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9. ABSTRACT

Motility of Plasmodium berghei ookinetes, which developed in primary and established cell line cultures obtained from Anopheles stephensi mosquitoes, was studied by using still photomicrographs and normal speed cinephotomicrography. At 18-72 hr after inoculation of P. berghei infected blood from hamsters or mice, motile ookinetes were seen in both mosquito cell cultures; the most active specimens were observed at 24-30 hr. Ookinetes underwent a sporadic forward gliding movement, during which a variable degree of rotation of the body upon its longitudinal axis usually occurred. Some specimens rotated repeatedly upon their axes without any forward progression. The direction of the gliding movement always coincided with the curvature of the ookinete body. In those specimens in which no rotation of the body occurred, a circular course resulted. Ookinetes covered a distance of as much as 50 μm during a single gliding movement. A few ookinetes undergoing locomotion appeared to leave a path or trail on the substrate. Occasionally, an ookinete penetrated a red cell with its slender anterior projection, resulting in lysis of the cell. After red cells had been penetrated by ookinetes, the parasites already within these cells fused with each other to form larger spheroidal bodies. Penetration of cultured cells was not observed.

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Motility of *Plasmodium berghei* Ookinetes in vitro[1]

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Motility of *Plasmodium berghei* ookinetes, which developed in primary and established cell line cultures obtained from *Anopheles stephensi* mosquitoes, was studied by using still photomicrographs and normal speed cinephotomicrography At 18-72 hr after inoculation of *P berghei* infected blood from hamsters or mice, motile ookinetes were seen in both mosquito cell cultures; the most active specimens were observed at 24-30 hr Ookinetes underwent a sporadic forward gliding movement, during which a variable degree of rotation of the body upon its longitudinal axis usually occurred. Some specimens rotated repeatedly upon their axes without any forward progression. The direction of the gliding movement always coincided with the curvature of the ookinete body In those specimens in which no rotation of the body occurred, a circular course resulted Ookinetes covered a distance of as much as 50 μm during a single gliding movement A few ookinetes undergoing locomotion appeared to leave a path or trail on the substrate Occasionally, an ookinete penetrated a red cell with its slender anterior projection, resulting in lysis of the cell After red cells had been penetrated by ookinetes, the parasites already within these cells fused with each other to form larger spheroidal bodies Penetration of cultured cells was not observed.

INTRODUCTION

Motility is characteristically present in various stages in the life cycle of *Plasmodium* spp. and related sporozoa. Microgametes move by flagella. However, the mechanism involved in swimming or gliding movements of sporozoites, merozoites, and ookinetes is not well understood. We report herein some light microscope observations of the movement of *P berghei* ookinetes, which developed in mosquito cell cultures (Rosales-Ronquillo and Silverman, 1974).

MATERIALS AND METHODS

The 10- to 28-day-old primary cell cultures (PCC), obtained from fragments of newly hatched larvae of *Anopheles stephensi* (Rosales-Ronquillo et al., 1972), as well as 4- to 7-day-old cultures of a cell line (SCL), obtained from *A. stephensi* and established by Schneider (1969), were grown on 10 × 50 mm coverslips in Leighton tubes at 21°C. The method described by Rosales-Ronquillo and Silverman (1974) was used to obtain in vitro development of *P. berghei* ookinetes. Cultures were inoculated with approximately 2 to 4 million hamster or mouse red cells containing *P. berghei* gametocytes and then incubated at 21°C. At 6-hr intervals between 12 and 96 hr after inoculation of infected blood, medium or coverslips were removed from Leighton tubes and examined in single- or double-coverslip preparations (Parker, 1961) with Zeiss-Nomarski interference-contrast microscopy. Ookinete motility was recorded on 35 mm Plus-X negative film or on 16 mm 7231 Eastman Plus-X negative film by using normal speed (960 frames/min) cinemicrography. Filmed sequences of specimens undergoing locomotion were then studied to determine details of ookinete motility.

