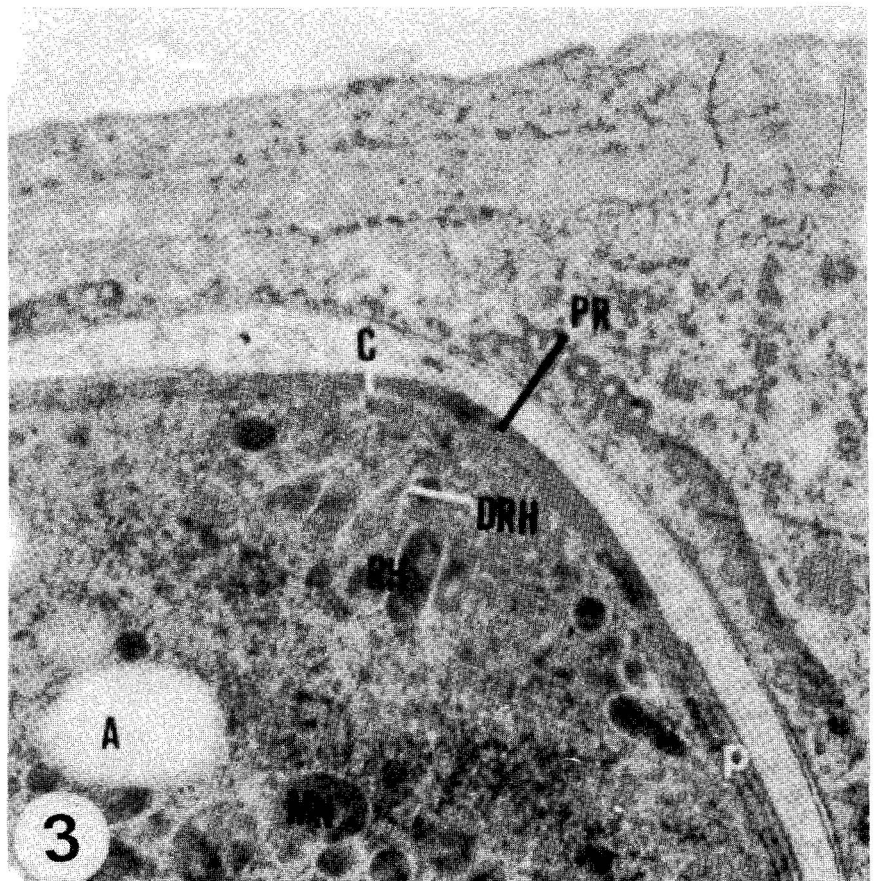


Fig. 3 : Section through the anterior region of the parasite showing the apical complex elements, conoid (C), polar ring (PR), rhoptries (RH) and ductules of rhoptries (DRH) as well as the pellicle (P) and its two membranes; some amylopectin granules (A) and micronemes (MN) are also seen. X 2800

