The fine structure of the gametocytes of an adeleine haemogregarine

Baker, J. R.** Lainson, R.***

SYNOPSIS

Gametocytes of an unidentified haemogregarine (Sporozoa, Telosporea, Coccidia, Eucoccida, Adeleina, Haemogregarinicae) from the frog Rana montezumae were examined by light and electron microscopy. The fully-grown gametocytes lay in a vacuole in the erythrocyte, surrounded by a sheath which was probably of parasitic origin. The gametocytes were bounded by a pellicle of two unit membranes, beneath which lay a ring of small longitudinal fibrils. At the anterior end there were two concentric layers of larger longitudinal fibrils, arranged in a truncated cone, lying between the pellicle and the cytoplasm of the parasite. The cytoplasm contained a few small mitochondria, many inclusions resembling lysosomes, and small vacuoles; there were also two larger, anterior inclusions, which did not appear to run to the tip of the organism. Numerous ribosomes were seen, but no endoplasmic reticulum. The single nucleus contained discrete, dense, granular masses, perhaps deoxyribonucleoprotein, and was apparently limited by only a single membrane.

Two Mexican frogs, infected with trypanosomes, were very kindly given to one of us (J. R. B.) by dr. F. Hawking and mr. M. J.

** Department of Parasitology, London School of Hygiene and Tropical Medicine, Keppel St., London, W.C.
*** Department of Parasitology, London School of Hygiene and Tropical Medicine, Keppel St., London, W.C.

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Worms of the National Institute for Medical Research, London. Both frogs were also infected, one very heavily, with a haemogregarine. Since, to our knowledge, no electron microscope observations had been made on organisms of this group, sections of the parasites were prepared for this purpose. This paper records our findings, together with additional observations made by light microscopy.

Examination of smears and sections of the viscera of the frogs revealed schizonts in smears prepared from the kidney and heart of the heavily-infected animal. To our regret, we have yet to find any of these bodies in sections, in spite of prolonged searching of material from these and other frogs (obtained through the courtesy of Dr. Monroy de Chevez). Consequently we cannot definitely identify the parasite at present. However, the intra-erythrocytic organisms contained no pigment and were far larger than the sporozoites of any known species of Lankesterella or Schellackia; we therefore believe that the parasite must belong to the superfamily Haemogregarinicae of the suborder Adeleina, order Eucoccida.

MATERIALS AND METHODS

Blood was collected from the heart of an anaesthetised female frog Rana montezumae with a pipette, and immediately expelled into excess ice-cold 1% solution of osmium tetroxide, buffered to pH 7.2°. After ten minutes, the material was centrifuged (1800g for five minutes) and the fixative poured off. The resulting pellet of parasites and blood cells was washed in the buffer used in preparing the fixative and passed thru a series of ethanol solutions (10%, 30%, 50%, and 70% for 5-10 minutes each). After storage overnight in 70% ethanol, the pellet was removed from the centrifuge tube and cut into small pieces. Dehydration was completed, the material was stained in 1% alcoholic tungstophosphoric acid, cleared in benzene, and embedded in ‘Araldite’ epoxy resin in gelatin capsules. Polymerization, block trimming and section cutting (on a Cambridge “Huxley” ultramicrotome, using glass knives) followed standard procedures. Sections were mounted on formvar-coated grids and examined in a Zeiss EM-9 electron microscope.
Some of the infected blood was examined by light microscopy, either immediately (using phase-contrast illumination) or after the preparation of dried, Giemsa-stained thin films.

**OBSERVATIONS**

1 **Light microscopy**

No matter how rapidly blood was collected from living frogs and examined, a number of extracellular gametocytes was seen. (This was also true of the material examined by electron microscopy, which had been fixed almost instantaneously). The free forms moved with the gliding motion characteristic of haemogregarines. Indications of some specialization of the protruding anterior end of the organisms was seen (Fig. 1a-c) in living specimens, but not in those which had been dried and stained. Intracellular parasites were surrounded by a thin membrane, which could also be seen in erythrocytes recently vacated by the organism (Fig. 1f). The hind end of the parasite was sometimes bent forward at an acute angle (Fig. 1d). Dried films stained with Giemsa’s stain revealed no detectable sexual dimorphism (Fig. 1g-k). In such films the free forms measured about 25-30 × 6.5µ and the intracellular parasites about 19 × 6.5µ; the latter usually lay alongside the erythrocyte nucleus, which was often displaced laterally (Fig. 1h-k).