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RESULTS

After inoculation of PCC and SCL cultures with infected blood, microgametocytes exflagellated within 7-10 min, fertilization of macrogametocytes occurred soon thereafter and within 18 hr zygotes developed to mature ookinetes by a process similar to that described previously for *P. berghei* (Rosales-Ronquillo and Silverman, 1974).

In PCC and SCL cultures, motile

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ookinetes were first seen at 18 hr after inoculation of infected blood. Ookinetes were most active at 24–30 hr and thereafter progressively decreased in activity until movement ceased at 40–72 hr. Some individual ookinetes were observed to undergo intermittent locomotion for a period as long as 15 hr.

Ookinetes moved by a sporadic forward gliding movement. The direction of this gliding movement always corresponded with the curvature of the body and usually was accompanied by a longitudinal rotation of the body; this resulted in forward progression in a spiral course (Figs. 1–11). The degree of motility varied considerably among various specimens. In the most active ookinetes, the body appeared to rotate approximately 180° on its longitudinal axis while advancing forward one body length (Figs. 1–7). Usually, this required 5–20 sec. Less active ookinetes rotated about 360° while moving forward one body length or less, and some rotated repeatedly on their

FIGS 1–18 Photomicrographs of ookinetes of *Plasmodium berghei* undergoing locomotion. All are of living specimens photographed with Zeiss-Nomarski interference-contrast microscopy, × 1800. Type of culture in which ookinetes developed, passage number of culture, and interval in hours after inoculation of cultures with *P berghei*-infected blood are listed in parentheses Abbreviations A, anterior end of ookinete, IRBC, infected red blood cell; N, nucleus of ookinete, O, ookinete, RBC, red blood cell, RM, red blood cell membrane.

FIGS 1–7 Ookinete undergoing helical gliding movement, note that as ookinete progresses forward approximately one body length, it rotates upon its longitudinal axis about 180° (PCC, O, 65).

FIG 1 Ookinete with its anterior end adjacent to red blood cell

FIG 2 Ookinete has advanced forward a distance of about one half its body length while its body rotated about 90°.

FIG 3 Ookinete has progressed one full body length and rotated about 180°

FIG 4 Ookinete has traveled two body lengths and rotated 360°

FIG 5 Ookinete has progressed approximately three body lengths and rotated 540°.

FIG 6 Ookinete has rotated another 120° while advancing an additional distance equal to approximately ⅔ its body length.

FIG 7 Ookinete has progressed a total distance of approximately 4 5 body lengths and rotated twice (720°) upon its axis.

FIGS 8–13 Two ookinetes, one of which is undergoing a helical gliding movement (Figs. 8–11), the other a circular gliding movement (Figs 8, 9, 12, 13) (SCL, 191, 24).

FIG 8 One ookinete is undergoing a helical gliding movement, the other specimen (arrow) is circling about an infected red cell

FIG 9 Gliding ookinete has progressed only slightly while the "circling" ookinete has moved to a 9 o'clock position

FIG 10 Gliding ookinete has rotated 180° on its axis and advanced one body length.

FIG 11 Gliding ookinete has undergone a 360° rotation while advancing two body lengths.

FIG 12 The ookinete (arrow) in Figs 8 and 9 has now revolved 1 5 times around the infected red cell.

FIG 13 Ookinete (arrow) is now situated at a 4 o'clock position

FIGS 14–18 Ookinete undergoing a circular gliding movement which then penetrates a red cell with its anterior projection (PCC, O, 43)

