

REPORT DOCUMENTATION PAGE				
AD-A198 947		1d. RESTRICTIVE MARKINGS		
		3. DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited		
4. PERFORMING ORGANIZATION REPORT NUMBER(S) NMRI 87-86		5. MONITORING ORGANIZATION REPORT NUMBER(S)		
6a. NAME OF PERFORMING ORGANIZATION Naval Medical Research	6b. OFFICE SYMBOL (if applicable)	7a. NAME OF MONITORING ORGANIZATION Naval Medical Command		
6c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055		7b. ADDRESS (City, State, and ZIP Code) Department of the Navy Washington, D.C. 20372-5120		
8a. NAME OF FUNDING/SPONSORING ORGANIZATION Naval Medical Research and Development Command	8b. OFFICE SYMBOL (if applicable)	9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER		
8c. ADDRESS (City, State, and ZIP Code) Bethesda, Maryland 20814-5055		10. SOURCE OF FUNDING NUMBERS		
		PROGRAM ELEMENT NO. 61102A	PROJECT NO. 3M16110ZBS	TASK NO. AF 427
				WORK UNIT ACCESSION NO. DA301616
11. TITLE (include Security Classification) <u>PLASMODIUM FALCIPARUM: FREEZE-FRACTURE OF THE GAMETOCYTE PELLICULAR COMPLEX</u>				
12. PERSONAL AUTHOR(S) C.A.M. Meszoely; E.F. Erbe; R.L. Steere; J. Trospen; R.L. Beaudoin				
13a. TYPE OF REPORT Report No. 6	13b. TIME COVERED FROM 1986 TO 1987	14. DATE OF REPORT (Year, Month, Day) 1987	15. PAGE COUNT 10	
16. SUPPLEMENTARY NOTATION Reprinted from: Experimental Parasitology 64: 300-309, 1987				
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)		
FIELD	GROUP	SUB-GROUP		
			Plasmodium falciparum freeze-fracture	
			parasitic protozoa gametocyte	
			human malaria structure	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)				
<p>DTIC ELECTED AUG 04 1988</p>				
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input checked="" type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION Unclassified		
22a. NAME OF RESPONSIBLE INDIVIDUAL Phyllis Blum, Information Services Division		22b. TELEPHONE (include Area Code) 202-295-2188	22c. OFFICE SYMBOL ISD/ADMIN/NMRI	

Plasmodium falciparum: Freeze-Fracture of the Gametocyte Pellicular Complex

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(Accepted for publication 4 February 1987)

MESZOELY, C. A. M., ERBE, E. F., STEERE, R. L., TROSPER, J., AND BEAUDOIN, R. L., 1987. *Plasmodium falciparum*: Freeze-fracture of the gametocyte pellicular complex. *Experimental Parasitology* 64, 300-309. Freeze-fracturing has been used to study the architecture of the pellicular complex of the gametocytes of *Plasmodium falciparum*. The gametocyte is surrounded by three membranes and a layer of subpellicular microtubules. During freeze-fracturing, each of the three membranes is split along its hydrophobic interior to yield a total of six fracture faces. The most obvious feature of each fracture face is the presence of globular intramembranous particles on their surfaces. The six fracture faces differ from one another in arrangement, size, and density of these intramembranous particles. In gametocytes, unlike in sporozoites, the intramembranous particles are always distributed randomly and lack any definite pattern or orientations. A unique feature of gametocytes revealed by the freeze-fracturing technique is the presence of several transverse sutures on the middle membrane that encircle the gametocyte and give it a segmented appearance. © 1987 Academic Press, Inc.

INDEX DESCRIPTORS AND ABBREVIATIONS: *Plasmodium falciparum*; Protozoa, parasitic; Malaria, human; Freeze-fracture; Gametocyte; Structure; Protoplasmic face of vacuolar membrane (PV); External face of the vacuolar membrane (EV); External face of middle membrane (EM); Protoplasmic face of middle membrane (PM); Protoplasmic face of inner membrane (PI); External face of inner membrane (EI).