In dried, Giemsa-stained smears of heart and kidney, schizonts were seen as large, more-or-less circular masses of cytoplasm, up to 45µ in diameter; the largest seen contained about 100 nuclei, each approximately 3µ in diameter. The schizonts were not obviously enclosed in a host cell, although one or more large nuclei adjacent to some of them might have represented host cell nuclei; it is impossible to speculate about the identity of these (supposed) host cells.

2 **Electron microscopy**

Young gametocytes could be seen lying within erythrocytes with no space intervening between the parasite’s pellicle and the host
cell’s cytoplasm (Fig. 1-2). In one section an apparent channel through the red cell’s cytoplasm might have represented the route of entry of the young parasite (Fig. 2, arrowed). Larger gametocytes (Fig. 3), however, lay in a vacuole within the erythrocyte, the vacuole being appreciably larger than the parasite. The hind end of the parasite was sometimes bent forward at an acute angle, a feature also seen in the living organism examined by phase contrast microscopy (Fig. 1d). In addition to its pellicle, such a mature gametocyte was loosely enclosed within a membrane which was not seen to surround the younger organisms; the membrane, which perhaps is a single unit membrane, was sometimes thrown into long folds (Fig. 3, OM). Organisms seen free in the plasma were no longer surrounded by this outer membrane, which again is in agreement with the appearance under the light microscope (Fig. 4)
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Fig. 1 – Light microscope drawings:
a-f – free-hand sketches of living gametocytes (phase-contrast illumination, x about 1,200)
g-k – camera lucida drawings of dried preparations stained with Giemsa’s stain (x 700)
a, b, c, g – extracellular gametocytes (arrow indicates anterior end in a, b and c)
d, e – young intracellular forms.
f – erythrocyte from which gametocyte has emerged.
h-k – fully grown intracellular forms.

Legend:
OM – Outer membrane (sheath)
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Fig. 2 – Young intracellular gametocyte, showing possible route of entry (arrowed), x about 7,000.

Legend:

N – Nucleus.

(The line on figure represents one micron)
Fig. 3 – Full-grown intracellular gametocyte; note extensions of sheath (OM) within vacuole inhabited by parasite and into red cell cytoplasm, x 15,000.

Legend:
N     – Nucleus.
OM    – Outer membrane (sheath)
PI    – Paired inclusions.
Electron micrographs of sections of gametocytes

Fig. 4 – Extracellular gametocyte, x about 10,500.

Legend:
- PI – Paired inclusions.
- N – Nucleus.
- V – Vacuoles.
Electron micrographs of sections of gametocytes

Fig. 5 – Longitudinal sections thru the anterior end of two extracellular gametocytes, x 40,000.

Legend:

I    – Lysosome-like inclusions.
IF   – Inner fibrils.
OF   – Outer fibrils at anterior end.
P    – Pellicle.
Electron micrographs of sections of gametocytes

Fig. 6 – Longitudinal sections thru the anterior end of 2 extracellular gametocytes, x 40,000.

Legend:
1 – Lysosome-like inclusions.
IF – Inner fibrils.
OF – Outer fibrils at anterior end.
P – Pellicle.
Electron micrographs of sections of gametocytes

Fig. 7 – Part of a transverse section thru the anterior end, x 40,000.

Legend:
- I  – Lysosome-like inclusions.
- IF – Inner fibrils.
- OF – Outer fibrils at anterior end.
- P  – Pellicle.
The pellicle of the gametocyte consisted, over most of the body, of two unit membranes and was $20-25\mu m$ thick (Fig. 8, 10 a; P). At the front end of the parasite, however, further differentiation had occurred. Except for the extreme anterior tip, the pellicle was separated by a short distance (about $60-70\mu m$) from the parasite’s cytoplasm, which was bounded by what appeared in longitudinal section to be a thick line. The intervening space was occupied by what appeared in longitudinal section as an additional line (Fig. 5, 6). In transverse section, however, these two structures were seen to be discontinuous (Fig. 7). We interpret them as two concentric rings of fibrils, the outer (OF in the figures) being flattened radially and measuring about $10 \times 50\mu m$, and the inner (IF) being more ovoid, flattened tangentially, and measuring about $50 \times 25\mu m$ (see the reconstruction in Fig. 11). Two fibrils, one of each kind, were arranged on each radius of the organism and there were about 100 such pairs around each parasite. Transverse sections in other regions of the gametocyte contained a similar number of small sub-pellicular fibrils (Fig. 8, F); it is possible that the larger paired anterior fibrils are derived from these. In sections where this anterior region had been cut obliquely, the fibrils gave the appearance of a thick “cellular” pellicle (Fig. 9). Thus the anterior portion of the parasite was, as it were, reinforced by a truncated cone (composed of the paired fibrils), lying between the pellicle and the cytoplasm; at the extreme front, however, the cytoplasm protruded slightly through the truncated end of the cone and again came into contact with the simple pellicle. Suggestions of this structure were seen under the light microscope before our observations with the electron microscope were begun (Fig. 1a-c)