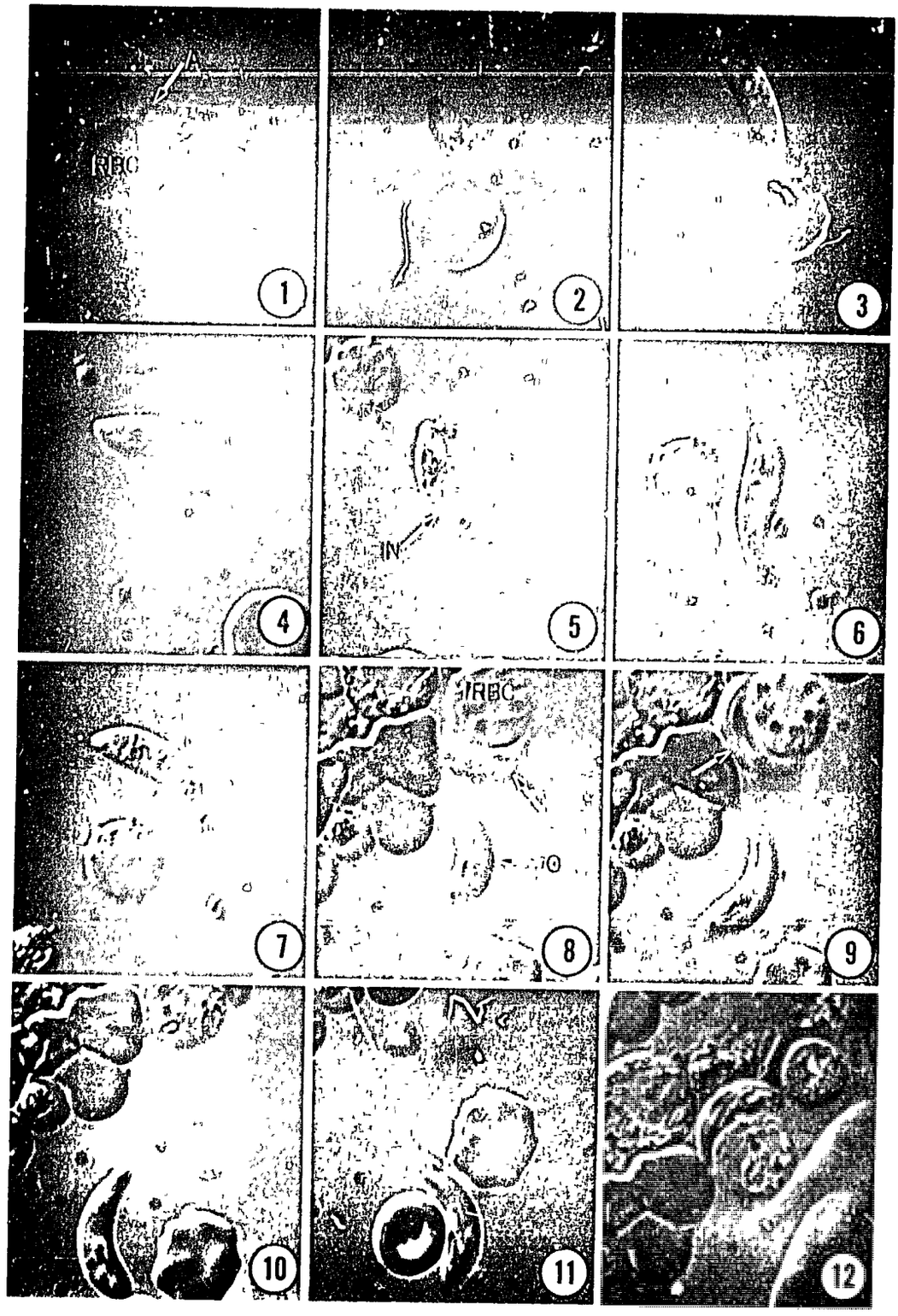
FIG 14 Ookinete with anterior projection (arrow)

FIG 15. Ookinete has moved closer to the red cell.

FIG 16 Ookinete has indented the red cell with its anterior end

FIG 17 The red cell has lysed, note that the anterior projection (arrow) of the ookinete is visible within the red cell and that the cell contains three separate parasites (double arrow).