INTRODUCTION

The first ultrastructural study on the gametocytes of the malarial parasite was carried out by Duncan and his colleagues in 1959 using the avian parasite *Plasmodium cathemerium*. Since that early report, several other transmission electron microscope studies have appeared. Garnham and

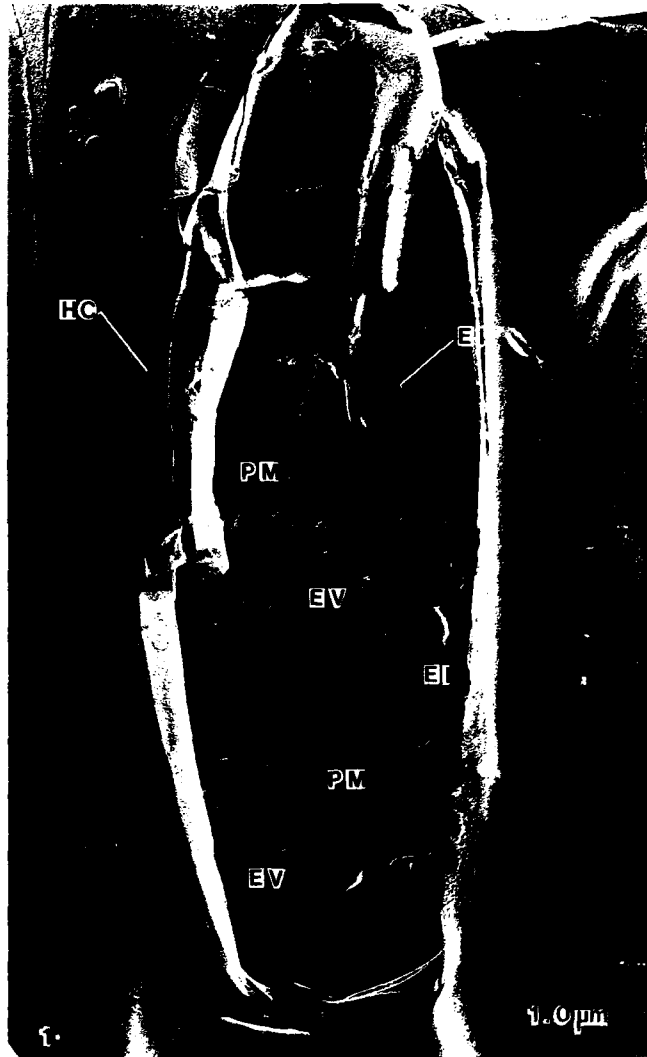
his co-workers (1967) examined the fine structure of *P. berghei*, Rudzinska and Trager (1968) that of *P. coatneyi*, and Aikawa and Jordan (1968) that of *P. floridense*. More recently, Sterling and Aikawa (1973) reviewed the ultrastructure of the avian Haemosporidia, and Sinden and his co-workers (1976) studied the gametogenesis in the rodent malaria *P. yoelii*. All of these studies were limited to the exami-

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nation of ultrathin stained sections except for that by Sinden (1975) where the scanning microscope was also used to examine microgametogenesis in the above species.

In contrast to the numerous conventional transmission electron microscopic studies

on gametocytes of the malarial parasite, no detailed study to date has been carried out using the techniques of freeze-fracturing on the sexual stages of the life cycle. Freeze-fracturing furnishes detailed information about the membrane anatomy not obtain-



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FIGS. 1-8. Abbreviations for all figures: PV, protoplasmic face of the *Plasmodium falciparum* vacuolar membrane; EV, external face of the vacuolar membrane; EO, external face of the outer membrane; PO, protoplasmic face of the outer membrane; PM, protoplasmic face of the middle membrane; EM, external face of the middle membrane; EI, external face of the inner membrane; PI, protoplasmic face of the inner membrane; HC, host cytoplasm; PF, protoplasmic face of the host erythrocyte membrane; FV, food vacuole; N, nucleus.

FIG. 1. Intraerythrocytic gametocyte of *Plasmodium falciparum* showing three fracture faces and transverse sutures (arrow) dividing the middle membrane of the parasite into at least nine segments.

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able by other methods and has been used to elucidate the membrane architecture in several stages of the malaria parasite's life cycle: the asexual erythrocytic stage was studied by Ladda and Steere (1969), Seed *et al.* (1971), Meszoely *et al.* (1972), and McLaren *et al.* (1977, 1979), and exoerythrocytic stages by Meszoely *et al.* (1975). The mosquito stages have been examined by Dubremetz *et al.* (1979), Aikawa *et al.* (1979), and Meszoely *et al.* (1982). To date, the only freeze-fracture study on the gametocytes is a brief report by Meszoely *et al.* (1982). The present study was undertaken to describe in detail for the first time the architecture of the pellicular complex in gametocytes of the malaria parasite of man, *P. falciparum*.