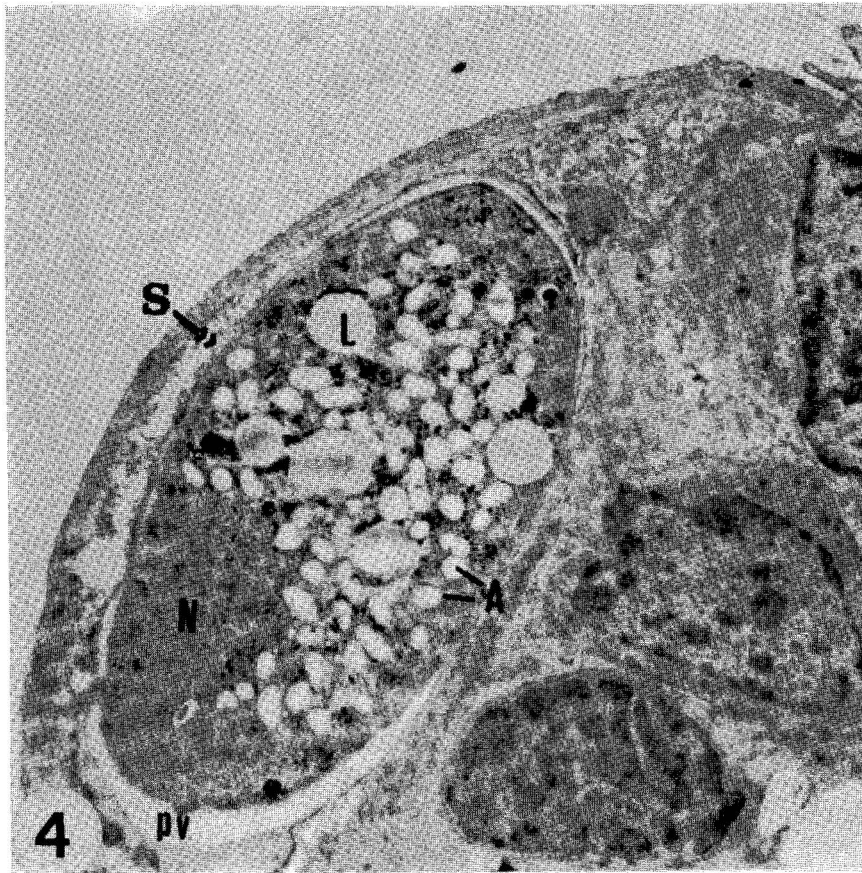


Fig. 4 : A developing schizont (S) which had just invaded the host cell. The parasite still has the features of the blood stages but becoming more rounded, meanwhile the parasitophorous vacuole (PV) begins to group around it. Amylopectin granules (A) as well as lipid (L) vesicles are also seen. X 28000