FIG. 18 Ookinete has moved forward slightly, note that the three parasites in Fig. 17 have fused to form a single body (arrow)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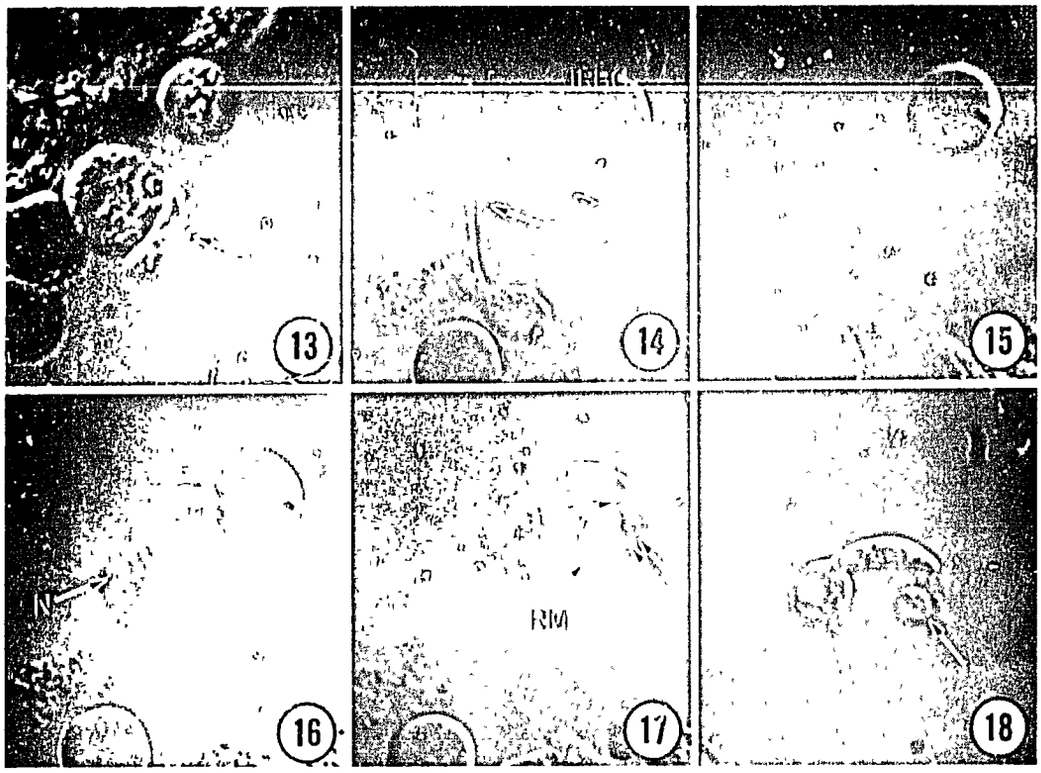

longitudinal axis without any forward progression. This latter type of movement is similar to the pivoting movement characteristically present in sporozoites of *Eimeria* spp., which attach to the substrate with the posterior end and rotate on their longitudinal axis (Fayer and Hammond, 1967). The time required for a 360° rotation of the ookinete body varied considerably, requiring only 10–20 sec or as much as several minutes. When no rotation of the body occurred, a circular course resulted. The diameter of the spiral motion varied with the degree of curvature of the body. A large spiral path occurred in those specimens with a greater body curvature. Specimens with only a slight curvature traveled in a relatively straight path. The distance covered in each gliding movement varied from a few to more than 50 μm. Some ookinetes appeared capable of moving equally well against the flow of media across the slide. Occasionally, a path or trail on the substrate could be seen for a short distance behind a moving ookinete. Ookinetes which were observed moving among several blood cells rotated longitudinally, conforming to the convex surface of a single cell and then to the depression between two adjacent cells. Some specimens closely adhered to the surface of some cells and repeatedly circled about these cells (Figs. 8, 9, 12, 13). Immature ookinetes which had a relatively large bulbous-like structure (Figs. 14–18) at the posterior end were also capable of moving; however, longitudinal rotation of the body appeared to be more difficult. Flexing of the body was not observed in any ookinetes.

Some ookinetes had an anterior projection which underwent lateral probing movements. Ookinetes in the process of moving occasionally indented the surface of blood cells before pushing the cells to one side. Occasionally, an ookinete penetrated a red cell with its anterior end, resulting in lysis of the cell (Figs. 14–18). Immediately

after parasitized red cells had lysed due to penetration by an ookinete, parasites within these red cells appeared to fuse together to form a larger spheroidal body (compare Fig. 17 with Fig. 18). Ookinete penetration of cultured cells was not seen.