MATERIALS AND METHODS

The erythrocytic stages of the NF54 strain of *Plasmodium falciparum* obtained from the University of Nijmegen and the Brazilian clone 7G8 line provided by the Walter Reed U.S. Army Institute of Research were used as sources of *P. falciparum* parasites. Gametocytes were produced *in vitro* by static culture techniques slightly modified from those previously described (Ifediba and Vanderberg 1981). Parasites in asexual stages were killed by treatment with mitomycin C 3 days prior to fixation (Sinden *et al.* 1984). This provided pure cultures of nearly mature stage IV and mature stage V gametocytes.

The parasitized red blood cells were fixed in 2% glutaraldehyde and cryoprotected in 40% glycerol. This preparation was frozen and fractured in a modified Denton DFE-2 freeze-etch module. Both fracture faces of the specimen were shadowed simultaneously to obtain complementary replicas as described by Steere (1973). Stereo pairs with specimen tilt of 10° between electron micrographs were obtained with the JEM-100-B transmission electron microscope equipped with 60° top entry goniometer stage.

RESULTS

The mature intraerythrocytic gametocytes, stages IV and V of *Plasmodium falciparum*, appear cigar shaped and sausage shaped, respectively, in freeze-fractured preparations filling most of the cytoplasm of the host erythrocyte (Figs. 1, 3, 6a, 7a, b). When the parasite is fractured transversely, it is roughly circular in outline. At this time, micro- and macrogametocytes cannot be readily differentiated from one another in freeze-fractured preparations.

The pellicular complex of the gametocyte consists of three membranes as well as a single row of subpellicular microtubules (Figs. 4, 8). During freeze-fracturing, each of the membranes fractures along its hydrophobic interior, yielding a total of six fracture faces. Intramembranous particles of different size and distribution cover

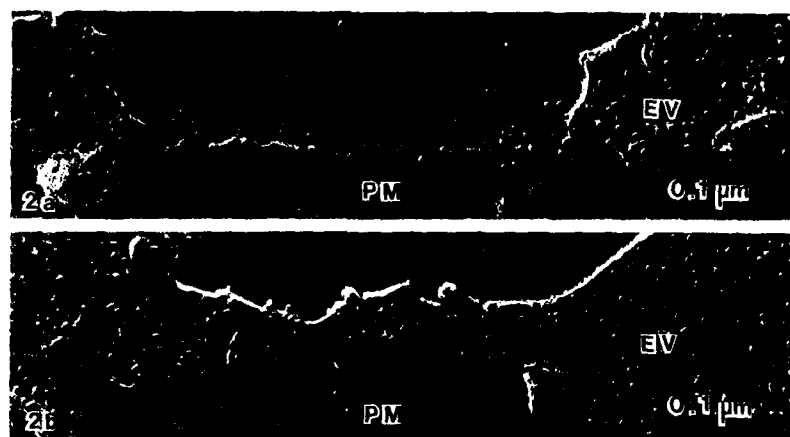


FIG. 2. *Plasmodium falciparum*: Gametocyte with transverse suture. (a) Transverse suture segmenting the middle membrane. (b) EV face of the outer membrane adhering to the suture and covering it. Note that the outer membrane shows no trace of the suture.