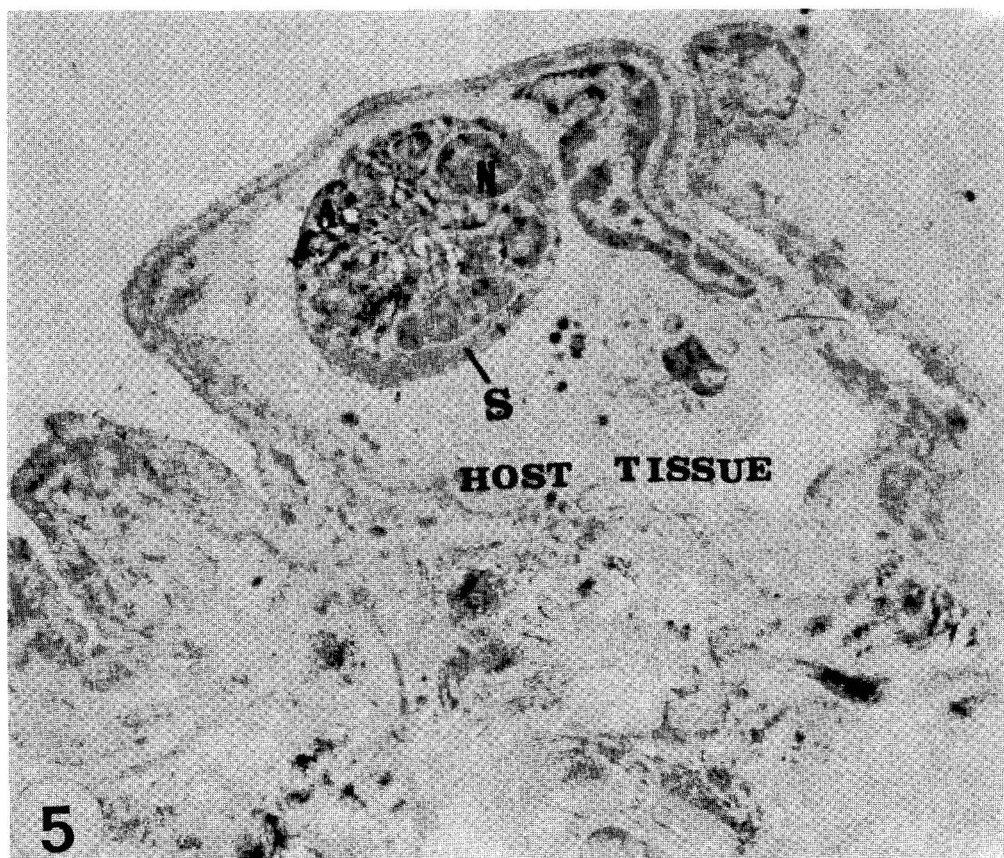


Fig. 5 : An early schizont with peripheral daughter nuclei (N) and amylopectin granules (A). X 3600

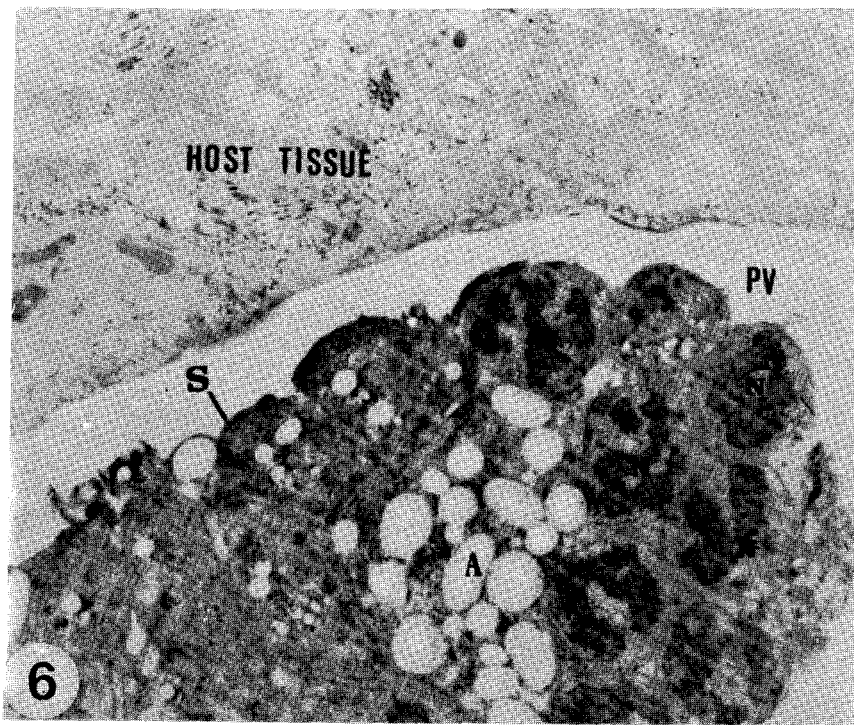


Fig. 6 : A schizont (S) showing a single membrane, the peripheral localization of the daughter nuclei (N) and the accumulation of amylopectin granules (A) in its cytoplasm. The schizont is surrounded by a parasitophorous vacuole (PV). X 6000



Fig. 7 : An enlarged portion of a merozoite (ME) showing that the merozoite has structural similarity to the blood stages having amylopectin granules (A) and micronemes (MN). The developing rhoptries (RHD) and the nucleus (N) are also seen. X 1700