DISCUSSION

Little is known about the mechanism of locomotion in sporozoans. There are no apparent specialized organelles in sporozoites, merozoites, or ookinetes which might provide locomotion. Schaudinn (1903) observed the presence of a gelatinous material at the posterior end of *P. vivax* ookinetes and sporozoites undergoing locomotion. Occasionally, in the present study, we observed a similar trail of material immediately behind some moving ookinetes. Rhoptries which are located in the anterior one half of the parasite (Canning and Sinden, 1973) may be involved in the formation of such a substance. This seems questionable, however, since Aikawa (1966) and Hepler et al (1966) found that during the early stages of host cell penetration the rhoptries of the merozoite decrease in size and disappear, indicating that these organelles are spent during the penetration process

Since ookinetes have an apical complex, multilayered pellicle, and subpellicular microtubules (Garnham et al., 1969), which are also present in sporozoites and merozoites (Aikawa, 1971), movement in these stages may occur by a similar process These stages also have a somewhat similar shape and undergo a similar type of helical gliding movement. Speer et al. (1974) observed spiral waves on the surface of *P. berghei* ookinetes which had been prepared for scanning electron microscopy. They suggested that such waves may result from twisting of the ookinete body or may actually be involved in locomotion Roberts et al. (1971, 1972) found similar wave-like elevations of the pellicle along the entire length of *Eimeria larimerensis* and *Isospora canis* sporozoites, respectively, which were believed to have been fixed while in the process of gliding. In a light microscope study of motility in ookinetes of four species of *Plasmodium*, Freyvogel (1966) observed prominent annular waves of contraction in *P. gallinaceum* ookinetes which formed at the anterior end and then moved posteriorly. In an electron microscope study, Garnham et al. (1969) also found wave-like elevations in the pellicle of *P. berghei voelii* ookinetes. In the present study, such waves were not observed in ookinetes of *P. berghei*, probably due to inherent limitations of the light microscope. The above evidence suggests, however, that these waves of contraction which apparently originate at the anterior end and then pass along the surface of the ookinete as well as other motile stages of the parasite may play an important role in locomotion.

In the present study, we observed fusion of several parasites with one another to form a larger body within red cells, which had just been penetrated by an ookinete. Within the parasitophorous vacuole of cultured kidney cells, several *E. magna* macrogametocytes were observed by Speer and Hammond (1974) to fuse together, resulting in the formation of a multinucleated organism. Fusion of these intracellular parasites with one another in these in vitro systems is apparently abnormal

We did not observe penetration of cultured cells by *P. berghei* ookinetes, but did observe penetration of red cells by the anterior end of some specimens, which resulted in lysis of the cell. Since ookinetes normally penetrate mucosal cells of the midgut of mosquitoes, the significance of penetration of a red cell is difficult to explain. The mechanism by which ookinetes enter cells is not clearly understood Normally macrogametes are fertilized in the midgut of the mosquito and subsequently develop into ookinetes, which then penetrate the mucosa and migrate to the external border of the gut, where development into the oocyst stage occurs (Garnham et al., 1962). Stimulation of ookinetes by a substance present in the midgut of mosquitoes may be necessary for penetration of the mucosa and

subsequent development of oocysts within the host. Rosales-Ronquillo and Silverman (1974) have obtained development of ookinetes in mosquito cell cultures but not beyond. Speer et al. (1970) found that bile salts or bovine bile stimulated motility in merozoites of several species of *Eimeria* and that a greater number of bile-treated *E callospermophili* merozoites entered cultured cells than untreated ones. Perhaps treatment of ookinetes with a substance from mosquitoes may play an important role in their further in vitro development A better understanding of motility and penetration of cells by these organisms should help in attempts to interfere with completion of the life cycles of malarial parasites as well as other sporozoa.

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