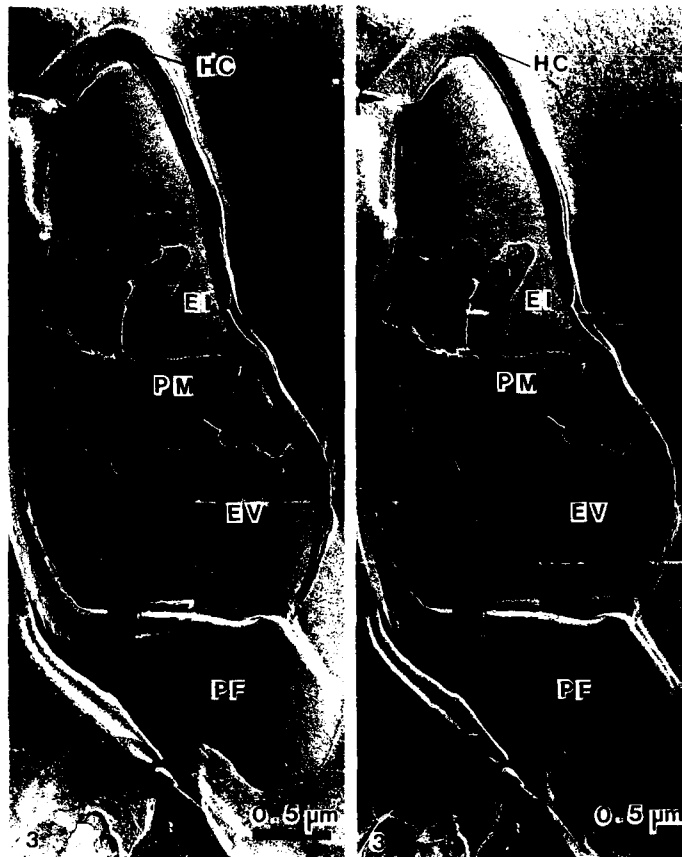


FIG. 3. *Plasmodium falciparum*: Gametocyte showing three fracture faces and also showing the protoplasmic face (PF) of the host erythrocyte. Suture (arrow).

these fracture faces (Figs. 4, 5). Nearly all the fracture faces differ from one another except EV and EI, which closely resemble one another. The nomenclature for labeling is based on that of Branton *et al.* (1975) and McLaren *et al.* (1979). The faces are described in order from the outside toward the interior of the gametocyte. The outermost membrane of the pellicular complex of the gametocyte is derived from the host erythrocyte, and consequently its fracture faces (PV and EV) are referred to as vacuolar. The outer plasma membrane of the host erythrocyte has been studied by freeze-fracture techniques by McLaren *et al.* (1977) and is not considered here.

The PV is covered by particles of approximately 10 nm in diameter. The density of these particles per unit area is about one-fourth the number seen on the opposing EV, which is densely covered with particles of approximately the same size as those found on PV. Particle size varies with most particles in the 10-nm range to a few down to 5 nm. The EM is covered by numerous particles of much smaller size (5 nm) than seen on the previous fracture faces PV and EV, but the density of these particles per unit area is about the same as observed for EV. The PM is nearly smooth with only a few widely scattered particles ranging in size from 5 to 10 nm present on

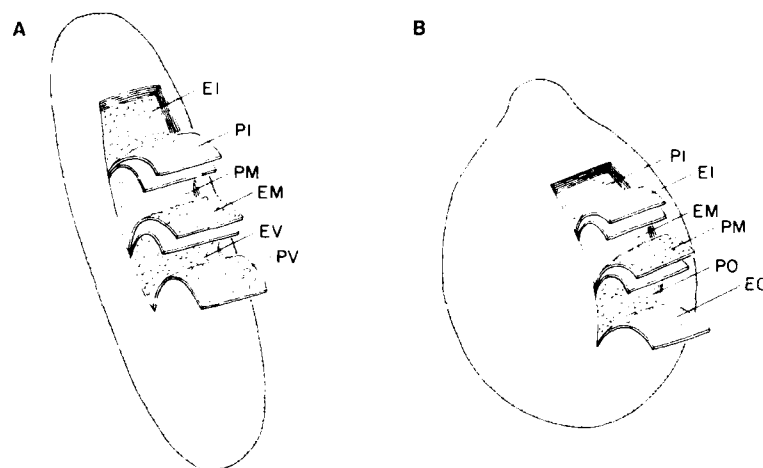


FIG. 4. *Plasmodium falciparum*: Diagrammatic reconstruction of the fracture faces of (A) the intraerythrocytic gametocyte (shown without the host erythrocyte) and (B) the free merozoite. Note that while the fracture faces of the outer membranes of the two stages look very similar, the two are not homologous. The outer membrane of the gametocyte is derived from the host erythrocyte while that of the merozoite is of parasite origin.

its surface. The PI is also nearly smooth, but the size of the few particles present is only in the 5 nm range. The EI resembles very closely that of EV in both the size and density of its particles.