The cytoplasm of the gametocyte contained many oval or elongate electron-dense inclusions (Fig. 5, 6, 8, 10 and others, I), which somewhat resembled the lysosomes of certain mammalian cells, and rather fewer small vacuoles (Fig. 3, 10 and others, V). There were also two larger electron-dense inclusions lying in the anterior region of the gametocyte (Fig. 3, 4, 8, 9; PI). In one section we saw what appeared to be small mitochondria (Fig. 8, M). The cytoplasm contained numerous small ribosome-like particles, but no endoplasmic reticulum was apparent.
The nucleus of the parasite (Fig. 3, 10; N) contained a number of apparently discrete dense granular masses, perhaps of deoxyribonucleoprotein; the nuclear membrane appeared to consist of only a single unit membrane (Fig. 10 a, NM)

DISCUSSION

From the frequency with which extracellular parasites were seen in blood, no matter how rapidly it was examined, we believe that the organisms must spend part of their life free in the plasma under natural conditions in the living host.

The “reinforcing” of the conical anterior end of the gametocytes, seen in the electron microscope, is suggestive of a penetrative mechanism. It is not known whether the organisms leave and re-enter erythrocytes in the vertebrate host; if they do, the anterior end may perhaps be used in this process. It is, however, difficult to imagine so large an organism being able to penetrate a red blood cell without damaging it. We believe that it is more likely that the parasites, once they have left the red cells, do not return to them. The penetrative anterior structures (if indeed this be their function) may be used during the parasite’s subsequent development in its invertebrate host (possibly a leech); it may be significant that the anterior end of the ookinete of Plasmodium is apparently organized rather similarly to that of the haemogregarine gametocytes described above.

The lysosome-like bodies seen throughout the cytoplasm of the gametocytes resemble structures described (under various names) from the sporozoites and ookinetes of malaria parasites. The larger paired bodies seen in the gametocytes bear a superficial resemblance to the “paired organelle” described from the sporozoites of Plasmodium, especially in transverse section, but there was nothing to suggest that they extended to the anterior tip of the gametocytes; we have no evidence as to their nature. The numerous small vacuoles may represent lipid droplets.
Electron micrographs of sections of gametocytes

Fig. 8 – Transverse section thru another gametocyte, behind that shown in Fig. 7, x 30,000.

Legend:
F – Subpellicular fibrils.
I – Lysosome-like inclusions.
M – Mitochondria.
P – Pellicle.
PI – Paired inclusions.
Electron micrographs of sections of gametocytes.

Fig. 9 – Oblique longitudinal section thru anterior end, x 20,000.

Legend:
- **P** – Pellicle
- **OF** – Outer fibrils at anterior end
- **IF** – Inner fibrils
- **PI** – Paired inclusions
- **N** – Nucleus

(The line on figure represents one micron)
Electron micrographs of sections of gametocytes

Fig. 10 – Transverse section thru nucleus, x 20,000.
Legend:
V – Vacuoles.
N – Nucleus.
I – Lysosome-like inclusions.
Electron micrographs of sections of gametocytes

Fig. 10a—Part of Fig. 10 at higher magnification to show apparent single nuclear membrane (NM), x 50,000.

Legend:
P – Pellicle
NM – Nuclear membrane
Fig. 11 – Diagrammatic reconstruction of sagittal half of anterior end of gametocyte; only peripheral structures are shown in detail.

Legend:

- **F** – Subpellicular fibrils.
- **P** – Pellicle.
- **OF** – Outer fibrils at anterior end.
- **IF** – Inner fibrils.
The outer membrane surrounding the intracellular parasites presumably represents the “sheath” often seen under the light microscope to surround this and other haemogregarines; its origin is obscure, but since the space between it and the parasite contained somewhat electron-dense material, it seems likely to us that it is a product of the parasite rather than the host-cell.

REFERENCES

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