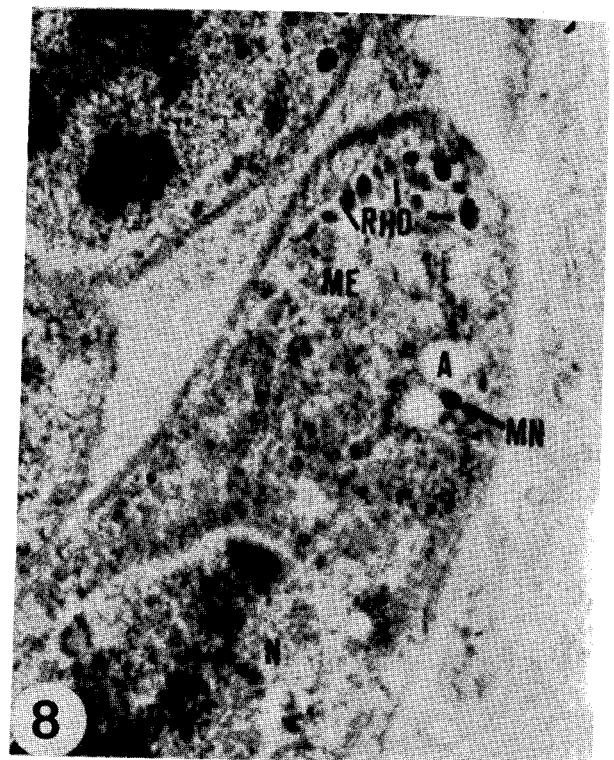


Fig. 8 : An enlarged portion of a merozoite (ME) showing that the merozoite has structural similarity to the blood stages having amylopectin granules (A) and micronemes (MN). The developing rhoptries (RHD) and the nucleus (N) are also seen. X 1700



Fig. 9 : A schizont (S) showing transverse sections of merozoites (ME) and the presence of a parasitophorous vacuole membrane (PVM) enclosing the merozoites and surrounding the parasitophorous vacuole (PV). X 5000

Fig. 10 : An enlarged transverse section of a merozoite showing the nucleus (Nu), micronemes (MN) and amylopectin granules (A). X 10000

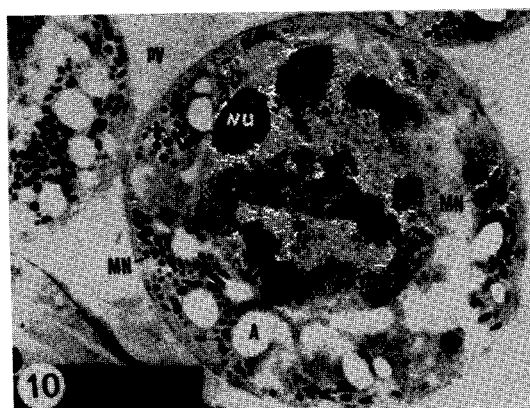
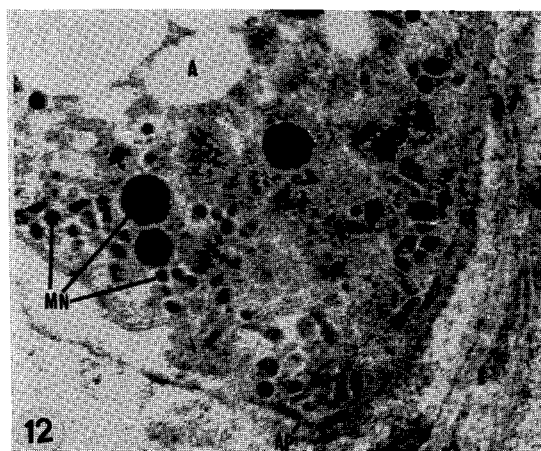


Fig. 11 : A merozoite showing its structural similarity to that of the erythrocytic parasites. X 10000

Fig. 12 : An enlarged end of a merozoite showing the developing rhoptries (RHD) and the amylopectin granules (A) and the micronemes (MN).



The presently recorded thickenings and protrusions of the schizonts, surface membranes, at sites where nuclei in the developing schizonts were present, has been noted by various authors and described as ectomerogony [5, 8, 20].

In the developing merozoites, the conoid, subpellicular microtubules, rhoptries and micronemes appear while the pellicle extend posteriorly enclosing the nucleus. Furthermore, the developing merozoites incorporates its cytoplasmic inclusions before it separates from the residual body of the schizont [5].

In the present work, the fine structure of the free merozoites is closely similar to the intra - erythrocytic stages of the parasite, namely gametocytes, except for the presence of more amylopectin granules in the free forms. This finding is in agreement with previous descriptions of other haemogregarines [4, 5, 6, 13].

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