A unique feature of the gametocyte visible only in freeze-fracture preparations is the presence of several transverse suture lines in the middle membrane that appear to divide the parasite into several segments (Figs. 1, 2, 3). Up to 12 segments and 11 sutures have been observed in a single parasite. The distance between sutures is rather uniform, measuring on the average around 0.7 μm . The suture is not present on either the outer or the inner membranes of the gametocyte, but its presence is marked on the inner membrane by a faint depression or groove. Also, the outer membrane shows strong affinity to the middle membrane along the suture line and remains attached to this area, even though the greater part of this membrane is fractured away (Fig. 2b).

In addition to the fracture faces of the membrane, subpellicular microtubules are also visible in crossfractured gametocyte

preparations (Fig. 8), as well as other cell organelles including nuclei (Fig. 7), cytochrome (Fig. 5a), and food vacuoles (Fig. 8).

DISCUSSION

A pellicular complex consisting of three membranes and row of subpellicular microtubules can be clearly identified in the freeze-fractured preparations of *Plasmodium falciparum* gametocytes. The six fracture faces resulting from the splitting of these membranes are distinguishable from one another by size and number of the intramembranous particles on their surfaces.

Unlike sporozoites of *P. yoelii*, *P. berghei*, and *P. knowlesi* (Dubremetz *et al.* 1979; Aikawa *et al.* 1979; Meszoely *et al.* 1982) and trophozoites of *P. knowlesi* (McLaren *et al.* 1979), the intramembranous particles of the gametocytes of *P. falciparum* are not organized into a distinct pattern but are randomly distributed on all six of its fracture faces. The pellicular system of the gametocyte in regard to particle distribution surprisingly resembles that of the free merozoite (McLaren *et al.* 1979). This is unexpected since the free

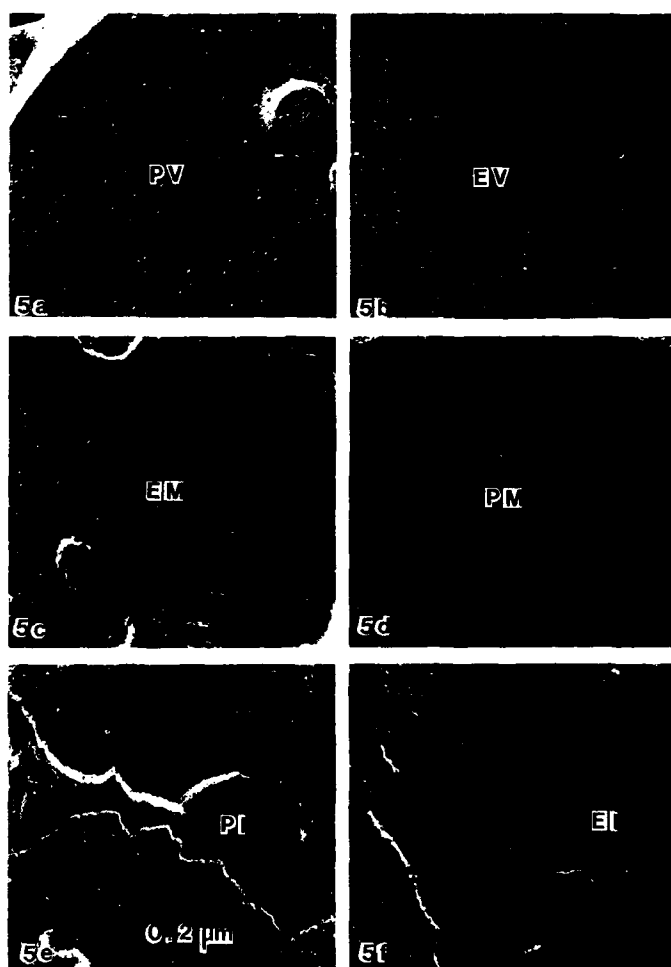


FIG. 5. *Plasmodium falciparum*: The six fracture faces of the gametocyte arranged from (a) to (f) in order toward the interior of the gametocyte. The plug-like structure in the right hand corner of (a) is a cytostome.

merozoite's outer membrane is presumed to become the gametocyte's middle membrane as the parasite invades the erythrocyte and carries the red cell's plasma membrane with it, transforming it into the outer or vacuolar membrane of the pellicular complex of the gametocyte (Sterling and Aikawa 1973). Little is known about the early development of the gametocyte, but considerable rearrangement of intramembranous particles would have to take place in these membranes for them to be homolo-

gous. Also, the outer or vacuolar membrane of the gametocyte bears little resemblance to that of the trophozoite, and the two are regarded as homologous. These observations strongly suggest that major rearrangements take place in the pellicular complex when the malarial parasite enters another stage of its life cycle, and that the architecture of the pellicular system is stage specific and is most likely related to a specific function and location in the host.

Another striking morphological feature

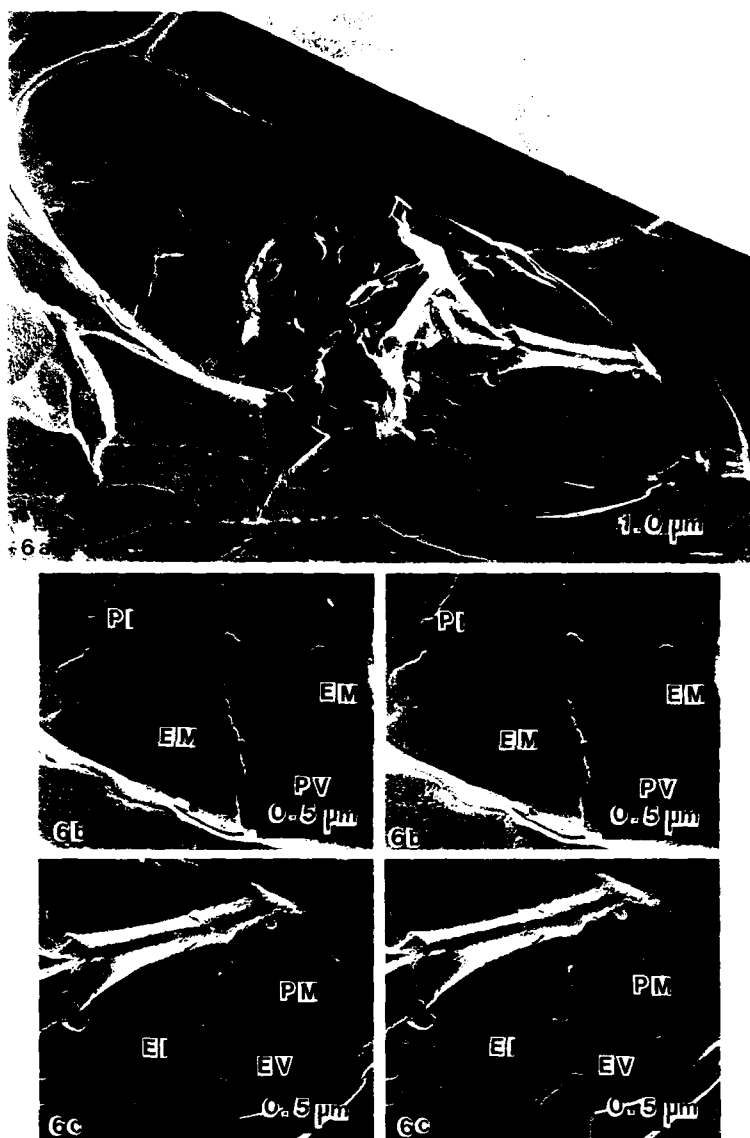


FIG. 6. *Plasmodium falciparum*: (a) Gametocyte showing all six fracture faces and transverse sutures. (b) Stereo pair of the above parasite showing the area to the left. (c) Stereo pair of the area to the right.

found was a series of transverse sutures on the gametocyte middle membrane. These sutures subdivide the middle membrane into several ring-like plates giving the parasite a segmented appearance. The transverse sutures on the gametocyte are unlike the single longitudinal suture reported for

the sporozoite (Meszoely *et al.* 1982) both in their appearance and in their orientation. In the sporozoite, only a single suture occurs, which runs parallel to the long axis of the parasite, whereas in the gametocyte, up to 11 of these transverse sutures have been observed, and they are always ori-

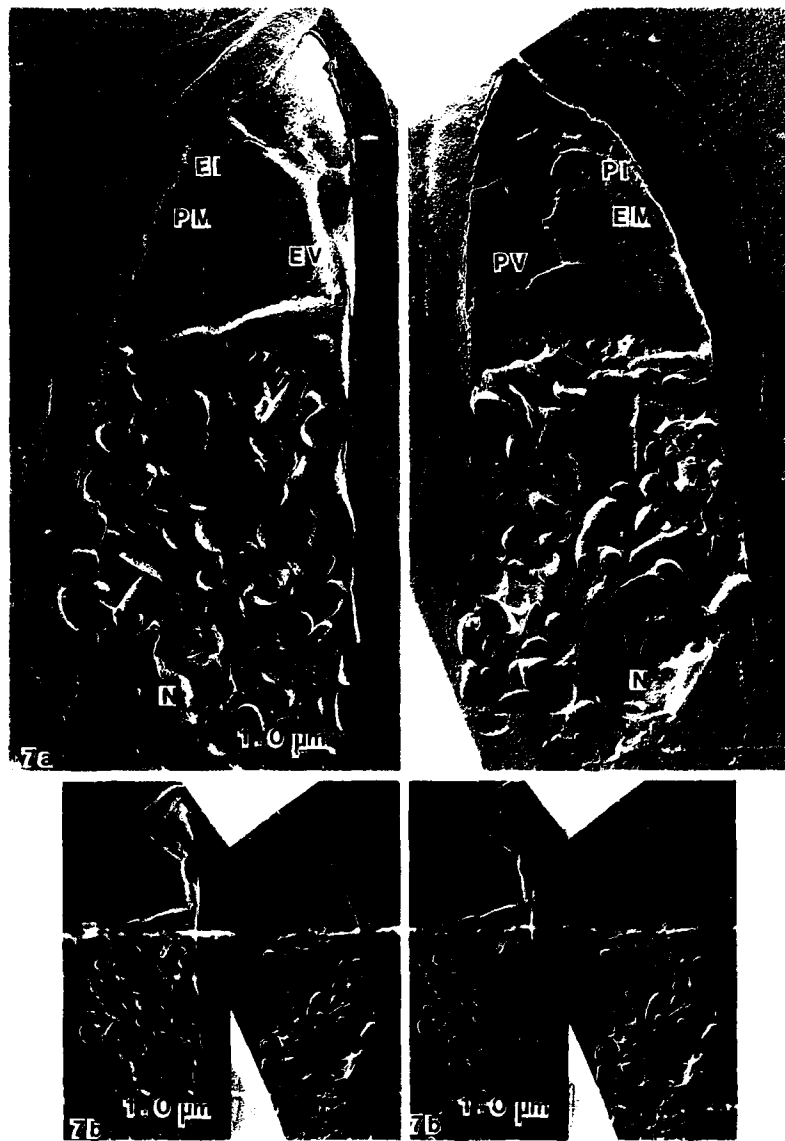


FIG. 7. *Plasmodium falciparum*: (a) Complementary replica of a gametocyte showing all six fracture faces and some of its organelles. (b) Stereo complementary pair of the same.

ented at a right angle to the long axis of the parasite. Also, the longitudinal suture in the sporozoite involves both the middle and the inner membrane, whereas the transverse ring sutures of the gametocyte are solely on the middle membrane. It is interesting to note that pieces of the outer membrane frequently remain attached to

the middle membrane in the vicinity of the suture, while most of the remaining outer membrane fractures away, which suggests some special affinity the outer membrane has for the middle membrane at the level of the suture.

As pointed out above, evidence from this and other freeze-fracture studies indicates

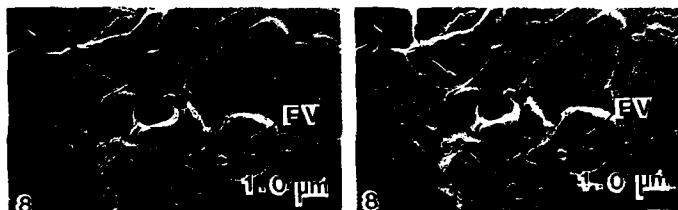


FIG. 8. *Plasmodium falciparum*: Stereo pair of the gametocyte showing subpellicular microtubules (arrows).

that the parasite pellicular structure undergoes major changes during its life cycle and especially that there are major rearrangements in intramembranous particle distribution. Since intramembranous particles are generally regarded as membrane proteins, it follows that significant rearrangements of these proteins occur as the parasite goes from one stage of its life cycle to another. This suggestion is supported by observations that antigens of the different *Plasmodium falciparum* life cycle stages also differ and are stage specific.

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